The
OXOID MANUAL
9th Edition 2006

Compiled by E. Y. Bridson
(substantially revised)
(former Technical Director of Oxoid)

Price: £50

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CONTENTS

1
INTRODUCTION

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INTRODUCTION

The origins of OXOID Ltd go back to the beginnings of the science of microbiology.

Justus von Liebig (a famous chemist who clashed with Louis Pasteur about the microbiological cause of fermentation) had long been concerned about malnutrition in the poor of Europe. In 1860 he devised a concentrated meat extract which could be stored at room temperature without risk of spoilage. He called it "Extractum carnis" and he hoped it could be made available to everyone. This hope could not be achieved in Europe because of the high price of meat. However, in 1861, George Christian Giebert, a Belgian engineer working in Uruguay, read of this work and of Liebig's promise to help anyone who could produce the Extract to the same high standards. Both men knew that in South America, cattle were being slaughtered in thousands, solely for their hides and fat, the meat being abandoned to rot. Giebert visited Liebig in his Munich factory, learned the process and raised money in Antwerp to create a meat extract factory at Fray Bentos in Uruguay. Liebig approved of Giebert's product and allowed it to be called Liebig Extract of Meat. By 1865, production was so successful that the company was running out of money. This problem was solved when the Liebig Extract of Meat Company was formed and registered in London that same year. Both scientist and engineer had succeeded in their tasks. When Liebig died in 1873, he knew that his excellent extract was available to all in Europe. When Giebert died, a year later, he knew that he had established a sound industrial basis for the production of high quality products. Later more factories were established in South America, with surrounding ranches to breed cattle.

After Liebig's death, it was no longer possible to protect the great man's name on the bottle of Extract. Inferior Liebig Extracts began to appear on the market. To overcome this problem the Liebig Extract of Meat Company registered the trade mark LEMCO, from its initials.

Whilst sales of LEMCO and its by product Corned Beef continued to rise, the Company expanded its product range. Another meat extract, OXO was developed for English taste which preferred its high salt, low fat piquant flavour. It was this product which formed the penny OXO cube, a cheap and convenient form of nourishment. The commencement of the First World War in 1914 severed all links with Belgium and the Liebig marketing company Oxo Limited was formed in London that same year to sell products in the UK.

In 1924 Oxo Limited formed a Medical Division to sell glandular products to doctors under the trade name OXOID. About this time, LAB-LEMCO was developed for use in culture media. It was formulated from pale-coloured, low fat meat extracts which were more appropriate for the growth of micro-organisms. This was also the period when Liebig-Oxo increased investigation into enzymic- and acid-hydrolysis of meat and vegetable proteins to increase flavour and amino-nitrogen content of OXO. This work would eventually lead to the familiar peptones, such as Bacteriological Peptone L37.

The Second World War in 1939 presented new challenges and opportunities for change. The formation of the Emergency Pathology Service (EPS) to combat epidemics and the threat of biological warfare, meant that bacteriological investigations increased greatly. The development of penicillin in the 1940s gave further impetus to this activity. The EPS was transformed into the Public Health Laboratory Service and penicillin was followed by many other antibiotics. The Medical Division of Oxo Ltd., began to turn its attention to this rapidly growing market where infectious disease diagnosis and the industrial production of allied biologicals were of increasing importance. When, in 1957, it was decided to stop sales of pharmaceutical products, the replacement products (Oxoid Culture Media) were already being developed. Experience in the War had shown the value of dehydrated media and it was decided that this would be the main activity of the Oxoid Division. So successful was this decision that in 1965, Oxoid Limited was created as a separate subsidiary company of Liebig Extract of Meat Company.

In 1968 Liebig Extract of Meat Company merged with Brooke Bond Limited. The merged company was given the name Brooke-Bond Oxo and trade in culture media continued under Oxoid Limited.

In 1984 Brooke-Bond Oxo was purchased by Unilever Plc and for the first time in its history Oxoid was separated from Oxo. This prepared the way for all Unilever's medical products companies to be relaunched under a single international corporate identity, UNIPATH.

Finally, in 1996 Unilever decided to concentrate more on its core businesses and as a result Oxoid became an independent company for the first time in its history.

During the entire period of the Company's development outlined above the science of bacteriology was itself evolving with considerable speed. Early observers of microscopic life forms including Needham (1745) had recognised the need for the preparation of suitable nutrient fluids for their growth but there was a lack of uniformity in the methods followed.

The study of nutrient media possessing more exact composition was initiated by Pasteur in 1860. Cohn developed this work and published the formula for his 'normal bacterial liquid' in 1870. Klebs noted Needham's early observations that saprophytic and putrefactive bacteria grew particularly well in a watery

extract of meat and used a culture fluid of this nature when he commenced study of pathogenic bacteria in 1871. Nageli first described 'peptone' in 1880. He has been credited as the first bacteriologist to discover that chemo-organotrophic organisms grow best in culture media containing a partially digested protein. Robert Koch quickly took up this development and in 1881 described his culture medium which was made from an aqueous meat extract to which was added peptone and sodium chloride. To this day this simple formula is the basic one for culture media.

In the following year (1882) Heuppe described the labour saving convenience of substituting commercial meat extract for Koch's watery extract of fresh meat. By 1902 an American text book of bacteriology was recommending the use of LEMCO for this purpose. This is quite possibly the first record of exporting culture media ingredients by the company.

It will be seen that the business of manufacturing dehydrated culture media was a natural consequence of the converging commercial activities of Oxoid and the development of the science of microbiology. The early history explains why OXOID is one of the very few producers of culture media that actually manufactures its own extracts and hydrolysates.

The OXOID Quality Policy

It is the policy of OXOID, Basingstoke to manufacture and sell OXOID products which are fit for the purpose for which they are intended and are safe in use.

OXOID Ltd (Basingstoke) is registered with the BSI to BS EN ISO 9001 (Reg No. FM 09914) with extended scope to include BS EN 46001: 1997. This standard endorses our quality management system for products manufactured at the Basingstoke site and includes: Dehydrated Culture Media, Selective Supplements, Sterile Reagents, Biochemical Reagents, Laboratory Preparations, Signal Blood Culture System bottles, Susceptibility Discs in cartridges, Diagnostic Reagents, Salmonella Rapid Test and Listeria Rapid Test.

Ready Prepared Media and Lab Ready Media are manufactured by G. M. Procter and are covered under BS EN ISO 9002 Reg No. FM 27644.

The essential elements of the Oxoid Quality management System include:

- product lot testing according to a defined protocol
- documented complaints and technical enquiries procedure
- policy for raw material procurement
- good manufacturing practice combined with in-process control
- comprehensive training for staff at all levels
- conformance to statutory Health and Safety and Environmental requirements

The Oxoid Quality Policy functions through all procedures described above and maintains the company's high reputation for the performance of its products.

Storage of OXOID Microbiological Products

To ensure optimum performance from OXOID products they must be stored under specified conditions and for no longer than the allocated shelf-life. The storage conditions and expiry date of each product are shown on the label, container or product insert. Products should be used in order of their lot/batch numbers.

Light
All prepared culture media should be stored away from light and exposure to direct sunlight avoided at all times.

Humidity
Sealed glass and plastic containers are unaffected by normal laboratory humidity. Opened containers of dehydrated powders will be affected by high humidity. Hot, steamy media preparation rooms are unsuitable environments to store containers of culture media; particularly containers which are frequently opened and closed. An adjacent cooler room or an adequate storage cupboard are preferable storage areas.

Temperature and time
The temperature storage conditions of culture media and their components vary widely. The following product groupings will help to differentiate the various requirements.

Prepared Agar and Broth Media (PM, R products)
Store at 2–8ºC. do not allow the products to freeze.
Shelf life 3 months to 2 years.

Biochemical Reagents (BR products)
Store at 2–8ºC.
Shelf life 1 to 5 years.

Gas Generating Kits
Store at 2–25ºC. in a dry place. Do not store these kits at a higher temperature for long periods.
Shelf life 3 years.

20 months	20 months	20 months
Anaerogen™	Campygen™	CO_2Gen™

Selective and Sterile Reagents (SR products, Selective supplements)
Store at 2–8ºC.

except

Horse Serum SR35	store at –20 to +8ºC
Nitrocefin SR112	
Reconstitution fluid SR112A	store at –20 to +8ºC
Penase SR129	store at –20ºC

Shelf life 1 week to 2 years.

Culti Loops
Store at 2–8ºC or frozen for *Campylobacter* sp.
Shelf life 6–10 months (except *Campylobacter jejuni* – 4–6 months)

Toxin Detection Kits
Store at 2–8ºC — Shelf life 1 year

Salmonella Rapid Test
Store at room temperature 15–25ºC — Shelf life 1 year to 15 months

Listeria Rapid Test
Store at 2–8ºC — Shelf life maximum 18 months

Dip Slides
Store at 10–15ºC — Shelf life 6–9 months

Quanticult
Store at 2–8ºC — Shelf life 6–10 months

Diagnostic Reagents (DR products)
Store at 2–8ºC, do not freeze — Shelf life 9 months to 2 years

Diagnostic Discs (DD range)
Store at –20ºC but keep working stock at 2–8ºC — Shelf life 1 to 2 years

DRYSPOT
Store at room temperature 15–25ºC — Shelf life 2 years

Susceptibility Discs
Store at –20ºC but keep working stock at 2–8ºC — Shelf life 1 to 3 years

Dehydrated Culture Media (CM, L products)
Sealed, unopened containers should be stored at room temperature 15–20ºC.

Opened containers should have the cap carefully and securely replaced. It is important that opened containers are stored in a dry atmosphere at room temperature.

Shelf life 1 to 5 years.

Prepared Plates of Culture Media
Poured plates of agar media are especially vulnerable to infection, dehydration and chemical degradation. Aseptic preparation and storage are essential to protect plates from microbial infection.

Water losses on storage can be minimised by impermeable wrapping and/or storage at 2–8ºC.

Chemical degradation e.g. oxidation or antimicrobial loss, can be retarded by protection from light, heat and dehydration.

Therefore storage of prepared plates at 2–8ºC (unless otherwise stated) in the absence of light and protected against moisture loss will minimise agar media deterioration from these defects.

It is important, however, to monitor the storage of prepared plates by quality control tests so that any deterioration can be detected and the storage period accurately determined. Simple weighing tests of fresh and stored plates will determine the rate of moisture loss. Greater than 5% loss of weight will indicate a significant loss of water.

PRECAUTIONS IN MICROBIOLOGY

Manipulations with micro-organisms may release some of them into the environment and lead to laboratory-acquired infections. Such release may be entirely accidental or it may be intrinsic in the technique or equipment used. Even the most careful worker, using the best methods and the correct equipment, is not immune from accidents and errors. Over 4500 such infections have been reported so far this century[1].

Accidents that release micro-organisms include spillage and breakage. Activities that frequently release micro-organisms include opening cultures, using inoculating needles and loops, using hypodermic needles, pipetting, mixing, homogenising, and centrifuging[1].

Micro-organisms released into the environment may enter the bodies of workers and other people in and around the laboratory and initiate infections. Those most at risk are clinical laboratory and research staff. Even in industry, e.g. in food testing laboratories, pathogens that are present in small numbers in samples submitted for examination may be concentrated by culture into infectious doses.

ROUTES OF INFECTION

Micro-organisms may enter the human body by any of several routes: through the respiratory tract, the alimentary tract, the skin, and the conjunctivae.

The Respiratory Tract – Inhalation

Very small droplets of liquids – aerosols – that may contain micro-organisms are generated when films of liquids are broken, e.g. when cultures are opened or broken, liquids are pipetted violently, bursting bubbles, splashes, falling drops impacting on surfaces, and breakages in centrifuges. The smallest of these droplets, those less than 5µm in size, remain suspended in the air and dry rapidly. The organisms they contain then become “droplet nuclei” and are moved around the room or to other parts of the building by air currents. If they are inhaled they are small enough to reach the alveoli, where they may initiate an infection. Larger droplets sediment rapidly under the influence of gravity and may contaminate benches, equipment or the hands. If they are inhaled they are trapped and removed in the upper air passages.

The Alimentary Tract – Ingestion

Workers’ hands may be contaminated by spillage and by the larger aerosol droplets. The organisms may then be transferred to the mouth by the fingers, or by contaminated pencils, pipettes, food etc.

The Skin

Although the intact skin is a good barrier against micro-organisms, the exposed parts, e.g. the hands and face, are frequently damaged by small cuts and abrasions, many of which may not be visible to the naked eye. These are portals of entry for micro-organisms.

In addition, ‘sharps’ injuries are not uncommon in laboratories[2]. Pricks and cuts with needles, knives, broken glass, etc. will allow the entry of micro-organisms.

The Conjunctivae

The very thin membranes surrounding the eyes are readily penetrated by micro-organisms in splashes or from contaminated fingers. Some people touch their eyes several times an hour.

CLASSIFICATION OF MICRO-ORGANISMS ON THE BASIS OF HAZARD

It is obvious that not all micro-organisms have the same capacity to cause infections, and that infections vary in their incidence, their severity, and the availability of prophylaxis and therapy. By international agreement micro-organisms are now classified into groups or classes according to the hazard they offer to workers and the community. There are four groups, ranging from the relatively harmless to the very hazardous. The wording varies slightly from state to state and that used in Europe[3] is shown in Table 1.

Lists of bacteria, viruses, fungi and parasites in Groups 2, 3 and 4 have been published by various national and international agencies, e.g. the European Union[3,4]. Micro-organisms not listed in these Groups are assumed to be in Group 1, although some of them may be responsible for allergies. There are inevitable disagreements, globally, because of differences in the geographical distribution, incidence, and local significance[5].

TABLE 1

Classification of micro-organisms on the basis of hazard and laboratory containment level

Class	*Description*	*Laboratory*
1	Unlikely to cause human disease.	Level 1
2	May cause human disease; might be a hazard to laboratory workers; unlikely to spread in the community; laboratory exposure rarely causes infections; effective prophylaxis and therapy available.	Level 2
3	May cause serious human disease; may be serious hazard to laboratory workers; may spread in the community; effective prophylaxis and therapy available.	Level 3
4	Causes severe human disease; serious threat to laboratory workers; high risk of spread in the community; no effective prophylaxis and therapy	Level 4

Based on the classification of the UK Advisory Committee on Dangerous Pathogens[6].
Classes (also known as Groups) 2, 3 and 4 include known pathogens.
Class 4 contains only viruses.

CLASSIFICATION OF LABORATORIES ACCORDING TO HAZARD GROUP

It follows from the classification of micro-organisms on the basis of hazard that precautions against laboratory-acquired infections should vary from minimal for those in Group 1 to maximum security for those in Group 4. Such precautions and safety requirements have been codified as Containment of Biosafety Levels[1,3–5]. These are outlined in Table 2. General precautions are considered below.

Where there are disagreements in classifications the local system should be regarded as the minimum, but there is no reason why microbiologists, if they think fit, should not use higher levels of precautions than those prescribed nationally.

TABLE 2

Summary of laboratory design features for laboratory containment levels

	Containment Level			
	1	***2***	***3***	***4***
Laboratory isolated and sealable for decontamination	—	—	+	+
Directional ventilation (inward)	—	D	+	+
Filtered air exhaust	—	—	+	+
Double door entry	—	—	O	+
Airlock with shower	—	—	—	+
Autoclave on site	+	+	+	+
in workroom	—	—	O	+
double ended	—	—	—	+
Microbiological safety cabinets				
Class I or II available	—	+	+	+
in workroom	—	—	+	+
Class III	—	—	O	+

Based on WHO Laboratory Biosafety Manual[5]
Key: — not required; + essential; D desirable; O optional

GENERAL PRECAUTIONS AGAINST LABORATORY-ACQUIRED INFECTIONS

There are several international and national guidelines and codes of practice[1,5–10]. Only outlines can be given here.

Personal Protection

Protective clothing should be worn at all times in the laboratory. Gowns, coats and overalls should be fastened at the sides or back, cover the chest and neck areas and fit closely at the wrists. Workers should remove this clothing before leaving the laboratory and not wear it in rest rooms, offices, libraries etc. Gloves should be worn if there is a risk of contaminating the hands, especially with blood. Disposable (latex) gloves should be worn once only and then autoclaved with other laboratory wastes. Re-usable gloves should be washed while still on the hands and then disinfected before re-use5. Safety spectacles should be worn during microbiological and chemical manipulations. Hands should be washed often and always before leaving the laboratory.

Laboratory Equipment

Inoculating Loops

Long wires vibrate and shed droplets, as do large and poorly made loops. The wires should be no longer than 6cm and the loops not more than 2 mm in diameter and completely closed. Plastic disposable loops are to be preferred as they do not need flaming but may be placed in disinfectant immediately after use.

Glassware

Chipped and scratched glassware is hazardous and should never be used.

Pasteur Pipettes

Glass Pasteur pipettes should not be used as they are often responsible for cuts and punctures of the skin. Soft plastic pipettes are safer.

Hypodermic Needles
To avoid 'needlestick' accidents pipettes and cannulas should be used instead of hypodermic needles. Opening devices for vaccine and septum-capped bottles are available.

Centrifuges
Accidents with centrifuges may release massive aerosols. They are often the result of improper handling. Centrifuges should be placed on low benches so that all operators can see the inside of the bowl when loading them. Buckets and trunnions should be inspected regularly for evidence of corrosion and hairline cracks; any suspect parts should be discarded. When not in use buckets should be placed upside down in racks to drain any fluid used in balancing. Buckets should be paired by weight and labelled accordingly. Paired buckets should be placed opposite one another for use. At least 2 cm clear space should be left between the top of the fluid in a centrifuge tube and its rim. Centrifuge tubes should be stoppered and sealed buckets used for any material that is potentially infectious. Paired buckets, with tubes in situ, should be balanced by adding 70% alcohol (NOT saline, which may corrode metal, leading to mechanical failure) to the space between the tube and the bucket. Instructions for use of centrifuges and action to be taken if a centrifuge tube breaks, usually indicated by a sudden change in sound and/or visible imbalance of the machine, should be posted adjacent to each machine.

Physical hazards associated with centrifuges are discussed in detail by Kennedy[11].

Water Baths
The water in water baths may become contaminated from the outsides of culture tubes or the leakage of their contents. These baths, even those operated at temperatures >60ºC should be emptied when not in use or a deposit may form in which micro-organisms can grow. A disinfectant that does not attack metals may be added to the water in baths that are in continuous use (hypochlorites should not be used; see below).

Homogenisers and Shakers
Bench-mounted models may generate aerosols and should be covered, (e.g. by clear plastic boxes) when in use. These covers should be disinfected after use. Hand-held homogenisers should be held in a wad of cotton wool in case they break. Homogenisers and containers from shakers should be opened in microbiological safety cabinets.

Pipetting
Pipetting by mouth, even water, should be banned. Pipetting devices should be provided. Pipettes should not be blown out vigorously, otherwise bubbles and aerosols may be formed.

Microbiological Safety Cabinets
These should conform to national standards and should be tested regularly by independent engineers to ensure that their performance is in accordance with the requirements of that standard. These cabinets are designed to protect the user from the inhalation of infectious aerosols and air-borne particles. They give no protection against spillages of cultures or against chemicals. Class II and Class III cabinets also protect the test or product from external air-borne contamination.

Microbiological safety cabinets should be used only by experienced personnel who have received proper instructions about their limitations. They must not be used as fume cupboards or for work with flammable or toxic substances.

They should be decontaminated at regular intervals by qualified staff who follow manufacturers', or other recognised procedures[1,5,10].

Laminar Outflow (clean air) Cabinets
These are NOT microbiological safety cabinets. They are designed to protect the work from external air-borne contamination and do not protect the worker, whose face and respiratory tract receive air that has passed over the workpiece. (See Cell and Tissue culture, below).

Fume Cupboards
Fume cupboards are designed to protect workers and the environment from toxic chemical fumes and gases. They should not be used for micro-organisms or other living material.

SPILLAGE AND BREAKAGE

Spillage of cultures and chemicals and breakage of vessels containing them must be reported immediately to the supervisor or local safety office. If the spillage is considerable the room should be vacated pending decontamination by qualified staff (see below).

Instructions for dealing with small-scale spillages and breakages should be posted in each laboratory, and should include the following:

- wear heavy-duty gloves
- cover the spillage/breakage with absorbent material, e.g. large paper towels
- pour disinfectant (see Table 3) over the paper towels and leave for at least 15 minutes
- scoop up the paper towels with a dust pan or stiff cardboard and place them along with the dust pan or cardboard, along with any broken glass into a laboratory discard container
- pick up any residual broken glass with forceps and add it to the discard container
- cover the area again with paper towels and pour on more disinfectant. Leave for 30 minutes before any further cleaning up
- autoclave the discard container.

TABLE 3

Properties of some disinfectants

	Active against							*Inactivated by*					*Toxicity*		
	Fungi	*Bacteria*		*Myco-bacteria*	*Spores*	*Viruses*		*Protein*	*Materials*		*Hard water*	*Deter-gent*	*Skin*	*Eyes*	*Lungs*
		G+	*G—*			*Lipid*	*Non lipid*		*Natural*	*Man-made*					
Phenolics	+++	+++	+++	++	—	+	v	+	++	++	+	C	+	+	—
Hypochlorites	+	+++	+++	++	++	+	+	+++	+	+	+	C	+	+	+
Alcohols	—	+++	+++	+++	—	+	v	+	+	+	+	—	—	+	—
Formaldehyde	+++	+++	+++	+++	+++a	+	+	+	+	+	+	—	+	+	+
Glutaraldehyde	+++	+++	+++	+++	+++b	+	+	NA	+	+	+	—	+	+	+
Iodophors	+++	+++	+++	+++	+	+	+	+++	+	+	+	A	+	+	—
QAC	+	+++	++	—	—	—	—	+++	+++	+++	+++	A(C)	+	+	—

+++ Good: ++ Fair: + Slight: — Nil: V Depends on virus: a Above 408C: b Above 208C: C Catonic: A Anionic

From Collins, C.H. (1993) *Laboratory Acquired Infections*. 3rd.edn. by permission of the publishers Butterworth-Heinemann, Oxford

PRECAUTIONS AGAINST BLOOD-BORNE INFECTIONS

In addition to the precautions listed above personnel who handle blood specimens or blood-stained material should wear high quality disposable gloves and also plastic disposable aprons over their normal protective clothing. Guidelines for the safe handling in laboratories of materials that may contain hepatitis and/or the human immunodeficiency virus have been published[1,7,8,12,13].

PRECAUTIONS WITH CELL AND TISSUE CULTURE

Separate accommodations should be provided to minimise contamination of cultures.

Some cells and tissue cultures may contain adventitious and unidentified micro-organisms or viruses from which the operator must be protected. All work with cells and cell lines should therefore be conducted in Class II microbiological safety cabinets. Laminar outflow cabinets (see above) must NOT be used.

STERILISATION, DISINFECTION AND DECONTAMINATION

These terms are not interchangeable. In microbiology:

Sterilisation – implies the complete destruction of all micro-organisms.

Disinfection – is the destruction or inactivation, usually by chemicals, of the vegetative forms of micro-organisms and the spores of some of them. Not all spores are inactivated. Not all spores are inactivated by chemical disinfectants.

Decontamination – usually means making equipment, materials and waste free from infectious agents.

Sterilisation
Here, this is restricted to autoclaving. For other methods, e.g. hot air, standard textbooks should be consulted[1,10].

The hazard most frequently encountered in autoclaving is failure to sterilise, i.e. to achieve and maintain the temperature/time ratio that is known to kill micro-organisms. (The physical hazards of autoclaving are described elsewhere[11]).

Autoclaves should be used only by personnel specifically trained and employed for that purpose. Infected materials and "clean" articles should be treated in separate loads and preferably separate autoclaves. Autoclaves should not be tightly packed: space must be left between articles in the load to enable steam to circulate freely.

The 'Holding time at temperature' (HTAT) for steam sterilisation is normally 20 minutes at 121ºC. The time begins when the temperature in the load has reached 121ºC as indicated by the recorder of the thermocouple in that load, NOT when the drain temperature reaches that temperature[1,10].

Higher temperatures are required for the treatment of material containing 'unconventional agents' (e.g. scrapie, CJD, etc).

Control of Sterilisation
In modern autoclaves this is achieved by instrumentation (thermocouple probes and recorders). It is advisable, however, to include some form of indicator, e.g. 'autoclave tape' in each load, and to check the HTAT independently at regular intervals. Alternatively, or in addition, biological tests may be used in the form of strips that contain Bacillus stearothermophilus[1,10].

Chemical Disinfection
Disinfectants vary in the action against bacteria, spores, fungi and viruses and should be chosen in accordance with the intended use. Most disinfectants are toxic, in varying degrees, and precautions, e.g. eye protection, should be taken when stock solutions are diluted.

Table 3 summarises the properties of some commonly used chemical disinfectants.

Disinfectants should be diluted according to the manufacturers' instructions. It is best to prepare dilutions daily as some deteriorate if use-dilutions are stored. For most purposes hypochlorites are adequate and should be diluted to contain 1,000–2,500 ppm available chlorine for normal work and 10,000 ppm for blood and high concentrations of protein. Industrial hypochlorite solutions usually contain 100,000 ppm available chlorine and should be diluted 1–2.5% or 10%.

Bench discard jars and containers
A discard jar containing an appropriate disinfectant should be provided at every work station to receive small items such as slides, Pasteur pipettes and plastic loops. Large jars, for pipettes are also needed. Plastic containers are safer than glass. Articles placed in these containers should be completely submerged in the disinfectant. Discard containers should be emptied and replaced daily.

Containers for discarded cultures should also be provided at each work station. These should not leak, be shallow – not more than 25 cm deep to facilitate steam penetration during autoclaving, and preferably of heat-resistant plastic. Plastic bags, usually blue or transparent with blue lettering, are used in some (mostly UK) laboratories. They should be supported in the containers described above.

Decontamination of Benches, Equipment and Rooms
Benches should be wiped down with a suitable disinfectant at the end of the working day (gloves should be worn). The accessible parts of equipment may similarly be disinfected but not with hypochlorites as they may attack metals.

Equipment to be serviced must also be decontaminated in this way and clearly labelled to indicate that this has been done and that it should not be used until after servicing.

The working surfaces and inner walls of microbiological safety cabinets should be swabbed with a suitable disinfectant, and the cabinets be fumigated with formaldehyde, as indicated above before filters are changed or maintenance carried out.

Rooms rarely need disinfection unless a major accident has released massive aerosols. Formerly this was done by formaldehyde fumigation, but this is now regarded as hazardous and uncertain. Spraying or washing with disinfectant/detergent mixtures is safer and more effective.

DISPOSAL OF INFECTED WASTE

Infected laboratory waste is included in the definitions of clinical waste and must ultimately be incinerated. Table 4 lists the materials that should be regarded as infectious in microbiological and clinical laboratories. As these are likely to be the most heavily infected of all such waste and may have to travel along the public highway, often for long distances. It is prudent to autoclave it first[1,14,15].

TABLE 4

Infected and potentially infected waste from microbiological laboratories

Disposables other than sharps

- Specimens or their remains (in their containers) submitted for tests containing blood, faeces, sputum, urine, secretions, exudates, transudates, other normal or morbid fluids but not tissues.
- All cultures made from these specimens, directly or indirectly.
- All other stocks of micro-organisms that are no longer required.
- Used diagnostic kits (which may contain glass, plastics, chemicals and biologicals).
- Used disposable transfer loops, rods, plastic Pasteur pipettes.
- Disposable cuvettes and containers used in chemical analyses.
- Biologicals, standards and quality control materials.
- Food samples submitted for examination in outbreaks of food poisoning.
- Paper towels and tissues used to wipe benches and equipment and to dry hands.
- Disposable gloves and gowns.

Sharps

- Hypodermic needles (syringes attached if custom so requires).
- Disposable knives, scalpels, blades, scissors, forceps, probes.
- Glass Pasteur pipettes; slides and cover glasses.
- Broken glass, ampoules and vials.

Tissues and animal carcasses

Bedding from animal cages

Adapted from Collins and Kennedy[14] by permission of the authors and publisher.

CONCLUSIONS

Every microbiological laboratory should have written safety policy and instructions that describe in full the safety precautions deemed necessary by the Director and Safety Officer.

All members of the staff should be aware of the authorised procedures for containing and destroying micro-organisms.

A schedule of regular microbiological safety cleaning should be maintained for all working surfaces and adjacent areas.

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2
CULTURE MEDIA

2006

CULTURE MEDIA QUALITY ASSURANCE

All manufacturing operations are conducted according to protocols which describe such procedures as the monitoring, maintenance, cleaning and calibration of equipment; plant sanitation; warehouse control of incoming materials and materials under test; labelling control and handling, storage and distribution of finished goods. The master formula and accompanying documents for each lot/ batch of product includes manufacturing control and packaging information pertaining to the product.

Quality tests on raw materials include identity tests, tests for performance and compatibility with other ingredients in a pre-production laboratory mix of the medium components. Additional tests are performed where required. For example, peptones are examined physically, chemically and microbiologically. Agars are tested for clarity, gel strength, diffusion characteristics etc.

Dehydrated media mixtures are examined for appearance, homogeneity and moisture content. Representative samples are reconstituted and examined for colour, clarity, pH, gel strength (if agar is present), compatibility with post-sterilisation additives and for microbiological performance. The medium is challenged with a specified inoculum of appropriate reference and target organisms, to measure recovery of growth, colony size and morphology, colour reactions, differentiation and selectivity. Testing complies, where appropriate, with the requirements of standards eg the International Pharmacopoeias, ISO 11133 Microbiology of food and animal feeding stuffs (Guidelines on quality assurance and performance testing of culture media) and the National Committee for Clinical Laboratory Standards (NCCLS) Guidelines.

Special procedures such as antimicrobial susceptibility tests are performed where appropriate for the recommended use of the medium.

All tests are performed in parallel with a previously approved reference/standard batch of the medium. This use of a standard medium with each test ensures uniformity in reading the results. An additional non-selective control medium is used to quantify the inoculum level.

Samples of each manufactured lot/batch are retained for the total shelf-life of the product. Stability testing on prepared and dehydrated culture media is undertaken as real-time storage with the product stored at the extremes of its recommended temperature for the length of its shelf-life. This protocol follows the guidelines proposed by BS EN 13640:2002 Stability Testing of in vitro diagnostic reagents.

FORMULATION OF CULTURE MEDIA: DEVELOPMENT AND MANUFACTURE

The formulation of all Oxoid culture media are published in Section 2.7 and the components can be divided into different roles or functions:

1. **Nutrients:** proteins/peptides/amino-acids.
2. **Energy:** carbohydrates.
3. **Essential metals and minerals:** calcium, magnesium, iron, trace metals: phosphates, sulphates etc.
4. **Buffering agents:** phosphates, acetates etc.
5. **Indicators for pH change:** phenol red, bromocresol purple etc.
6. **Selective agents:** chemicals, antimicrobial agents.
7. **Gelling agent:** usually agar.

There is often an overlap of functions of some media components, thus protein hydrolysates will supply amino-nitrogen, energy, some metals/minerals and

act as buffering agents. Phosphate buffers are important suppliers of minerals and agar contributes metals.

1. Nutrients

Naegeli is credited with the earliest publications (1880/82) describing the requirements of microorganisms for a protein component which he called ‘peptone’.

Later work showed that the group of bacteria, now defined as chemo-organotrophs, required aminonitrogen compounds as essential growth factors in their culture media.

Meat infusions contain water-soluble fractions of protein (amino-acids and small peptides) along with other water-soluble products such as vitamins, trace metals, minerals and carbohydrates (glycogen). Such infusions or extracts may have been regarded as ‘peptones’ but their amino-nitrogen content was usually too low to sustain the growth of large numbers of bacteria.

It was not until deliberate attempts were made to hydrolyse proteins with acids or enzymes that sufficiently high concentrations of water-soluble protein fractions (peptides) were made available for bacterial growth.

Many nutrient media usually contain a mixture of protein hydrolysate (peptone) and meat infusion (meat extract/Lab-Lemco).

The difficulties associated with the production of protein hydrolysates were soon recognised and commercial suppliers of peptones became established by the 1920s. The commercial supply of dried peptone eventually led to complete culture media being produced in the form of dehydrated media.

Although meat was the first and most obvious protein to hydrolyse, other proteins were tried later and some showed specific advantages which ensured their retention in culture media to this day. Casein hydrolysate with its pale colour and high tryptophan content and soya peptone with its high energy carbohydrate content are popular examples of nonmeat peptones.

In recent years, increasing concerns over the inclusion of animal and genetically modified products has led to the development of Veggietones. These are a highly nutritious alternative to conventional culture media and are ideal for use in fermentation and other pharmaceutical processes where genetically modified or animal-based products must be avoided. All the Veggietones are quality control tested to meet US, British, European and Japanese Pharmacopoeia performance standards, as well as those of the NCCLS.

A detailed description of these products is given in Section 3.1 ‘Peptones-Hydrolysates’.

The nutrient components of culture media are carefully selected to recover the required spectrum of organisms in the sample e.g. coliforms or anaerobes. General purpose media such as blood agar in its various forms will often contain mixtures of peptones to ensure that peptides of sufficient variety are available for the great majority of organisms likely to be present. However, more demanding organisms will require supplemental growth factors to be added and examples of such requirements can be seen in media for *Legionella* species.

Most of the components used for the nutrition of microorganisms are undefined and require extensive testing with careful selection to ensure a reasonable degree of uniformity. Would it not be better to use wholly defined peptides and amino-acids to produce a totally defined medium? Whilst such media would improve uniformity, experience has shown that they lack good performance as general purpose media. They would also be very expensive compared with undefined media. The use of totally defined culture media is an understandable goal of most microbiologists but defined media have yet to prove themselves equal in performance to currently used complex mixtures of meat and plant protein hydrolysates.

2. Energy

The most common substance added to culture media as a source of energy to increase the rate of growth of organisms is glucose. Other carbohydrates may be used as required.

Carbohydrates added to media at 5-10 grammes per litre are usually present as biochemical substrates to detect the production of specific enzymes in the identification of organisms. It is usual to add pH indicators to such formulations.

3. Essential Metals and Minerals

The inorganic essential components of culture media are many and can be divided on a semi-quantitative basis:

Typical macro-components (gm/litre): Na, K, Cl, P, S, Ca, Mg, Fe.

Typical micro-components (mgm-microgm/litre): Zn, Mn, Br, B, Cu, Co, Mo, V, Sr, etc.

As previously mentioned, a formulation may not have specific metals and minerals listed in its formulation. In such cases it is assumed that all the factors required are present in the hydrolysates, buffers and agar components.

4. Buffering Agents

It is important that the pH of a culture medium is poised around the optimum necessary for growth of the desired microorganisms. The use of buffer compounds at specific pK values is especially necessary when fermentable carbohydrates are added as energy sources. Phosphates, acetates, citrates, zwitterion compounds and specific amino-acids are examples of buffering agents that may be added to culture media.

A side effect of such compounds is their ability to chelate (or bind) divalent cations (Ca++ and Mg++). Polyphosphate salts, sometimes present in sodium phosphate, are compounds which can bind essential cations so firmly that they are made inaccessible to the micro-organisms.

The effect of these binding or chelating agents will be seen in diminished growth or failure to grow at all, unless care has been taken to supplement the essential cations in the formulation. Opacity forming in a medium, after heating or on standing at 50°C for several hours, is commonly caused by phosphate interaction with metals. Such phosphate precipitates can very effectively bind Fe and lower the available amount of this essential metal in the medium.

5. Indicator Substances

The addition of coloured indicator substances is a very effective way of detecting fermentation of specific carbohydrates in a culture medium. Such compounds should change colour distinctly and rapidly at critical pH values.

Most of the compounds used e.g. phenol red, bromocresol purple, fuchsin, etc., are toxic and it is essential to use low concentrations of pre-screened batches/lots. Known sensitive strains of microorganisms are used in the screening tests.

6. Selective Agents

Chemicals or antimicrobials are added to culture media to make them selective for certain micro-organisms. The selective agents are chosen and added at specific concentrations to suppress the growth of unwanted organisms in a polymicrobial sample. It is, of course, essential to have established that the selective agents, at the appropriate concentration, will allow uninhibited growth of the desired organisms.

Common chemical selective agents are: bile salts, dyestuffs, selenite, tetrathionate, tellurite and azide. Antimicrobial agents are commonly used in mixtures when suppressing polymicrobial contaminating flora. Antimicrobials are more specific in their selective action than the chemical agents shown above. However, the critical weighing and heat-lability of most antimicrobials demand special care and post sterilisation addition. To reduce some of these problems a range of freeze dried antibiotic supplements has been manufactured. These are accurate preparations of antimicrobials designed to add to defined media to create specific, selective formulations.

The wide variety of organisms and their almost infinite ability to adapt to changing conditions makes a truly selective medium unlikely. Selective media can be said to suppress most of the unwanted organisms and allow most of the desired organisms to grow. The final formulation is usually a compromise which achieves the best of these criteria.

7. Gelling Agents

Although gelatin is still used for a few specific media and carrageenans, alginates, silica gel and polyacrylamides are sometimes used as gelling agents, the outstanding gel-forming substance used in culture media is agar.

Hesse, a worker in Robert Koch's laboratory, is credited with its first use in culture media, although Frau Hesse gave him the idea from its use in table-jellies in hot climates.

Its inertness to microbial action, the unique setting and melting temperatures (38°C and 84°C respectively) the high gel strength which allows low concentrations of agar to be used, its clarity and low toxicity have contributed to its wide popularity with microbiologists. Its ability to retain its gel structure at 60°C makes agar of special value to culture media which have to be incubated at this temperature to isolate thermophilic organisms.

Agar is obtained from agarophyte sea-weeds mainly *Gelidium*, *Gracilaria* and *Pterocladia* species. It is extracted as an aqueous solution at greater than 100°C, decolourised, filtered, dried and milled to a powder.

Agar is not an inert gelling agent; it contributes nutrients and/or toxic agents to culture media, depending on the chemical processing carried out by the suppliers.

Microbiological agar is specially processed to yield a low toxicity, high clarity, low mineral and high diffusion gel.

Other Components

There are many other substances added to culture media for specific purposes e.g. growth factors for fastidious organisms, Eh-reducing compounds for anaerobic organisms (thioglycollate and cysteine), whole blood to detect haemolytic enzymes and encourage the growth of organisms which are vulnerable to oxidation products.

Development and Manufacture of Culture Media

The development of dehydrated culture media is a process leading to the large-scale manufacture of a reproducible, stable product. The initial development

of the formulation is usually carried out by microbiologists who wish to create a novel medium with specific characteristics or who wish to improve the performance of an existing product. Such work is usually written up in microbiological journals, having first been judged by some form of peer review and proved to be of special value by other workers in the field.

Simple conversion of the published formula into a mixture of dehydrated components is seldom achieved. Usually the peptone/hydrolysate base has to be adapted and variations in concentration of other components may be required. Laboratory mixes of the medium are prepared as R&D trials and after testing

in the laboratory are sent to the originator for comment. Opportunity may also be taken to get the views of other experts in this field. Special strains of organisms may be required to check the finer points of performance.

Subject to good report, a trial batch will be manufactured and this will be used for larger trials and wider-scale testing. During these trials QC testing and performance criteria will be established and the specifications of the components will be determined. Bought-in components will have buying specifications and in-house components will have manufacturing specifications and standard-operating-processes produced. Stability trials will begin if there is confidence that the final formulation has been achieved.

The reports on the larger and wider-spread trials are studied and if the results are satisfactory preparation will be made to manufacture a full production batch/lot. All the components of the medium, including special protein hydrolysates which may have to be specially manufactured, are assembled and a laboratory mix tested to see that it meets the performance specification. Finally the components are milled, mixed and blended to produce a finely divided, homogeneous powder which is held in large containers for further testing before release.

All this work, plus literature, labels and product inserts is carried out under the aegis of R&D/Marketing. Subsequent production lots are manufactured under our Quality Management System which includes process monitoring and end-product testing by the Product Performance Department.

No product can be released without clearance from The Quality Department.

SPECIAL FIELDS OF CULTURE MEDIA APPLICATION

EXAMINATION OF CLINICAL AND VETERINARY SAMPLES

In both clinical and veterinary microbiology the purpose of examining samples of tissue, fluids or excreta is to isolate and identify pathogenic organisms.

Although both fields of investigation have common interests and common organisms, they are separate specialist activities. Reference should be made to the appropriate specialist publications in either field to obtain specific guidance.

It should be stressed that every specimen must be evaluated, many laboratories cannot cover the whole microbiological field, the various infective agents should be taken into consideration and, if necessary, material referred to the appropriate reference laboratory.

Poor specimen samples can only yield poor or misleading results. It is important that personnel collecting or taking samples are instructed by the laboratory to prevent faulty collection procedures.

Satisfactory samples, collected without extraneous contamination and before antimicrobial therapy should be transferred to the laboratory with minimal delay. If transportation is required then appropriate transport media should be used to protect delicate organisms. Where quantitative results are important e.g. urine cytology and bacteriology, or where commensal overgrowth should be prevented, refrigeration of samples at 2-8°C is essential.

All samples should be clearly labelled and sent in leak-proof, satisfactory containers. Sealed, transparent plastic bags, containing the sample container and the request form attached to but not inside the plastic bag, is the most acceptable method of sending pathological samples to the laboratory.

BLOOD CULTURES

A full description of the Oxoid Signal Blood Culture System and the Isolator Blood Culture System is to be found in the Blood Culture Section. Examination of blood for infectious agents is one of the most important and often most urgent examinations requested. All the various systems of blood culturing require blood samples to be collected with scrupulous care to avoid extravenous contamination. The blood/broth medium should be subcultured to appropriate media either at fixed time intervals or whenever changes in appearance of the medium are noted e.g. turbidity, darkening, lysis etc. Subculture after 24 hours incubation, regardless of appearance, is recommended to detect early evidence of bacteraemia. All subcultures must be made with great care to avoid contaminating the blood/broth medium.

Associated pathogens

Staphylococci (coagulase positive and negative)
Streptococci (alpha/beta/non-haemolytic strains)
Coliform organisms (including other enteric organisms)
Non-fermentative organisms (*Pseudomonas* and *Acinetobacter* species)

Anaerobes (*Clostridia*, *Bacteroides*, *Fusobacterium* species and anaerobic cocci)
Neisseria species
Haemophilus influenzae
Brucella species

Immune-compromised patients are subject to bloodborne infections by any opportunistic organism: mycobacteria, fungi and rare/exotic organisms should be anticipated.

Commensal organisms
None.

CEREBROSPINAL FLUID (CSF)

It is very important that all samples of CSF are examined with minimal delay. A description of the appearance of the sample must be made e.g. colour, clarity, clots etc., the cells, protein and sugar content should then be measured. The following results are indications of infection:

raised polymorphs/low sugar - indicates bacterial infection raised

lymphocytes/normal sugar - indicates viral infection raised

lymphocytes/high protein/low sugar -indicates mycobacterial infection.

If a fibrin clot is present then particular attention should be paid to *Mycobacterium tuberculosis*.

Cell counts are of little validity when clots are present.

Centrifuge a portion of the CSF and make three films of sufficiently small area so that the whole may be examined under the microscope. Stain one film by Gram's stain, one by Leishmann or Giemsa stain and one by an acid-fast bacilli stain. In purulent samples *Haemophilus influenzae* may be difficult to see under Gram's stain. Carbol-thionin or a similar nucleic-acid stain may be helpful to see the bacteria in such circumstances.

Inoculate a portion of the centrifuged sample (taking suitable aseptic precautions) on blood agar (incubate aerobically and anaerobically), 'chocolate' Columbia Agar (incubate in a 5% CO_2 atmosphere) examine after 18-24 hours incubation at 35°C. Carry out antimicrobial susceptibility tests on any organisms isolated. Select appropriate antimicrobials for blood/brain infections. Culture for *Mycobacterium tuberculosis* if the examination results indicate tuberculosis.

Direct tests to identify common bacterial antigens in CSF are available.

Associated pathogens
Haemophilus influenzae
Neisseria meningitidis
Streptococcus pneumoniae
Mycobacterium tuberculosis
Listeria monocytogenes

Nocardia species and *Bacillus Cryptococcus neoformans Coliform* bacilli, Pseudomonas species and Group B streptococci occur in neonates.

Patients involved in surgical manipulations e.g. shunts, valves etc., can become infected with *Staphylococcus epidermidis* and micrococci.

Commensal organisms
None.

SPUTUM

Samples of sputum are often the poorest samples received. The ideal of obtaining discharge from the bronchial tree, without contamination from saliva, is seldom achieved.

Obvious samples of saliva should be rejected. Washing the sample with sterile saline to separate purulent material may be necessary to reduce salival contamination. Homogenisation with Sputosol SR0233 will also help assess the significant flora which may be localised in one small part of the sample. Make films for a Gram's stain and an acid-fast bacilli stain. Inoculate blood agar media and incubate in 5% CO2 atmosphere at 35°C overnight.

A MacConkey Agar (CM0007) plate will help distinguish the coliforms and streptococci frequently found in sputum. If legionellosis is suspected inoculate Legionella BMPA Medium (CM0655 + SR0110 + SR0111).

If mycetoma or other fungal diseases are suspected inoculate Sabouraud Dextrose Agar (CM0041) or Dermasel Agar Base (CM0539+ SR0075).

Associated pathogens
Staphylococcus aureus
Streptococcus pneumoniae
Haemophilus influenzae
Coliform bacilli
Klebsiella pneumoniae
Pasteurella species/*Yersinia* species
Mycobacterium tuberculosis
Branhamella catarrhalis
Mycoplasma species
Legionella species
Candida/*Aspergillus*/*Histoplasma*/*Cryptococcus*/*Blastomyces* species

Commensal organisms
Staphylococcus epidermidis, micrococci, non-pathogenic neisseria, *Streptococcus viridans*, small numbers of *Candida* and coliform bacilli.

EAR, NOSE AND THROAT SWABS

The ENT department will send good samples to the laboratory but samples taken elsewhere may be less satisfactory and care should be taken that staff are instructed on how to take satisfactory ENT swabs.

Ear swabs: make films and stain with Gram's solutions and with methylene blue if diphtheria is suspected. Inoculate blood agar plates and incubate aerobically and anaerobically for 18-24 hours at 35°C.

Inoculate tellurite medium if the swab is from a child of school age or if diphtheria is suspected for other reasons.

Associated pathogens
Staphylococcus aureus
Streptococcus pyogenes
Haemophilus species
Corynebacterium diphtheriae
Pseudomonas aeruginosa
Coliform bacilli
Bacteroides/*Fusobacterium* species
Fungi

Commensal organisms
Micrococci, diphtheroids and *Staphylococcus epidermidis*.

Nose swabs: anterior nasal swabs or pernasal swabs may be sent depending on the organisms suspected. Direct films are of little value. Inoculate blood agar and tellurite media, incubate 18-24 hours at 35°C. Pernasal swabs for *Bordetella pertussis* should be inoculated on to Charcoal Agar (CM0119 + SR0082).

Associated pathogens
Staphylococcus aureus
Streptococcus pyogenes
Neisseria meningitidis
Bordetella pertussis
Haemophilus species
Corynebacterium diphtheriae

Commensal organisms
Diphtheroids, *Stapylococcus epidermidis*, nonpathogenic neisseria, *Bacillus* species, small numbers of coliform bacilli.

Throat swabs: make a film and stain with dilute carbol-fuchsin, examine for Vincent's organisms, yeasts and mycelium.

Inoculate blood agar and incubate aerobically and anaerobically for 18-24 hours at 35°C. Inoculate tellurite medium and incubate for 48 hours at 35°C.

Associated pathogens
Streptococcus pyogenes
Corynebacterium diphtheriae
Corynebacterium ulcerans

Staphylococcus aureus
Neisseria meningitidis
Candida albicans
Borrelia vincenti

Commensal organisms
Streptococcus viridans, Staphylococcus epidermidis, diphtheroids, *Streptococcus pneumoniae, Haemophilus influenzae* non-type B, non-pathogenic neisseria.

URINE

Specimens of urine for microbiological examination are usually 'mid-stream' samples, more rarely catheter collected samples or supra-pubic aspirations. All samples should be delivered quickly to the laboratory, or preserved for short periods at 2-8°C, or a small amount of boric acid can be added.

Dip Slides have the advantage that they can be immersed in fresh urine or the patient can micturate directly on to the agar surface of the Dip Slide. Thus the bacterial colonies seen after transport and incubation reflect accurately the original microbial ecology.

Examination of urine includes counting white cells, red cells and urinary casts, estimating the number of bacteria per ml and identifying the organisms grown.

Samples of urine can be inoculated on to MacConkey Agar CM0007 and CLED Medium CM0301 using a calibrated loop (0.01 ml) or filter paper inoculation. Incubate overnight at 35°C, and count the number of colonies developed.

<20 colonies = $<10^4$ orgs/ml
20-200 colonies = 10^4-10^5 orgs/ml
>200 colonies = $>10^5$ orgs/ml

The question of significance of growth depends on the flora grown and the clinical history of the patient. The criteria proposed by Kass (significance = $>10^5$ cfu/ml) for asymptomatic patients does not apply universally to all patients.

Associated pathogens
Escherichia coli
Enterobacter and *Proteus* species
Staphylococci (coagulase positive and negative)
Enterococcus
Mycobacterium tuberculosis

Commensal organisms
When in doubt contact the physician or repeat the sample.

PUS AND WOUND SWABS

Samples of pus or properly taken swabs of wound exudates should be sent quickly to the laboratory.

Pus samples should be diluted with sterile saline to detect the 'sulphur granules' of *Actinomyces israelii*. Inoculate the granules on to blood agar and incubate aerobically and anaerobically. To avoid Proteus species spreading across the plates use chloral hydrate in one of the plates or take equivalent precautions. Wilkins-Chalgren Anaerobe Agar CM06l9 or Anaerobe Basal Agar CM0972 + selective supplements can be used to isolate anaerobes. Inoculation into Thioglycollate Broth is helpful to enrich the growth of anaerobes and aerobes.

Examine Gram-stained films and acid-fast bacilli stained films. Superficial wounds may be infected with atypical mycobacteria (*Myco. marinum*, *Myco. ulcerans*, *Myco. chelonei*), culture on Lowenstein-Jensen medium and incubate at 30°C. Wounds from burns, although infected with *Staphylococcus aureus* and *Streptococcus pyogenes*, may also be heavily colonised with Gram-negative organisms - especially *Pseudomonas* species.

Examination of films and inoculation on to blood agar plates containing Staph/Strep Supplement SR0070, chloral hydrate or phenethyl alcohol should help separate the infecting organism. Incubate aerobically and anaerobically at 35°C.

Examine the plates soon after removal from the incubator because *Proteus* species become more motile at room temperature.

Associated pathogens
Staphylococcus aureus
Streptococcus pyogenes
Anaerobic cocci
Clostridia species
Bacteroides species
Pasteurella species
Yersinia species
Actinomyces species
Mycobacterium species
Bacillus anthracis
Listeria monocytogenes
Proteus and *Pseudomonas* species
Nocardia and other fungi

Commensal organisms
Pus - none

Wound swabs - small numbers of skin commensal organisms.

EYE SWABS (purulent discharges)

Eye discharge swabs should arrive in transport media but preferably the eye discharge should be sampled directly on to culture media.

Examine smears for *Neisseria gonorrhoeae* and *Chlamydia trachomatis*, using Gram's stain and Giemsa stain or immuno-fluorescent reagents.

Inoculate blood agar plates and incubate aerobically and anaerobically at 35°C overnight.

Inoculate a Columbia 'chocolate' blood agar and incubate in a 5% CO_2 atmosphere at 35°C overnight. Prolong the incubation for 48 hours if the Gram film is doubtful.

Associated pathogens
Staphylococcus aureus
Streptococcus pneumoniae
Neisseria gonorrhoeae
Haemophilus species
Chlamydia trachomatis
Moraxella species
Corynebacterium diphtheriae
Pseudomonas aeruginosa
Coliform organisms

Commensal organisms
Staphylococcus epidermidis
Micrococcus species
Diphtheroids

FAECES, FAECAL AND RECTAL SWABS

Rectal swabs are of the least value compared with samples of faeces or faecal swabs. All samples should be sent to the laboratory quickly or placed in transport media.

There is a very wide range of culture media available to cultivate the growing list of enteric pathogens. It would not be cost-effective to use them indiscriminately therefore the clinical history of the patient is essential to focus attention on the most likely organisms.

Salmonellae and Shigellae: inoculate one or more enrichment medium (selenite/tetrathionate/RV broths) and at least two isolation media, one of which must be able to support the growth of shigella (DCLS, DCA, Hektoen, Modified SS, XLD). Incubate for 18-24 hours at 35°C although tetrathionate broth and RV broth can be incubated at 43°C to increase selectivity for salmonellae. Subculture on to appropriate media.

Enterotoxigenic Escherichia coli (ETEC): inoculate MacConkey Agar CM0007 and MacConkey Sorbitol Agar CM0813. Look for non-sorbitol fermenting colonies indicative of *Escherichia coli* O157:H7; confirm identity with serological tests. Look for *Staphylococcus aureus* also on MacConkey Agar in case the disease is staphylococcal enterocolitis.

Campylobacter: inoculate Campylobacter Selective media made with one of the various selective supplements available.

Vibrios: *V. cholerae* or *V. parahaemolyticus* may be suspected. Inoculate alkaline peptone water and TCBS Agar CM0333.

Yersinia: *Y. enterocolitica* may be isolated on Yersinia Selective Agar Base (CM0653 + SR0109). Inoculate the medium and incubate for 18-24 hours at 32°C.

***Clostridium perfringens*:** inoculate blood agar and incubate anaerobically (and aerobically as a control). Inoculate two tubes of Cooked Meat Broth and heat one at 80°C for 30 minutes to detect heat-resistant spores. Subculture to blood agar and incubate anaerobically and aerobically.

Aeromonas: *A. hydrophila* and *A. sobria* are associated with enteritis of children and adults. Inoculate Aeromonas Medium Base (Ryan) CM0833 + SR0136 or Blood Agar containing 20mgm per litre of Ampicillin. Incubate 18-24 hours at 35°C.

***Clostridium difficile*:** when this organism is isolated from antimicrobial-associated-colitis it is considered to be a pathogen. It can be found fairly commonly in infant stools where it is usually non-toxigenic. Inoculate alcohol-treated faeces on Clostridium Difficile Agar Base (CM0601 + SR0096) and incubate anaerobically at 35°C for 18-24 hours.

Associated pathogens
Bacillus cereus
Plesiomonas shigelloides
Clostridium botulinum

Commensal organisms
Coliform bacilli, *Proteus* species, *Pseudomonas* species, *Bacteroides* species and many *Clostridium* species.

SEXUALLY TRANSMITTED DISEASE SWABS

STD samples may come from the eye, throat, rectum, cervix, vagina or urethra.

Eye swabs: look for *Neisseria gonorrhoeae* and *Chlamydia trachomatis* as previously described.

Throat swabs: look specifically for *N. gonorrhoeae*. Vaginal/cervical swabs: examine a Gram's stained smear for N. gonorrhoeae and a ‘wet’ slide preparation for *Trichomonas vaginalis* and for ‘clue cells’ diagnostic for *Gardnerella vaginalis*. Yeast cells may be seen in either preparation. To isolate *G. vaginalis* inoculate Columbia Blood Agar Base containing 10% human, rabbit or horse blood plus G. vaginalis Selective Supplement (SR0119). Incubate at 35°C in a 7% CO_2 atmosphere for 48 hours.

Urethral swabs: as well as *N. gonorrhoeae*, include *C. trachomatis* in the smear examination using Giemsa stain or a specific immunofluorescent reagent.

Inoculate all swabs on Thayer-Martin Medium CM0367 + SR0090 + SR0091 or SR0101 or on Modified New York City Medium CM0367 + SR0105+ SR0095 or SR0104. Incubate in a 5% CO2 atmosphere at 35°C for 24-48 hours. Sabouraud Dextrose Agar CM0041 or Dermasel Agar CM0539 + SR0075 can be inoculated if Candida are suspected.

PUERPERAL INFECTIONS

High vaginal swabs from such conditions should be examined carefully for *Clostridium perfringens*. Nonsporing, square-ended Gram-positive rods which appear to be capsulated may be seen in the Gram stained film and should reported to the physician immediately.

Inoculate blood agar plates and incubate aerobically and anaerobically at 35°C for 18-24 hours.

Micro-organism/Group	Purpose	Culture Medium	Code
Aeromonas hydrophila	Selective isolation	Aeromonas Medium Base (Ryan)	CM0833
		Ampicillin Selective Supplement	SR0136
Anaerobes (general)	Cultivation and enumeration of anaerobic bacteria	Cooked Meat Medium	CM0081
	General growth of anaerobes, use in blood culture and susceptibility studies	Schaedler Anaerobe Agar	CM0437
		Schaedler Anaerobe Broth	CM0497
		Wilkins Chalgren Anaerobe Agar	CM0619
		Wilkins Chalgren Anaerobe Broth	CM0643
		Anaerobe Basal Agar	CM0972
		Anaerobe Basal Broth	CM0957
	Selective isolation	N-S Anaerobe Selective Supplement	SR0107
		G-N Anaerobe Selective Supplement	SR0108
		Neomycin Selective Supplement	SR0163
	RCM is recommended as the diluent in viable counts of anaerobes	Reinforced Clostridial Agar	CM0151
		Reinforced Clostridial Medium (RCM)	CM0149
	Isolation of *Clostridium difficile*	Clostridium difficile Agar Base	CM0601
		Clostridium difficile Selective Supplement	SR0096
		CDMN Selective Supplement	SR0173
	Confirmation and presumptive identification	*C. difficile* Test Kit	DR1107
		C. difficile Toxin A Test	TD0970
		An-Ident Discs	DD0006
	Confirmation of *Cl. perfringens* by the Nagler test	Blood Agar Base	CM0055
		Fildes Extract	SR0046
		Egg Yolk Emulsion	SR0047
	Enterotoxin detection	PET-RPLA	TD0900
Bacillus cereus	Selective isolation	Bacillus cereus Selective Agar Base (PEMBA)	CM0617
		Mannitol Egg Yolk Polymixin Medium (MYP)	CM0929
		Polymyxin Selective Supplement (for above)	SR0099
		Chromogenic Bacillus cereus Agar Base	CM1036
		Chromogenic Bacillus cereus Selective) Supplement	SR0230
	Demonstration of lecithinase activity	Egg Yolk Emulsion	SR0047
		Nutrient Agar	CM0003
	Enterotoxin detection	BCET-RPLA	TD0950
Bordetella species	Selective isolation	Charcoal Agar Base	CM0119
		Bordetella Selective Supplement	SR0082
Brucella species	Selective isolation	Brucella Medium Base	CM0169
		Blood Agar Base No. 2	CM0271
		Columbia Agar Base	CM0331
		Brucella Selective Supplement	SR0083
		Modified Brucella Selective Supplement	SR0209
Burkholderia cepacia	Selective isolation	Burkholderia cepacia Agar Base	CM0955
		Burkholderia cepacia Selective Supplement	SR0189
Campylobacter species	For the selective isolation *Campylobacter* species	Campylobacter selective isolation media:	
		Blood Agar Base No 2	CM0271
		Columbia Agar Base	CM0331
		Brucella Medium Base	CM0169
		Skirrow Selective Supplement	SR0069
		Butzler Selective Supplement	SR0085
		Modified Butzler SelectiveSupplement	SR0214
		Blaser-Wang Selective Supplement	SR0098
		Laked horse blood	SR0048
		Campylobacter Growth Supplement (FBP)	SR0232

Micro-organism/Group	Purpose	Culture Medium	Code
Campylobacter species *cont.*		Campylobacter Agar Base (Preston)	CM0689
		Preston Selective Supplement	SR0117
		Modified Preston Selective Supplement	SR0204
		Campylobacter Agar Base (Karmali)	CM0935
		Campylobacter Selective Supplement (Karmali)	SR0167
		Modified Karmali Selective Supplement	SR0205
		Campylobacter Blood Free Medium (modified CCDA)	CM0739
		CCDA Selective Supplement	SR0155
	For the selective enrichment of *Campylobacter* species	Nutrient Broth No 2	CM0067
		Preston Selective Supplement	SR0117
		Modified Preston Selective Supplement	SR0204
		Campylobacter Growth Supplement (FBP)	SR0232
	Confirmation	Campylobacter Test Kit	DR0150
Corynebacterium species	Selective isolation	Hoyle Medium Base	CM0083
		Potassium Tellurite 3.5%	SR0030
		Tinsdale Medium	CM0487
		Tinsdale Selective Supplement	SR0065
Diluents	Diluent or rinse fluid in bacteriological examination	Ringer Solution Tablet	BR0052
	Solvent diluent solution for calcium alginate swabs	Calgon Ringer Tablets	BR0049
	Diluent rinse after hypochlorites or other chlorine sources	Thiosulphate Ringer Tablets	BR0048
	Dilution	Maximum Recovery Diluent	CM0733
Enterobacteriaceae see also Salmonella and Shigella	Enrichment Medium	EE Broth (Buffered Glucose Broth)	CM0317
	Selective enumeration	MacConkey Agar No 3	CM0115
		MacConkey Agar	CM0007
		MacConkey Agar w/o Salt (CM7B)	CM0507
		MacConkey Agar No. 2	CM0109
	Identification and differentiation	SIM Medium	CM0435
		Triple Sugar Iron Agar	CM0277
		Lysine Decarboxylase Broth	CM0308
		Kligler Iron Agar	CM0033
		Desoxycholate Agar	CM0163
		Eosin Methylene Blue (EMB) Agar	CM0069
	Urease producers	Urea Agar Base	CM0053
		Urea Broth Base	CM0071
		Urea solution	SR0020
	Methyl red and VP test	MRVP Medium	CM0043
	Citrate Utilisation	Simmons Citrate Agar	CM0155
	Indole production	Tryptone Water	CM0087
		Kovacs Reagent	MB0209
	Spot indole	DMACA reagent	MB1448
	PYRase	OBIS PYR	ID0580
	Differentiation between lactose and non-lactose fermenting organisms	MacConkey Agar	CM0007
		MacConkey Agar w/o Salt (CM7B)	CM0507
Enterococcus species	Isolation and enumeration	Azide Blood Agar Base	CM0259
		KF Streptococcus Agar	CM0701

Micro-organism/Group	Purpose	Culture Medium	Code
Enterococcus species *cont.*		TTC 1% Solution	SR0229
		Slanetz and Bartley Medium (m-Enterococcus agar)	CM0377
		Todd Hewitt Broth	CM0189
		Edwards Medium Modified	CM0027
		VRE Broth	CM0984
		VRE Agar	CM0985
		Meropenem Selective Supplement	SR0184
		Gentamicin Selective Supplement	SR0185
		Vancomycin Selective Supplement	SR0186
	Confirmation	Streptococcal Grouping Kit	DR0585
		Dryspot Streptococcal Grouping Kit	DR0400
		Streptococcus Plus Kit	DR0575
Escherichia coli O157:H7 and other serogroups	Detection and isolation *Escherichia coli* O157:H7	Sorbitol MacConkey Agar	CM0813
		Cefixime Tellurite Selective Supplement	SR0172
		Modifed Tryptone Soya Broth	CM0989
		Vancomycin Cefixime Cefsulodin Supplement	SR0190
		Novobiocin Selective Supplement	SR0181
		EC Broth with Reduced Bile Salts	CM0990
		CR Sorbitol MacConkey Agar	CM1005
		Cefixime supplement	SR0191
	Confirmation of *Escherichia* coli O157	*E. coli* O157 Latex Test	DR0620
		Dryspot *E. coli* O157 Latex Test	DR0120
	Detection of O157 antibody in serum	O157 Check LPS Abtibody Kit	DR0190
	Toxin detection	VTEC RPLA	TD0960
		E. coli ST EIA	TD0700
		VET RPLA	TD0920
	Confirmation of *E. coli* serogroups 026, 191, 0103, 0111, 0128, 0145	Dryspot Seroscreen	DR0300
Gardnerella vaginalis	Selective isolation	Columbia Agar Base	CM0331
		Gardnerella vaginalis Selective Supplement	SR0119
Gonococci (GC) - see *Neisseria* species			
Gelatin liquefying organisms	Gelatin liquefaction is used as an aid in the identification of some organisms	Nutrient Gelatin (CM135a)	CM0635
Haemophilus species	Selective isolation	HTM Base	CM0898
		HTM Supplement	SR0158
Helicobacter pylori	Detection of antibody in serum	Oxoid Pylori Test	DR0130
	Selective isolation	Columbia Agar Base	CM0331
		H. pylori Selective Supplement (Dent)	SR0147
Infectious mononucleosis	Detection of IM heterophile antibodies	Infectious mononucleosis Test	DR0680
		Dryspot IM Test Kit	DR0180
Legionella species	Isolation	Legionella CYE Agar Base	CM0655
		BCYE Growth Supplement	SR0110
		BCYE Growth Supplement w/o L-cystine	SR0175
		Legionella BMPA Selective Supplement	SR0111
		Legionella GVPC Selective Supplement	SR0152
		Legionella GVPN Selective Supplement	SR0215
		Legionella MWY Selective Supplement	SR0118
	Confirmation	Legionella Latex Test	DR0800
		Dryspot Legionella Latex Test	DR0200

Micro-organism/Group	Purpose	Culture Medium	Code
Listeria monocytogenes	Selective isolation/ enumeration	Listeria Selective Agar Base (Oxford)	CM0856
		Oxford Listeria Selective Supplement	SR0140
		Modified Oxford Listeria Selective Supplement	SR0206
	Differentiation of *Listeria monocytogenes*	OBIS Mono	ID0600
	Identification by latex agglutination	Listeria Test Kit	DR1126
	Biochemical identification	Microbact 12L	MB1128
Mycoplasma species		Mycoplasma Agar Base	CM0401
		Mycoplasma Broth Base	CM0403
		Mycoplasma Supplement-G	SR0059
		Mycoplasma Supplement-P	SR0060
Neisseria species	Selective isolation - New York City medium	GC Agar Base	CM0367
		Laked Horse Blood	SR0048
		Yeast Autolysate Supplement	SR0105
		Liquid Yeast Autolysate	SR0182
		LCAT Selective Supplement	SR0095
		VCAT Selective Supplement	SR0104
	Selective isolation - Thayer Martin medium	GC Agar Base	CM0367
		Soluble Haemoglobin	LP0053
		Vitox Supplement	SR0090
		VCN Selective Supplement	SR0101
		VCNT Selective Supplement	SR0091
Salmonella species	Selective Enrichment	Selenite Broth Base	CM0395
		Sodium biselenite	LP0121
		Selenite Cystine Broth Base	CM0699
		Mannitol Selenite Broth Base	CM0399
		Selenite Broth (10 ml)	LR0039
		Tetrathionate Broth Base	CM0029
		Tetrathionate Broth (USA)	CM0691
		Muller-Kauffmann Tetrathionate Broth Base	CM0343
		Muller-Kauffmann Tetrathionate-Novobiocin Broth Base (MKTT-n)	CM1048
		Novobiocin Selective Supplement	CM0181
	Isolation and Enumeration	Bismuth Sulphite Agar	CM0201
		Brilliant Green Agar	CM0263
		Brilliant Green Agar (Modified)	CM0329
		Sulphamandelate Supplement	CM0087
		DCLS agar	CM0393
		Desoxycholate Agar	CM0163
		Desoxycholate Citrate Agar	CM0035
		Desoxycholate Citrate Agar (Hynes)	CM0227
		Hektoen Enteric Agar	CM0419
		MacConkey Agar	CM0007
		MacConkey Agar No. 3	CM0115
		XLD Medium	CM0469
		SS Agar	CM0099
		SS Agar Modified	CM0533
		MLCB agar	CM0783
		Chromogenic Salmonella Agar Base	CM1007
		Chromogenic Salmonella Selective Supplement	SR0194
	Enrichment/Isolation/ Detection	Salmonella Elective Medium	CM0857
		Salmonella Rapid Test	FT0201

Micro-organism/Group	Purpose	Culture Medium	Code
Salmonella species *cont.*	Salmonella Rapid Test	Salmonella Latex test	FT0203
	Confirmation	Salmonella Latex test	DR1108
	Identification	OBIS Salmonella	ID0570
Sensitivity media		Diagnostic Sensitivity Test Agar	CM0261
		Iso-Sensitest Agar	CM0471
		Iso-Sensitest Broth	CM0473
		Mueller-Hinton Agar	CM0337
		Mueller-Hinton Broth	CM0405
		Sensitest Agar	CM0409
		HTM Base	CM0898
		HTM Supplement	SR0158
		HR Medium	CM0845
Shigella species		MacConkey Agar	CM0007
		Hektoen Enteric Agar	CM0419
		MacConkey Agar No 3	CM0115
		DCLS Agar	CM0393
		XLD Medium	CM0469
		SS Agar	CM0099
		SS Agar Modified	CM0533
		Desoxycholate Citrate Agar (Hynes)	CM0227
		Desoxycholate Citrate Agar	CM0035
Staphylococcus aureus	Enrichment	Salt Meat Broth	CM0094
	Isolation, enumeration	Staphyloccocus 110 Medium	CM0145
		Vogel Johnson Agar	CM0641
		Potassium Tellurite 3.5%	SR0030
		Mannitol Salt Agar (Chapman Medium)	CM0085
		Staph/Strep supplement (CNA)	SR0070
		Staph/Strep supplement Modified (Modified CNA)	SR0126
		Oxacillin Resistance Screening Agar Base (ORSAB)	CM1008
		ORSAB Selective Supplement	SR0195
	Differentiation		
	(a) DNase production	DNase Agar	CM0321
	(b) Phosphatase production	Blood Agar Base	CM0055
	(c) Coagulase Production	Staphylase Test	DR0595
	Latex agglutination tests	Dryspot Staphytect Plus	DR0100
		Staphytect Plus	DR0850
		PBP2' Latex Test	DR0900
	Enterotoxin detection	SET-RPLA	TD0900
Streptococcus species	Selective isolation of streptococci from dairy products containing mixed flora. Tryptose Phosphate broth can be used with added azide and agar (APHA)	Edwards Medium Modified	CM0027
		Tryptose Phosphate Broth	CM0283
		Columbia Blood Agar Base	CM0331
		Staph/Strep supplement (CNA)	SR0070
		Staph/Strep supplement Modified (Modified CNA)	SR0176
		Streptococcus supplement (COBA)	SR0126
	Isolation of Group B streptococci	GBS Agar Base (Islam)	CM0755
	Identification	Streptococcal Grouping Kit	DR0585
	Latex agglutination test	Dryspot Streptococcal Grouping Kit	DR0400
		Strep Plus Kit	DR0575
		Dryspot pneumo	DR0420
Syphilis	Antibody detection	VDRL Test Kit - 100 test	DR0525

Micro-organism/Group	Purpose	Culture Medium	Code
Syphilis *cont.*		VDRL Test Kit - 500 test	DR0526
		VDRL Carbon Antigen	DR0520
Transport media		Cary Blair	CM0519
		Stuart's Transport Medium	CM0111
		Amies Transport Medium	CM0425
Treponema pallidum	Antibody detection	TPHA Test	DR0530
Trichomonas species	Isolation and culture	Trichomonas Medium Base	CM0161
		Horse Serum	SR0035
		Trichomonas Medium No. 2 (5 ml)	LR0027
Urine culture	Selective isolation and enumeration	CLED Medium	CM0301
		CLED Medium with Andrades	CM0423
		Chromogenic UTI Medium	CM0949
		Chromogenic UTI Medium (Clear)	CM1050
Viable Organisms	General cultivation and maintenance as well as diluents	Blood Agar	CM0055
		Nutrient Agar	CM0003
		Tryptose Blood Agar Base	CM0233
		Tryptose Phosphate Broth	CM0283
		Azide Blood Agar Base	CM0259
		Sheep Blood Agar Base	CM0854
		Columbia Blood Agar Base	CM0331
		Blood Agar Base No. 2	CM0271
		Nutrient Broth No 2	CM0067
		Maximum Recovery Diluent	CM0733
		Buffered Peptone Water	CM0509
Vibrio species	Selective isolation and enumeration	TCBS Cholera Medium	CM0333
	Presumptive Identification	0129 Discs - 10 mcg/disc	DD0014
		0129 Discs - 150 mcg/disc	DD0015
	Toxin detection	VET RPLA	TD0920
Yeasts and Moulds	Cultivation, isolation and enumeration of yeasts and moulds	Potato Dextrose Agar	CM0139
		Sabouraud Dextrose Agar	CM0041
		Sabouraud Maltose Agar (CM41a)	CM0541
	Dermatophytes	Dermasel Agar	CM0539
		Demasel Selective Supplement	SR0075
Yersinia species	Selective isolation and enumeration	Yersinia CIN Agar	CM0653
		Yersinia CIN Selective Supplement	SR0109

EXAMINATION OF FOOD AND DAIRY PRODUCTS

There is no general agreement on methods for the laboratory examination of foods and dairy products. The standard reference books used are:

Compendium of Methods for the Microbiological Examination of Foods by the American Public Health Association. Washington D.C. 2001

Bacteriological Analytical Manual 8th Ed. Revision A by the Association of Official Analytical Chemists. Washington D.C. 8th Ed 1978.

Microorganisms in Foods Vols. 1 & 2 by the International Commission on Microbiological Specifications for Foods. Toronto University Press. 1988 with Revision.

In Europe, the Codex Alimentarius Commission is considering standard methods, aided by published standards from the International Organization for Standardization (ISO). The bacteriological examination of food and dairy products falls into one or more of the following four categories:

1. **Total Viable Count:** this is an attempt to measure the total number of bacteria, yeasts and moulds in a product by inoculating dilutions of suspensions of the sample into various culture media and incubating them for fixed periods at temperatures varying from 22-55°C. The resulting colony counts are then calculated as organisms per gram of product. The results obtained are compared with expected figures and the product is passed or failed. It is not an accurate process and fairly gross changes in numbers are looked for which indicate unsatisfactory raw materials, processing or storage conditions.
2. **Indicator Organism Count:** specific organisms are sought, most often coliforms (lactose-fermenters) or Enterobacteriaceae (glucose-fermenters) using selective media. See section on Violet Red Bile Agar and Violet Red Bile Glucose Agar. These organisms indicate the standard of hygiene used in the manufacture of the food products.
3. **Detection of Specific Spoilage organisms:** spoilage organisms are usually associated with taints and off-flavours in stored products. They are the major factor in determining the shelf-lives of food products and are now considered to be of more relevance than total viable counts. Moulds and psychrotrophic Gram-negative rods are specifically sought, using selective culture media and low temperature incubation.
4. **Detection of Food Poisoning Organisms:** Hazard analysis critical control point technique (HACCP) is a systematic approach to hazard identification, assessment and control. The hazards are determined, the critical control points of those hazards are identified and procedures to monitor the critical control points are established. An HACCP audit is an essential stage in the implementation of this process. [ICMSF (1989) 'Micro-organisms in Foods, 4. Application of hazard analysis critical control point (HACCP) system to ensure microbiological safety and quality'. Blackwell Scientific Publications, Oxford.]

Table

Micro-organism Group	Purpose	Culture Medium	Code
Aeromonas hydrophila	Selective isolation	Aeromonas medium base (Ryan) Ampicillin Selective Supplement	CM0833 SR0136
Anaerobes (general)	Cultivation and enumeration of anaerobic bacteria	Cooked Meat Medium Liver Broth	CM0081 CM0077
	RCM is recommended as the diluent in viable counts of anaerobes	Reinforced Clostridial Agar Reinforced Clostridial Medium (RCM)	CM0151 CM0149
	Detection and enumeration of thermophillic anaerobes causing sulphide spoilage	Iron Sulphite Agar	CM0079
	Diagnostic examination of canned food samples	Crossley Milk Medium	CM0213
	Selective isolation of anaerobes from dried/ frozen foods	Schaedler Anaerobe Agar Schaedler Anaerobe Broth	CM0437 CM0497
Bacillus cereus	Selective isolation	Bacillus cereus Selective Agar (PEMBA) Mannitol Egg Yolk Polymixin Agar (MYP) Polymixin Selective Supplement (for above) Chromogenic Bacillus cereus Agar	CM0617 CM0929 SR0099 CM1036

Micro-organism Group	Purpose	Culture Medium	Code
Bacillus cereus *cont.*		Chromogenic Bacillus cereus Selective Supplement	SR0230
	Demonstration of Lecithinase activity	Egg Yolk Emulsion	SR0047
		Nutrient Agar	CM0003
	Enterotoxin detection	BCET-RPLA	TD0950
Brochothrix thermosphacta	Selective isolation	STAA Agar base	CM0881
		STAA Selective Supplement	SR0151
		STA Selective Supplement	SR0162
Campylobacter species	For the selective isolation of *Campylobacter* species	Campylobacter selective isolation media:	
		Blood Agar Base No 2	CM0271
		Columbia Agar Base	CM0331
		Brucella Medium Base	CM0169
		Campylobacter Selective Supplement (Skirrow)	SR0069
		Campylobacter Selective Supplement (Butzler)	SR0085
		Modified Butzler ISO Selective Supplement	SR0214
		Campylobacter Selective Supplement (Blaser-Wang)	SR0098
			SR0048
		Laked horse blood	SR0232
		Campylobacter growth supplement	CM0689
		Campylobacter Agar Base (Preston)	SR0117
		Preston Campylobacter Selective Supplement	CM0935
		Modified Preston Campylobacter Selective Supplement	SR0167
		Campylobacter Agar Base (Karmali)	
		Campylobacter Selective Supplement (Karmali)	SR0205
		Modified Karmali Selective Supplement	
		Campylobacter Blood Free selective Agar Base (modified CCDA)	CM0739
		CCDA selective supplement	SR0155
	For the selective enrichment of *Campylobacter* species	Bolton Broth Base	CM0983
		Bolton Broth Selective supplement	SR0183
		Modified Bolton Selective supplement	CM0208
		Nutrient Broth No 2	CM0067
		Preston Campylobacter Selective Supplement	SR0117
		Modified Preston Campylobacter Selective Supplement	SR0204
	Identification by latex agglutination	Campylobacter test kit	DR0150
Clostridium perfringens	Selective isolation of *Cl. perfringens*	Perfringens Agar Base (OPSP)	CM0543
		OPSP supplement A	SR0076
		OPSP supplement B	SR0077
		Perfringens Agar Base	CM0587
		Perfringens (TSC) Selective Supplement	SR0088
		Perfringens (SFP) Selective Supplement	SR0093
	Confirmation of *Cl. perfringens* by the Nagler test	Blood Agar Base	CM055
		Fildes Extract	SR0046
		Egg Yolk Emulsion	SR0047
	Enterotoxin detection	PET-RPLA	TD0900
Coliform group	*Enterobacter sakazakii*	Chromogenic Enterobacter sakazakii Agar	CM1055
	Isolation and enumeration of coliforms including *E. coli*	Lactose Broth	CM0137
		Lauryl Tryptose Broth	CM0451
		Lauryl Sulphate Broth	CM0451
		MacConkey Broth Purple	CM0505(5a)
		MacConkey Broth	CM0005

Micro-organism Group	Purpose	Culture Medium	Code
Coliform group *cont.*		Violet Red Bile (Lactose) Agar	CM0107
		Chromogenic E. coli/coliform Medium	CM0956
		Chromogenic E. coli/coliform Selective Medium	CM1046
		Brilliant Green Bile 2% Broth	CM0031
		EC Broth	CM0853
		Minerals Modified Glutamate Medum	CM0607
		Sodium glutamate	LP0124
		Desoxycholate agar	CM0163
	Escherichia coli confirmation	TBX Medium	CM0945
		Tryptone Bile Agar	CM0595
		MacConkey Broth	CM0005
	Differentiation between lactose and non-lactose fermenting organisms	MacConkey Agar	CM0007
		China Blue Lactose Agar	CM0209
		Endo Agar Base	CM0479
		Basic Fuchsin Indicator	BR0050
	Differentiation and enumeration of coliforms	Tergitol 7 Agar	CM0739
		TTC supplement	SR0148
		Desoxycholate Agar	CM0163
		Eosin Methylene Blue Agar	CM0069
		Violet Red Bile (Lactose) Agar	CM0107
		MUG supplement	BR0071
		VRBA with MUG	CM0978
		Lauryl Sulphate Broth Modified with MUG and added tryptophan	CM0967
		Lauryl Sulphate Broth with MUG	CM0980
		EC Broth with MUG	CM0979
	Confirmation of presumptive coliform tests	Endo Agar Base	CM0479
		Basic Fuchsin	BR0050
		Eosin Methylene Blue Agar	CM0069
	Differentiation of coliform group		
	(a) Methyl red and VP test	MRVP Medium	CM0043
	(b) Citrate Utilisation	Simmons Citrate Agar	CM0155
	(c) Indole production	Tryptone Water	CM0087
		Kovacs Reagent	MB0209
	Spot indole	DMACA reagent	MB1448
	Detection of *Escherichia coli* O157:H7	Sorbitol MacConkey Agar	CM0813
		Cefixime Tellurite Selective Supplement	SR0172
		Buffered Peptone Water	CM0509
		Vancomycin Cefixime Cefsulodin Supplement	SR0190
		Modifed Tryptone Soya Broth	CM0989
		Novobiocin Selective Supplement	SR0181
		EC Broth (Reduced Bile Salts)	CM0990
		Cefixime Rhamnose Sorbitol MacConkey Agar	CM1005
		Cefixime Supplement	SR0191
	Toxin detection	VTEC RPLA	TD0960
		E. coli ST EIA	TD0700
		VET RPLA	TD0920
Enterobacteriaceae (see also coliforms and Salmonella/Shigella)	Resuscitation of stressed cells e.g. in preserved foods	Buffered Peptone Water	CM0509
		Tryptone Soya Broth	CM0129
		Buffered Peptone Water (ISO)	CM1049
	Enrichment Medium	EE Broth (Buffered Glucose Broth)	CM0317
	Selective enumeration	MacConkey Agar No 3	CM0115
		Violet Red Bile Glucose Agar	CM0485

Micro-organism Group	Purpose	Culture Medium	Code
Enterobacteriaceae *cont.*	Differentiation	Simmons Citrate Agar	CM0155
		SIM Medium	CM0435
		Triple Sugar Iron Agar	CM0277
		Lysine Decarboxylase Broth	CM0308
	Urease producers	Kligler Iron Agar	CM033
		Urea Agar Base	CM0053
		Urea Broth	CM0071
		Urea solution	SR0020
	PYRase	OBIS PYR	ID0580
Enterococcus species	Isolation and enumeration of *Enterococcus* species	Azide Blood Agar Base	CM0259
		Kanamycin Aesculin Azide Medium	CM0591
		Kanamycin Sulphate Selective Supplement	SR0092
		KF Streptococcus Agar	SR0701
		Slanetz and Bartley Medium (m-Enterococcus agar)	CM0377
		TTC Supplement	SR0229
	Serotyping	Streptococcal Grouping Kit	DR0585
		Dryspot Streptococcal Grouping Kit	DR0400
		Strep plus kit	DR0575
Gelatin liquefying organisms	Gelatin liquefaction is used as an aid in the identification of some organisms	Nutrient Gelatin (CM135a)	CM0635
Hygiene and dilution	Diluent or rinse fluid in bacteriological examination of food products and plant	Ringer Solution Tablets	BR0052
	Solvent diluent solution for calcium alginate swabs	Calgon Ringer Tablets	BR0049
	Chlorine Neutralising Ringer solution to counteract the bactericidal effects of hypochlorite and other chlorine solutions	Thiosulphate Ringer Tablets	BR0048
	Dilution	Maximum Recovery Diluent	CM0833
Lactobacillus species	For the selective isolation and enumeration of *Lactobacillus* species from meat and yoghurts	MRS Agar	CM0361
		MRS Broth	CM0539
		Tomato Juice Agar	CM0113
		Rogosa Agar	CM0627
		Orange Serum Agar	CM0657
Lecithinase Producing organisms	Lecithin activity for e.g. *Bacillus* species	Egg Yolk Emulsion	SR0047
		Nutrient Agar	CM0003
Lipolytic organisms	Isolation of contaminating lipolytic organisms from dairy products. Also for the examination of activity of moulds in mould ripened cheese	Tributyrin Agar	PM0004
Listeria monocytogenes	Selective Enrichment	Buffered Listeria Enrichment Broth	CM0897
		Listeria Selective Enrichment Supplement	SR0141
		Fraser Broth Base	CM0895
		Fraser Supplement	SR0156
		Half Fraser Supplement	SR0166

Micro-organism Group	Purpose	Culture Medium	Code
Listeria monocytogenes cont.		Listeria Enrichment Broth	CM0862
		Listeria Enrichment Supplement	SR0141
		Modified Listeria Enrichment Supplement	SR0213
		Listeria Enrichment supplement Modified 10 mg/l of acriflavine	SR0149
		Listeria Enrichment Broth Base (UVM)	CM0863
		Listeria Primary Selective Enrichment Supplement (UVMI)	SR0142
		Listeria Secondary Enrichment Selective Supplement (UVMII)	SR0143
		Novel Enrichment Broth (ONE) Listeria	CM1066
		ONE Broth Listeria Selective Supplement	SR0234
	Selective isolation/ enumeration	Listeria Selective Agar Base (Oxford)	CM0856
		Oxford Listeria Selective Supplement	SR0140
		Modofied Oxford Listeria Selective Supplement	SR0206
		Palcam Agar Base	CM0877
		Palcam Selective Supplement	SR0150
		Chromogenic Listeria Agar Base	CM1080
		Chromogenic Listeria Selective Supplement	SR0227
		Chromogenic Listeria Differential Supplement	SR0228
		Chromogenic Listeria Agar Base (ISO)	CM1084
		Chromogenic Listeria Selective Supplement (ISO)	SR0226
	Differentiation of *Listeria monocytogenes*	OBIS Mono	ID0600
	Identification by latex agglutination	Listeria Test Kit	DR01126
	Biochemical identification	Microbact 12L	MB1128
	Isolation and confirmation	Oxoid Rapid Listeria Test	FT0401
Micrococci	Enumeration and differentiation of lactose and non-lactose fermenting organisms including micrococci	China Blue Lactose Agar	CM0209
Plate count	General media for performing heterotrophic plate counts in foods and for testing the suitability of water for food preparation. Nutrient Gelatin is used for the plate count of psychrophilic organisms such as *Pseudomonas* species and for testing for gelatinase activity	Nutrient Gelatine (CM135a)	CM0635
		Tryptone Glucose Extract Agar	CM0127
		Tryptone Soya Agar	CM0131
		Yeast Extract Agar	CM0019
		Plate Count Agar	CM0325
		Standard Plate Count Agar (APHA)	CM0463
		Milk Plate Count Agar with antibiotic free skimmed milk	CM0681
		Milk Agar	CM0021
		PPCT Selective Supplement	SR0159
		R2A agar	CM0906
		Water Plate Count Agar (ISO)	CM1012
Pseudomonas species	Isoation of pseudomonads	Pseudomonas Agar Base	CM0559
		CFC Supplement	SR0103
Salmonella species	Pre-enrichment	Buffered Peptone Water	CM0509
		Buffered Peptone Water (ISO)	CM1049
	Selective Enrichment	Selenite Broth Base	CM0399
		Sodium biselenite	LP0121
		Tetrathionate Broth Base	CM0029

Micro-organism Group	Purpose	Culture Medium	Code
Salmonella species *cont.*		Rappaport Vassiliadis (RV) Enrichment Broth	CM0669
		Rappaport Vassiliadis Soya (RVS) Enrichment Broth	CM0866
		Selenite Cystine Broth Base	CM0699
		Tetrathionate Broth (USA)	CM0691
		Muller-Kauffmann Tetrathionate Broth Base	CM0343
		MKTT-n Broth	CM1048
		Novobiocin Selective Supplement	SR0181
	Isolation and Enumeration	Bismuth Sulphite Agar	CM0201
		Brilliant Green Agar	CM0263
		Brilliant Green Agar (Modified)	CM0329
		DCLS Agar	CM0393
		Desoxycholate Citrate Agar (Hynes)	CM0227
		Hektoen Enteric Agar	CM0419
		XLD Medium	CM0469
		XLT-4 Agar	CM1016
		XLT-4 Selective Supplement	SR0237
		MLCB agar	CM0783
		Salmonella Chromogenic Agar	CM1007
		Salmonella Chromogenic Selective Supplement	SR0194
		MSRV Medium	CM0910
		Novobiocin Selective Supplement	SR0181
	Enrichment/Isolation	Elective Medium	CM0857
	Detection	Rapid Test	FT0201
	Salmonella Rapid Test	Latex test	FT0203
	Confirmation	Salmonella latex test	DR1108
	Identification	OBIS Salmonella	ID0570
Shigella species		MacConkey Agar	CM0007
		Hektoen Enteric Agar	CM0419
		MacConkey Agar No 3	CM0115
		XLD Medium	CM0469
Staphylococcus aureus	Enrichment	Salt Meat Broth Tablets	CM0094
		Giolitti Cantoni Broth	CM0523
		Potassium Tellurite 3.5%	SR0030
	Isolation, enumeration differentiation	Baird Parker Agar Base	CM0275
		Egg Yolk Tellurite Emulsion	SR0054
		Egg Yolk Emulsion	SR0047
		Baird-Parker (RPF) Base	CM0961
		RPF supplement	SR0122
		Staphyloccus Medium No. 110	CM0145
		Vogel-Johnson Agar	CM0641
		Mannitol Salt Agar (Chapman Medium)	CM0085
		Staph/Strep Selective Supplement (CNA)	SR0070
		Staph/Strep Modified CNA Supplement	SR0176
	Differentiation		
	(a) DNase production	DNase Agar	CM0321
	(b) Phosphatase production	Blood Agar Base	CM0055
	(c) Coagulase Production	Staphylase Test	DR0595
	Latex agglutination tests	Dryspot Staphytect Plus	DR0100
		Staphytect plus	DR0850
	Biochemical Identification	Microbact Staph 125	MB1561
Streptococcus species	Selective isolation of streptococci from dairy products containing	M17 Agar	CM0785
		M17Broth	CM0817
		Edwards Medium Modified	CM0027

Micro-organism Group	Purpose	Culture Medium	Code
Streptococcus species *cont.*	mixed floar. Tryptose phosphate Broth can be used with added azide and agar (APHA)	Trytose Phosphate Broth	CM283
		Columbia Blood Agar Base	CM0331
		Staph/Strep supplement (CNA)	SR0070
		Staph/Strep supplement Modified (Modified CNA)	SR0176
		Streptococcus supplement (COA)	SR0126
	Identification Latex agglutination test	Streptococcal Grouping Kit	DR0585
Thermophilic flat sour	Detection and enumeration of flat sour organisms in canned foods, sugar, etc	Dextrose Tryptone Agar	CM0075
		Dextrose Tryptone Broth	CM0073
		Tryptone Glucose Yeast Extract Agar	CM0127
Viable Organisms (see also Plate Count)	General cultivation and maintenance as well as diluents	Blood Agar	CM0055
		Nutrient Agar	CM0003
		Nutrient Broth No 2	CM0067
		Maximim Recovery Diluent	CM0733
		Buffered Peptone Water	CM0509
Vibrio species	Selective isolation and Enumeration	Enrichment-Alkaline Peptone Water	CM1028
		TCBS Cholera Medium	CM0333
	Isolation of *Vibrio vulnificus*	SPS Agar Base	CM1083
		Polymixin	SR0099
	Presumptive Identification	0129 Discs - 10 mcg/disc	DD0014
		0129 Discs - 150 mcg/disc	DD0015
	Toxin detection	VET RPLA	TD0920
Yeasts and Moulds	Cultivation, isolation and enumeration of yeasts and moulds	Malt Extract Agar	CM0059
		Malt Extract Broth	CM0057
		OGYE Agar Base	CM0545
		OGYE Selective Supplement	SR0073
		Potato Dextrose Agar	CM0139
		Rose-Bengal Chloramphenicol Agar	CM0549
		Chloramphenicol Selective Supplement	SR0078
		Yeast and Mould Agar	CM0920
		Dichloran Glycerol (DG18) Agar Base	CM0729
		Dichloran Rose Bengal Chloramphenicol Agar	CM0727
		Sabouraud Dextrose Agar	CM0041
		Sabouraud Maltose Agar	CM0541
		Wort Agar	CM0247
	Aspergillus flavus/ parasiticus	AFPA Medium	CM0731
Yersinia species	Selective isolation and enumeration	Yersinia Selective Agar Base (CIN)	CM0653
		Yersinia Selective Supplement	SR0109

PHARMACEUTICAL PRODUCTS

The safety tests of pharmaceutical and biological products include procedures to measure:

1. the absence of viable micro-organisms (sterile products)
2. the absence or presence within limits of specific organisms eg. *salmonella*, *pseudomonas*, coliforms and *staphylococci*.
3. the microbial flora of raw materials and natural substances (the 'bioburden').

Before carrying out these tests it is important that the appropriate reference texts are consulted for the full descriptions of the methods required. There are no universally approved standards and each country has national standards which must be followed.

Examples of publications which offer complete, detailed test procedures and interpretations of results are:

The United States Pharmacopoeia 27 and The National Formulary 22. 2004.

Official Methods of Analysis of the AOAC 17th Edn. Washington D.C. 2003.

British Pharmacopoeia 2003.

European Pharmacopoeia 4th Edn. 2004.

The Pharmacopoeia of Japan, Tokyo. Society of Japanese Pharmacopoeia. 14th Ed. 2001.

Many pharmaceutical and biological reagents contain preservatives and, when testing them for the presence of viable organisms, it is important to add neutralising agents to the recovery media to overcome residual antimicrobial effects. Some sterility test media contain antagonists to specific preservatives in their formulation.

Preservative	**Neutralising Agent**
Halogens	1% sodium thiosulphate
Aldehydes	2% sodium sulphite
Hexachlorophenes and Quaternaries	5% Tween 80 1% lecithin
Phenols/alcohols	Dilute 1:100 with nutrient broth.

To overcome the bacteriostatic effects of antimicrobial compounds, a filtration technique is used in which the product is passed aseptically through a 0.22 micron membrane filter. The filters are washed with sterile diluent to remove residues of antimicrobials on the filter; they are then cut with sterile scissors and distributed aseptically among various media. This technique can also be used for other preservative compounds.

Oily substances and some insoluble powders will require treatment with sterile Tween 80 to make them suitable for microbial examination. Incubation of inoculated anaerobic and aerobic media should be extended up to 7 days at 35°C before final examination and subculture. Incubation at 30-32°C for the same period is usually recommended for yeasts and moulds.

Test	**Organism**	**Medium**	**Code**
Antibiotic Assay Media	Medium for seed layer	Antibiotic Medium No 1	CM0327
	Assay broth for penicillin	Antibiotic Medium No 3	CM0287
General Media	Maintenance and growth	Columbia Blood Agar Base	CM0331
		Sabouraud Dextrose Agar	CM0041
	Anaerobes	Brain Heart Infusion Agar	CM0375
		Brain Heart Infusion Broth	CM0225
		Cooked Meat Medium	CM0081
	Dilutions	Buffered Sodium Chloride Peptone Solution	CM0982
		Maximum Recovery Diluent	CM0733
Environmental Testing	General - settle plates and contact plates	Tryptone Soya Agar	CM0131
		Sabouraud Dextrose Agar	CM0041
		R_2A Agar	CM0906
		Yeast Extract Agar	CM0019
Media fill trials		Cold filterable TSB	CM1065
		Cold Filterable Vegetable Peptone Broth	VG0104
Raw material and finished product testing	Anaerobes	Reinforced Clostridial Medium	CM0149
	Burkholderia cepacia	Burkholderia cepacia Agar Base	CM0995
		Burkholderia cepacia Selective Supplement	SR0189

Test	Organism	Medium	Code
Raw material and finished product testing *cont.*	*Escherichia coli*/coliforms	Lactose Broth	CM0137
		MacConkey Agar No 3	CM0115
		MacConkey Broth Purple	CM0005a (CM505)
	Enterobacteriaceae	EE Broth	CM0317
		Eosin Blue Methylene Blue Agar	CM0069
		Violet Red Bile Glucose Agar	CM0485
	Salmonella species selective enrichment	Selenite Cystine Broth	CM0699
		Sodium biselenite	LP0121
		Tetrathionate Broth (USA)	CM0671
		Brilliant Green Agar	CM0263
		Bismuth Sulphite Agar	CM0201
	Identification	Lysine Iron Agar	CM0381
		Triple Sugar Iron Agar	CM0277
		Urea Broth	CM0071
		Urea solution	SR0020
		MRVP	CM0043
		Simmons Citrate Agar	CM0155
		Lysine Decarboxylase Broth	CM0308
		Trytone Water	CM0087
		Indole Reagent	MB0209
	Pseudomonas aeruginosa	Cetrimide Agar	CM0579
	Isolation of *Staphylococcus aureus*	Baird Parker Agar Base	CM0275
		Egg yolk tellurite emulsion	SR0054
		Vogel Johnson Agar	CM0641
		Potassium tellurite Solution (3.5%)	SR0030
		Giolitti-Cantoni Broth	CM0321
		Mannitol Salt Agar	CM0085
	Yeasts and Moulds	Sabouraud Dextrose Agar	CM0041
		Chloramphenicol Supplement	SR0078
		Potato Dextrose Agar	CM0139
		Malt Extract Agar	CM0059
Sterilty Testing		Clausen Medium	CM0353
		Tryptone Soya Broth	CM0129
		Thioglycollate Medium USP	CM0173
		Thioglycollate Medium Alternative	CM0391
		Vegetable Peptone Broth	VG0101

BREWING

The fermentation of hop-flavoured extracts of barley malt (wort) with 'top-fermenting' strains of *Saccharomyces cerevisiae* for English beers or 'bottom-fermenting' strains of *S. carlsbergensis* for continental lagers, is a major industry in most parts of the world.

The most important concern of the brewing microbiologist is the establishment and maintenance of good plant hygiene. Infection of the brew with bacteria will cause 'off-flavours' and lead to considerable losses. Lowering the pH helps prevent infection by most bacteria but *Lactobacillus* and *Pediococcus* species are not affected and may still cause spoilage of the beer.

The microbiologist is equally concerned with the quality and purity of the `pitching' yeast i.e. the yeast inoculum used for the specific fermentation. Constant monitoring of the fermentation is required to detect the occurrence of `wild' or non-specific yeasts which may appear during the brewing process. The fortunes of large brewing houses rest on the production of optically bright solutions of standardised colour and unvarying taste for what are, perhaps, the most critical consumers in the world. It follows, therefore, that every effort is made to control the brewing, filtration and bottling/canning stages of this most critical product.

MEDIA FOR BREWING

Micro-organism Groups	Culture Medium	Code
Coliforms	Lactose Broth	CM0137
	MacConkey Agar	CM0007
	MacConkey Broth Purple	CM0505(5a)
Lactic acid spoilage bacteria	MRS Broth	CM0359
	MRS Agar	CM0361
	Tomato Juice Agar	CM0113
	Raka Ray Agar	CM0777
	Universal Beer Agar	CM0651
	Cycloheximide Solution (0.1%)	SR0222
Total contaminating bacteria in yeast	Actidione Agar	PM0118
	WL Nutrient Agar	CM0309
	Cycloheximide Solution (0.1%)	SR0222
	MacConkey Agar	CM0507(7b)
	Wort Agar	CM0247
Total count of bacteria	Yeast Extract Agar	CM019
	WL Nutrient Agar	CM0309
	WL Nutrient Broth	CM0501
	Cycloheximide Solution (0.1%)	SR0222
Culture Yeast	Malt Extract Agar	CM0059
	OGYE Agar	CM0545
	OGYE Selective Supplement	SR0073
	WL Nutrient Agar	CM0309
	WL Nutrient Broth	CM0501
	Wort Agar	CM0247
	Yeast and Mould Agar	CM0920
	Czapek Dox Agar	CM0095
	Potato Dextrose Agar	CM0139
'Wild' Yeast contaminants	Lysine Medium	CM0191
	Yeast and Mould Agar (+ copper)	CM0920
General Media for the cultivation of non-fastidious organisms	Nutrient Broth	CM0001
	Nutrient Agar	CM0003

For water testing refer to separate section.

WATER SUPPLY AND SEWAGE DISPOSAL

The close connection between water fit for drinking and sewage disposal is best illustrated by the large towns which sit astride the major rivers in central USA. Each town draws water for consumption up-stream and discharges sewage effluent down-stream. The last town in such a chain may be drawing water containing the effluents of seven or eight large conurbations.

Such practices, which operate in all major countries, are safe, providing great care is taken in filtering and chlorinating the in-coming water. Equally, the processing of sewage must be safely operated so that pathogen-free and chemically clean effluent of low biological-oxygen-demand (BOD) is released back into the river down-stream.

Drinking water

Stored and river water may contain a wide variety of organisms, mainly saprophytic bacteria with optimal temperatures of growth around 22°C. Filtration and chlorination of the water, before distribution to the public, removes most of these organisms.

Microbiological tests are carried out to make sure that the quality of the treated water meets the specifications required by the Regulatory Authorities such as (The Microbiology of Drinking Water (2002) Methods for the examination of Water and Associated Materials. American Public Health Association. 1998. Standard Methods for the Examination of Water and Wastewater. 20th Edn. Washington D.C.)

Bacterial pollution of water may originate from individuals with clinical symptoms of disease or from symptomless carriers of enteric pathogens such as Salmonella typhi. Such pathogens are difficult to detect in a water supply because their numbers are often few and their incidence sporadic. Therefore, indicator organisms of intestinal contamination are looked for because they are present in much larger numbers and they persist much longer than pathogens in polluted water.

From a public health point of view, the coliform test is the most important as the presence of *Escherichia coli* at >5 bacilli per 100 ml of unchlorinated water indicates a less than satisfactory supply.

The quantitative assessment used is either a multiple tube, most probable number (MPN) or a membrane filtration method. The exact techniques and media used are cited in the references mentioned or in other national reference publications. *Clostridium perfringens* and *Enterococcus faecalis* can persist in water supplies for long periods. Their presence in water, when coliform organisms are absent, indicates faecal contamination at a more remote time.

Sewage disposal

In highly industrialised countries where large communities have developed, the disposal of industrial and domestic waste is an increasing problem.

International opinion is against untreated sewage being discharged into coastal or estuarine waters and the use of efficient treatment plants to process sewage before discharge is now recommended.

Untreated sewage consists mainly of water containing organic and inorganic dissolved and suspended substances, together with many micro-organisms. After preliminary screening to remove solid matter, the liquid is treated by one of three common methods:

(i) activated sludge process - this involves vigorous stirring or aeration by other means to reduce the BOD and cause separation of the organic matter.

(ii) biological filtration - in this process the liquid is filtered through large beds of sand and the micro-organisms are trapped in the zoogleal slime which forms during filtration.

(iii) oxidation ponds - settled sewage is held in ponds or lagoons for 30 days before the supernatant fluid is released.

All three processes utilise living organisms to reduce the BOD of the effluent to levels where it can be discharged into waterways without causing pollution.

LEGIONNAIRE'S DISEASE

This disease, caused by inhalation of large numbers of *Legionella* species, is essentially a water-borne infection. The infection starts in air-conditioning plants where large volumes of water are recirculated and cooled by blowing air through the water. Such warm circulated water quickly grows large quantities of legionellae and the aerosol of organisms caused by the air-cooling system spreads down-wind to infect passers-by. Not everyone exposed develops the disease of legionellosis and the characteristics of susceptible victims are still being determined but a major factor is the quantity of organisms inhaled. A large inhaled dose of legionellae will inevitably lead to atypical pneumonia.

The most severe form of the disease is caused by *Legionella pneumophila* SG1 and it can be rapidly fatal without prompt antimicrobial treatment.

The organisms can easily be isolated from the water using specific legionella media as described in this manual. Isolation of the organism from the patient is more difficult and most infections are diagnosed by immunological tests.

It is now advised that all recirculating, air-cooled water systems are treated at regular and frequent intervals with bactericidal compounds to prevent the build-up of large numbers of legionellae.

MEDIA FOR WATER AND SEWAGE MICROBIOLOGY

Micro-organism Groups	Purpose	Medium	Code
Aeromonas hydrophila	Selective isolation	Aeromonas Medium Base (Ryan)	CM0831
	Identification	Ampicillin Selective Supplement	SR0136
		O129 discs	DD0014
Campylobacter species	Selective Isolation	Campylobacter selective isolation media:	
		Blood Agar Base No 2	CM0271
		Columbia Blood Agar Base	CM0331
		Brucella Medium Base	CM0169
		Campylobacter Selective Supplement (Skirrow)	SR0069
		Campylobacter Selective Supplement (Butzler)	SR0085
		Modified Butzler ISO Selective Supplement	SR0214
		Laked Horse Blood	SR0048
		Campylobacter Growth Supplement	SR0232
		Campylobacter Selective Agar (Preston)	CM0689
		Preston Campylobacter Selective Supplement	SR0017
		Modified Preston Campylobacter Selective Supplement	SR0204
		Campylobacter Blood Free Selective Agar (modified CCDA)	CM0739
		CCDA Selective Supplement	SR0155
	Identification	Campylobacter test kit	DR0150
Clostridium perfringens	Detection of *C. perfringens* indicating remote or intermittent water pollution	Perfringens Agar Base	CM0587
		Perfringens TSC Selective Supplement	SR0088
		m-CP Agar Base	CM0992
		m-CP Selective Supplement	SR0188
	Confirmation by Nagler Test	Blood Agar Base	CM0055
		Fildes Extract	SR0046
		Egg Yolk Emulsion	SR0047
Coliform group	Detection of coliforms including *Escherichia coli*	Lactose Broth	CM0137
		Lauryl Trytose Broth	CM0451
		MacConkey Broth (purple)	CM0505a
		Minerals Modified Medium	CM0607
		Sodium glutamate	LP0124
		Endo Agar Base	CM0479
		Basic Fuchsin Indicator	BR0050
		MacConkey Agar	CM0007/ CM0507(7b)
		MacConkey Agar No 3	CM0115
		MUG supplement	BR0071
		Violet Red Bile (Lactose) Agar	CM0107
		Violet Red Bile Glucose Agar	CM0485
		EC Broth	CM0853
		EC Broth with MUG	CM0979
		Brilliant Green (2%) Bile Broth	CM0031
	Membrane filtration technique	M-ENDO Agar LES	MM0551
		Basic Fuchsin Indicator	BR0050
		mLGA	CM1031
		Membrane Lauryl Sulphate Broth	MM0615

Micro-organism Groups	Purpose	Medium	Code
Coliform group *cont.*	*E. coli* O157 Isolation and selective enumeration	Sorbitol MacConkey Agar	CM0813
		Cefixime Tellurite Selective Supplement	SR0172
		Buffered Peptone Water	CM0509
		Buffered Peptone Water	CM1049
		Modifed Tryptone Soya Broth	CM0989
		Novobiocin Selective Supplement	SR0181
	Identification Serology	Dryspot *E. coli* O157	DR0120
	Differentiation of coliform group		
	(a) Methyl red and VP test	MRVP Medium	CM0043
	(b) Citrate Utilisation	Simmons Citrate Agar	CM0155
	(c) Indole production	Tryptone Water	CM0087
	(d) Citrate utilisation	Kovacs Reagent	MB0209
		Simmons Citrate Agar	CM0155
Enterococcus species	Detection, isolation and enumeration of faecal enterococci	Azide Blood Agar	CM0259
		Azide Dextrose Broth	CM0868
		Kanamycin Aesculin Azide Agar Base	CM0591
		Kanamycin Íulphate Selective Supplement	SR0092
		KF Streptococcus agar	CM0701
		TTC supplement	SR0229
		Slanetz and Bartley Medium (m-Enterococcus agar)	CM0377
		Bile Aesculin Agar	CM0888
		Brain Heart Infusion Agar	CM0375
		Brain Heart Infusion Broth	CM0225
	Identification	Dryspot Streptococcal Grouping Kit	DR0400
		Streptococcal Grouping Kit	DR0585
General Media	Preparation of dilutions etc	Saline Solution Tablets	BR0053
		Ringers Solution Tablets	BR0052
		Phosphate Buffered Saline	BR0014
		Thiosulphate Ringers Solution	BR0048
		Maximum Recovery Diluent	CM0733
	Subculture and maintenance	Nutrient Broth	CM0067
		Nutrient Agar	CM0033
		Lab Lemco Agar	CM0017
Legionella species	Isolation and identification of *Legionella* species	Legionella CYE Agar Base	CM0655
		Legionella (BCYE) Supplement	SR0110
	Selective supplements	MWY Selective Supplement	SR0118
		BMPA Selective Supplement	SR0111
		GVPC Selective Supplement	SR0152
		GVPN Selective Supplement	SR0215
	Identification of *Legionella* Serotyping	BCYE supplement without L-cysteine	SR0175
			DR0800
			DR0200
		Legionella latex test	DR0210
		Dryspot Legionella	DR0220
Plate count	General heterotrohic plate counts	R2A Agar	CM0906
		Plate Count Agar (APHA)	CM0463
		Yeast Extract Agar	CM0019
		Water Plate Count Agar (ISO)	CM1012
		Milk Agar	CM0021
		Plate Count Agar (Tryptone Glucose Yeast Agar)	CM0325

Micro-organism Groups	Purpose	Medium	Code
Pseudomonas species	Isoation of pseudomonads	Pseudomonas Agar Base	CM0559
	from water	CN Selective Supplement	SR0102
	Identification	Oxidase Sticks	BR0064
Salmonella species	Pre-enrichment	Buffered Peptone Water	CM0509
		Buffered Peptone Water (ISO)	CM1049
	Selective Enrichment	Selenite Broth Base	CM0399
		Sodium Biselenite	LP0121
		Tetrathionate Broth Base	CM0029
		Rappaport Vassiliadis (RV) Enrichment Broth	CM0669
		Rappaport Vassiliadis Soya Broth (RVS)	CM0866
		Enrichment	CM0699
		Selenite Cystine Broth Base	CM0691
		Tetrathionate Broth (USA)	CM0343
		Muller Kauffmann Tetrathionate Broth Base	SR0181
		MKTT-n Broth	
		Novobiocin Selective Supplement	
	Isolation from selective	Bismuth Sulphite Agar	CM0201
	enrichment	Brilliant Green Agar	CM0263
		Brilliant Green Agar (Modified)	CM0329
	Identification	XLD Medium	CM0469
		Salmonella Chromogenic Agar	CM1007
		Salmonella Chromogenic Selective	SR0194
		Supplement	
		Lysine iron Agar	CM0381
		Triple Sugar Iron Agar	CM0277
		Urea Broth	CM0071
		Urea solution	SR0020
		Salmonella latex kit	DR01108
Shigella species	Isolation	Hektoen Enteric Agar	CM0419
		Novobiocin Selective Supplement	SR0181
Staphylococci	Isolation of staphylococci	Baird Parker Agar Base	CM0275
		Egg Yolk Tellurite Emulsion	SR0054
		Mannitol Salt Agar	CM0085
	Identification	Staphytect Plus	DR0850
	Latex agglutination	Dryspot Staphytect Plus	DR0100
	Biochemical Identification	Microbact Staph 125	MB1561
Yersinia species	Selective isolation	Yersinia Selective Agar Base	CM0653
		Yersinia Selective Supplement	SR0109
	Identification	Triple Sugar Iron Agar	CM0277
		Urea Broth	CM0071
		Urea solution	SR0020
Vibrio species	Selective Isolation	TCBS Cholera Medium	CM0333
	Identification	Oxidase sticks	BR0064
		O129 discs	DD0014/
			DD0015
Yeasts and Moulds	Isoation and Enumeration	Czapek Dox Agar	CM0097

CULTURE MEDIA PRODUCT DESCRIPTIONS

AEROMONAS MEDIUM BASE (RYAN)

Code: CM0833

A selective diagnostic medium for the isolation of Aeromonas hydrophila from clinical and environmental specimens when used with Ampicillin Selective Supplement.

Formula	***gm/litre***
Proteose peptone	5.0
Yeast extract	3.0
L. Lysine monohydrochloride	3.5
L. Arginine monohydrochloride	2.0
Sorbitol	3.0
Inositol	2.5
Lactose	1.5
Xylose	3.75
Bile Salts No. 3	3.0
Sodium thiosulphate	10.67
Sodium chloride	5.0
Ferric ammonium citrate	0.8
Bromothymol blue	0.04
Thymol blue	0.04
Agar	12.5
Final pH 8.0 + 0.1	

AMPICILLIN SELECTIVE SUPPLEMENT

Code: SR0136

Vial contents (each vial is sufficient for 500 ml of medium)	***per vial***	***per litre***
Ampicillin	2.5 mg	5.0 mg

Directions
Suspend 29.5 g in 500 ml of distilled water. Bring gently to the boil. DO NOT AUTOCLAVE. Cool to 50°C and aseptically add one vial of Ampicillin Selective Supplement reconsituted as directed. Mix well and pour plates.

Description
Ryan[1] modified the formulation of XLD Medium so that it would support the growth of *Aeromonas* spp. and *Plesiomonas* spp. as well as the usual Enterobacteriaceae. It could therefore be used as a universal medium in the investigation of enteric disease. However, to improve its performance in the isolation of aeromonads, the addition of ampicillin at 5 mg/l is recommended. The effectiveness of ampicillin as a selective agent for *Aeromonas* spp. has been reported by several workers[2,3,4,5,6]. The value of Aeromonas Medium Base (Ryan) is that the recommended level of ampicillin is well below that which can cause inhibition of some strains of aeromonad[7].

The utility of Aeromonas Medium (Ryan) and its superiority over some other formulae for detection of *Aeromonas* spp. in tap water, bottled water and foods including meat, poultry, fish and seafoods has been reported[8,9,10]. Aeromonas Medium (Ryan) is specified by the MAFF/DHS Steering Group on the Microbiological Safety of Food for detection and enumeration of *Aeromonas hydrophila* in clinical specimens[11]. *Aeromonas* spp. occur widely in soil and water, where they cause diseases in fish and amphibians. They also occur in untreated and chlorinated drinking water, raw foods and raw milk[11,12]. It is considered that the major cause of gastrointestinal infections by *Aeromonas* spp.[12,13] is from ingesting infected water[14,15].

The role of these organisms in gastrointestinal disease is still subject to debate but a rapidly expanding body of literature suggests that *Aeromonas* spp. can cause a wide spectrum of enteric symptoms in adults

as well as children[5,16]. It would therefore be a useful diagnostic aid to include this selective medium when investigating diarrhoeal disease.

Aeromonas Medium Base has been developed to improve the enumeration and isolation of *Aeromonas* spp. from clinical and environmental specimens.

Technique

1. Prepare the medium according to directions and pour into sterile dishes. The prepared medium may be stored at 2-8°C up to 5 days.
2. Inoculate the plates with a suspension of food, faeces etc., diluted to form single colonies on the inoculated plate.
3. Incubate the plates aerobically at 30-35°C for 24 hours. If further incubation is required hold at room temperature (22-25°C).
4. Examine the plates for the presence of dark green, opaque colonies with darker centres. Confirm the identity with biochemical tests.

The typical colonial appearance of *Aeromonas* isolates on this medium is as follows:

***Aeromonas* species:**	Dark green, opaque with darker centre, diameter 0.5-1.5 mm
***Pseudomonas* species:**	Blue/grey translucent, diameter from pinpoint to 0.25 mm

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared plates of medium 2-8°C.

Appearance

Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Green coloured gel.

Quality control

Positive control:	***Expected result***
Aeromonas hydrophila ATCC® 7966*	Good growth; opaque green colonies with dark centres
Negative control:	
Escherichia coli ATCC® 11775*	No growth

*This organism is available as a Culti-Loop®

Precautions

Although *Aeromonas* and *Plesiomonas* spp. will grow on the medium if ampicillin is omitted, it will be more difficult to distinguish them from the other organisms present on the plate. Suspected colonies of *Aeromonas* spp. must be confirmed by biochemical tests.

References

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AFPA Base

Code: CM0731

A selective identification medium for the detection of Aspergillus flavus and Aspergillus parasiticus.

Formula	***gm/litre***
Peptone	10.0
Yeast Extract	20.0
Ferric ammonium Citrate	0.5
Dichloran	0.002
Agar	15.0
pH 6.3 + 0.2	

Directions
Suspend 22.75 g in 500 ml of distilled water and heat to dissolve completely. Rehydrate one vial of Chloramphenicol Selective Supplement SR0078 as directed and add to the AFPA Base. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C. Mix well and pour into sterile Petri dishes.

Description
Ideally culture media for isolating and enumerating yeasts and moulds in foods should support recovery of all viable propagules, restrict spreading moulds, inhibit bacterial growth and aid in the identification of the fungi[1]. AFPA Base comes close to this ideal.

Aspergillus flavus and *Aspergillus parasiticus* are fungi which can potentially produce highly dangerous toxic residues known as aflatoxins. They especially affect oilseeds, edible nuts and cereals in subtropical and tropical regions throughout the world due to inadequate storage conditions.

The toxins are particularly carcinogenic in humans and eating contaminated food may result in liver cancer, amongst other diseases. Liver cancer takes time to develop but the aflatoxins also act as an immuno-suppressant so that affected individuals become susceptible to a wide range of diseases. Livestock are also at risk and poultry are particularly susceptible: over 200,000 chickens died in 1994, in Andhra Pradesh, India, after eating contaminated feeds. Cattle are not so susceptible but, if they are fed on contaminated feed, the toxin may pass into the milk.

Besides endangering human health, aflatoxin contamination seriously affects the export potential of high-value commodity crops, such as edible nuts (groundnut, pistachio, cashew and almond) and spices (turmeric and chillies), which could provide an important source of income for farmers in the semi-arid tropics. Diagnosing, or even preventing, aflatoxin contamination will enable subsistence farmers to benefit from increased trade. It will also contribute to an improvement in the general health of people, often the poor, who consume contaminated foods.

The FDA has established specific guidelines on acceptable levels of aflatoxins in human food and animal feed by establishing action levels that allow for the removal of contaminated lots from the food chain. The action level for human food is 20 ppb total aflatoxins, with the exception of milk which has an action level of 0.5 ppb for aflatoxin M1. The action level for most feeds is also 20 ppb. However, it is very difficult to accurately estimate aflatoxin concentration in a large quantity of material because of the variability associated with testing procedures; hence, the true aflatoxin concentration in a lot cannot be determined with 100% certainty.

AFPA Base is based on the formulation of Pitt, Hocking and Glenn[2]. It is recommended for the rapid detection and enumeration of these two species of *Aspergillus*, which are potential aflatoxin producers. AFPA Base is a modification of Aspergillus Differential Medium[3] and shows the following advantages over this and other mycological media.

1. Improved colour production on the reverse of the plate due to the optimal concentration of a more soluble iron salt and the addition of yeast extract. Colonies of *Aspergillus flavus* and *Aspergillus*

parasiticus develop an intense yellow/orange colouration on the reverse of the colonies, and this is a differential characteristic for these species.

2. Improved growth rate of *Aspergillus flavus* due to optimal balance of peptone and yeast extract.
3. Improved inhibition of bacteria and rapidly growing fungi due to a mixture of dichloran and chloramphenicol.

Technique

1. Process the food sample in a Stomacher using 40 g in 200 ml of 0.1% peptone water (Maximum Recovery Diluent CM0733). Alternatively add the sample to 0.1% peptone water and shake periodically for 30 minutes.
2. Dilute the sample 1:10, 1:20 and 1:40 in 0.1% peptone water.
3. Surface plate 0.1 ml of each dilution.
4. Incubate at 30°C and examine after 42-43 hours.
5. Count all colonies‡ that show the reverse, yellow/orange pigmentation.
6. Report the results as a number of colonies of *Aspergillus flavus* and *Aspergillus parasiticus* per gram of food.

‡*Aspergillus oryzae* can produce the same yellow/orange pigmentation. It is important in the production of Asian fermented foods, particularly soy sauce, and is only rarely isolated from other sources.

Aspergillus niger produces colonies of similar size and texture to *Aspergillus flavus* at 30°C. However, on the reverse the colonies may appear pale yellow but will never be yellow/orange. After 43 hours or longer incubation, colonies of *Aspergillus niger* remain pale yellow but begin production of black conidial heads which enables clear differentiation to be made from *Aspergillus flavus*.

Please note:

Moulds and fungi growing on this medium may produce mycotoxins. Therefore, as well as the normal precautions taken to avoid disseminating infection, the plates should be carefully handled and disposed of safely. This precaution would also apply to positive food samples.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared plates at 2-8°C.

Appearance

Dehydrated Medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured gel.

Quality control

Positive controls:	***Expected results***
Aspergillus flavus ATCC® 22547	White mycelium, buff spores, orange underside
Aspergillus parasiticus ATCC® 28285	White mycelium, cream spores, orange underside
Negative control:	
Aspergillus niger ATCC® 16404*	White mycelium, black spores, yellow underside
Escherichia coli ATCC® 25922*	No growth

*This organism is available as a Culti-Loop®

References

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ALKALINE PEPTONE WATER

Code: CM1028

A broth medium for the enrichment of Vibrio species from food, water and clinical samples.

Formula	***gm/litre***
Peptone	10.0
Sodium Chloride	20.0
pH 8.6 ± 0.2	

Directions
Add 30 g to one litre of distilled water. Mix well, distribute into final containers and sterilize by autoclaving at 121°C for 15 minutes.

Description
Alkaline Peptone Water is for the enrichment of *Vibrio cholera* and *Vibrio* species from food, water and clinical samples. This broth can also be used for direct microscopic examination of samples using the hanging drop method.

Alkaline Peptone Water was first formulated by Shread, Donovan and Lee[1] to be used as a non-selective enrichment broth for the cultivation of *Aeromonas* species. Cruickshank reported that when the pH is raised, the medium can be used to effectively cultivate Vibrio species[2].

The 2% (w/v) sodium chloride incorporated in this medium promotes the growth of *Vibrio cholerae*, while the alkalinity of this medium inhibits most of the unwanted background flora.

Technique
There are various methods available for the isolation of *Vibrio* species from environmental, food and clinical samples. These generally involve a pre-enrichment step followed by plating onto a solid medium and morphological, biochemical and serological identification. Many different enrichment media have been described but of these only Alkaline Peptone Water has achieved wide acceptance.

Refer to the appropriate guidlelines or standards for formulations and exact methodology.

Clinical Samples
Inoculate swab specimens directly into Alkaline Peptone Water. Material not being cultured directly from a swab may be transferred into the medium using a sterile microbiological loop. For faecal specimens, aseptically transfer approximately 1 g of the sample to the medium and mix well. The inoculated broths are generally incubated at 35-37°C for 5-6 hours or 18-20 hours at 18-20°C[3].

Food and Water Samples
Refer to the appropriate standard such as APHA[4,5] FDA-BAM[6] and ISO[7].

For all methods plating media should be incubated overnight and then inspected for typical colonies:

Sodium Dodecyl Sulphate Polymixin B Sucrose Medium (SPS Medium): Sucrose positive vibrios such as *Vibrio cholerae* and *Vibrio alginolyticus* are yellow in colour. Sucrose negative species such as *Vibrio parahaemolyticus* and *Vibrio vulnificus* produce blue green colonies. Organisms producing sulphatase e.g. *Vibrio vulnificus* are also usually surrounded by a halo of precipitation.

Cholera Medium TCBS (CM0333): Sucrose positive vibrios such as *Vibrio cholerae* and *Vibrio alginolyticus* are yellow in colour on TCBS. Sucrose negative species such as *Vibrio parahaemolyticus* and *Vibrio vulnificus* produce blue green colonies.

MacConkey Agar (CM0007): Lactose negative *Vibrio* species produce colourless colonies.

Biochemical Confirmation: *Vibrio* spp. are oxidase positive and ferment glucose with the production of acid only. As oxidase testing may lead to false negative results on media containing carbohydrates (such as TCBS) subculture to nutrient or blood agar before testing.

Storage conditions and Shelf life
The dehydrated medium should be stored at 10-30°C and used before the expiry date on the label. Store the prepared medium may be stored for up to 1 month at room temperature

Appearance
Dehydrated Medium: Straw coloured, free-flowing powder.

Prepared medium: Clear, straw coloured liquid

Quality Control

Positive control:	***Expected results***
Vibrio parahaemolyticus ATCC® 17802*	Turbid growth
Vibrio vulnificus ATCC® 27562*	Turbid growth
Vibrio furnissii ATCC® 11218*	Turbid growth
Negative control:	
Uninoculated medium	No change

References

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AMIES TRANSPORT MEDIUM

Code: CM0425

An improved transport medium, containing charcoal to prolong the viability of pathogenic organisms.

Formula	***gm/litre***
Charcoal pharmaceutical	10.0
Sodium chloride	3.0
Sodium hydrogen phosphate	1.15
Potassium dihydrogen phosphate	0.2
Potassium chloride	0.2
Sodium thioglycollate	1.0
Calcium chloride	0.1
Magnesium chloride	0.1
Agar	4.0
pH 7.2 + 0.2	

Directions

Suspend 20 g in 1 litre of distilled water. Bring to the boil to dissolve the agar completely. Distribute into small, screwcap bottles, stirring meanwhile to keep the charcoal evenly suspended. Screw down the caps firmly on the completely filled bottles. Sterilise by autoclaving at 121°C for 15 minutes. Invert the bottles whilst cooling to distribute the charcoal uniformly. Store in a cool place.

Description

Amies[1] modified Stuart's Transport Medium[2,3,4] by replacing glycerophosphate with an inorganic phosphate buffer and adding charcoal to the medium.

The metabolism of glycerophosphate by coliform organisms and other Gram-negative rods in Stuart's original formulation resulted in the proliferation of these organisms from wound swabs and faecal specimens.

A concentration of NaCl at 0.3% w/v was discovered by Amies to be optimal for the preservation of *Neisseria gonorrhoeae*.

Calcium and magnesium salts were added in the belief that these ions were of importance in controlling the permeability of the bacterial cells and so contributing to their survival.

Stuart[3] showed that the survival of *Neisseria gonorrhoeae* was increased by the use of charcoal swabs, but because they were black and dusty, they proved unpopular with the patients. Amies[1] overcame this problem by incorporating charcoal in this medium.

Survival of *N. gonorrhoeae* at 22°C

85 strains	*With charcoal*	*Without charcoal*
Time	***No. of strains surviving***	***No. of strains surviving***
24 hours	82	20
48 hours	70	0
72 hours	38	0

100 strains	*Stuart's Medium*	*Amies Medium*
Time	***No. of strains surviving***	***No. of strains surviving***
24 hours	91	98
48 hours	79	87
72 hours	56	77

(Tables taken from Amies[1]).

The agar concentration was increased from that proposed by Stuart because the presence of charcoal particles interferes with the gelling properties of the agar.

Amies removed the methylene blue indicator from Stuart's formulation considering it superfluous because of the presence of charcoal in the medium. Care should be taken to ensure that the prepared bottles of transport medium are not stored longer than 9 months from the date of preparation, or freshly steamed and the charcoal resuspended before use.

The value of these modifications was shown in two studies which tested the efficiency of various transport media[5,6]. Amies Transport Medium is recommended for the transport of specimens to be cultured for *Bacteroides ureolyticus*.[7]

Technique

Use sterile, cotton-tipped swabs on wooden sticks to collect the specimen. Push the swab down one third of the medium depth and cut the stick so that when the cap is screwed down, the swab is forced to the bottom of the medium.

Make sure the cap is screwed firmly on the bottle and keep cool during the transport period.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

The prepared medium, held in tightly screw-capped bottles, can be stored at room temperature.

Appearance

Dehydrated medium: Black coloured, free-flowing powder.

Prepared medium: Straw coloured semi-solid gel.

Quality Control

Positive control:	***Expected result at 35°C***
Staphylococcus aureus ATCC® 25923*	Good growth
Escherichia coli ATCC® 25922 *	Good growth
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

Precautions

It is important that the charcoal is properly suspended in the medium, invert the bottles when the bottles are cool but the agar still liquid.

During preparation of the medium, avoid prolonged heating in open flasks because thioglycollate is volatile.

Old medium should be freshly steamed and the charcoal resuspended before use.

Keep medium cool during transport but do not freeze.

References
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ANDRADE'S PEPTONE WATER – see PEPTONE WATER (ANDRADE)

ANAEROBE BASAL AGAR

Code: CM0972

A nutrient agar for the growth of anaerobic micro-organisms, particularly Bacteroides spp. and other fastidious anaerobes.

Formula	***gm/litre***
Peptone	16.0
Yeast extract	7.0
Sodium chloride	5.0
Starch	1.0
Dextrose	1.0
Sodium pyruvate	1.0
Arginine	1.0
Sodium succinate	0.5
L-cysteine HCl	0.25
Sodium bicarbonate	0.4
Ferric pyrophosphate	0.5
Haemin	0.005
Vitamin K	0.0005
Dithiothreitol	0.25
Agar	12.0
pH 6.8 ± 0.2	

Directions
Suspend 46 g in 1 litre of distilled water. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50-55°C and aseptically add 5-10% sterile Defibrinated Horse Blood SR0050.

Mix well and pour into sterile Petri dishes.

Description
Anaerobe Basal Agar contains peptones, carefully selected to support good growth of anaerobic bacteria and yeast extract as a vitamin source. Starch is present to absorb any toxic metabolites[1]. Sufficient arginine is added to ensure the growth of *Eubacterium lentum*[2], whilst haemin and vitamin K are growth factors required by many *Bacteroides* species[3]. Haemin is also required by *Porphyromonas* species. Sodium succinate improves the growth of *Prevotella melaninogenica* and *Bacteroides* species[4]. Sodium pyruvate is added as an energy source for asaccharolytic cocci such as *Veillonella*. It also acts similarly to catalase and degrades traces of hydrogen peroxide, which may be produced by the action of molecular oxygen on media components[5]. L-cysteine hydrochloride and dithiothreitol are reducing agents, and cysteine has also been shown to stimulate the growth of some anaerobes[6].

Technique
Inoculate the medium by surface plating to obtain single colonies. Incubate anaerobically for up to 5 days at 37°C. Anaerobic conditions can be achieved using the Oxoid AnaeroGen Atmosphere Generation System AN0025 with the Oxoid AnaeroJar AG0025.

The medium may be rendered selective for Gram-negative anaerobes by the addition of G-N Supplement SR0108 and for non-sporing anaerobes by the addition of N-S Supplement SR0107 with Tween 80. Neomycin Selective Supplement SR0163 can be added to select for Clostridia.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared plates of medium for up to 3 weeks at 2-8°C.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured gel.

Quality control

Positive controls:	***Expected result***
Peptostreptococcus anaerobius ATCC® 27337*	Good growth; grey coloured colonies
Prevotella melaninogenica ATCC® 25845*	Good growth; grey coloured colonies
Clostridium perfringens ATCC® 13124*	Good growth; grey coloured colonies
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

References

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3. Gibbons R. J. and MacDonnald J. B. Haemin and vitamin K compounds as required factors for the cultivation of certain strains of *Bacteroides melaninogenicus*. *J. Bact.* 1960: 80. 164-170.
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5. Neilson P. A. Role of reduced sulphur compounds in nutrition of *Proprionobacterium acnes*. *J. Clin*. *Micr.* 1983: 17. 276-9.
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ANAEROBE BASAL BROTH

Code: CM0957

A nutrient broth for the growth of anaerobic micro-organisms, particularly Bacteroides spp. and other fastidious anaerobes.

Formula	***gm/litre***
Peptone	16.0
Yeast extract	7.0
Sodium chloride	5.0
Starch	1.0
Dextrose	1.0
Sodium pyruvate	1.0
Arginine	1.0
Sodium succinate	0.5
L-cysteine HCl	0.5
Sodium bicarbonate	0.4
Ferric pyrophosphate	0.5
Haemin	0.005
Vitamin K	0.0005
Sodium thioglycollate	0.5
Dithiothreitol	1.0
pH 6.8 ± 0.2	

Directions
Suspend 35.4 g of Anaerobe Basal Broth in 1 litre of distilled water. Bring to the boil to dissolve completely. Distribute into final containers. Sterilise by autoclaving at 121°C for 15 minutes.

Description
Oxoid Anaerobe Basal Broth is formulated from a range of nutrients which have been selected to optimise the recovery and growth of the majority of anaerobic organisms of clinical importance.

The formulation includes yeast extract as a source of vitamins and starch is included to absorb toxic products[1]. Sufficient arginine is added to ensure growth of *Eubacterium lentum*[2], whilst haemin and vitamin K are present as they are essential for the growth of *Bacteroides* spp[3]. Pyruvate is present as an energy source for asaccharolytic cocci such as *Veillonella* spp. It also eliminates traces of hydrogen peroxide which may be produced by the action of molecular oxygen on medium constituents[4]. Sodium succinate improves the growth of *Prevotella melaninogenica* and *Bacteroides* spp[5]. It is included together with the reducing agent L-cysteine hydrochloride, which has been shown to stimulate directly the growth of some anaerobes[6].

Technique
It is preferable to use freshly reconstituted and sterile medium which is inoculated as soon as it has cooled to room temperature. Tubes which are not used on the day of preparation should be placed in a boiling water bath or steamer for approximately 15 minutes to remove dissolved oxygen. They should be allowed to cool without agitation and then inoculated.

Storage conditions and Shelf life
Anaerobe Basal Broth should be stored tightly capped in the original container at 10-30°C. When stored as directed, the medium will remain stable until the expiry date printed on the label.

Appearance
Dehydrated Medium: Straw coloured, free-flowing powder.

Prepared medium: Straw/green coloured solution.

Quality control

Positive controls:	***Expected results***
Peptostreptococcus anaerobius ATCC® 27337*	Turbid growth
Prevotella loescheii ATCC® 15930	Turbid growth
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

References

1. Ajello G. W. Geely J. C. Hayes P. S. *et al*. Trans-isolate medium: a new medium for primary culturing and transport of *Neisseria meningitidis*, Streptococcus pneumoniae and *Haemophilus influenzae*. *J. Clin. Micro.* 1984:20:55-8.
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4. Neilson P. A. Role of reduced sulphur compounds in nutrition of *Proprionobacterium acnes*. *J. Clin. Micr.* 1983:17:276-9.
5. Lev M. Keudell K. C. and Milford A. F. Succinate as a growth factor for *Bacteroides melaninogenicus*. *J. Bact*. 1971:108:175-8.
6. Shanson D. C. and Singh J. Effect of adding cysteine to brain-heart infusion broth on the isolation of *Bacteroides fragilis* from experimental blood cultures. *J. Clin. Path.* 1981:34:221-3.

ANTIBIOTIC MEDIUM No. 1

SEED AGAR

Code: CM0327

A medium recommended for the seed layer in the preparation of plates for the microbiological assay of antibiotics.

Formula	***gm/litre***
Peptone	6.0
Tryptone	4.0
Yeast extract	3.0
'Lab-Lemco' powder	1.5
Glucose	1.0
Agar No.1	11.5
pH 6.5 ± 0.2	

Directions

Suspend 27 g in 1 litre of distilled water and bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

Description

This is perhaps the most important medium in antibiotic assay work and it is in a specially modified form to take advantage of the properties of Oxoid Agar No. 1.

Hanus, Sands and Bennett[1] drew attention to the inhibitory properties which certain agars have towards some antibiotics, particularly streptomycin, kanamycin, polymyxin B and neomycin. Because Oxoid Agar No. 1 does not share these inhibitory properties it is especially suited to antibiotic assay work. In addition, this agar, with its superior diffusion properties, produces more clearly defined inhibition zones.

NB. In the Oxoid formulation, only 11.5 g of Agar No. 1 are used to give the equivalent gel strength of 15 g ordinary agar.

This medium is used as a seed agar with *Micrococcus flavus* for the plate assay of bacitracin; with *Sarcina lutea* for the plate assay of chloramphenicol and with *Staphylococcus aureus* for the assay of kanamycin sulphate, penicillin G, sodium methicillin and sodium oxacillin.

It is also employed as a base agar in the assay of the following drugs: chloramphenicol, kanamycin sulphate, colistin sulphate, sodium methicillin, sodium oxacillin and vancomycin hydrochloride.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared plates of medium at 2-8°C.

Appearance

Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured gel.

Quality control

Positive control:	***Expected results***
Bacillus subtilis ATCC® 6633*	Good growth; cream coloured colonies
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

Reference

1. Hanus F. J., Sands J. G. and Bennett E. O. (1967) *Applied Microbiology* 15(1). 31-34.

ANTIBIOTIC MEDIUM No. 3 ASSAY BROTH

Code: CM0287

Used in the serial dilution assay of penicillin and other antibiotics.

Formula	***gm/litre***
Peptone	5.0
Yeast extract	1.5
'Lab-Lemco' powder	1.5
Glucose	1.0
Sodium chloride	3.5
Dipotassium hydrogen phosphate	3.68
Potassium dihydrogen phosphate	1.32
pH 7.0 ± 0.2	

Directions

Add 17.5 g to 1 litre of warm (60°C) distilled water and mix well to dissolve. Distribute and sterilise by autoclaving at 121°C for 15 minutes.

Description

Medium No. 3 is used in the turbidimetric assay of penicillin and tetracycline with *Staphylococcus aureus*.

Taking advantage of modern technology, this medium is based on the original 'Penassay Broth' which has been withdrawn.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Appearance

Dehydrated Medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured solution.

Quality Control

Positive control:	***Expected results***
Staphylococcus aureus ATCC® 25923*	Turbid growth
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

ARCOBACTER BROTH

Code: CM0965

An enrichment broth for Arcobacter species

Formula	***gm/litre***
Peptone	18.0
Yeast extract	1.0
Sodium chloride	5.0
pH 7.2 ± 0.2	

Directions for use with CAT Supplement SR0174
Dissolve 12 g of Arcobacter Broth in 500 ml of distilled water. Sterilise at 121°C for 15 minutes.

Allow to cool to 50°C and add one vial of CAT Selective Supplement SR0174 reconstituted as directed.

Dispense into sterile containers.

Directions for use with CCDA Supplement SR0155
Dissolve 12 g of Arcobacter Broth in 500 ml of distilled water. Sterilise at 121°C for 15 minutes. Allow to cool to 50°C and add one vial of CCDA Selective Supplement SR0155 reconstituted as directed.

Dispense into sterile containers.

Incubate at 30°C aerobically for 24 hours.

Description
Oxoid Arcobacter Broth is intended for use with Cefoperazone, Amphotericin B, Teicoplanin (CAT) Selective Supplement SR0174 as a selective enrichment broth for the growth of *Arcobacter* species and with the more selective CCDA SR0155 for the selective enrichment of *Arcobacter butzleri*.

Peptones in the base medium are specifically designed to provide the ideal growth conditions for *Arcobacter* species. The incubation conditions and the absence of blood or charcoal supplements suppress the growth of *Campylobacter* species. Cefoperazone, Amphotericin B and Teicoplanin are added to suppress the growth of competing flora, but allow the growth of Arcobacter species. CCDA Selective Supplement SR0155 is substituted for CAT to selectively isolate *Arcobacter butzleri*[1,2].

Arcobacters are micro aerophilic, Gram-negative rods, which were formerly classified as Campylobacter[3].

Four Arcobacter species have been identified: *Arcobacter butzleri*, *Arcobacter cryaerophilus*, *Arcobacter skirrowii* and *Arcobacter nitrofigilis*, all of which have a greater propensity to grow in air than *Campylobacter* spp.

Arcobacter butzleri, *Arcobacter cryaerophilus*, and *Arcobacter skirrowii* have been associated with disease in humans[4,5], and typically are isolated from faecal samples.

Arcobacter butzleri has been isolated from patients with bacteraemia, peritonitis, endocarditis and diarrhoea. Patients with *Arcobacter butzleri*-associated diarrhoea typically suffer from abdominal pain and nausea, fever, chills, vomitting and malaise, but the organism has also been implicated in an outbreak of recurrent abdominal cramps without diarrhoea[6]. The source of infection is usually contaminated water or sewage[6].

Arcobacter cryaerophilus group 1B has been isolated from patients with bacteraemia and diarrhoea[4,5], although it is a much less common human isolate than *Arcobacter butzleri* [6].

Arcobacter nitrofigilis has only been isolated from marsh grass to date, never from humans or animals. It is not thought to be clinically significant[7].

Appearance
Dehydrated Medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured solution.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Quality Control

Positive control:	
Arcobacter butzleri ATCC® 12481	White/grey colonies
Negative control:	
Escherichia coli ATCC® 25922	Inhibited

References

1. Data on file
2. Lammerding, A. M., Harris, J. E., Lior, D. L. *et al*. Presented at the 81st annual meeting of IAMFES (1994).
3. Vandamme, P., Falsen, E., Rossau, R., Hoste, B., Segers, P., Tytgat, R., De Ley, J. (1991). *Int. J. Syst. Bacteriol.* 41:88-103.
4. Kiehlbauch, J. A., Brenner, D. J., Nicholson, M. A., Baker, C. N., Patton, C. M., Steigerwalt, A. G., Wachsmuth, I. K. (1991). *J. Clin. Microbiol.* 29:376-385.
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ASPERGIUS MEDIUM – see AFPA MEDIUM

AZIDE BLOOD AGAR BASE

Code: CM0259

A selective medium for the detection and isolation of streptococci and staphylococci from faeces, sewage and other specimens. With added blood the medium may be used for the determination of haemolytic reactions.

Formula	*gm/litre*
Tryptose	10.0
'Lab-Lemco' powder	3.0
Sodium chloride	5.0
Sodium azide	0.2
Agar	12.0
pH 7.2 ± 0.2	

Directions

Suspend 30 g in 1 litre of distilled water and bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. For azide blood agar, cool to 45-50°C and add 5% of sterile blood.

Description

A selective medium for the detection and isolation of streptococci from faeces, sewage, and other specimens containing a mixed flora. Azide Blood Agar Base is similar to the medium used by Edwards[1] for the isolation of mastitis streptococci. Sodium azide has a bacteriostatic effect on most Gram-negative organisms but permits growth of Gram-positive organisms such as streptococci and some strains of staphylococci. Proteus species are slightly more resistant than other *Enterobacteriaceae* but swarming is prevented (Snyder and Lichstein[2], Lichstein and Snyder[3]).

At the concentration and pH used, sodium azide exerts no appreciable effect on haemolysis so that the medium, with added blood, may be used for the simultaneous determination of haemolytic reactions.

Azide blood agar is recommended by the American Public Health Association[4] for the isolation of streptococci from cheese. The plates, inoculated with dilutions of emulsified cheese, are incubated at 35°C and representative colonies subcultured for subsequent identification.

There are variations in formula of Azide Blood Agar Base which have been recommended for different purposes:

1. Packer[5] increased the sodium azide concentration to 0.9 g per litre and added 0.002 g per litre of crystal violet. The pH was also adjusted to 6.8 ± 0.1. This is a more selective medium for faecal streptococci in foods[6].
2. Packer[5] and Wood[7] used the above formulation with 5% blood and crystal violet increased at 0.01 g per litre, for the isolation of *Erysipelothrix rhusiopathiae* and *Streptococcus pneumoniae*.
3. Dale[8] and Bohm[9] recommended the addition of phenol (1.0-2.5 g per litre) to Packer's formulation to isolate *Erysipelothrix rhusiopathiae*.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store prepared blood agar plates of medium at 2-8°C.

Appearance

Dehydrated Medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured gel.

Quality Control

Positive controls:	***Expected results***
Enterococcus faecalis ATCC® 29212*	Good growth; white/grey colonies
Staphylococcus aureus ATCC® 25923*	Good growth; white colonies
Negative control:	
Proteus vulgaris ATCC® 13315*	Inhibited
Escherichia coli ATCC® 25922*	Inhibited

*This organism is available as a Culti-Loop®

Precautions

Proteus and *Escherichia* species may not always be inhibited on the Edward's formulation.

Always use a light inoculum for best selective results.

Anaerobic incubation will enhance haemolytic reactions.

Haemolytic reactions will not be typical on Packer's modification of Azide Blood Agar Base. *Streptococcus lactis* will not grow on Packer's modification with 5% sheep blood.

Read the section on Hazard Precautions for azide-containing media.

References

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AZIDE DEXTROSE BROTH (ROTHE)

Code: CM0868

For the detection of enterococci in water.

Formula	***gm/litre***
Peptone	20.0
Glucose	5.0
Sodium chloride	5.0
Di-potassium hydrogen phosphate	2.7
Potassium dihydrogen phosphate	2.7
Sodium azide	0.2
Final pH 6.8 ± 0.2	

Direction
Add 35.6 g to one litre of distilled water for single strength broth or 71.2 g for double strength broth. Heat gently to dissolve. Dispense into final containers and sterilise by autoclaving at 121°C for 15 minutes.

Description
Azide Dextrose Broth (Rothe) is used for the detection of enterococci in water and sewage[1].

The presence of enterococci serves as an indicator of faecal contamination. Enterococci are better indicators than *Escherichia coli* of sewage pollution in chlorinated waters because they have a greater resistance to chlorine.

Mallmann and Seligmann[2] recommended Azide Dextrose Broth for the quantitative determination of enterococci in water, sewage, foods and other materials suspected of contamination with sewage.

A blend of peptone and glucose render Azide Dextrose Broth highly nutritious, and sodium chloride maintains osmotic equilibrium. The use of sodium azide as an inhibitor of Gram-negative organisms has been reported by several workers[2,3,4], and the concentration selected provides optimum protection for the enterococci while largely suppressing the Gram-negative flora. The phosphate buffer system controls pH.

Technique
Inoculate 10 ml of medium with 1 ml of the test sample. Inoculate a further three tubes with 0.1 ml, 0.01 ml and 0.001 ml sample respectively. For samples of 10 ml or more, use double strength broth. Incubate all tubes at 35°C and examine for turbidity after 24 and 48 hours. For a more detailed description please consult 'Standard Methods for the Examination of Water and Wastewater'.

The presence of enterococci in the sample is indicated by turbidity in the broth.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured solution.

Quality control

Positive control:	***Expected results***
Enterococcus faecalis ATCC® 29212*	Turbid growth
Negative control:	
Escherichia coli ATCC® 25922*	No growth

*This organism is available as a Culti-Loop®

Precautions
This product contains less than 1% azide and has low toxicity. However, when handling the powder, wear gloves, mask and eye protection. When washing azide products down sinks, use sufficient water to prevent accumulation of azide in the plumbing.

References
1. Greenberg A. E. *et al.* (ed). (1998) *Standard Methods for the Examination of Water and Wastewater*, 20th edn. APHA, Washington, DC.
2. Mallmann W. L. and Seligmann E. B. (1950) *Am. J. Public Health* 40. 286.
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BACILLUS CEREUS SELECTIVE AGAR BASE

Code: CM0617

A selective and diagnostic medium for the isolation and enumeration of Bacillus cereus.

Formula	***gm/litre***
Peptone	1.0
Mannitol	10.0
Sodium chloride	2.0
Magnesium sulphate	0.1
Disodium hydrogen phosphate	2.5
Potassium dihydrogen phosphate	0.25
Bromothymol blue	0.12
Sodium pyruvate	10.0
Agar	15.0
pH 7.2 ± 0.2	

BACILLUS CEREUS SELECTIVE SUPPLEMENT

Code: SR0099

Vial contents (each vial is sufficient or 500 ml of medium)	***per vial***	***per litre***
Polymyxin B	50,000 IU	100,000

Directions
Suspend 20.5 g in 475 ml of distilled water and bring gently to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C and aseptically add the contents of one vial of Oxoid Bacillus Cereus Selective Supplement reconstituted as directed, then add 25 ml of sterile Egg Yolk Emulsion SR0047. Mix well and pour into sterile Petri dishes.

Description
Bacillus Cereus Selective Agar, is based on the highly specific diagnostic and selective PEMBA medium, developed by Holbrook and Anderson[1] for the isolation and enumeration of *Bacillus cereus* in foods. It meets the requirements for a medium that is sufficiently selective to be able to detect small numbers of *Bacillus cereus* cells and spores in the presence of large numbers of other food contaminants. The medium is also sufficiently diagnostic that colonies of *Bacillus cereus* are readily identified and confirmed by microscopic examination.

The role of *Bacillus cereus* in food poisoning, particularly from the consumption of contaminated rice, is now well documented[2,3,4]. The organism has also been implicated in eye infections[5,6] and a wide range of other conditions including abscess formation, meningitis, septicaemia and wound infection. *Bacillus cereus* is recognised as a significant pathogen in post-operative and post-traumatic wounds of orthopaedic patients[7].

Amongst veterinarians, *Bacillus cereus* is a known cause of disease, especially mastitis, in ewes and heifers[8].

In the formulation of Bacillus Cereus Selective Agar a peptone level of 0.1% and the addition of sodium pyruvate improve egg yolk precipitation and enhance sporulation. Bromothymol blue is added as a pH indicator to detect mannitol utilisation. The medium is made selective by addition of Bacillus Cereus Selective Supplement, which gives a final concentration of 100 IU of polymyxin B per ml of medium. Polymyxin B, as a selective agent for the isolation of *Bacillus cereus* has been previously suggested by Donovan[9] and found to be satisfactory by Mossel[10]. It is recommended that, where large numbers of fungi

are expected in the inoculum, cycloheximide (SR0222) is added to the medium at a final concentration of 40 mg/litre.

The primary diagnostic features of the medium are the colonial appearance, precipitation of hydrolysed lecithin and the failure of *Bacillus cereus* to utilise mannitol.

The typical colonies of *Bacillus cereus* are crenated, about 5 mm in diameter and have a distinctive turquoise to peacock blue colour surrounded by a good egg yolk precipitate of the same colour.

These features distinguish *Bacillus cereus* from other *Bacillus* species except *Bacillus thuringiensis*. Other egg yolk-reacting organisms which can grow on the medium, including *Staphylococcus aureus*, *Serratia marcescens* and *Proteus vulgaris* are distinguished from *Bacillus cereus* by colony form and colour. These organisms also produce an egg yolk-clearing reaction in contrast to egg yolk precipitate produced by *Bacillus cereus*.

Microscope examination for presence of lipid globules in the vegetative cells is recommended as a rapid and confirmatory test for *Bacillus cereus* and replaces the need for biochemical testing. Holbrook and Anderson[1] have confirmed that only *Bacillus cereus* of the *Bacillus* species are capable of possessing lipid globules in their vegetative cells when grown on the selective medium. One further advantage of this test is that strains of *Bacillus cereus* that react only weakly or not at all with egg yolk can be detected and confirmed.

Technique

1. Homogenise 10g of the food sample for 30 seconds in 90 ml of 0.1% Peptone Water CM0009 using a Stomacher 400[11]. Dried foods should first be rehydrated by soaking 20 g in 90 ml of Tryptone salt solution (Tryptone LP0042 0.3% and sodium chloride 0.8%, pH 7.3) for 50 minutes at room temperature. Add a further 90ml of 0.1% peptone water to give a final dilution of 10-1. Homogenise for 30 seconds using the Stomacher 400.
2. Further dilutions of the homogenate should be made in 0.1% peptone water.
3. Inoculate 0.1 ml amounts of the 10-1 and higher dilutions on to the surface of the medium.
4. Incubate the plates at 35°C for 24 hours.
5. Examine for typical colonies of *Bacillus cereus*.
6. Leave the plates for a further 24 hours at room temperature in order to detect all the *Bacillus cereus* colonies.
7. Confirm the presumptive identification of *Bacillus cereus* by the Rapid Confirmatory Staining Procedure.
8. Report the results as the number of *Bacillus cereus* colonies per gram weight of the food sample.

The medium may also be used for detecting *Bacillus cereus* in milk. When necessary, decimal dilutions of the samples should be made in 0.1% peptone water. Undiluted and diluted samples are inoculated directly onto plates of agar and incubated. An incubation temperature of 30°C for 18 hours is recommended as optimal for promoting the growth of *Bacillus cereus* relative to that of other organisms[9].

For examining clinical specimens plates may be inoculated in the usual way.

Rapid Confirmatory Staining Procedure

This staining method was developed by Holbrook and Anderson[1] combining the spore stain of Ashby[12] and the intracellular lipid stain of Burdon[13]. For reasons of safety, Citroclear* replaces xylene in the original technique.

Procedure

1. Prepare films from the centre of a 1-day-old colony or from the edge of a 2-day colony.
2. Air-dry the film and fix with minimal heating.
3. Flood the slide with aqueous 5% w/v malachite green and heat with a flaming alcohol swab until steam rises. Do not boil.
4. Leave for 2 minutes without re-heating.
5. Wash the slide with running water and blot dry.
6. Flood the slide with 0.3% w/v Sudan black in 70% ethyl alcohol. Leave for 15 minutes.
7. Wash the slide with running Citroclear* from a wash bottle for 5 seconds.
8. Blot dry using filter paper.
9. Flood the slide with aqueous 0.5% w/v safranin for 20 seconds.
10. Wash under running water.
11. Blot dry and examine under the microscope using the oil immersion lens. A blue filter may be used to accentuate the appearance of the lipid granules but this will give a blue colour cast to the red of the cytoplasm.

*Citroclear is available from: H.D. Supplies, Aylesbury, Buckinghamshire. Tel: +44 (0)1296 431920

Characteristic appearance of *B. cereus* vegetative cells

(i) Cells are 4-5 micron long and 1.0-1.5 micron wide with square ends and rounded corners.

(ii) The spores stain pale green to mid green, are central or paracentral in position and do not swell the sporangium.

(iii) Lipid globules are black and the vegetative cytoplasm red.

The appearance, together with the typical colony form, confirms the identification of *Bacillus cereus*.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

The prepared medium may be stored at 2-8°C.

Appearance

Dehydrated medium: Dark straw/yellow coloured, free-flowing powder.

Prepared medium: Dark blue coloured gel.

Quality control

Positive control:	***Expected results***
Bacillus cereus ATCC® 10876	Good growth; pale blue colonies with precipitate and peacock blue medium
Negative controls:	
Bacillus subtilis ATCC® 6633*	Growth; straw coloured colonies
Escherichia coli ATCC® 25922*	Inhibited

*This organism is available as a Culti-Loop®

Precautions

On this medium *Bacillus cereus* is indistinguishable from *Bacillus thuringiensis*.

Identify *Bacillus cereus* by colony form, colour, egg yolk hydrolysis and confirm with cell and spore morphology[14].

Occasional strains of *Bacillus cereus* show weak or negative egg yolk reactions.

References

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BACILLUS CEREUS MYP AGAR – see MYP AGAR

BACILLUS CEREUS CHROMOGENIC AGAR – see CHROMOGENIC BACILLUS CEREUS AGAR

BAIRD-PARKER AGAR BASE

Code: CM0275

A selective and diagnostic medium for the isolation and enumeration of Staphylococcus aureus in foods.

Formula	***gm/litre***
Tryptone	10.0
'Lab-Lemco' powder	5.0
Yeast extract	1.0
Sodium pyruvate	10.0
Glycine	12.0
Lithium chloride	5.0
Agar	20.0
pH 6.8 ± 0.2	

Directions

Suspend 63 g in one litre of distilled water and boil to dissolve the medium completely. Dispense into tubes or flasks and sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C and aseptically add 50 ml of Egg Yolk Tellurite Emulsion SR0054. Mix well before pouring.

Prepared plates may be stored at 4°C.

Description (with E-Y-T Emulsion SR0054)

Baird-Parker[1] developed this medium from the tellurite-glycine formulation of Zebovitz *et al*.[2] and improved its reliability in isolating *Staphylococcus aureus* from foods.

Baird-Parker added sodium pyruvate, to protect damaged cells and aid their recovery[3] and egg yolk emulsion as a diagnostic agent. It is now widely recommended by national and international bodies for the isolation of *Staphylococcus aureus*[4].

The selective agents glycine, lithium and tellurite have been carefully balanced to suppress the growth of most bacteria present in foods, without inhibiting *Staphylococcus aureus*.

Egg yolk emulsion makes the medium yellow and opaque. *Staphylococcus aureus* reduces tellurite to form grey-black shiny colonies and then produces clear zones around the colonies by proteolytic action. This clear zone with typical grey-black colony is diagnostic for *Staphylococcus aureus*. On further incubation, most strains of *Staphylococcus aureus* form opaque haloes around the colonies, and this is probably the action of a lipase. Not all strains of *Staphylococcus aureus* produce both reactions. Some strains of *Staphylococcus saprophyticus* produce both clear zones and opaque haloes but experienced workers can distinguish these from *Staphylococcus aureus* by the longer incubation time required[5].

Colonies typical of *Staphylococcus aureus* but without an egg yolk reaction should also be tested for coagulase production[6].

Egg yolk reaction negative strains of *Staphylococcus aureus* may occur in some foods, especially cheese.

Smith and Baird-Parker[7] found that the addition of 50 mg of sulphametazine per ml of medium suppressed the growth and swarming of *Proteus* species. Small numbers of *Staphylococcus aureus* could then be recovered from specimens containing mixed *Proteus* strains.

Baird-Parker and Davenport[8] showed that the recovery of damaged staphylococci was greater on Baird-Parker medium than on other recovery media tested.

Broeke[9] and de Waart *et al*.[10] found Baird-Parker medium valuable in ecological studies on foods incriminated in staphyloenterotoxicosis. 97.5% of the 522 strains of *Staphylococcus aureus* tested, isolated from human and food origins developed characteristically and quantitatively on Baird-Parker medium.

Colony characteristics of typical organisms on Baird-Parker Egg Yolk-Tellurite Medium

Organism	***Growth***	***Colony***
Staphylococcus aureus	Good	Grey-black shiny convex 1-1.5 mm diameter (18 hours) up to 3 mm (48 hours) narrow white entire margin surrounded by zone of clearing 2-5 mm
Staphylococcus epidermidis	Variable	Not shiny black and seldom produces clearing
Staphylococcus saprophyticus	Variable	Irregular and may produce clearing Wide opaque zones may be produced in 24 hrs
Micrococcus species	Variable	Very small in shades of brown and black. No clearing
Bacillus species	Variable	Dark brown matt with occasional clearing after 48 hrs
Escherichia coli.	Variable	Large brown black
Proteus species	Variable	Brown black with no clearing
Yeasts	Variable	White, no clearing

Technique

1. Dry the surface of agar plates for a minimal period of time prior to use.
2. With a glass spatula, spread 0.1 ml aliquots of food dilutions made up in Buffered Peptone Water on the agar surface until it is dry. Up to 0.5 ml may be used on larger dishes (24 cm).
3. Incubate the inverted dishes at 35°C. Examine after 24 hours and look for typical colonies of *Staphylococcus aureus*. Re-incubate negative cultures for a further 24 hours.

Quantitative results

Incubate the dishes for 48 hours and select those with 20-200 colonies.

Count the *Staphylococcus aureus*-like colonies and test them for coagulase reaction.

Report *Staphylococcus aureus* results per gram of food.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Prepared plates of medium are best used freshly prepared[11].

Appearance

Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured gel.

Quality control

Positive control:	***Expected Results (48 hours)***
Staphylococcus aureus ATCC® 25923*	Good growth; black shiny colonies with white and clear zones
Negative control:	
Staphylococcus epidermidis ATCC® 155	Inhibited or no growth

*This organism is available as a Culti-Loop®

Precautions

Regard all suspicious colonies as *Staphylococcus aureus* regardless of negative reactions in the medium and carry out further tests.

Colonies of some contaminating organisms growing in close proximity to the coagulase positive colonies may partially digest the coagulase halo reaction.

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BAIRD-PARKER AGAR BASE (RPF)

Code: CM0961

An improved base medium for use with RPF Supplement. This conforms to ISO 6888 Part 2 for the enumeration of coagulase-positive staphylococci.

Formula	***gm/litre***
Pancreatic digest of casein	10.0
Meat extract	5.0
Sodium pyruvate	10.0
Yeast extract	1.0
Glycine	12.0
Lithium chloride	5.0
Agar	20.0
pH 7.2 ± 0.2	

RPF SUPPLEMENT

Code: SR0122

Vial contents (per vial sufficient for 100 ml of medium)	***per vial***	***per litre***
Bovine fibrinogen	0.375 g	3.75 g
Rabbit plasma	2.5 ml	25.0 ml
Trypsin inhibitor	2.5 mg	25.0 mg
Potassium tellurite	2.5 mg	25.0 mg

Directions
Suspend 6.3 g of Baird-Parker Agar Base (RPF) in 90 mls of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121ºC for 15 minutes. Cool to 48ºC and add 1 vial of RPF Supplement (SR0122), reconstituted as directed. Mix well and pour plates.

NB: Baird-Parker Agar Base (RPF) should only be used with RPF Supplement.

Description
Staphylococcus aureus is a Gram-positive coccus capable of producing enterotoxin which can induce food poisoning. The organisms may be present in small numbers in many foods and if allowed to multiply unchecked may produce highly heat-resistant enterotoxins. The ability of *Staphylococcus aureus* to produce lecithinase and lipase has been recognised for many years and the detection of these enzymes in egg yolk medium has become a widely used procedure for the identification of this organism. The ability of *Staphylococcus aureus* to produce coagulase using a similar basal formulation enables confirmatory diagnosis with the incorporation of rabbit plasma into the base medium.

Rabbit Plasma Fibrinogen Agar (RPF Agar) is based on the formulation described by Beckers *et al.*[1]. This

medium is a modification of Baird-Parker Medium and is recommended for the selective isolation, enumeration and confirmation of *Staphylococcus aureus* from food and other specimens[2].

The RPF Agar formulation retains the Baird-Parker Agar Base which has been specifically formulated to resuscitate injured cells[3]. This medium differs from Baird-Parker Medium in that the egg yolk emulsion has been replaced by fibrinogen, rabbit plasma and trypsin inhibitor. The fibrinogen was added to enhance the coagulase reaction in the RPF Agar[4]. Rabbit plasma was selected and it was found to be more specific for the coagulase activity when compared to other sources of plasma[1]. Trypsin inhibitor was added to prevent fibrinolysis.

The RPF Agar supplement has been modified in one respect from the original formulation in that the potassium tellurite content has been reduced four-fold, i.e. from 0.01% to 0.0025% w/v. This reduction was necessary as it was discovered in the Oxoid laboratory that some strains of *Staphylococcus aureus* were sensitive to potassium tellurite when used at 0.01% w/v in RPF Agar[5]. This modification of RPF Agar was found to give comparable growth and selectivity to that achieved on Baird-Parker Medium. The improved productivity of RPF Agar has also been confirmed by other laboratories[6,7]. The reduction in potassium tellurite concentration in RPF Agar results in *Staphylococcus aureus* strains forming white or grey or black colonies, which are surrounded by an opaque halo of precipitation, i.e. the coagulase reaction.

Technique

Surface Inoculation Method

1. Prepare the RPF Agar plates as directed.
2. Process the food sample in a stomacher or Waring blender using the recommended sample size and diluent.
3. Separate plates are inoculated with 0.1 ml of the prepared samples and the subsequent decimal dilutions of them.
4. Incubate at 35°C and examine after 24 and 48 hours incubation.
5. Count all the colonies that have an opaque halo of precipitation around them. Do not limit the count to black colonies.
6. Report as number of coagulase positive staphylococcus isolated per gram of food.

Pour Plate Method

1. Prepare the RPF Agar as directed and hold at 48°C.
2. Process the food sample in a stomacher or Waring blender using the recommended sample size and diluent.
3. Add 1 ml of the prepared sample (initial suspension and subsequent decimal dilution) into each sterile Petri dish.
4. Add aseptically 20 ml of sterile RPF Agar and prepare pour plates.
5. Incubate at 35°C and examine after 24-48 hours.
6. Count all the colonies that have an opaque halo of precipitation around them.
7. Report as number of coagulase positive staphylococcus isolated per gram of food.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Prepared plates of medium are best used freshly prepared[1,2].

Appearance

Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured gel.

Quality control

Positive control:	***Expected results***
Staphylococcus aureus ATCC® 25923*	Good growth: grey/black colonies with coagulase zones
Negative controls:	
Staphylococcus epidermidis ATCC® 12228*	Inhibited growth: grey/black colonies with no zones
Bacillus subtilis ATCC® 6633*	No growth

*This organism is available as a Culti-Loop®

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BCYE – see BUFFERED CHARCOAL YEAST EXTRACT

BiGGY AGAR

Code: CM0589

For the isolation and presumptive identification of Candida species.

Formula	***gm/litre***
Yeast extract	1.0
Glycine	10.0
Glucose	10.0
Sodium sulphite	3.0
Bismuth ammonium citrate	5.0
Agar	13.0
pH 6.8 ± 0.2	

Directions

Suspend 42 g in 1 litre of distilled water and bring gently to the boil to dissolve the agar. Allow to cool to 50-55°C. Mix gently to disperse the flocculant precipitate and pour into sterile Petri dishes. **DO NOT AUTOCLAVE THE MEDIUM.**

Description

BiGGY, Bismuth Sulphite Glucose Glycine Yeast Agar, is based on the formulation developed by Nickerson[1] and may be used for the isolation and presumptive identification of *Candida* species.

In a study of sulphite reduction by yeasts, the ability of many yeasts to reduce a bismuthyl hydroxy polysulphite was noted. This was demonstrated to be most evident in *Candida* species, but strong reducing ability was confined to *Candida albicans*, *Candida krusei* and *Candida tropicalis*. Growth on an acidic or neutral medium containing bismuth sulphite produced black colonies because of the extra-cellular reduction of the bismuth sulphite, to bismuth sulphide.

The bismuth sulphite complex confers a high degree of selectivity to the medium, and most strains of bacteria are inhibited on BiGGY Agar.

Barr and Collins[2] described the addition of neomycin sulphate to the medium at 2 mg per litre to improve inhibition of accompanying bacterial flora.

The medium may be used for the isolation and presumptive identification of *Candida albicans* and *Candida tropicalis* from sputum[2,3] and vaginal smears[4]. It is a recommended medium for the quality assessment of pharmaceutical and cosmetic products[5].

Technique
Reconstitute the medium as directed and pour into sterile Petri dishes to contain approximately 20 ml of medium.

Freshly prepared plates should be used. Reactions on slant cultures are unsatisfactory[1].

Incubate the plates at 28-30°C and examine daily for evidence of sulphite reduction.

Colony appearance on BiGGY Agar (48 hours)

	Colony morphology
C. albicans	Smooth, circular brown black, slight mycelial fringe; no colour diffusion into surrounding medium; no sheen.
C. tropicalis	Smooth, dark brown with black centres; slight mycelial fringe; diffuse blackening of medium after 72 hours; sheen.
C. krusei	Large, flat, wrinkled silvery brown black with brown peripheries; yellow halo diffused into medium.
C. pseudotropicalis	Medium size, flat, dark reddish brown glistening; slight mycelial fringe; no diffusion.
C. parakrusei	Medium size, flat, wrinkled, glistening dark reddish brown with lighter periphery; extensive yellow mycelial fringe.
C. stellatoidea	Medium size, flat, dark brown; very light mycelial fringe.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Medium should be freshly prepared just prior to use.

Quality control

Positive controls:	***Expected results***
Candida albicans ATCC® 10231*	Good growth; brown coloured colonies
Candida tropicalis ATCC® 750*	Good growth; brown coloured colonies
Negative control:	
Escherichia coli ATCC® 25922*	No growth

*This organism is available as a Culti-Loop®

Precautions
Carry out further tests to confirm identity of isolated yeasts.

Do not use slants of medium because the reactions are unsatisfactory.

The flocculent precipitate present in the molten medium must be evenly suspended whilst dispensing the agar.

References

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BILE AESCULIN AGAR

Code: CM0888

A differential medium for the isolation and presumptive identification of enterococci/Group D streptococci.

Formula	***gm/litre***
Peptone	8.0
Bile salts	20.0
Ferric citrate	0.5
Aesculin	1.0
Agar	15.0
pH 7.1 ± 0.2	

Directions
Suspend 44.5 g in 1 litre of distilled water and bring gently to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

Description
The major use of Bile Aesculin Agar is to differentiate between enterococci/Group D streptococci and non Group D streptococci. It may also be used for the presumptive identification of other groups of organisms.

Enterococci/Group D streptococci hydrolyse aesculin to form aesculetin and dextrose. Aesculetin combines with ferric citrate in the medium to form a dark brown or black complex which is indicative of a positive result. Bile salts will inhibit Gram-positive bacteria other than enterococci/Group D streptococci.

The value of bile tolerance together with hydrolysis of aesculin as a means of presumptively identifying enterococci/Group D streptococci is widely recognised[1,2,3,4,5].

The use of these parameters forms the basis of Bile Aesculin Agar and was described by Swan[6] who concluded that the use of this medium is a valid alternative to Lancefield grouping for the recognition of enterococci/Group D streptococci.

Facklam[7] further confirmed its usefulness in differentiating enterococci/Group D streptococci from non Group D streptococci while other workers have used the medium for presumptive identification of the Klebsiella-Enterobacter-Serratia group amongst the Enterobacteriaceae[8,9,10].

Technique
Using a sterile loop inoculate the medium with 4-5 colonies and incubate at 37°C for 18-24 hours.

The result is positive for bile salt tolerance and aesculin hydrolysis if blackening of the medium occurs.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw brown coloured gel.

Quality control

Positive controls:	***Expected result***
Enterococcus faecalis ATCC® 29212*	Good growth; brown coloured colonies with aesculin hydroysis
Enterobacter aerogenes ATCC® 13048*	Good growth; brown coloured colonies with aesculin hydrolysis
Negative control:	
Streptococcus pyogenes ATCC® 19615*	No growth

*This organism is available as a Culti-Loop®

References

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BISMUTH SULPHITE AGAR

Code: CM0201

A modification of the original Wilson and Blair Medium for the isolation of Salmonella typhi and other salmonellae. It is particularly useful for the isolation of lactose-fermenting salmonellae.

Formula	***gm/litre***
Peptone	5.0
'Lab-Lemco' powder	5.0
Glucose	5.0
Disodium phosphate	4.0
Ferrous sulphate	0.3
Bismuth sulphite indicator	8.0
Brilliant green	0.016
Agar	12.7
pH 7.6 ± 0.2	

Directions
Suspend 20 g in 500 ml of distilled water in a 1 litre flask. Heat gently with frequent agitation until the medium just begins to boil and simmer for 30 seconds to dissolve the agar. Cool to 50-55°C, mix well to disperse suspension and pour thick plates (25 ml medium per plate). Allow the medium to solidify with the dish uncovered. Larger volumes may be prepared if great care is taken and adequate head space provided.

Dry the plates before use but take care to avoid overdrying. Correctly prepared plates should have a smooth, cream-like opacity with a pale straw colour. There should be no sedimentation of the indicator. **DO NOT OVERHEAT – DO NOT AUTOCLAVE**

Description
Bismuth Sulphite Agar is a modification of the original Wilson and Blair[1] selective medium for the isolation and preliminary identification of *Salmonella typhi* and other salmonellae from pathological material, sewage, water supplies, food and other products suspected of containing these pathogens. In this medium freshly precipitated bismuth sulphite acts together with brilliant green as a selective agent by suppressing the growth of coliforms, whilst permitting the growth of salmonellae. Sulphur compounds provide a substrate for hydrogen sulphide production, whilst the metallic salts in the medium stain the colony and surrounding medium black or brown in the presence of hydrogen sulphide.

Atypical colonies may appear if the medium is heavily inoculated with organic matter. Such a situation may be prevented by suspending the sample in sterile saline and using the supernatant for inoculation.

The freshly prepared medium has a strong inhibitory action[2] and is suitable for heavily contaminated samples. Storing the poured plates at 4°C for 3 days causes the medium to change colour to green, making it less selective with small numbers of salmonellae being recovered[3]. However, for *Salmonella typhi* recovery the latter technique is not recommended[4].

Where the number of salmonellae is expected to be small, enrichment methods may be employed.

The use of this medium is advocated by several authorities[5,6,7].

Technique
Bismuth Sulphite Agar may be used in conjunction with other selective enteric agars for the isolation of salmonellae by direct plating or from enrichment media[8]. Thus the following scheme may be adopted. Inoculate directly on Bismuth Sulphite Agar and one or more of the following:

Desoxycholate Citrate Agar CM0227 or DCLS Agar CM0393
XLD Agar CM0469

Brilliant Green Agar CM0329
MacConkey Agar No. 3 CM0115

At the same time inoculate an enrichment broth, such as Selenite Broth Base CM0395 + Sodium Biselenite LP0121 or Tetrathionate Broth CM0343. Sub-culture on to Bismuth Sulphite Agar and any other selective medium after 12-18 hours incubation. Examine the plates after 18 hours incubation and sub-culture suspect colonies to identification media, e.g. Kligler Iron Agar CM0033.

All negative plates should be incubated for 48 hours.

Salmonella typhi

Appearance

Black 'rabbit-eye' colonies with a black zone and metallic sheen surrounding the colony after 18 hours. Uniformly black after 48 hours incubation.

Other *Salmonella* species

Appearance

Variable colony appearance after 18 hours, they may be black, green or clear and mucoid. Uniformly black colonies are seen after 48 hours, often with widespread staining of the medium and a pronounced metallic sheen.

Other organisms, e.g. coliform bacteria, *Serratia*, *Proteus* species

Appearance

Usually inhibited but occasional strains give dull green or brown colonies with no metallic sheen or staining of the surrounding medium.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label. Note the following comments:

Due to its contents of reactive and hygroscopic substances, dehydrated Bismuth Sulphite Agar quickly deteriorates when exposed to the atmosphere. This is usually indicated by aggregation into a solid non-friable mass, and by the development of a brown coloration. Medium reconstituted from such material is brown, does not become green on storage, and is characterised by loss of differential and selective properties. For this reason the powder should be stored in a cool, dry place and after use the container should be properly closed.

Prepared medium

It is recommended that the medium should be used on the day of preparation.

Appearance

Dehydrated medium: Pale green coloured, free-flowing powder.

Prepared medium: Pale green coloured gel.

Quality control

Salmonella typhi should be used only in a Class II laboratory, not for routine testing or in food laboratories.

Positive controls:	***Expected results***
Salmonella poona NCTC 4840*	Good growth; black coloured colonies with metallic sheen
Salmonella typhimurium ATCC® 14028*	Good growth; black coloured colonies with metallic sheen
Negative controls:	
Escherichia coli ATCC® 25922*	Inhibited or no growth
Citrobacter freundii ATCC® 8090*	Inhibited or no growth

*This organism is available as a Culti-Loop®

Precautions

Prepared plates of medium should not be stored for longer than two days at 2-8°C; after which time the dye oxidises to give a green medium that can be inhibitory to some salmonellae.

Shigella species are usually completely inhibited.

Salmonella sendai, *Salmonella cholera-suis*, *Salmonella berta*, *Salmonella gallinarum* and *Salmonella abortus-equi* are markedly inhibited[9].

It is important that the spreading technique yields well separated colonies. The typical colonial characteristics will not develop if the growth is too heavy or confluent; *Salmonella typhi* colonies will appear light green in these circumstances. Therefore, when in doubt, almost any growth on the medium should be subject to further tests.

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BLASER-WANG SELECTIVE MEDIUM

A campylobacter medium which can inhibit the growth of Candida albicans.

COLUMBIA BLOOD AGAR BASE

Code: CM0331

Formula	***gm/litre***
Special peptone	23.0
Starch	1.0
Sodium chloride	5.0
Agar	10.0
pH 7.3 ± 0.2	

Directions

Add 39 g to 1 litre of distilled water. Boil to dissolve and sterilise by autoclaving at 121°C for 15 minutes.

CAMPYLOBACTER SELECTIVE SUPPLEMENT (BLASER-WANG)

Code: SR0098

Vial contents (each vial is sufficient for 500 ml of medium)	***per vial***	***per litre***
Vancomycin	5.0 mg	10.0 mg
Polymyxin B	1,250 IU	2,500 IU
Trimethoprim	2.5 mg	5.0 mg
Amophotericin B	1.0 mg	2.0 mg
Cephalothin	7.5 mg	15.0 mg

Directions

Reconstitute one vial as directed add the contents of one vial to 500 ml of sterile nutrient medium cooled to approximately 50°C prepared from Columbia Agar or Blood Agar Base No. 2, with 10% defibrinated horse/sheep blood or 5-7% laked horse blood SR0050, SR0051 or SR0048. Mix gently and pour into sterile Petri dishes.

Description
Campylobacter Selective Supplement Blaser-Wang is based on the formulation of Skirrow[1], but with the addition of amphotericin B and cephalothin[2].

The inclusion of amphotericin B inhibits the growth of *Candida albicans* and cephalothin improves the selectivity of the supplement.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Opaque red coloured gel.

References
1. Skirrow M. B. (1977) *BMJ* 2. 9-11.
2. Blaser M. J., Hardesty H. L., Powers B. and Wang W. L. L. (1980) *J. Clin. Micro.* 11. 309-313.

BLEB – see BUFFERED LISTERIA ENRICHMENT BROTH

BLOOD AGAR BASE

Code: CM0055

A non-selective general purpose medium which may be enriched with blood or serum.

Formula	***gm/litre***
'Lab-Lemco' powder	10.0
Peptone neutralised	10.0
Sodium chloride	5.0
Agar	15.0
pH 7.3 ± 0.2	

Directions
Suspend 40 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

For blood agar, cool the Base to 50°C and add 7% of Defibrinated Horse Blood SR0050. Mix with gentle rotation and pour into Petri dishes or other containers.

Description
Oxoid Blood Agar Base is a non-selective general purpose medium widely employed for the growth of pathogenic and non-pathogenic bacteria:

(i) Without additions, the medium may be employed as a nutrient agar (a richer medium than Nutrient Agar CM0003), or as a medium for the short-term maintenance of stock cultures.

(ii) With added serum or other enrichments, the medium becomes suitable for the cultivation of many fastidious organisms. Serum and other thermolabile enrichments should be added to the sterilised medium cooled to 45-50°C.

(iii) With added blood, the medium is not only enriched, but becomes suitable for the determination of the typical haemolytic reactions which are important diagnostic criteria for streptococci, staphylococci, and other organisms. For blood agar, 7% of sterile blood should be added to the sterilised medium cooled to 45-50°C.

Blood Agar Base was used during investigations on irradiated *Escherichia coli* and other bacteria[1,2]. It was the most suitable medium for investigating the phages of *Clostridium perfringens*[3] and as the basis of a selective medium for *Clostridium perfringens*[4]. It was used with added phenolphthalein phosphate for the detection of phosphatase-producing staphylococci[5] and with added salt and agar for the assessment of surface contamination on equipment and pig carcasses[6]. It was used for determining the salinity range of growth of marine flavobacteria[7].

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared plates of medium at 2-8°C.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured gel.

Quality control

Positive controls:	***Expected results***
Staphylococcus aureus ATCC® 25923*	Good growth; grey white coloured colonies
Streptococcus pyogenes ATCC® 19615*	Good growth; pale colonies; beta haemolysis
Streptococcus pneumoniae ATCC® 6303*	Good growth; green grey coloured colonies; alpha heamolysis.
Negative control:	
Uninoculated plate	No change

*This organism is available as a Culti-Loop®

Precautions
The haemolytic reactions of organisms inoculated onto this medium will be affected by the animal blood used e.g. horse or sheep and the incubation conditions e.g. aerobic, capnoeic or anaerobic[8].

When horse blood is added to the medium *Haemophilus haemolyticus* colonies will produce beta-haemolysis and mimic *Streptococcus pyogenes*[8].

References

1. Alper T. and Gillies N. E. (1960) *J. Gen. Microbiol.* 22. 113-128.
2. Hodgkins Brenda and Alper T. (1963) *J. Gen. Microbiol.* 30. 307-315.
3. Williams Smith H. (1959) *J. Gen. Microbiol.* 21. 622-630.
4. Noble W. C. (1961) *J. Path. Bact.* 81. 523-526.
5. Noble W. C. (1962) *J. Clin. Path.* 15. 552-558.
6. Hansen N. H. (1962) *J. Appl. Bact.* 25. 46-53.
7. Hayes P. R. (1963) *J. Gen. Microbiol.* 30. 1-19.
8. Facklam R. R. (1980) in *Manual of Clinical Microbiology.* Eds. Lennette E. H., Balows A., Hausler W. J. & Truant J. P. 3rd edn. *Amer. Soc. for Microbiology.* Washington DC. pp.88-110.

BLOOD AGAR BASE No. 2

Code: CM0271

An improved Blood Agar Base possessing enhanced nutritional properties suitable for the cultivation of fastidious pathogens and other micro-organisms.

Formula	***gm/litre***
Proteose peptone	15.0
Liver digest	2.5
Yeast extract	5.0
Sodium chloride	5.0
Agar	12.0
pH 7.4 ± 0.2	

Directions
Suspend 40 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 45-50°C and add 7% sterile blood.

Mix with gentle rotation and pour into sterile dishes or other containers.

Description

Oxoid Blood Agar Base No. 2 was developed to meet the demand for an especially nutritious blood agar base which would permit the maximum recovery of delicate organisms without interfering with their haemolytic reactions. In comparison with fresh digest agar, Blood Agar Base No. 2 may be shown to have equal or superior growth promoting properties and chromogenic bacteria grown on the Oxoid medium show enhanced pigment formation. Comparison with many other blood agars has shown that with Oxoid Blood Agar Base No. 2 growth of many bacteria – especially the fastidious streptococci and pneumococci – is considerably improved, as shown by luxuriant and early colonial development.

Oxoid Blood Agar Base No. 2 is specified by the American Food and Drug Administration for the preparation of sheep blood agar[1].

Phillips[2] described an improved medium for sporulation of *Clostridium perfringens* based on Blood Agar Base No. 2 to which are added lysed horse blood, bile, sodium bicarbonate and quinoline.

The medium induced significant sporulation in all of 100 strains of *Clostridium perfringens* isolated from human faeces.

Brucella:

To prepare a selective medium add Brucella Selective Supplement SR0083 or Modified Brucella Selective Supplement SR0209 to 500ml of sterile, molten Blood Agar Base No. 2 containing 5-10% v/v inactivated horse serum and 1% w/v dextrose[2,3].

Campylobacter:

To prepare a selective medium add Campylobacter Supplement (Skirrow)[5] SR0069 or Campylobacter Supplement (Butzler)[6] SR0085 or Modified Butzler (ISO) Campylobacter Supplement SR0214 or Campylobacter Supplement (Blaser-Wang)[7] to 500 ml of sterile, molten Blood Agar Base No. 2 containing Campylobacter Growth Supplement SR0084 or SR0232 as required and 5-7% v/v horse or sheep blood (SR0048, SR0050 or SR0051).

Haemophilus:

For the primary isolation of *Haemophilus* species from specimens containing a mixed flora, use Blood Agar Base No. 2 with added Defibrinated Horse Blood SR0050. Even better results may be obtained using the horse blood agar plates with half of each spread with 2 drops of 10% saponin[9]. Where haemolytic reactions are not important, for example when dealing with pure cultures, the Base may be used to prepare chocolate agar. Add 10% of Defibrinated Horse Blood code SR0050 to the Base at 80°C and maintain at this temperature for 5 to 10 minutes, agitating frequently. Cool to 50°C, mix well and pour plates.

Roberts, Higgs and Cole used Blood Agar Base No. 2 as the basis of a medium which is selective for *Haemophilus* spp. in primary culture of clinical specimens. The medium distinguishes *Haemophilus influenzae* and *Haemophilus parainfluenzae* by differences in colony colour[10].

A selective chocolate blood agar for the culture of *Haemophilus influenzae* from respiratory secretions of cystic fibrosis patients has been described[11]. The medium is based on Blood Agar Base No. 2 to which 7% v/v horse blood and 8mg/litre of cefsulodin is added. Growth of *Pseudomonas aeruginosa* and *Staphylococcus aureus* on this medium is inhibited.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared plates of medium at 2-8°C.

Appearance

Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured gel.

Quality control

Positive controls:	***Expected results***
Blood Agar	
Staphylococcus aureus ATCC® 25923*	Good growth; white/grey colonies
Streptococcus pyogenes ATCC® 19615*	Good growth; pale straw coloured colonies; β-haemolysis
Haemophilus influenzae ATCC® 35056	Good growth; colourless colonies
Brucella Agar	
Brucella abortus ATCC® 4315	Good growth
Campylobacter Agar	
Campylobacter jejuni ATCC® 29428*	Good growth; grey/brown colonies
Negative controls:	
Blood Agar	
Uninoculated medium	No change
Brucella Agar and Campylobacter Agar	
Escherichia coli ATCC® 25922*	Inhibited

*This organism is available as a Culti-Loop®

Precautions

Brucella cultures are highly infective and must be handled under properly protected conditions. Incubate in 5-10% carbon dioxide atmosphere for 24-48 hours.

References

1. F.D.A. *Bacteriological Analytical Manual* (1998) 8th Edition. F.D.A. Washington DC.
2. Phillips K. D. (1986) *Lett. Appl. Microbiol.* 3. 77-79.
3. Farrell I. D. and Robinson L. (1972) *J. Appl. Bact.* 35. 625-630.
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6. Butzler J. P. and Skirrow M. B. (1979) *Clins. Gastroenterol.* 8. 737-65.
7. Blaser M. J., Hardesty H. L., Powers B. and Wang W. L. L. (1980) *J. Clin. Microbiol.* 11. 309-313.
8. George H. A., Hoffman P. S. and Krieg N. R. (1978) *J. Clin. Microbiol.* 8. 36-41.
9. Waterworth Pamela M. (1955) *Brit. J. Exp. Path.* 36. 186-194.
10. Roberts D. E., Higgs E. and Cole P. J. (1987) *J. Clin. Pathol.* 40. 75-76.
11. Smith A. and Baker M. (1997) *J. Med. Microbiol.* 46. 883-885.

BMPA SELECTIVE MEDIUM

A semi-selective medium for the isolation of Legionella pnumophila from clinical and environmental specimens.

LEGIONELLA CYE AGAR BASE

Code: CM0655

Formula	***gm/litre***
Activated charcoal	2.0
Yeast extract	10
Agar	13.0

LEGIONELLA BMPA SELECTIVE SUPPLEMENT

Code: SR0111

Vial contents	*Per 100 ml vial*	*Per 500 ml vial*	*Per litre*
Cefamandole	0.4 mg	2.0 mg	4.0 mg
Polymyxin B	8,000 IU	40,000 IU	80,000 IU
Anisomycin	8 mg	40 mg	80 mg

Direction
Suspend 2.5 g of Legionella CYE Agar Base in 90 ml of distilled water and bring gently to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Allow to cool to 50°C and aseptically add the contents of one vial of Legionella BYCE Growth Supplement SR0110 and one vial of Legionella BMPA-α Selective Supplement reconstituted as directed. Mix gently and pour into sterile Petri dishes. The final pH of both media should be 6.9 ± 0.2.

Description
The discovery of the causative organism of Legionnaires' disease has been reviewed by Fallon[1]. Since that review further progress has been made in culturing the organism from clinical specimens and also in the enumeration of Legionella species from environmental samples. Feeley *et al*.[2] described a modification of F-G Agar[3] in which acid hydrolysed casein was replaced by yeast extract as the source of protein and starch was replaced by activated charcoal (Norit A) at a final concentration of 0.2% (w/v). This medium, which they named CYE Agar[2] has been further supplemented with ACES Buffer and α-ketoglutarate and is described in the literature as BCYE-α Medium[4]. BCYE-α Medium has been shown to yield optimal recovery of Legionellaceae in a shorter incubation period from environmental samples and clinical specimens[5].

Oxoid BCYE Medium is based on the formulation of Edelstein[4] and is prepared from Legionella CYE Agar Base and Legionella BCYE Growth Supplement SR0110. The sterile lyophilised supplement contains ACES Buffer/potassium hydroxide, α-ketoglutarate, ferric pyrophosphate and L-cysteine HCl. When added to CYE Agar Base it stabilises the pH of the medium at 6.9 ± 0.2 and provides essential growth factors.

Technique
For each sample, three plates should be inoculated: one after pretreatment with heat, one after pretreatment with acid and one that has received neither pretreatment.

Heat pre-treatment
1. Take 10 ml of concentrated sample and place in a water bath at 50°C for 30 minutes.

Acid pre-treatment
1. Take 10 ml of concentrated sample and centrifuge in sealed buckets at 2,500 rpm for 20 minutes.
2. Decant the supernatant to leave approximately 1 ml of fluid.
3. Add 9 ml of HCl-KCl buffer (see below) and resuspend by gentle shaking. Leave to stand for 5 minutes and inoculate without further delay.

HCl-KCl Buffer
3.9 ml of 1.2M HCl
25 ml of 0.2M KCl
Adjust to pH 2.2 using 1M KOH

Directions

Environmental Samples
1. Take 10 ml of the concentrated sample and centrifuge at 2,500 rpm for 20 minutes (using sealed buckets).
2. Remove the supernatant to leave approximately 1 ml of fluid. Resuspend the deposit. This constitutes the inoculum.
3. Spread 0.1 ml on to plates of BCYE Medium with and without selective agents using a sterile spreader.
4. Add 9 ml of HCl-KCl buffer* (pH 2.2); shake gently and leave for 5 minutes.

*HCl-KCl buffer: 3.9 ml of 0.2 M HCl; 25 ml of 0.2 M KCl; Adjust the pH to 2.2 using 1M KOH.

Alternatively
Heat 10 ml of the sample concentrate in a 50°C water bath for 30 minutes.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.
Store the selective supplement in the dark at 2-8°C and use before the expiry date on the label.

Appearance
Dehydrated medium: Black free flowing powder
Prepared medium: Black coloured gel.

References

1. Fallon J. Oxoid Limited. *Culture September* 1979, P. 3-4.
2. Feeley J. C., Gibson R. J., Gorman G. W., Langford N. C., Rasheed J. W., Mackel D. C. and Baine W. B. (1979) *J. Clin. Micro.* 10. 437-441.
3. Feeley J. C. Gorman G. W., Weaver R. E., Mackel D. C. and Smith H. W. (1978) *J. Clin. Micro.* 8. 320-325.
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5. PHLS Communicable Diseases Report (1983) CDR 83/49.

BLOOD AGAR BASE SHEEP BLOOD – see SHEEP BLOOD AGAR BASE

BLOOD AGAR BASE WITH TRYPTOSE – see TRYPTOSE BLOOD AGAR BASE

BOLTON SELECTIVE ENRICHMENT BROTH

BOLTON BROTH

Code: CM0983
A medium for the selective pre-enrichment of Campylobacter organisms in food samples.

Formula	*gm/litre*
Meat peptone	10.0
Lactalbumin hydrolysate	5.0
Yeast extract	5.0
Sodium chloride	5.0
Alpha-ketoglutaric acid	1.0
Sodium pyruvate	0.5
Sodium metabisulphite	0.5
Sodium carbonate	0.6
Haemin	0.01
pH 7.4 ± 0.2	

BOLTON BROTH SELECTIVE SUPPLEMENT

Code: SR0183

Vial contents (each vial is sufficient to supplement 500 ml of medium)

	per vial	*per litre*
Cefoperazone	10.0 mg	20.0 mg
Vancomycin	10.0 mg	20.0 mg
Trimethoprim	10.0 mg	20.0 mg
Cycloheximide	25.0 mg	50.0 mg

Directions
Add 13.8 g of Bolton Broth to 500 ml of distilled water. Sterilise by autoclaving at 121°C for 15 minutes. Cool

to 50°C. Aseptically add 25 ml Laked Horse Blood SR0048 and 1 vial of Bolton Broth Selective Supplement, reconstituted as directed. Mix well and distribute into sterile screw-top containers.

Description

Bolton Selective Enrichment Broth is intended for the pre-enrichment of *Campylobacter* in food samples. *Campylobacter* are Gram-negative, spirally-shaped microaerophilic organisms which may be present in raw milk, untreated water, improperly handled food and undercooked meats, poultry and shellfish. Human consumption of these organisms can result in a range of clinical illnesses from transient asymptomatic colonisation to severe dysentery. The symptoms of *Campylobacter enteritis* include diarrhoea, stomach pain, nausea, fever, headache and muscle pain. Complications of infection by *Campylobacter jejuni* may include unnecessary appendectomies as a result of abdominal pain, reactive arthritis or Guillian-Barré syndrome[1]. *Campylobacter* infection is recognised as one of the most common causes of bacterial gastroenteritis in humans, and the minimum infective dose may be as low as 500-800 cells[1].

Since awareness of the apparent role of *Campylobacter* in human disease was heightened by Skirrow in 1977[2], a great number of culture media have evolved in response to the need to optimise performance. There was early recognition of the need for enrichment culture when examining food samples to overcome the damaging effects that food processing and preservation techniques can have on *Campylobacter* cells. Use of lower incubation temperatures in the early stages of enrichment is now widely established as an aid to cell recovery[3]. This principle was employed by Bolton in the development of his enrichment broth[4].

Campylobacter can be injured by food processing and preservation procedures[3]. This makes them susceptible to selective agents which are tolerated by undamaged cells. False negative results are avoided through use of recovery medium such as Bolton Selective Enrichment Broth which increases the number of cells available for culture, first by resuscitating injured organisms and then encouraging them to multiply.

Bolton Selective Enrichment Broth contains nutrients to aid resuscitation of sub-lethally injured cells, and is formulated to avoid the need for a microaerobic atmosphere. Initial incubation is carried out at 30°C-37°C, depending on the type of food to be examined. After the pre-enrichment, the incubation temperature is raised to 42°C to increase the selective pressures on competing organisms.

Inclusion of sodium metabisulphite and sodium pyruvate in Bolton Broth quenches toxic compounds that may form in the culture medium. These additions also increase the aero-tolerance of the culture. The antibiotics contained in Bolton Broth Selective Supplement optimise selectivity for *Campylobacter* spp. Vancomycin – active against Gram-positives. Cefoperazone – predominantly active against Gram-negatives. Trimethoprim – active against a wide variety of Gram-negative and Gram-positive organisms. Cycloheximide – active against yeasts.

Technique

One method of use is as follows:

Place 25 g of food sample in 225 ml Bolton Selective Enrichment Broth (prepared as described above) and homogenise the mixture using a Stomacher (or similar device). Bolton Selective Enrichment Broth does not require incubation in a microaerobic environment, but must be used in screw-topped containers which are filled to within 20 mm of the top[4]. Incubate for 4 hours at 37°C, followed by further incubation at 42°C. The broth can be sub-cultured after 24 hours and 48 hours onto either Modified CCDA (CM0739 + SR0155) or Preston Agar (CM0689 + SR0117 + SR0048)[4].

For other methods please refer to BAM[5].

Storage conditions and Shelf Life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C for up to 2 weeks.

Appearence

Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Broth: Straw coloured solution containing small black particles.

Quality control

Positive control:	***Expected results:*** when sub-cultured on modified CCDA
Campylobacter jejuni ATCC® 29428*	Good growth; grey coloured colonies
Negative control:	
Escherichia coli ATCC® 25922*	No growth

*This organism is available as a Culti-loop®

References
1. National Advisory Committee on Microbiological Criteria for Foods (1193). *Journal of Food Protection* 57. 1101-1121.
2. Skirrow M. B. (1977) *British Medical Journal* 2. 9-11.
3. Post D. E. (1995) *Food-Borne Pathogens Monograph Number 3 Campylobacter.*
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BRAIN HEART INFUSION AGAR

Code: CM0375

A solid medium which contains the highly nutritious infusions recommended for the cultivation of fastidious organisms.

Formula	***gm/litre***
Calf brain infusion solids	12.5
Beef heart infusion solids	5.0
Proteose peptone	10.0
Sodium chloride	5.0
Glucose	2.0
Disodium phosphate	2.5
Agar	10.0
pH 7.4 ± 0.2	

Directions
Suspend 47 g in 1 litre of distilled water. Boil to dissolve the medium completely. Distribute into tubes or flasks and sterilise by autoclaving at 121°C for 15 minutes.

Description
Brain Heart Infusion Agar may be recommended for the cultivation of streptococci, *Neisseria* and other fastidious organisms.

Seth[1] described the use of Oxoid Brain Heart Infusion with agar for the isolation of *Neisseria gonorrhoeae*. Oxoid Brain Heart Infusion Agar was designed to be equivalent in performance. The addition of 10% v/v horse blood plus vancomycin 3.0 mg/ml, colistin methane sulphonate 7.5 mg/ml, nystatin 12.5 IU/ml and trimethoprim lactate 8.0 mg/ml produced a specific medium which prevented the growth of *Proteus* species without significantly affecting *Neisseria gonorrhoeae*.

The addition of blood and antibiotics also makes Brain Heart Infusion Agar suitable for the isolation of the tissue phase of *Histoplasma capsulatum* and other pathogenic fungi, including *Coccidioides immitis*[2,3].

For the selective isolation of fungi, without blood, cyclohexamide 0.5 mg/ml and chloramphenicol 0.05 mg/ml of Brain Heart Infusion Agar may be added[4,5].

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared plates of medium at 2-8°C.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured gel.

Quality control

Positive controls:	***Expected results***
with blood	
Neisseria meningitidis ATCC® 13090*	Good growth; grey brown coloured colonies
Streptococcus pneumoniae ATCC® 6303*	Good growth; grey green coloured colonies
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

Precautions
When using this medium to isolate *Histoplasma capsulatum, Coccidioides immitis* or other pathogenic fungi which can produce free infective spores, extreme care must be taken to avoid dissemination of infective particles in the laboratory. The cultures should be examined only in a closed, filtered air cabinet.

References

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2. Howell A. (1948) *Public Health Reports* 63. 173-178.
3. Creitz J. R. and Puckett T. F. (1954) *Amer. J. Clin. Path.* 24. 1318-1323.
4. Ajello L., Georg L. K., Kaplan W. and Kaufman L. (1960) in *Laboratory Manual for Medical Mycology (CDC) Atlanta Ga.* US.DHEW. Center for Disease Control.
5. McDonough E. S., Georg L. K., Ajello L. and Brinkman A. (1960) *Mycopath. Mycol. Appl.* 13. 113-116.

BRAIN HEART INFUSION BROTH

Code: CM0225

A highly nutritious infusion medium recommended for the cultivation of streptococci, pneumococci, meningococci and other fastidious organisms. Suitable for blood culture work.

Formula	***gm/litre***
Calf brain infusion solids	12.5
Beef heart infusion solids	5.0
Proteose peptone	10.0
Glucose	2.0
Sodium chloride	5.0
Disodium phosphate	2.5
pH 7.4 ± 0.2	

Directions
Dissolve 37 g in 1 litre of distilled water. Mix well and distribute into final containers. Sterilise by autoclaving at 121°C for 15 minutes.

Description
A versatile liquid infusion medium which is suitable for the cultivation of streptococci, pneumococci, meningococci, and other fastidious organisms. This medium is recommended for blood culture work and, with the additions described below, for the isolation and cultivation of pathogenic fungi.

Oxoid Brain Heart Infusion is essentially a buffered infusion broth giving similar results to the brain dextrose broths originally employed for the cultivation of streptococci[1], and for the cultivation of dental pathogens[2].

The addition of 0.1% agar will serve to reduce convection currents and so create conditions of varying oxygen tension which favour the growth and primary isolation of aerobes and anaerobes[3], while even easily cultivated organisms show improved growth[4].

Brain Heart Infusion was used in a test for the pathogenicity of streptococci[5,6] and the same medium was enriched with ascitic fluid for the cultivation of gonococci[7].

Oxoid Brain Heart Infusion is especially useful as a growth and suspension medium for staphylococci which are to be tested for coagulase production; Newman[8] employed a similar medium for this purpose in an investigation of food poisoning caused by dairy products.

A satisfactory medium for blood culture can be prepared by adding 1 g of agar per litre of Brain Heart Infusion. Ensure that the agar is uniformly distributed in the sterile broth before dispensing into bottles. More conveniently, add 1 Agar Tablet CM0049 to each 100 ml of Brain Heart Infusion and sterilise by autoclaving for 15 minutes at 121°C. Cool to 60-70°C and mix gently to ensure uniform distribution of the agar.

Tubes of Brain Heart Infusion which are not used the same day as sterilised should be placed in a boiling water bath for several minutes to remove absorbed oxygen, and cooled rapidly without shaking, just before use.

Further supplements to improve the recovery of organisms from blood can be added before sterilisation or aseptically post-sterilisation. Co-enzyme[1] (NAD), penicillinase and p-amino benzoic acid are examples.

Brain Heart Infusion supplemented with yeast extract, haemin and menadione was consistently better in producing heavy growth of five species of Bacteroides than three standard anaerobic broths. Furthermore, microscopy of overnight cultures showed normal morphology in Brain Heart Infusion but abnormal morphology in the three anaerobic broths[9].

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store tubed or bottled medium in the dark and below 20°C.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured solution.

Quality control

Positive controls:	***Expected results***
Streptococcus pneumoniae ATCC® 6303*	Turbid growth
Candida albicans ATCC® 10231*	Turbid growth
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

References

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2. Haden R. L. (1923) *Arch. Internal Med.* 32. 828-849.
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5. Chapman G. H. *et al*. (1944) *Am. J. Clin. Path.* 9: Tech. Suppl. 3. 20-26.
6. Chapman G. H. (1946) *Am. J. Digestive Diseases* 13. 105-107.
7. Reitzel R. J. and Kohl C. (1938) *J. Am. Med. Assoc.* 110. 1095-1098.
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BREWER'S THIOGLYCOLLATE MEDIUM – see THIOGLYCOLLATE MEDIUM (BREWER)

BRILLIANT GREEN AGAR

Code: CM0263

A selective medium for the isolation of salmonellae, other than Salmonella typhi.

Formula	***gm/litre***
Proteose peptone	10.0
Yeast extract	3.0
Lactose	10.0
Sucrose	10.0
Sodium chloride	5.0
Phenol red	0.08
Brilliant green	0.0125
Agar	12.0
pH 6.9 ± 0.2	

Directions
Suspend 50 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

Description
Brilliant Green Agar was first described as a selective isolation medium for *Salmonella* species by Kristensen *et al*.[1] Kauffmann[2] modified their formula to give a highly selective plating medium for the isolation and identification of salmonellae from faeces and other pathological material, and from food and dairy products. This medium was not designed for the isolation of *Salmonella typhi* or *Shigella* species and where these may be encountered, Brilliant Green Agar should be used in parallel with other selective plating media such as Desoxycholate Citrate Agar (Hynes) CM0227, Hektoen Enteric Agar CM0419, XLD Agar CM0469. Bismuth Sulphite Agar (Modified) CM0201 is specifically recommended for *Salmonella typhi*.

The use of enrichment/selective broths prior to sub-culture on Brilliant Green Agar will improve the probability of isolating salmonellae. Tetrathionate Broth Base CM0029, Tetrathionate Broth USA CM0671, Selenite Broth Base CM0395 and Muller-Kauffmann Tetrathionate Broth Base CM0343 may be used in conjunction with Brilliant Green Agar.

Brilliant Green Agar corresponds to the medium recommended by the APHA[3,4] and the AOAC[5].

The addition of sulphonamides to Brilliant Green Agar helps improve the isolation of salmonellae[6]. To one litre of Brilliant Green Agar add 1.0 g of sulphapyridine or 0.8 g sulphadiazine and sterilise in the normal way.

Technique
Examination of faeces, or similar material, for salmonellae:

1. Heavily inoculate a Brilliant Green Agar plate. At the same time, inoculate other plating media and tubes of Selenite Broth and Tetrathionate Broth.
2. Incubate the Brilliant Green Agar plate for 18-24 hours at 35°C.
3. Examine the plates and identify suspect colonies using differential tests for serological methods.
4. If no non-lactose-fermenters are observed on the primary plate cultures, inoculate Brilliant Green Agar and other media with the enrichment cultures – then proceed as in point 3.

Examination of Foods

1. Pre-enrich four 25 g aliquots of food in 75 ml of Buffered Peptone Water CM0509 and incubate at 35°C for 4-6 hours.
2. Add to each sample 75 ml of double-strength Selenite Cystine Broth CM0699 and incubate at 43°C for 24 hours.
3. Sub-culture to plates of Brilliant Green Agar and Bismuth Sulphite Agar (Modified) CM0201.
4. Incubate the plates at 35°C and examine the Brilliant Green Agar after 24 hours and the Bismuth Sulphite Agar after 48 hours.
5. Look for colonies with *Salmonella* characteristics and confirm their identity with biochemical and serological tests.

Examination of food for salmonellae (enumeration)[4]
This is carried out by adding equal volumes of decimal dilutions of the homogenised sample to tubes of

double strength Selenite Broth. After incubation, a loopful from each tube is plated on Bismuth Sulphite Agar and Brilliant Green Agar.

Colonies with salmonellae characteristics are identified and the most probable number of salmonellae per gram of sample is calculated from the three highest sample dilutions which yield salmonellae on sub-culture.

Examination of dairy products for salmonellae[3]
Milk and liquid milk products, dried milk, cheese, eggs and egg products – Brilliant Green Agar is employed, with and without an enrichment phase, in conjunction with other selective media for enteric bacteria.

Colonial Characteristics

Non-lactose/sucrose-fermenting organisms
Red-pink-white opaque coloured colonies surrounded by brilliant red zones in the agar – most probably *Salmonella* (but not *Salmonella typhi*).

***Proteus* and *Pseudomonas* species**
These may grow as small red colonies.

Lactose/sucrose-fermenting organisms (normally inhibited).

Yellow to greenish yellow coloured colonies surrounded by intense yellow-green zones in the agar – *Escherichia coli* or *Klebsiella*/*Enterobacter* group.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared plates of medium at 2-8°C.

Appearance
Dehydrated medium: Straw green coloured, free-flowing powder.

Prepared medium: Green brown coloured gel.

Quality control

Positive control:	***Expected results***
Salmonella typhimurium ATCC® 14028*	Good growth; red coloured colonies: red medium
Negative control:	
Escherichia coli ATCC® 25922*	Inhibited or no growth

*This organism is available as a Culti-Loop®

Precautions
Lactose-fermenting *Salmonella* (*Salmonella arizona*) may be present in foods[7].

Salmonella typhi and *Shigella* species may not grow on this medium, use the cited alternative media.

Proteus, *Citrobacter* and *Pseudomonas* species may mimic enteric pathogens by producing small red colonies.

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BRILLIANT GREEN AGAR (MODIFIED)

Code: CM0329

A selective and diagnostic agar for salmonellae (other than Salmonella typhi) from food and feeds.

Formula	***gm/litre***
'Lab-Lemco' powder	5.0
Peptone	10.0
Yeast extract	3.0
Disodium hydrogen phosphate	1.0
Sodium dihydrogen phosphate	0.6
Lactose	10.0
Sucrose	10.0
Phenol red	0.09
Brilliant green	0.0047
Agar	12.0
pH 6.9 ± 0.2	

Directions

Suspend 52 g in 1 litre of distilled water. Heat gently with occasional agitation and bring just to the boil to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 50°C, mix well and pour plates.

SULPHAMANDELATE SUPPLEMENT

Code: SR0087

Vial contents (each vial is sufficient for 500 ml of medium)	***per vial***	***per litre***
Sodium sulphacetamide	500 mg	1000 mg
Sodium mandelate	125 mg	250 mg

Directions

Reconstitute one vial of Sulphamandelate supplement as directed. Avoid frothing. Add the solution to 500 ml of sterile Oxoid Brilliant Green Agar (Modified) cooled to 50-55°C. Mix gently and pour into sterile Petri dishes.

Description

Brilliant Green Agar (Modified) was developed from a formula supplied by the Rijks Instituut voor de Volksgezondheid (National Institute for Public Health), Utrecht[1,2].

The medium has been widely assessed in Europe and has been used in the ISO standards[3,4,5].

The advantages claimed for the medium are the greater inhibition of *Escherichia coli* and *Proteus* species than other formulations: the restriction of growth of *Pseudomonas* species, whose colonies may resemble salmonellae on Brilliant Green Agar and cause confusion or much extra work to confirm their identity: the absence of inhibitory properties towards small numbers of salmonellae[6].

SELECTIVE BRILLIANT GREEN AGAR (MODIFIED)

Watson and Walker[7] incorporated a combination of sulphacetamide (at 1.0 mg/ml) and mandelic acid (at 0.25 mg/ml) into Oxoid Brilliant Green Agar (Modified) to obtain maximum recovery of salmonellae from Muller-Kauffmann Tetrathionate Broth whilst giving maximum suppression of contaminating organisms.

Oxoid Salmonella Sulphamandelate Supplement, used for the isolation and enumeration of salmonellae from sewage and sewage sludge, is based on the formulation of Watson and Walker[1,7]. These authors showed that the use of Brilliant Green Agar (Modified) incorporating a combination of sulphacetamide (1.0 mg/ml) and mandelic acid (0.25 mg/ml) incubated at 43°C resulted in maximum recovery of salmonellae from Muller-Kauffmann Tetrathionate Broth.

The method described[7] has been shown to be a quick and reliable technique for the isolation of sub-lethally damaged salmonellae from treated sewage and sewage sludge.

Use of antibiotic supplemented Brilliant Green Agar is made necessary because the pre-enrichment of the sewage in phosphate buffered peptone (PBP) water will encourage not only the growth of stressed salmonellae but many competing organisms.

The inhibitory properties of Muller-Kauffmann Tetrathionate Broth are not sufficient by themselves to suppress the growth of the latter. The advantage claimed for Selective Brilliant Green Agar is its greater inhibition of contaminating organisms and a lower incidence of false positives.

This advantage was confirmed by Fricker and his co-workers when using Brilliant Green Agar (Modified) containing sodium sulphacetamide and sodium mandelate for plating enrichment cultures in Rappaport Broth, from sewage and sewage polluted water[8,11], seagull faeces[9] and chicken[10,12].

Vassilliadis *et al*.[13] added 2.5 g of sodium desoxycholate LP0057 to one litre of Brilliant Green Agar (Modified) to prevent swarming by *Proteus hauseri*, during examination of sewage effluents. They found desoxycholate to be superior to sulphonamides in suppressing swarming without affecting the growth of a wide range of salmonellae serotypes.

Colonial Characteristics

Salmonellae	Red colonies surrounded by bright red medium
Lactose/Sucrose fermenters	Inhibited to a certain extent, but producing yellow green colonies when growth is evident
Proteus	Almost completely inhibited, those colonies that grow produce red colonies without swarming
Pseudomonas	Inhibited growth of small, crenated red colonies

Techniques

Technique for food and feeds

An outline of the method used by Edel and Kampelmacher[2] in their trials is as follows:

1. One part of the food sample was added to 20 parts of Muller-Kauffmann Tetrathionate Medium CM0343.
2. After agitation, the flask of broth was placed into a 45°C waterbath for 15 minutes only.
3. The flask was then transferred to a 43°C incubator.
4. The broth was sub-cultured to Brilliant Green Agar (Modified) after 18 and 48 hours.

   A single loopful of broth was used to streak inoculate either two 9 cm diameter plates (without recharging the loop between plates) or one 14 cm diameter plate.
5. The plates were incubated at 35°C for 18-24 hours.
6. Red colonies, resembling salmonellae, were picked off the plates and sub-cultured to Lysine Decarboxylase Broth CM0308 and Triple Sugar Iron Agar CM0277. These media were incubated at 35°C for 18-24 hours.

   If the reactions on these media were positive for salmonellae then slide agglutination tests were carried out on the surface growth of the Triple Sugar Iron Agar.

Technique for sewage[7]

1. Take a representative sample of sewage or sludge for examination.
2. Homogenise a suitable volume in a macerator or stomacher.
3. Inoculate five 10 ml samples into 35 ml of Buffered Peptone Water CM0509, five 1 ml samples and five 0.1 ml samples into 10 ml of Buffered Peptone Water. Incubate at 35°C overnight.
4. Transfer 10 ml portions into 35 ml of Muller-Kauffmann Tetrathionate Broth and incubate at 43°C.
5. Sub-culture the broths on to Brilliant Green Agar (Modified) containing Sulphamandelate Selective Supplement after 24 and 48 hours incubation.
6. Incubate the Brilliant Green Agar plates overnight at 43°C.
7. Identify suspicious (red) colonies using further diagnostic tests.

   The Sulphamandelate Selective Supplement inhibits competing organisms which multiply during the resuscitation and recovery stages in Buffered Peptone Water.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared plates of medium at 2-8°C.

Appearance
Dehydrated medium: Pale green coloured, free-flowing powder.

Prepared medium: Green brown coloured gel.

Quality control

Positive control:	***Expected results***
Salmonella typhimurium ATCC® 14028*	Good growth; red coloured colonies and media
Negative controls:	
Escherichia coli ATCC® 25922*	Inhibited or no growth; yellow green coloured colonies
Proteus mirabilis ATCC® 29906*	Inhibited or no growth; pink coloured colonies

*This organism is available as a Culti-Loop®

Precautions
Lactose-fermenting salmonellae may be present in foods.

Salmonella typhi and *Shigella* species may not grow on this medium.

Proteus, *Citrobacter* and *Pseudomonas* species may mimic enteric pathogens by producing small red colonies.

References

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BRILLIANT GREEN BILE (2%) BROTH

Code: CM0031

This medium is used to detect or confirm the presence of members of the coli-aerogenes group; the brilliant green content suppresses anaerobic lactose fermenters, such as Clostridium perfringens, and the medium is recommended for the 44°C confirmatory test for Escherichia coli.

Formula	***gm/litre***
Peptone	10.0
Lactose	10.0
Ox bile (purified)	20.0
Brilliant green	0.0133
pH 7.4 ± 0.2	

Directions
Dissolve 40 g in 1 litre of distilled water. Mix well, distribute into containers fitted with Durham's tubes and sterilise by autoclaving at 121°C for 15 minutes.

An alternative procedure is to heat the dissolved broth at 100°C for 30 minutes, a recommended procedure when preparing double-strength broth[1].

Description

This medium was formulated by Durham and Schoenlein[2] to select organisms of the coli-aerogenes group. The bile and brilliant green components inhibit the Gram-positive organisms, whilst the coli-aerogenes group are recognised by the rapid formation of gas during lactose fermentation[3].

It is important that the inhibitory agents in the medium are balanced with the nutrient and mineral components, so that Clostridia and Bacillus spores will not give false positive reactions in the medium i.e. gas formation.

Brilliant Green Bile Broth is used in water, dairy and food analysis[4,5,6,7,8].

MUG Reagent BR0071 – The addition of 4-methylumbelliferyl-β-D-glucuronide (MUG) BR0071 to this medium will enhance the detection of *Escherichia coli*. See MUG Reagent BR0071 under Biological Reagents for further details.

Technique

To indicate the presence of *Escherichia coli*, Brilliant Green Bile Broth is incubated at 44 ± 1°C for 48 hours. Turbidity in the broth and gas production in the inverted tube are positive signs. An indole production test at 44°C is also carried out in Tryptone Water CM0087 or Peptone Water CM0009 to confirm the identity of *Escherichia coli*.

In water plant control tests where <1 ml to 10 ml volumes of water are used, it is important not to overdilute the medium. Thus 1 ml or less volumes of water can be added to 10 ml of Brilliant Green Bile Broth. For 10 ml volumes of water, double-strength Brilliant Green Bile Broth should be used in equal volumes.

When incubated at 35°C for 48 hours, gas formation presumptively indicates coli-aerogenes organisms.

Food macerates are decimally diluted and added to the broth in the proportion 1:10. Double strength broth can be used for large volume samples.

Incubation is carried out at 44°C for 48 hours to detect *Escherichia coli.* At 32°C for 25-48 hours to detect coli-aerogenes organisms[1,10] or at 4°C for 10 days to detect psychrotrophic coliform organisms.

The medium becomes turbid and yellowish-green in colour when bacteria are growing and when accompanied by copious gas formation, there is presumptive evidence of coli-aerogenes organisms. Confirmatory tests should be carried out.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared tubes of broth at 2-8°C.

Appearance

Dehydrated medium: Pale green, free-flowing powder.

Prepared medium: Green coloured solution.

Quality control

Positive controls:	***Expected result***
Escherichia coli ATCC® 25922*	Turbid growth; gas
Enterobacter aerogenes NCTC 9735*	Turbid growth; gas
Negative control:	
Staphylococcus aureus ATCC® 25923*	No growth

*This organism is available as a Culti-Loop®

Precautions

Do not autoclave double-strength broth.

Gram-positive sporing organisms may produce gas if the bile/brilliant green inhibition is attenuated by food material.

References

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BRUCELLA MEDIUM BASE

Code: CM0169

For the preparation of serum-dextrose-antibiotic medium for the cultivation and isolation of Brucella using Brucella Selective Supplement SR0083 or SR0209. Without antibiotics, it may be used in conjunction with the Cruickshank dyestrip method for differentiation between strains.

Formula	***gm/litre***
Peptone	10.0
'Lab-Lemco' powder	5.0
Glucose	10.0
Sodium chloride	5.0
Agar	15.0
pH 7.5 ± 0.2	

Directions
Suspend 45 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C and add 5% of inactivated Horse Serum (i.e. Horse Serum held at 56°C for 30 minutes). Mix well before pouring.

BRUCELLA SELECTIVE SUPPLEMENTS

Vial contents (each vial is sufficient for 500 ml of medium)	**Code: SR0083**	**Code: SR0209**
Polymyxin B	2,500 IU	2,500 IU
Bacitracin	12,500 IU	12,500 IU
Cycloheximide	50 mg	
Nalidixic acid	2.5 mg	2.5 mg
Nystatin	50000 IU	50000 IU
Vancomycin	10 mg	10 mg
Natamycin		25 mg

Directions
Reconstitute supplement as directed. Incubate for 10-15 minutes at 35°C. Mix thoroughly and immediately add the vial contents to 500 ml of sterile Oxoid Brucella Medium Base CM0169, cooled to 50°C together with 5-10% v/v sterile inactivated horse serum SR0035 and 1-5% w/v of a filter-sterilised 10% solution of dextrose. Mix well and pour into sterile Petri dishes.

Description
Brucella Medium Base may be used to prepare the serum-dextrose-antibiotic medium described by Jones and Brinley Morgan[1] for the cultivation and isolation of *Brucella*, including fastidious types. Brucella medium with antibiotics has advantages over the media described by Kuzdas and Morse[2] and Renoux[3] in that it will support growth of fastidious types and it is more effective as a selective medium. During investigations, Jones and Brinley Morgan showed that serum-glucose agar with antibiotics gave excellent growth of all *Brucella* strains and permitted better growth of *Brucella abortus* biotype 2 – a strain which had been difficult or impossible to cultivate.

Technique
The addition of dyes (i.e. malachite green and gentian violet) as selective agents, is not recommended, as it may result in poor growth of many *Brucella* strains. Where a non-selective medium is required, the medium may be employed with the addition of serum only (i.e. without antibiotics): for subsequent differentiation between strains of *Brucella*. This medium is recommended for use in conjunction with the Cruickshank dyestrip method[4]:

1. Impregnate filter paper strips with 1:200 Basic Fuchsin or 1:600 Thionin. Dry.
2. Place the strips parallel on the surface of the serum-dextrose agar and then cover with a thin layer of the same medium. Then allow the medium to solidify.
3. Make stroke inoculations of the *Brucella* strains to be tested, at right angles to the strips.
4. Incubate in 10% carbon dioxide for 2-3 days at 35°C.
5. Examine. Resistant strains grow right across the strip, but sensitive strains show inhibition of growth up to 10 mm from the strip. Typical growth patterns are then as follows:

	Basic Fuchsin 1:200	**Thionin 1:600**
Brucella abortus	growth	no growth
Brucella melitensis	growth	growth
Brucella suis	no growth	growth

However, there are exceptions to the above and it is therefore advisable to base identification on many characteristics[5].

The slow growth of *Brucella* species, combined with their requirement for highly nutritious media means that selective agents must be incorporated to prevent overgrowth of contaminant organisms from milk or veterinary tissues.

Media containing bacteriostatic dyes are inhibitory to strains of *Brucella abortus* biotype 2 and other fastidious strains. Antibiotics used in place of dyes enabled growth of all biotypes *Brucella* species to appear on selective media[1]. However, Leech *et al*.[6] showed that the serum-glucose-antibiotic formulation[1] was not sufficiently selective and was less efficient than guinea-pig inoculation.

Barrow and Peel[7] modified a selective medium devised by Mair[8]. This contained both antibiotics and gentian violet. Failure of some strains of *Brucella abortus* to grow confirmed their sensitivity to very low concentrations of the dye recognised by Mair.

Farrell[9] developed a highly selective antibiotic-containing nutrient medium which incorporated bacitracin 25 iu/ml, vancomycin 20 mg/ml, cycloheximide 100 mg/ml and nystatin 100 iu/ml, in a serum-glucose agar base.

In comparative trials[10] the medium was shown to give a rate of isolation equivalent to that achieved by guinea-pig inoculation. It also supported the growth of *Brucella abortus* biotype 2 strains.

Brucella Selective Supplement is based on the superior formulation of Farrell. Its greater efficiency at suppressing bacterial contamination than either serum-glucose agar or Barrow and Peel's Medium was shown in a further trial[11].

1. For direct culture of *Brucella* species from milk transfer the samples to sterile tubes and hold overnight at 40°C.
2. Withdraw an aliquot of gravity cream with a spiral wire and spread over a plate of supplemented agar with a bent sterile glass rod.
3. Incubate the plates at 35°C in an atmosphere containing 10-20% (v/v) carbon dioxide and examine every two days for ten days.
4. *Brucella* colonies appear as 1-2 mm diameter convex colonies with round entire edges, and may be identified by slide agglutination.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared agar plates at 2-8°C.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured gel.

Quality control

Positive control: (w/o antibiotics)	***Expected results***
Bordetella bronchiseptica ATCC® 4617*	Good growth; small clear colonies
Negative control: (with antibiotics)	
Staphylococcus aureus ATCC® 25923*	No growth

*This organism is available as a Culti-Loop®

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BUFFERED GLUCOSE BRILLIANT GREEN BILE BROTH – see EE BROTH

BUFFERED LISTERIA ENRICHMENT BROTH

Code: CM0897

A selective enrichment medium for the detection of Listeria monocytogenes when prepared from Buffered Listeria Enrichment Broth base and Listeria Selective Supplement SR0141.

BUFFERED LISTERIA ENRICHMENT BROTH

Formula	***gm/litre***
Tryptone soya broth	30.0
Yeast extract	6.0
Potassium di-hydrogen orthophosphate	1.35
Disodium hydrogen orthophosphate	9.60
Final pH 7.3 ± 0.2	

LISTERIA SELECTIVE ENRICHMENT SUPPLEMENT

Code: SR0141

Vial contents: (each vial is sufficient for 500 mls of medium)	***per vial***	***per litre***
Nalidixic acid	20.0 mg	40.0 mg
Cycloheximide	25.0 mg	50.0 mg
Acriflavine hydrochloride	7.5 mg	15.0 mg

MODIFIED LISTERIA SELECTIVE ENRICHMENT SUPPLEMENT

Code: SR0213

Vial contents: (each vial is sufficient for 500 mls of medium)	***per vial***	***per litre***
Nalidixic acid	20.0 mg	40.0 mg
Amphotericin B	5.0 mg	10.0 mg
Acriflavine hydrochloride	7.5 mg	15.0 mg

Directions

Add 23.5 g to 500 ml of distilled water and mix well to dissolve. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C and aseptically add the contents of 1 vial of Listeria Selective Enrichment Supplement SR0141 or the alternative Modified Listeria Selective Supplement SR0213 reconstituted as directed. Mix well and aseptically distribute into sterile containers in volumes as required.

Description

Listeria Selective Enrichment Broth CM0862 is based on the formulation described by Lovett *et al*.[1] and is recommended for the enrichment of *Listeria* species in food. Subsequent work has concluded that the enrichment properties can be improved by increasing the buffering capacity of the medium by the addition of potassium di-hydrogen orthophosphate and disodium hydrogen orthophosphate. Buffered Listeria Enrichment Broth is therefore a modification of the original medium.

Techniques

1. Add 25 g or 25 ml samples to 225 ml of Buffered Listeria Enrichment Broth. Homogenise if required.
2. Incubate at 30°C for 48 hours.
3. Subculture from the Buffered Listeria Emrichment Broth onto Listeria Selective Agar plates (See Note) after 24 and 48 hours by:
   (i) Direct plating onto Listeria Selective Agar plates.
   (ii) Adding 1 ml of the Buffered Listeria Enrichment Broth to 9 ml of 0.5% KOH, vortex mixing, and plating onto Listeria Selective Agar plates.

Note

Suitable Listeria Selective Media are:

1. Listeria Selective Medium (Oxford formulation) (Oxoid CM0856 and Oxoid SR0140).
2. PALCAM Medium (Oxoid CM0877 and Oxoid SR0150)

Storage conditions and Shelf life

Buffered Listeria Enrichment Broth should be stored tightly capped in the original container in a cool, dry place away from bright light. When stored as directed the medium will remain stable until the expiry date printed on the label.

Listeria Selective Enrichment Supplement SR0141 as supplied should be stored at 2-8°C. When stored as directed the reagents are stable until the expiry date printed on the label.

Appearance

Dehydrated Medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured solution.

Quality Control

Positive controls:	***Expected results***
Listeria monocytogenes ATCC® 19117	Turbid growth
Negative control:	
Staphylococcus aureus ATCC® 25923*	Inhibited

*This organism is available as a Culti-Loop®

Precautions

Listeria Selective Enrichment Supplement contains cycloheximide and is toxic if swallowed, inhaled or by skin contact. As a precaution when handling wear gloves and eye/face protection.

Acriflavine is activated by light and may form compounds inhibitory for Listeria.

References

1. Lovett J., Francis D. W. and Hunt J. M. (1987) *J. Food Prot.* 50. 188-192.
2. Curtis G. D. W., Nichols W. W. and Falla T. J. (1989) *Lett. Appl. Micro.* 8. 169-172.

BUFFERED CHARCOAL YEAST EXTRACT AGAR (EDELSTEIN BCYE MEDIUM)

LEGIONELLA CYE AGAR BASE

Code: CM0655

Charcoal Yeast Extract Agar for the isolation of Legionellaceae when used with Legionella BCYE Growth Supplement SR0110 (Edelstein BYCE Agar).

Formula	***gm/litre***
Activated charcoal	2.0
Yeast extract	10.0
Agar	13.0

LEGIONELLA BCYE GROWTH SUPPLEMENT

Code: SR0110

Vial contents	***Supplement 100 ml***	***Supplement 500 ml***	***Supplement per litre***
Buffer/Potassium hydroxide	1.0 g	5.0 g	10 g
Ferric pyrophosphate	0.025 g	0.125 g	0.25 g
L-cysteine HCl	0.04 g	0.2 g	0.4 g
α-Ketoglutarate	0.1 g	0.5 g	1.0 g

Directions to prepare BCYE Agar

Suspend 2.5 g of Legionella CYE Agar Base in 90 ml of distilled water and bring gently to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Allow to cool to 50°C and aseptically add the contents of one vial of Legionella BCYE Growth Supplement reconstituted as directed. Mix gently and pour into sterile Petri dishes. The final pH of the medium should be 6.9 ± 0.2.

Description

The discovery of the causative organism of Legionnaires' disease has been reviewed by Fallon[1]. Since that review further progress has been made in culturing the organism from clinical specimens and also in the enumeration of *Legionella* species from environmental samples. Feeley *et al*.[2] described a modification of F-G Agar[3] in which acid hydrolysed casein was replaced by yeast extract as the source of protein and starch was replaced by activated charcoal (Norit A) at a final concentration of 0.2% (w/v). This medium, which they named CYE Agar[2] has been further supplemented with ACES Buffer and α-ketoglutarate and is described in the literature as BCYE-α Medium4. BCYE-a Medium has been shown to yield optimal recovery of Legionellaceae in a shorter incubation period from environmental samples and clinical specimens[5].

Oxoid BCYE Medium is based on the formulation of Edelstein[4] and is prepared from Legionella CYE Agar Base CM0655 and Legionella BCYE Growth Supplement SR0110. The sterile lyophilised supplement contains ACES Buffer/potassium hydroxide, α-ketoglutarate, ferric pyrophosphate and L-cysteine HCl. When added to CYE Agar Base it stabilises the pH of the medium at 6.9 ± 0.2 and provides essential growth factors.

Additionally, a medium omitting L-cysteine may be prepared from Legionella CYE Agar Base CM0655 and BYCE Growth Supplement SR0175.

Legionellaceae have an absolute nutritional requirement for L-cysteine. Presumptive Legionella spp. colonies can be subcultured onto both BCYE Medium with L-cysteine CM0655 and SR0110, and BCYE Medium without L-cysteine CM0655 and SR0175.

All plates are incubated at 35°C. Colonies which have grown on BCYE Medium with L-cysteine, but not on BCYE Medium without L-cysteine, can be regarded as presumptive *Legionella* spp.

Storage conditions and Shelf life
Store the dehydrated medium below 30°C and use before the expiry date on the label.
Store the prepared plates at 2-8°C away from light.

Appearance
Dehydrated medium: Black, free-flowing powder.
Prepared medium: Black gel.

Quality control

Positive controls:	***Expected results***
Legionella pneumophila ATCC® 33152*	Good growth; grey/white coloured colonies
Legionella pneumophila NCTC 12821	Good growth; grey/white coloured colonies
Negative control:	
Uninoculated medium	No change
Selective media	
Positive controls:	
Legionella pneumophila ATCC® 33152*	Good growth; grey/white-blueish coloured colonies
Legionella pneumophila NCTC 12821	Good growth; grey/white-blueish coloured colonies
Negative control:	
Staphylococcus epidermidis ATCC® 12228*	Inhibited

*This organism is available as a Culti-Loop®

References

1. Fallon J. Oxoid Limited. *Culture September* 1979, P. 3-4.
2. Feeley J. C., Gibson R. J., Gorman G. W., Langford N. C., Rasheed J. W., Mackel D. C. and Baine W. B. (1979) *J. Clin. Micro.* 10. 437-441.
3. Feeley J. C. Gorman G.W., Weaver R. E., Mackel D. C. and Smith H. W. (1978) *J. Clin. Micro*. 8. 320-325.
4. Edelstein P. H. (1981) *J. Clin. Micro.* 14. 298-303.
5. PHLS Communicable Diseases Report (1983) CDR 83/49.

BUFFERED PEPTONE WATER

Code: CM0509

A pre-enrichment medium to be used prior to selective enrichment for the isolation of Salmonella species from foods.

Formula	***gm/litre***
Peptone	10.0
Sodium chloride	5.0
Disodium phosphate	3.5
Potassium dihydrogen phosphate	1.5
pH 7.2 ± 0.2	

Directions
Add 20 g to 1 litre of distilled water. Mix well and distribute into final containers. Sterilise by autoclaving at 121°C for 15 minutes. It is extremely important that the distilled water used is of a high quality with a low mineral content/conductivity.

Description

Oxoid Buffered Peptone Water may be used as a pre-enrichment medium, prior to selective enrichment in the isolation of salmonellae from foods. It also provides conditions for resuscitation of cells that have been injured by processes of food preservation.

It was noted by Edel and Kampelmacher[1] that sub-lethal injury to salmonellae may occur in many food processes. In a survey involving isolation of salmonellae from meat that had been artificially contaminated with sub-lethally injured organisms, pre-enrichment in buffered peptone water at 37°C for 18 hours before selection in Brilliant Green-Tetrathionate-Bile Broth showed superior results compared with a direct selection method.

Pietzsch[2] found that isolation of salmonellae was much improved by pre-enrichment of egg samples in buffered peptone water at 37°C for 18 hours followed by incubation of 10 ml of this sample in 100 ml Selenite Cystine Broth CM0699 or Muller-Kauffmann Tetrathionate Broth CM0343 at 43°C for 48 hours.

Sadovski[3] reported that in experiments involving isolation of salmonellae from frozen vegetables the rapid drop in pH when using lactose broth[4] as a pre-enrichment medium was detrimental to the recovery of salmonellae. This was due to the enhanced sensitivity to low pH of freeze-injured salmonellae which may contaminate frozen vegetables. Pre-enrichment with buffered peptone water maintained a high pH over a period of 24 hours incubation. Vegetable tissue has a low buffering capacity and the medium overcame this problem.

A shortened enrichment time of 6 hours was investigated[5] but in circumstances where heavily contaminated materials were examined, the addition of 0.1 g of malachite green per litre of Buffered Peptone Water was advised.

The addition is important where small numbers of salmonellae may have their generation time increased because of competitive growth and may not reach the minimum number for successful isolation.

For cocoa products the inclusion of casein in the pre-enrichment medium is necessary to inhibit bactericidal substances present in cocoa[6]. A comparative collaborative study confirmed the value of adding casein and malachite green to buffered peptone water when examining cocoa bean dust and chocolate for *Salmonella*[7].

Technique for the isolation of *Salmonella*[5]

Buffered Peptone Water may be used according to published standard methodologies, e.g. ISO.

Storage conditions and Shelf life

Store dehydrated medium at 10-30°C and use before the expiry date of the label.

Store prepared medium at 2-8°C.

Appearance

Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Light straw coloured solution.

Quality control

Positive controls:	***Expected result***
Salmonella typhimurium ATCC® 14028*	Turbid growth
Salmonella poona NCTC 4840*	Turbid growth
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

Precautions

Observe the safety precautions required when cultivating salmonellae.

Liquid cultures are more infective than plates and special care should be taken if the 43°C incubation takes place in a water bath.

Do not use malachite green if *Salmonella typhi* may be present in the test material.

References

1. Edel W. and Kampelmacher E. H. (1973) *Bull. Wld Hlth Org.* 48. 167-174.
2. Pietzsch O., Kretschmer F. J. and Bulling E. (1975) *Zbl. Bakt. Abt. I. Orig.* 232. 232-246.
3. Sadovski A. Y. (1977) *J. Food Technol.* 12. 85-91.
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5. van Schothorst M. and Renaud A. M. (1985) *J. Appl. Bact.* 59. 223-230.
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BUFFERED PEPTONE WATER (ISO)

Code: CM1049

Formula	***gm/litre***
Enzymatic digest of casein	10.0
Sodium chloride	5.0
Disodium hydrogen phosphate (anhydrous)	3.5*
Potassium dihydrogen phosphate	1.5
pH 7.0 ± 0.2	

*Equivalent to 9.0 g of disodium hydrogen phosphate dodecahydrate

Directions
Add 20 g of Buffered Peptone Water (ISO) to 1 litre of distilled water. Mix well and distribute into final containers. Sterilise by autoclaving at 121°C for 15 minutes.

Description
Oxoid Buffered Peptone Water (ISO) is a non-selective pre-enrichment medium for the isolation of *Salmonella* from food and associated samples.

Oxoid Buffered Peptone Water (ISO) is a pre-enrichment broth designed to be used prior to selective enrichment in MKTTn Broth (CM1048 + SR0181) and RVS Medium (CM0866), for the isolation of *Salmonella* species from foods according to ISO 6579:2002[1].

Low numbers and/or sub-lethally injured *Salmonella* may be present in the test sample. Pre-enrichment in Buffered Peptone Water (ISO) allows the cells time to repair and multiply, before being introduced into selective culture, improving the chances of recovering *Salmonella* from the sample.

The test sample is added to Buffered Peptone Water (ISO) at a ratio of 1:10, and incubated at 37°C ± 1°C for 18 hours + 2 hours.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Prepared medium may be kept for up to 2 weeks at room temperature.

Appearence
Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Light straw coloured solution.

Quality control

Positive control:	***Expected result***
Salmonella typhimurium ATCC® 14028*	Turbid growth
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

References
1. Anon BS EN ISO 6579:2002. Microbiology of food and animal feeding stuffs. Horizontal method for the detection of *Salmonella* spp.

BUFFERED SODIUM CHLORIDE PEPTONE SOLUTION

Code: CM0982

An isotonic diluent made to the EP specification.

Formula	***gm/litre***
Peptone	1.0
Sodium chloride	4.3
Potassium dihydrogen phosphate	3.56
Disodium hydrogen phosphate $2H_2O$	5.77
pH 7.0 ± 0.2	

Directions

Suspend 14.63 g of Buffered Sodium Chloride Peptone Solution in 1 litre of distilled water. If required, add 1-10 ml Tween® 20 or 80 per litre of medium. Mix well and distribute into final containers. Sterilise by autoclaving at 121°C for 15 minutes.

Description

Buffered Sodium Chloride Peptone Solution is intended for the dilution of samples in the analysis of non-sterile products for microbial contaminants.

This solution conforms to the European Pharmacopoeia[1].

The combination of phosphate buffer, sodium chloride and peptone increases the viability of sensitive micro-organisms by providing an isotonic environment. This is of particular importance when trying to recover cells that may be stressed or sensitive to osmotic pressure.

Storage conditions and Shelf Life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store prepared media at room temperature.

Appearence

Dehydrated medium: Light straw coloured, free-flowing powder.

Prepared medium: Light straw coloured solution.

Quality control

Positive controls:	***Expected result***
Escherichia coli ATCC® 8739*	No decrease in colony count within 4 hours
Staphylococcus aureus ATCC® 653P*	No decrease in colony count within 4 hours
Negative control:	
Uninocluated medium	No change

*This organism is available as a Culti-Loop®

Reference

1. *European Pharmacopoeia* 2004, 4th Edition.

BURKHOLDERIA CEPACIA AGAR BASE

Code: CM0995

A medium for the selective isolation of Burkholderia cepacia from the respiratory secretions of patients with cystic fibrosis and for routine testing of non-sterile inorganic salt solutions containing preservative.

Formula	***gm/litre***
Peptone	5.0
Yeast Extract	4.0
Sodium pyruvate	7.0
Potassium dihydrogen phosphate	4.4
Disodium hydrogen phosphate	1.4
Bile salts	1.5
Ammonium sulphate	1.0
Magnesium sulphate	0.2
Ammonium ferrous sulphate	0.01
Phenol red	0.02
Crystal violet	0.001
Agar	12.0
pH 6.2 ± 0.2	

BURKHOLDERIA CEPACIA SELECTIVE SUPPLEMENT

Code: SR0189

Vial contents (each vial is sufficient for 500 ml of medium)	***per vial***	***per litre***
Polymixin B	75,000 IU	155,000 IU
Gentamicin	2.5 mg	5.0 mg
Ticarcillin	50.0 mg	100.0 mg

Directions

Suspend 18.25 g of Burkholderia cepacia Agar Base in 500 ml of distilled water, mix well and sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C and aseptically add the contents of one vial of Burkholderia cepacia Selective Supplement, reconstituted as directed. Mix well and distribute into sterile Petri dishes.

Description

Burkholderia cepacia (formerly known as *Pseudomonas cepacia*) is a motile aerobic oxidase positive Gram-negative bacillus commonly found in liquid reservoirs and moist environments. The cells are 0.5 to 1.0 mm wide and 5 mm in length. It is an important opportunistic pathogen and causes pulmonary infection among individuals with cystic fibrosis (CF). Isolates from CF patients often display multidrug resistance and as many as 20% of colonised individuals will succumb to *Burkholderia cepacia* syndrome, a necrotizing pneumonia associated with fever that culminates into a rapid and fatal clinical deterioration[1].

Originally isolated from onions, *Burkholderia cepacia* can survive for long periods and multiply in hostile environments such as antiseptic and disinfectant solutions, distilled water, whirlpool baths, nebulizers and commercially packaged urinary catheter kits[2]. An outbreak in Arizona in 1998 due to contaminated alcohol-free mouthwash, was investigated by the Food and Drug Administration (FDA), who suggested an association with the deionisation procedure of the water used to prepare the product[3]. The organism may be present in low numbers in many non-sterile products used in hospitals. It has been isolated from various water sources and can grow in distilled water with a nitrogen source due to its ability to fix carbon dioxide from air[4]. Suction catheters rinsed in acetic acid solution have reduced incidence of transmission of *Burkholderia cepacia* and other pseudomonads.

The slower growing *Burkholderia cepacia* can be missed on conventional media such as blood or MacConkey Agar due to overgrowth caused by other faster growing organisms found in the respiratory tract of CF patients such as mucoid Klebsiella species, *Pseudomonas aeruginosa* and *Staphylococcus* species. This may lead to the infection being missed or wrongly diagnosed.

Technique
Take a routine respiratory sample from the patient e.g. sputa, deep pharyngeal swabs or bronchial washings. Dilute the sample, if necessary, in Ringer's solution to give a 1:2 dilution. Streak onto Burkholderia cepacia Medium and incubate at 37°C for 48 to 72 hours.

Examine after 48 hours for sage green colonies and the medium turning from straw-green to bright pink. All colonies should be further identified and confirmed. Re-incubate for a further 24 hours if necessary.

Typical colonies of *Burkholderia cepacia* are circular, entire, sage green colonies with the medium changing from a straw-green to bright pink.

Storage conditions and Shelf Life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared plates of medium at 2-8°C.

Appearence
Dehydrated medium: Straw pink, free-flowing powder.

Prepared medium: Orange coloured gel.

Quality Control

Positive control:	***Expected Results***
Burkholderia cepacia ATCC® 25608*	Good growth, green colonies
Negative Control:	
Psudomonas aeruginosa ATCC® 27853*	Inhibited

*This organism is available as a Culti-Loop®

References
1. Whitby P. W. (1998) *Journal of Clinical Microbiology* 36: 1642-1645
2. Geftic S. G., Heymann H. and Adair F. W. (1979) *Applied and Environmental Microbiology* 37: 505-510
3. Matrician L. (1998) Virtual Hospital: *Morbidity and Mortality Weekly Report Volume* 47: No. 43
4. Koneman E. W. *et al* (1997) *Color Atlas and Textbook of Diagnostic Microbiology* Fifth Ed.: 269-272

CANDIDA CHROMOGENIC AGAR – see CHROMOGENIC CANDIDA AGAR

CARY-BLAIR MEDIUM

Code: CM0519
A transport medium for Gram negative and anaerobic organisms.

Formula	***gm/litre***
Disodium hydrogen phosphate	1.1
Sodium thioglycollate	1.5
Sodium chloride	5.0
Calcium chloride	0.09
Agar	5.6
pH 8.4 ± 0.2	

Directions
Suspend 13.3 g in 1 litre of distilled water and bring gently to the boil to dissolve the agar. Distribute into small, screw-cap bottles and sterilise by immersing in free-steam for 15 minutes. Allow to cool and tighten the screw caps to prevent water loss.

Description
Oxoid Cary-Blair Medium is a transport medium for the collection and shipment of clinical specimens based on the formulation of Cary and Blair[1].
The low nutrient content of the medium and utilisation of phosphate as a buffering agent instead of sodium glycerophosphate, prevents bacterial overgrowth by *Escherichia coli*, *Citrobacter freundii* and *Klebsiella aerogenes*.

This sometimes happens when using Stuart Transport Medium CM0111 because these organisms possess specific glycerophosphate dehydrogenases[2]. The low oxidation-reduction potential of the medium ensures bacterial survival over long periods[3].

Cary and Blair[1] reported recovery of cholera vibrios up to 22 days, salmonellae and shigellae after 49 days and Yersinia pestis up to 75 days storage at 28°C.

Cary-Blair Medium is particularly suitable in field epidemiological surveys for *Vibrio parahaemolyticus*, especially where rectal swabs are to be transported to a central diagnostic laboratory[4,5]. Survival of *Vibrio parahaemolyticus* in Cary-Blair medium has been reported after a 35-day period at a temperature of 70-80°F[6].

The medium can be modified to improve the transport and survival of *Campylobacter* species.

(i) by the addition of 1% w/v sodium pyruvate (10 g/litre) to the formula[7].

(ii) reducing the agar content from 5 g to 1.6 g per litre[8].

For the transport of fastidious anaerobic bacteria the medium may be prepared as directed and filled into long narrow screw-capped tubes[9]. It may also be prepared as a pre-reduced anaerobic sterilised medium (PRAS)[10]. Methods of producing PRAS media are described by Holdeman and Moore[11].

Technique

Use sterile, cotton-tipped swabs on wooden sticks to collect the specimen. Push the swabs down one third of the medium depth and cut the stick. Screw the cap firmly on the bottle.

Label the bottle and send it to the laboratory without delay.

The recovery of *Shigella* species is higher when the transport medium is held at 4°C or frozen[12].

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

The prepared medium should be stored away from light at 2-8°C or at room temperature (22-25°C) up to 19 months[13].

Appearance

Dehydrated Medium: Off white coloured, free-flowing powder.

Prepared medium: Light straw coloured, semi-solid gel.

Quality control

Positive controls:	***Expected results***
Shigella sonnei ATCC® 25931*	Good growth on subculture
Vibrio parahaemolyticus NCTC 11344	Good growth on subculture
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

Precautions

The medium should not be incubated to check sterility, prior to use. This should be carried out on separate quality control samples.

The medium can maintain the viability of fastidious organisms for transport purposes but it should not be used as a storage or enrichment medium.

The results obtained from the medium are dependent on the quality of the specimen material. Commensal anaerobic organisms may overgrow in the medium and cause misleading results.

References

1. Cary S. G. and Blair E. B. (1964) *J. Bact.* 88. 96-98.
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3. Stuart R. D. (1959) *Public Health Reports* 74. 431-438.
4. Cary S. G., Fusillo M. H. and Harkins C. (1965) *Am. J. Clin. Path.* 43. 294-295.
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12. Wells J. G. and Morris G. K. (1981) *J. Clin. Microbiol*. 13. 789-791.
13. Morris G. K and Heck J. (1978) *J. Clin. Microbiol.* 8. 616-617.

CAMPYLOBACTER BLOOD-FREE SELECTIVE MEDIUM (MODIFIED CCDA-PRESTON)

A medium, which when prepared from Campylobacter Blood-Free Selective Agar Base CM0739 and CCDA Selective Supplement SR0155, can be used for the isolation of Campylobacter jejuni, Campylobacter coli and Campylobacter laridis.

CAMPYLOBACTER BLOOD-FREE SELECTIVE AGAR BASE

Code: CM0739

Formula	***gm/litre***
Nutrient Broth No. 2	25.0
Bacteriological charcoal	4.0
Casein hydrolysate	3.0
Sodium desoxycholate	1.0
Ferrous sulphate	0.25
Sodium pyruvate	0.25
Agar	12.0
pH 7.4 ± 0.2	

CCDA SELECTIVE SUPPLEMENT

Code: SR0155

An improved selective supplement for Campylobacter Blood-Free Selective Agar Base.

Vial contents (each vial is sufficient for 500 ml of medium)	***per vial***	***per litre***
Cefoperazone	16 mg	32 mg
Amphotericin B	5 mg	10 mg

Directions
Suspend 22.75 g of Campylobacter Blood-Free Selective Agar Base in 500 ml of distilled water and bring to the boil to dissolve. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C. Aseptically add 1 vial of CCDA Selective Supplement SR0155 reconstituted as directed. Mix well and pour into sterile Petri dishes.

Description
Modified CCDA Medium is based on the original formulation described by Bolton *et al.*[1] which was developed to replace blood with charcoal, ferrous sulphate and sodium pyruvate. Improved selectivity was achieved when cephazolin in the original formulation was replaced by cefoperazone as the selective agent[2]. More recent work has shown an increased isolation rate can be achieved if the plates are incubated at 37°C rather than 42°C[3].

Amphotericin B has been added to the formula to suppress the growth of yeast and fungal contaminants that may occur at 37°C.

Modified CCDA medium and Campy-BAP medium were equal in performance in a rapid colony-lift procedure for detection of thermophilic campylobacters in which membranes are blotted on agar cultures and then subjected to immunoassay[5].

In a study of healthy puppies and kittens for carriage of *Campylobacter* species[6], modified CCDA medium was found to be a suitable medium and more productive for *Campylobacter upsaliensis* in this application than CAT medium. Modified CCDA medium has been confirmed as suitable for isolation of *Campylobacter* spp. from non-clinical samples following enrichment in Exeter broth[7].

The use of Campylobacter Blood-Free Medium is specified by the U.K. Ministry of Agriculture, Fisheries and Food (MAFF) in a validated method for isolation of *Campylobacter* from foods[4].

Technique

1. Prepare Campylobacter Blood-Free Selective Agar as described in the directions.
2. Emulsify approximately 0.5 g of the specimen in 5 ml of sterile 0.1% peptone water to form an approximate 1:10 dilution.
3. Inoculate onto the selective medium with cotton tipped swabs so that single isolated colonies are formed.
4. Incubate the plates in an atmosphere consisting of approximately 5-6% oxygen, 10% carbon dioxide and 84-85% nitrogen for 48 hours at 37°C. This can best be achieved by using the Oxoid Gas Generating Kit for campylobacter (BR0056) in conjunction with the Oxoid Anaerobic Jar (HP0011) and an active catalyst (BR0042). For jars of smaller capacity AnaeroJar (2.5 litres) use the Oxoid Gas Generating Kit for Campylobacters (BR0060). Alternatively use CampyGen CN0025A or CN0035A which does not require the use of a catalyst or the addition of water.

The colonial morphology of campylobacters can be used as a guideline for identification to species level. *Campylobacter jejuni* strains produce grey, moist flat spreading colonies. Some strains may have a green hue or a dry appearance, with or without a metallic sheen. *Campylobacter coli* strains tend to be creamy-grey in colour, moist, slightly raised and often produce discrete colonies.

Colonies tend to swarm when initially isolated from clinical specimens.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the selective supplement in the dark at 2-8°C and use before the expiry date on the label.

The prepared medium may be stored for up to 2 weeks at 2-8°C .

Appearance

Dehydrated Medium: Black coloured, free-flowing powder.

Prepared medium: Black coloured gel.

Quality control

Incubation at 37°C for 48 hours.

Positive controls:	***Expected results***
Campylobacter jejuni ATCC® 33291*	Good growth; grey coloured colonies
Negative control:	
Escherichia coli ATCC® 25922*	No growth

*This organism is available as a Culti-Loop®

References

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CEFOPERAZONE, AMPHOTERICIN B, TEICOPLANIN SUPPLEMENT (CAT)

Code: SR0174

A selective supplement for the isolation of thermophilic Campylobacter spp. and improved recovery of Campylobacter upsaliensis from faeces.

Vial contents (each vial is sufficient to supplement 500 ml of medium)	***per vial***	***per litre***
Cefoperazone	8.0 mg	16.0 mg
Teicoplanin	4.0 mg	8.0 mg
Amphotericin B	10.0 mg	20.0 mg

Directions

Prepare 500 ml of sterile Blood-Free Campylobacter Agar Base as directed. Cool to 50°C and aseptically add one vial of SR0174 reconstituted as directed.

Mix well and pour the resulting CAT medium into sterile Petri dishes. Incubate cultures at 37°C for 48-72 hours in a microaerobic atmosphere.

Description

Because of the sensitivity of *Campylobacter upsaliensis* to a wide range of antibiotics, isolation of the organism from faeces using selective media has hitherto been difficult. The recommended isolation method uses a membrane filter culture technique on non-selective agar. This does not give good recovery from faeces containing less than 105 CFU/g[5], and is a technically demanding method which is relatively slow to perform.

CAT Supplement SR0174 is based on the formulation described by Aspinall *et al*.[7]. When added to Blood-Free Campylobacter Agar Base which contains charcoal, it gives good isolation of thermophilic *Campylobacter* spp. The isolation of *Campylobacter upsaliensis* on a selective medium is possible because CAT Supplement contains reduced levels of cefoperazone compared to other campylobacter supplements. This inhibits most Enterobacteriaceae, but not enterococci. Teicoplanin is included to inhibit enterococci. Amphotericin B is added as an antifungal agent.

Further work confirmed the effectiveness of CAT medium as an alternative to membrane filtration culture for selective isolation of thermophilic campylobacters including *Campylobacter upsaliensis*[8].

Atabay, Corry and On[9] isolated a previously unknown catalase-negative, urease-positive Campylobacter from cattle faeces using CAT medium. This organism could not be cultured on blood-free Campylobacter medium (CCDA).

A study in which the productivity of CAT medium, blood-free media and semi-solid medium were compared, showed that CAT medium, used in parallel with membrane filtration on non-selective blood agar, is likely to be the most productive method for recovery of the greatest number of Campylobacter and Arcobacter species[10].

Storage conditions and Shelf life

CAT Supplement SR0174 should be stored at 2-8°C in the dark. When stored as directed, the reagents remain stable until the stated expiry date shown on the packaging.

Quality control

Positive controls:	***Expected results***
Campylobacter upsaliensis ATCC® 43954*	Good growth; pale colonies
Campylobacter jejuni ATCC® 33291*	Good growth; grey coloured colonies
Negative control:	
Enterococcus faecalis ATCC® 33186	Inhibited

*This organism is available as a Culti-Loop®

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CDMN – see CLOSTRIDIAL DIFFICLE MOXALACTAM NORFLOXACIN MEDIUM

CEFIXIME RHAMNOSE SORBITOL MACCONKEY AGAR (CR-SMAC Agar Base)

Code: CM1005

A selective, differential medium based on Sorbitol MacConkey Agar with added rhamnose and cefixime. This medium provides a selective base with improved differentiation of Escherichia coli O157.

Formula	***gm/litre***
Peptone	20.0
Sorbitol	10.0
Bile Salts No. 3	1.5
Sodium chloride	5.0
Rhamnose	5.0
Neutral red	0.03
Crystal violet	0.001
Agar	15.0
pH 7.1 ± 0.2	

CEFIXIME SUPPLEMENT

Code: SR0191

Vial Contents (each vial is sufficient for 500 ml of medium)	***per vial***	***per litre***
Cefixime	0.025 mg	0.05 mg

Directions

Suspend 28.25 g in 500 ml of distilled water. Mix well and sterilise by autoclaving at 121°C for 15 minutes. Cool to approximately 50°C and aseptically add the contents of one vial of Cefixime Supplement SR0191, reconstituted as directed. Mix well and pour into sterile Petri dishes.

To reconstitute Cefixime Supplement, aseptically add 2 ml sterile distilled water to 1 vial of supplement. Mix gently to dissolve.

Description

The addition of rhamnose to Sorbitol MacConkey Agar (SMAC) has been shown to aid the differentiation of *Escherichia coli* O157 from background flora. Approximately 60% of non-sorbitol fermenting *Escherichia coli* of serogroups other than O157 ferment rhamnose (Sheffield PHL, unpublished data). Cefixime has been shown to reduce the level of competing flora, particularly *Proteus* spp., that often account for large numbers of non-sorbitol fermenting colonies[1].

Escherichia coli O157 do not usually ferment sorbitol or rhamnose, so will appear as straw coloured colonies. However, rhamnose is fermented by most sorbitol negative *Escherichia coli* of other serogroups. These colonies will be pink/red and will not be counted as presumptive *Escherichia coli* O157 colonies[1].

Cefixime Rhamnose Sorbitol MacConkey Agar (CR-SMAC) is a selective and differential medium for the detection of *Escherichia coli* O157. Field trials have shown that using CR-SMAC, in conjunction with the more selective CT-SMAC, leads to a higher overall recovery of *Escherichia coli* O157 from enrichment broths[2,3].

Technique
Inoculate the plates with a suspension of the sample. to produce separated colonies. Incubate for 24 hours at 35-37°C.

Examine the plates for straw coloured colonies; these are sorbitol and rhamnose negative organisms. Confirm suspected *Escherichia coli* O157 with the *Escherichia coli* O157 Latex Test DR0620 or Dryspot *Escherichia coli* O157 DR0120.

Coloured colonies may also occur:

sorbitol positive	pink to red
rhamnose positive	pink to red

Storage conditions and Shelf Life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium for up to 2 weeks at 2-8°C.

Appearance
Dehydrated Medium: Straw pink coloured, free-flowing powder.

Prepared medium: Red coloured gel

Quality control

Positive controls:	***Expected results***
Escherichia coli O157:H7 NCTC 12900* (sorbitol negative, rhamnose negative)	Good growth, straw coloured colonies
Negative controls:	
Escherichia coli ATCC® 25922* (sorbitol positive)	Good growth, pink colonies
Proteus mirabilis ATCC® 29906 (sorbitol negative, rhamnose negative)	Inhibited

*This organism is available as a Culti-Loop®

References
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CETRIMIDE AGAR – see PSUDOMONAS CETRIMIDE AGAR

CFC AGAR – see PSUDOMONAS AGAR (CFC FORMULATION)

CHAPMAN MEDIUM – see MANNITOL SALT AGAR

CHARCOAL AGAR

Code: CM0119

A medium for the cultivation and isolation of Bordetella pertussis and Haemophilus influenzae.

Formula	***gm/litre***
'Lab-Lemco' powder	10.0
Peptone	10.0
Starch	10.0
Charcoal bacteriological	4.0
Sodium chloride	5.0
Nicotinic acid	0.001
Agar	12.0
pH 7.4 ± 0.2	

Directions

Suspend 51 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C, add 10% of defibrinated blood and mix gently. The medium is made selective for the isolation of *Bordetella pertussis* and *Bordetella parapertussis* by the addition of Bordetella Selective Supplement SR0082.

Add the contents of one vial of Bordetella Selective Supplement reconstituted as directed to 500 ml of sterile, molten Charcoal Agar CM0119, cooled to 50°C, together with 10% v/v defibrinated horse blood SR0050. Mix well before pouring into sterile Petri dishes.

For *Haemophilus influenzae*, omit the selective agents and convert to 'chocolate' agar.

Transport Medium for *B. pertussis*

The vial contents may be added to 500 ml of half-strength Charcoal Agar + 10% v/v defibrinated horse blood SR0050 for use as a transport medium for *Bordetella pertussis*.

Description

Charcoal Agar was developed by Oxoid to provide a non-blood-containing medium for the cultivation of *Bordetella pertussis* and *Haemophilus influenzae*. Proom[1] showed that nicotinic acid was an essential growth factor for the bordetellae. Ensminger *et al*.[2] used a charcoal medium for the growth of *Bordetella pertussis* in vaccine production and found that the medium could replace Bordet-Genou. Mishulow *et al*.[3] used charcoal agar for *Bordetella pertussis* cultivation.

Haemophilus influenzae is cultivated on the medium containing 10% 'chocolated' blood but no antibiotics. The inoculated plate is incubated for 2 to 3 days at 37°C. The colonies are usually small, transparent and droplet-like, but some transformation to the 'rough' type colony may occur. Species differentiation is performed by examination of the need for X and V growth factors, on Blood Agar Base CM0055.

The greatest problem in the isolation of *Bordetella* species from naso-pharyngeal secretions is the suppression of unwanted flora during the long incubation period on very nutritious media.

Fleming's first *in vitro* demonstration of penicillin was to show that it could help isolate *Bordetella pertussis* on media[4]. Lacey[5] confirmed this but found that the penicillin-resistant flora still caused problems. He supplemented penicillin with 2 µg/ml 4,4'diamidino-diphenylamine dihydrochloride (M & B 938) thereby increasing the selectivity of this medium.

Broome *et al*.[6] found methicillin to be superior to penicillin in suppressing unwanted naso-pharyngeal flora but the earlier publication of Sutcliffe and Abbott[7] where cephalexin (40 µg/ml) was shown to be superior to penicillin, has proved to be the most significant advance.

The benefits of cephalexin as a selective agent for *Bordetella pertussis* have been confirmed[8,9,10,11]. The ability to recover stressed cells and the much longer shelf life (6-8 weeks) are added benefits to its superiority at suppressing unwanted naso-pharyngeal growth.

Regan and Lowe[8] showed that half-strength Oxoid Charcoal Agar, supplemented with 40 µg/ml cephalexin SR0082 v/v lysed, defibrinated horse blood was an excellent enrichment and transport medium.

The efficacy of this transport medium has been confirmed by other workers[12].

Technique

The following technique for the laboratory diagnosis of *Pertussis* is recommended[11].

1. Collect pernasal swabs in the early stage of the illness and place in tubes of half-strength Charcoal Agar supplemented with 10% v/v lysed, defibrinated horse blood and 40 mg/ml cephalexin.
2. Generously inoculate the swabs on to thick layers of Charcoal Agar containing 10% v/v defibrinated horse blood and 40 µg/ml cephalexin (SR0082).

   A non-selective medium in which the cephalexin is omitted may be used in addition.
3. Perform direct fluorescent antibody (DFA) tests on the secretions, using *Bordetella pertussis* and *Bordetella parapertussis*-conjugated antisera, to help make an earlier diagnosis.
4. Replace the swabs in the original transport medium and hold at room temperature. If the culture plates become overgrown with commensal flora or fungi, use the swabs to inoculate fresh plates of medium.
5. Incubate the plates at 35°C in a moist atmosphere (60-70% humidity) for up to six days. Examine the plates after 40 hours incubation and twice-daily thereafter.
6. Look for small, shiny, greyish-white, round convex colonies. Suspicious colonies should be Gram stained, using a two-minute safranin counterstain. Some pleomorphic cells may be seen, caused by the cephalexin in the selective medium.
7. Confirm the identification with DFA tests on the suspicious colonies.

Precautions

Stuart's transport medium or similar formulation media should not be used for *Bordetella*-containing specimens[13].

Two pernasal swabs should be taken from each patient, one through each nostril[14].

Make sure the charcoal remains in suspension when dispensing the medium by gently swirling the flask.

Lysed horse blood is used in the transport medium but whole blood is used in the isolation medium.

Most naso-pharyngeal flora are inhibited by cephalexin but *Pseudomonas aeruginosa* and some fungi may grow through. Amphotericin B can be added (12 mg/ml) as an antifungal agent to prevent the growth of filamentous fungi. However, this level of amphotericin B can be inhibitory to *Bordetella pertussis* and should not be used routinely.

METRONIDAZOLE SUSCEPTIBILITY TEST FOR

Helicobacter pylori

Charcoal agar supplemented with a concentrate of essential growth factors has been reported to be a reliable testing medium for determining metronidazole resistance in *Helicobacter pylori*[15].

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared plates of medium at 2-8°C.

Appearance

Dehydrated medium: Black, free-flowing powder.

Prepared medium: Black gel.

With Cephalexin: ***Positive controls:***	***Expected results***
Bordetella pertussis ATCC® 8467	Good growth; grey coloured colonies
Bordetella parapertussis NCTC 10521	Good growth; grey coloured colonies
Negative controls:	
Staphylococcus aureus ATCC® 25923*	Inhibited
Klebsiella pneumoniae ATCC® 13883*	Inhibited
Without Antibiotics:	
Positive control:	
Haemophilus influenzae ATCC® 35056*	Good growth; grey coloured colonies
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

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CHINA BLUE LACTOSE AGAR

Code: CM0209

A standard, non-inhibitory solid medium for enumeration and differentiation of bacteria in dairy products.

Formula	***gm/litre***
Peptone	5.0
'Lab-Lemco' powder	3.0
Lactose	10.0
Sodium chloride	5.0
China blue	q.s.
Agar	12.0
pH 7.0 ± 0.2	

Directions
Suspend 35 g in 1 litre of distilled water. Boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

Description
China Blue Lactose Agar was formulated by Brandl and Sobeck-Skal[1]. A standard, non-inhibitory solid medium for the differentiation and enumeration of bacteria in milk, proposed by the Methodenkommission für Milchwirtschaft[2]. The china blue serves as a pH indicator, to differentiate between lactose fermenters and non-lactose fermenters, but does not suppress the growth of cocci; therefore this medium may be used for the detection of streptococci and staphylococci as well as the coli-aerogenes group.

Plates may be streak-inoculated or decimal dilutions of milk may be added to the molten, cooled medium in a pour-plate technique.

After 18 hours incubation at 35°C colony appearances are:

Colour	***Micro-organisms***
Blue	Lactose-fermenters e.g. *Escherichia coli* and coliform bacteria: 3-4 mm diameter
	Staphylococci: 1 mm diameter
	Streptococci: 0.5 mm diameter
Colourless	Non-lactose-fermenters e.g. *Salmonella*, *Serratia*, *Proteus* species and others

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance
Dehydrated medium: Straw/pale blue coloured, free-flowing powder.

Prepared medium: Blue coloured gel.

Quality control

Positive controls:	***Expected result***
Enterococcus faecalis ATCC® 29212*	Good growth; pale blue colonies
Escherichia coli ATCC® 25922*	Good growth; light blue coloured colonies with dark centres
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

Precautions
It is important to remember that Gram positive and negative cocci and bacilli can grow on this medium. Always confirm the organism morphology and Gram reaction.

References

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CHOLERA MEDIUM TCBS

Code: CM0333

A selective isolation medium for pathogenic vibrios.

Formula	***gm/litre***
Yeast extract	5.0
Bacteriological peptone	10.0
Sodium thiosulphate	10.0
Sodium citrate	10.0
Ox Bile	8.0
Sucrose	20.0
Sodium chloride	10.0
Ferric citrate	1.0
Bromothymol blue	0.04
Thymol blue	0.04
Agar	14.0
pH 8.6 ± 0.2	

Directions
Suspend 88 g in 1 litre of distilled water. Boil to dissolve the medium completely. DO NOT AUTOCLAVE.

Pour plates without further heating and dry before use.

Description
Kobayashi, Enomoto, Sakazaki and Kuwahara[1] developed TCBS media from the selective isolation agar of Nakanishi[2].

The Oxoid TCBS medium conforms to the formulation of Kobayashi *et al*., except that it contains specially processed ox bile, free from the defects noted by Nakanishi and Kobayashi.

The complexity of the composition of this medium means that uniformity of growth is a difficult standard to maintain. Several investigations have shown variation between batches of TCBS Medium made by different companies[3,4,5,6].

Quality control by the manufacturers of this medium is especially important because satisfactory inhibition of normal gut flora and lack of inhibition of certain *Vibrio* species is very critical. West *et al*.[7] showed that Oxoid TCBS Medium came closest to their criteria for a satisfactory product.

WHO has established a minimum acceptable guideline for the recovery of *Vibrio* species on TCBS Medium[8].

The Oxoid medium is suitable for the growth of *Vibrio cholerae*, *Vibrio parahaemolyticus*, and most other *Vibrios*[9].

Most of the Enterobacteriaceae encountered in faeces are totally suppressed for at least 24 hours. Slight growth of *Proteus* species and *Enterococcus faecalis* may occur but the colonies are easily distinguished from *Vibrio* colonies.

Oxoid TCBS Medium is complete and requires no additives or aseptic additions of blood. It therefore shows a considerable advantage over Lauryl Sulphate Tellurite Agar which requires further additions after sterilisation. Apart from this convenience factor, it also possesses superior growth characteristics for *Vibrio* species, compared with tellurite media. Whilst inhibiting non-vibrios, it promotes rapid growth of pathogenic vibrios after overnight incubation at 35°C. For the isolation of other vibrios from environmental samples, incubation at lower temperatures, 20-30°C is needed.

Colonial appearance of organisms on TCBS Medium
24 hours incubation at 35°C.

Organisms	***Colonies***
Vibrio cholera and El Tor type	Yellow, flat, 2-3 mm diameter
Vibrio parahaemolyticus	Blue-green, 3-5 mm diameter
Vibrio alginolyticus	Yellow, 3-5 mm diameter
Vibrio metschnikovii[10]	Yellow, 3-4 mm diameter
Vibrio fluvialis[11]	Yellow, 2-3 mm diameter
Vibrio vulnificus[12]	Blue-green, 2-3 mm diameter
Vibrio mimicus[13]	Blue-green, 2-3 mm diameter
Enterococcus species	Yellow, 1 mm diameter
Proteus species	Yellow-green, 1 mm diameter
Pseudomonas species	Blue-green, 1 mm diameter

Some strains of *Aeromonas hydrophila* grow producing yellow colonies but *Plesimonas shigelloides* does not usually grow well on TCBS.

Technique
Streak the faeces or a sub-culture from an enrichment medium, e.g. Alkaline Peptone Water, across the surface of Oxoid TCBS Cholera Medium. Incubate plates for 18-24 hours at 35°C for clinical specimens or lower temperature for environmental samples.

Cultures grown on TCBS should be examined quickly after removal from an incubator as the yellow colonies of *Vibrio* cultures e.g. *Vibrio cholerae*, may revert to a green colour when left at room temperature[9].

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance
Dehydrated medium: Straw/green coloured, free-flowing powder.

Prepared medium: Green coloured gel.

Quality control

Positive controls:	***Expected result***
Vibrio furnissii NCTC 11218 (a non-pathogenic strain[6])	Good growth; yellow colonies
Vibrio parahaemolyticus NCTC 10885	Good growth; green colonies
Negative control:	
Escherichia coli ATCC® 25922*	No growth

*This organism is available as a Culti-Loop®

Precautions
The identification of the various *Vibrio* species on TCBS Medium is presumptive and further tests are required for confirmation.

Yellow colonies on TCBS Medium will give unsatisfactory oxidase reactions.

Colonies taken from TCBS Medium are ‘sticky’ and react poorly in slide agglutination tests. Sub-culture to nutrient agar is required before slide agglutination tests can be carried out.

Some strains of *Vibrio vulnificus* produce better recovery at 30°C.

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CHRISTENSON AGAR – see UREA AGAR

CHRISTENSON BROTH – see UREA BROTH

CHROMOGENIC BACILLUS CEREUS AGAR

Code: CM1036

A chromogenic medium for the isolation and differentiation of Bacillus cereus from food samples.

Formula	***gm/litre***
Yeast extract	4.0
Peptone	10.0
Di-sodium hydrogen phosphate	2.52
Potassium di-hydrogen phosphate	0.28
Sodium pyruvate	10.0
Chromogenic mix	1.2
Agar	13.0
pH 7.2 ± 0.2	

CHROMOGENIC BACILLUS CEREUS SELECTIVE SUPPLEMENT

Code: SR0230

Vial contents (each vial is sufficient for 500 ml of medium):	***per vial***	***per litre***
Polymyxin B	53,000 IU	106,000 IU
Trimethoprim	5.0 mg	10.0 mg

Directions

Suspend 20.5 g in 500 ml of distilled water. Mix well and bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Cool the medium to 50°C and aseptically add 1 vial of Chromogenic Bacillus cereus Selective Supplement. Mix well and pour into sterile Petri dishes.

Description

Bacillus cereus, a Gram-positive, aerobic, spore-forming, rod-shaped bacterium, is widely distributed in nature. It is readily isolated from soil, dust, cereal crops, vegetation, animal hair, fresh water and sediments.

Therefore it is not surprising to find the organism associated with virtually every raw agricultural commodity. The ability to form spores ensures survival through all stages of food processing short of retorting and the organism is present in most raw materials used in food manufacture. Under normal circumstances *Bacillus cereus* is found at <103 cells per gram of food and does not cause any problems as the minimum level to cause illness is more than 105 cells per gram[1].

Bacillus cereus-associated gastroenteritis results from the ingestion of two distinct toxins (emetic toxin and enterotoxin) produced during the vegetative stage of growth, in foods that have been poorly refrigerated following cooking. Two types of illness are caused by the two toxins. The diarrhoeal type of illness is caused by a large molecular weight protein or enterotoxin. Onset is usually within 6-15 hours of ingestion of contaminated food. The vomiting (emetic) type of illness is believed to be caused by a low molecular weight, heat-stable peptide and symptoms can start to occur within 0.5-6 hours of ingestion[1].

A wide variety of foods including meats, milk, vegetables, and fish have been associated with the diarrhoeal-type food poisoning. The vomiting-type outbreaks have generally been associated with rice products; however, other starchy foods such as potato, pasta and cheese products have also been implicated. Food mixtures such as sauces, puddings, soups, casseroles, pastries, and salads have frequently been incriminated in food poisoning outbreaks[2].

Oxoid Chromogenic Bacillus cereus Agar incorporates the chromogenic substrate 5-bromo-4-chloro-3-indolyl-β-glucopyranoside, which is cleaved by the enzyme β-glucosidase present in *Bacillus cereus* resulting in the formation of blue/green colonies. Polymixin B inhibits most Gram-negative organisms and some Gram-positive organisms including some *Bacillus* other than *Bacillus cereus*. Trimethoprim, which is also added to the medium, blocks folic acid synthesis necessary for DNA production and is active against many Gram-positive bacteria including *Staphylococcus aureus*, *Enterococcus* spp. and some non-cereus *Bacillus* species. The combination of these two antibiotics has been shown to be more effective than the use of polymyxin B alone[3].

Because *Bacillus thuringiensis* is biochemically identical to *Bacillus cereus* it will also grow as blue/green colonies on this medium. *Bacillus thuringiensis* is known primarily as an insect pathogen but it has also been reported to have been linked to some human gastroenteritis outbreaks[4].

Technique

Please note that the following is only intended as a suggested method of use.

1. Prepare a 10% dilution (w/v) of the food sample to be tested in 0.1% Peptone Water (CM0009) or MRD (CM0733).
2. Homogenise the sample for 1 minute using an appropriate laboratory blender.
3. Inoculate 0.1 ml volumes of 10-1, 10-2 and 10-3 dilutions of the homogenate onto the surface of Chromogenic Bacillus cereus Agar plates.
4. Incubate the plates at 37°C for 24 hours.
5. Examine for typical colonies of *Bacillus cereus*.
6. Confirm the presumptive identification of *Bacillus cereus* by a validated method, e.g. oxidase, Gram-stain.
7. Report the results as the number of *Bacillus cereus* colonies per gram weight of the food sample.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label. Chromogenic Bacillus cereus Selective Supplement must be stored at 2-8°C.

Store the prepared medium for up to 2 weeks at 2-8°C.

Appearance

Dehydrated medium: Straw coloured, free-flowing powder.

Selective supplement: a white, freeze-dried pellet.

Prepared medium: a light straw coloured gel.

Quality control

Positive control:	***Expected results***
Bacillus cereus ATCC® 10876	Good growth blue/green colonies
Negative controls:	
Bacillus subtilis ATCC® 6633*	No growth
Escherichia coli ATCC® 25922*	No growth

*This organism is available as a Culti-Loop®

References

1. *Foodborne Pathogenic Microorganisms and Natural Toxins Handbook* Bacillus cereus *and other Bacillus* spp. (2003) U.S. Food & Drug Administration (C.F.S.A.N.).
2. *The Oxoid Manual* (1998) 8th Edition. Oxoid, UK.
3. Poster – Cloke J. M., Ring M., Campbell S., Smith E. and Stringer J. *Evaluation of a new Oxoid chromogenic medium for the Isolation of* Bacillus cereus *from foods* (2003) Oxoid & Burton's Foods, UK.
4. *Handbook of Culture Media for Food Microbiology* (2003) Volume 37. Chapter 4. Media for *Bacillus* spp. and related genera relevant to foods. Edited by Corry J. E. L., Curtis G. D. W. and Baird R. M. Publisher – Elsevier, Amsterdam.

CHROMOGENIC CANDIDA AGAR

Code: CM1002

A selective differential medium for the rapid isolation and identification of clinically important Candida species.

Formula	***gm/litre***
Peptone	4.0
Chromogenic mix	13.6
Agar	13.6
pH 6.0 ± 0.2	

CHROMOGENIC CANDIDA SELECTIVE SUPPLEMENT

Code: SR0231

Vial Contents (each vial is sufficient for 500 ml of medium)	***per vial***	***per litre***
Chloramphenicol	250 mg	500 mg

Directions

Suspend 15.6 g in 500 ml of distilled water. Add 1 vial of Chromogenic Candida Selective Supplement reconstituted as directed. Mix well and bring to the boil with frequent agitation. DO NOT AUTOCLAVE. Cool the medium to 45°C and pour into sterile Petri dishes.

Description

Serious infections due to *Candida* species are becoming increasingly prevalent. This poses particular problems because of the increasing incidence of non-*albicans* spp. and the emergence of non-*albicans* isolates resistant to both amphotericin B and the newer azoles[1].

Candida species are the fourth most commonly encountered nosocomial pathogens in bloodstream infections in the United States and candidiasis is associated with mortality rates as high as 60% in immuno-suppressed patients. Of the *Candida* spp. encountered in clinical practice, *Candida albicans* is the most common and this species is usually susceptible to the azole group of antifungal agents. However, it is the shift toward the isolation of more azole-tolerant species, such as *Candida glabrata*, *Candida tropicalis*, and *Candida krusei*, due to the increasing use of itraconazole and fluconazole as the antifungal drugs of first choice for candidiasis, which is causing greatest concern. Rapid identification of the *Candida* spp. causing infection is therefore critical for the clinician in determining the correct antifungal therapy[2].

Conventional identification of *Candida* spp. is based on an extensive series of tests, e.g. carbohydrate fermentation and assimilation, growth at 37°C and 42°C, colony and cell morphology and the ability to form germ tubes.

Rapid identification of micro-organisms in general has been shown to have a major impact on the morbidity, mortality, and duration of hospitalisation. For *Candida* species involved in bloodstream infections on ICUs, it was shown by Ibrahim *et al*.[3] that initial therapy was inadequate in 95% of the cases because no antifungal agent was administered. Due to this inadequacy in the initial treatment, a mortality rate of about 60% was observed in the patient group with *Candida* infections. Hence, early recognition of a *Candida* infection would help a clinician to select proper treatment. Combined with rapid identification of the causative organism, this treatment could be optimised to include a non-azole group antifungal agent, if required, at an early stage of the infection.

The medium incorporates two chromogens that indicate the presence of the target enzymes:

X-NAG (5-bromo-4-chloro-3-indolyl N acetyl β-D-glucosaminide) detects the activity of hexosaminidase.

BCIP (5-bromo-6-chloro-3-indolyl phosphate p-toluidine salt) detects alkaline phosphatase activity.

The typical enzyme patterns of *Candida* spp. are shown in Table 1. An opaque agent has been incorporated into the formulation to improve the colour definition on the agar. The broad-spectrum antibacterial agent chloramphenicol is added to the agar at 500 mg/l to inhibit bacterial growth on the plates.

Technique

Good laboratory practices for the appropriate collection and transport of specimens should be followed.

Clinical specimens should be inoculated directly onto the agar.

Incubate plates aerobically at 30°C. Inspect for the growth of *Candida* spp. at 24, 48 and 72 hours.

Table 1			
Chromogen Enzyme	***X-NAG Hexosaminidase***	***BCIP Alkaline phosphatase***	***Typical colony appearance***
C. tropicalis	+		Dark blue
C. albicans	+		Green*
C. dubliniensis			
C. krusei		+	Dry, irregular pink-brown
C. glabrata *C. kefyr*		Variable	Beige/yellow/ brown†
C. parapsilosis			
C. lusitaniae			

Notes:

*The green colour of *Candida albicans* and *Candida dubliniensis* is caused by the same chromogenic reaction as the dark blue colour of *Candida tropicalis*. However, other reactions caused by the medium (the nature of which are subject to a patent application) cause the colonies to appear green.

†*Candida glabrata*, *Candida kefyr*, *Candida parapsilosis* and *Candida lusitaniae* appear as a variety of beige/brown/yellow colours, due to the mixture of natural pigmentation and some alkaline phosphatase activity. Experienced users may be able to differentiate these species by colour and colony morphology.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at room 2-8°C.

Appearence

Dehydrated medium: straw coloured, free-flowing powder.

Prepared medium: cream coloured opaque gel.

Quality control

Positive controls:	***Expected result***
C. albicans ATCC® 10231*	Good growth, green colonies
C. krusei ATCC® 6258	Good growth, dry, irregular pink-brown colonies
Negative control:	
Escherichia coli ATCC® 25922*	Inhibited

*This organism is available as a Culti-Loop®

References

1. Sheehan D. J. *et al.* (1999) Current and Emerging Azole Antifungal Agents. *Clinical Microbiology Reviews* 12(1). 40-79.
2. Odds F. C. (1988) Candida and candidosis, 2nd ed. Baillière Tindall, London, England.
3. Ibrahim E. H. *et al.* (2001) The influence of inadequate antimicrobial treatment of bloodstream infections on patient outcomes in the ICU setting. *Chest*, 118(1). 146-55.

CHROMOGENIC E. COLI/COLIFORM MEDIUM

Code: CM0956

A chromogenic medium to aid differentiation between Escherichia coli and other coliforms in cultures produced from food and environmental samples.

Formula	***gm/litre***
Chromogenic mix	20.3
Yeast extract	3.0
Peptone	5.0
Lactose	2.5
Sodium chloride	5.0
Di-sodium hydrogen phosphate	3.5
Potassium di-hydrogen phosphate	1.5
Neutral red	0.03
Agar	15.0
pH 6.8 ± 0.2	

Directions
Suspend 55.8 g of Chromogenic E.coli/coli coliform medium in one litre of distilled water. Sterilise by autoclaving at 121°C for 15 minutes. Cool to approximately 50°C. Mix well and pour and into sterile Petri dishes.

Description
Chromogenic E.coli/coliform medium is a differential agar used for the presumptive identification of *Escherichia coli* and coliforms from food and environmental samples. The agar base uses two enzyme substrates to differentiate between *Escherichia coli* and other coliforms. One chromogenic substrate is cleaved by the enzyme glucuronidase which is specific for *Escherichia coli* and produced by approximately 97% of strains[1,2,3]. The second chromogenic substrate is cleaved by galactosidase, an enzyme produced by the majority of coliforms. This results in purple *Escherichia coli* colonies, as they are able to cleave both chromogenic substrates and pink coliform colonies as they are only able to cleave the galactosidase chromogen.

Technique
Dry the surface of the medium. Prepare the food sample by diluting as appropriate with 0.1% sterile peptone water CM0009 and homogenise in a stomacher or laboratory blender. Pipette 0.5 ml or 1.0 ml of the homogenate as appropriate on to the plate and spread over the surface with a glass spreader. Incubate the plates for 18-24 hours at 37°C.

Calculate the total number of coliforms per gram by multiplying purple and pink colonies by the dilution factor. The number of presumptive *Escherichia coli* is obtained by multiplying the number of purple colonies by the dilution factor.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance
Dehydrated Medium: Straw to straw/pink free flowing powder

Prepared medium: Opaque pink coloured gel.

Positive controls:	***Expected results***
Escherichia coli ATCC® 25922*	Good growth purple colonies
Klebsiella pneumoniae ATCC® 13883*	Good growth pink colonies
Negative control:	
Pseudomonas aeruginosa ATCC® 27853*	Good growth straw colonies

*This organism is available as a Culti-Loop®

Precautions
Refer to the material safety data sheet before handling this product.

Some pathogenic strains, such as *Escherichia coli* O157:H7 are usually glucuronidase negative and therefore will not give purple colonies on this medium.

References
1. Kilian M. and Bulow P. (1976). *Acta. Pathol. Microbiol. Scand.* Sect. B 84: 245-251.
2. Kilian M. and Bulow P. (1979). *Acta. Pathol. Microbiol. Scand.* Sect. B 87: 271-276.
3. Frampton E. W., Restaino L. and Blaszko N. (1988). *J. Food Prot.* 51(5): 402-404.

CHROMOGENIC E. COLI/COLIFORM SELECTIVE MEDIUM

Code: CM1046

A selective, chromogenic medium for the detection and enumeration of Escherichia coli and other coliforms from food and water samples.

Formula	***gm/litre***
Peptone	8.0
Di-sodium hydrogen phosphate	2.2
Sodium chloride	5.0
Potassium di-hydrogen phosphate	1.8
Sodium lauryl sulphate	0.1
Chromogenic mix	0.35
Agar	10.6
pH 6.7 ± 0.2	

Directions
Suspend 28.1 g of Selective E. coli/Coliform Chromogenic Medium in 1 litre of distilled water. Bring the medium gently to the boil, to dissolve completely. Either pour the medium into sterile Petri dishes or keep molten at 45ºC for pour plate technique.

Description
The recovery and enumeration of *Escherichia coli* and coliforms are important indicators of environmental and food hygiene. Detection of β-glucuronidase activity is widely used to differentiate *Escherichia coli* bacteria, as the enzyme, which is encoded by the uidA gene, is present in *Escherichia coli*, but not other members of the coliform group. As coliforms are lactose-positive, β-galactosidase activity, encoded by the *lacZ* gene, is then used to differentiate this group from other organisms able to grow on the selective medium. This results in purple *Escherichia coli*, as they are able to cleave both chromogens, with other coliforms giving pink colonies as they cleave only the galactoside chromogen.

Selective E. coli/Coliform Chromogenic Medium contains two chromogenic agents – Rose-Gal – which detects β-galactosidase activity; X-Glu – which detects β-glucuronidase activity. The medium also contains sodium lauryl sulphate which acts as a selective agent, inhibiting the growth of Gram-positive organisms.

Most organisms in the coliform group are able to ferment lactose, so will cleave the pink Rose-Gal chromogen, producing pink colonies. *Escherichia coli* strains can be differentiated from the other coliforms as they also possess the enzyme β-glucuronidase (which has been shown to be highly specific to *Escherichia coli*). The X-Glu chromogen is targeted by this enzyme. The ability of *Escherichia coli* species to cleave both chromogens means that typical colonies will be purple.

Organism	***β-glucuronidase***	***β-galactosidase***	***Colony colour***
E. coli	+	+	Purple
Coliforms		+	Pink
Other organisms	- +	- -	Colourless or Blue

Technique
Selective E. coli/Coliform Chromogenic Medium may be used for the detection and enumeration of *Escherichia coli* and coliforms in food and water samples.

Prepare food samples by diluting 1:5 or 1:10 (as appropriate) with 0.1% (w/v) sterile Peptone Water (CM0009), and homogenise in a Stomacher or a laboratory blender.

Heavily contaminated water samples should first be diluted in Ringers Solution (BR0052) or Maximum Recovery Diluent (CM0733) so that the number of colonies to be counted is of a readable number e.g. 20-100 colonies. Potable water should be concentrated either by centrifugation or by using the filter membrane method.

The following incubation techniques may be used:

1. Spread Plate
Dry the surface of the prepared plates. Pipette 0.1 ml of the prepared sample onto the plate and spread over the surface with a sterile spreader. Incubate plates for 24 hours at 37ºC.

2. Pour-Plate Method
Pipette 1 ml of the prepared sample into an empty Petri dish. Add 15-20 ml of medium, cooled to 45ºC. Gently swirl the plates to thoroughly mix and allow to set. Incubate for 24 hours at 37ºC.

3. Filter Membrane Method
Dry the surface of the prepared plates. Filter an appropriate volume of sample through the membrane. Place the membrane onto the surface of an agar plate and avoid trapping air-bubbles under the membrane. Incubate for 24 hours at 37°C.

For all methods count the numbers of pink and purple colonies. Multiply the numbers of colonies by the dilution factor and express the result as the number of coliforms and *Escherichia coli* per gram of food or volume of water.

Storage conditions and Shelf life
Dehydrated medium: store tightly capped in the original container at 10-30°C.

Prepared medium will be stable for up to 2 weeks when stored at 2-8ºC.

Appearence
Dehydrated medium: light coloured, free-flowing powder.

Prepared medium: light straw coloured, transparent gel.

Quality control

Positive controls:	***Expected result***
Escherichia coli ATCC® 25922*	Good growth, purple colonies
Klebsiella pneumoniae ATCC® 13883*	Good growth, pink colonies
Negative control:	
Staphylococcus aureus ATCC® 25923*	Inhibited

*This organism is available as a Culti-Loop®

References
1. Kilian M. and Bulow P. (1976) *Acta Pathol. Microbiol. Scand.* Sect. B 84, pp.245-251.
2. Kilian M. and Bulow P. (1979) *Acta Pathol. Microbiol. Scand*. Sect. B 87, pp.271-276.
3. Frampton E. W., Restaino L. and Blaszko N. (1988) *J. Food Prot.* Vol: 51(5). pp.402-404.

CHROMOGENIC ENTERBACTER SAKAZAKII AGAR (DFI formulation)

Code: CM1055

Chromogenic Enterobacter sakazakii Agar (DFI formulation) is intended for the differentiation and enumeration of Enterobacter sakazakii from infant formula and other food samples.

Formula	***gm/litre***
Tryptone	15.0
Soya peptone	5.0
Sodium chloride	5.0
Ferric ammonium citrate	1.0
Sodium desoxycholate	1.0
Sodium thiosulphate	1.0
Chromogen	0.1
Agar	15.0
Final pH 7.3 ± 0.2	

Directions

Suspend 43.1 g of Chromogenic Enterobacter sakazakii Agar in 1 litre of distilled water. Mix well and bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Cool the medium to 50°C. Mix well and pour into sterile Petri dishes.

Description

Chromogenic Enterobacter sakazakii Agar (DFI formulation[1]) is based on the α-glucosidase reaction which is detected by incorporating the chromogenic substrate 5-bromo-4-chloro-3-indolyl-α-D-glucopyranoside in the medium. The enzyme α-glucosidase, present in *Enterobacter sakazakii*, hydrolyses the substrate producing blue-green colonies on this pale yellow medium. *Proteus vulgaris* is also weakly α-glucosidase positive and could grow to give colonies of a similar colour to *Enterobacter sakazakii.* However, on this medium, *Proteus* spp. grow as grey colonies: they produce hydrogen sulphide in the presence of ferric ions forming ferrous sulphide. Desoxycholate inhibits the growth of most Gram-positive organisms.

Enterobacter sakazakii is a Gram-negative rod-shaped bacterium that rarely causes disease in healthy adults but has been implicated in outbreaks of disease in premature infants (neonates). Research suggests that neonates or those infants who have other medical conditions are more susceptible to this infection. Most reported cases of infection are severe, including sepsis (bacteria in the blood), meningitis, or necrotising enterocolitis (severe intestinal infection). Neurological damage may be permanent, and the death rate is reported to be as high as 40-80%[2].

Enterobacter sakazakii is an opportunistic pathogen which has been isolated at low levels from powdered infant formulas. The organism's high tolerance to desiccation provides a competitive advantage for *Enterobacter sakazakii* in dry environments, as found in milk powder factories, and thereby increases the risk of post-pasteurisation contamination of the finished product[3].

The current FDA method[3] for the detection of *Enterobacter sakazakii* is based on yellow pigment production and originated from pioneering work of Muytjens *et al*[4]. Samples are incubated in water overnight then enriched in EE Broth (CM0317), followed by plating on VBRGA (CM0485) to isolate Enterobacteriaceae. Five colonies are selected and streaked on Tryptone Soya Agar (CM0131), incubated for up to 3 days and observed for yellow colonies, typical of *Enterobacter sakazakii*. However, this method does not select for *Enterobacter sakazakii* and the combined use of EE Broth and VRBGA could allow other Enterobacteria to outgrow *Enterobacter sakazakii* and give false negative results. It is not possible to select for *Enterobacter sakazakii* colonies from VRBGA plates on the basis of colony morphology as they will appear the same as other Enterobacteria.

Technique

Please note that the following is only a suggested method of use. Refer to current guidelines and recommendations.

Oxoid Chromogenic Enterobacter sakazakii Agar has been shown to increase the recovery of *Enterobacter sakazakii* from powdered infant formula and other food samples when it is used to replace the two plating media in the FDA method 2002[1].

1. Using an inoculating loop remove 10 µl from the incubated EE Broth and streak or spread onto the surface of a Chromogenic Enterobacter sakazakii Agar plate.

2. Incubate the plate at 35-37°C for 24 hours and observe for blue-green colonies.
3. Confirm presumptive blue-green colonies as *Enterobacter sakazakii* biochemically. This may be carried out using systems such as Microbact™ 24E (MB1131A or MB1074A).

Appearance:
Chromogenic Enterobacter sakazakii Agar Dehydrated powder: a straw coloured, free-flowing powder.

Prepared medium: a light yellow coloured gel.

Storage conditions and Shelf life
The dehydrated medium should be stored at 10-30°C and used before the expiry date on the label.

Prepared medium may be stored for up to 2 weeks at 2-8°C.

Quality control

Positive controls:	***Expected results***
Enterobacter sakazakii ATCC® 29004	Good growth: blue-green colonies
Escherichia coli ATCC® 25922*	Growth: straw colonies
Negative control:	
Staphylococcus aureus ATCC® 25923*	Inhibited

*This organism is available as a Culti-Loop®

Reference

1. Iversen C., Druggan P. D. and Forsythe S. *J. Int. J. Food Microbiology* 2003. In Press.
2. Nazarowec-White M. and Farber J. M. *Int. J. of Food Microbiol.* 1997; 34. 103-113.
3. Breeuwer P., Lardeau A., Peterz M. and Joosten H. M. *J. of App. Microbiol.* 2003; 5. 967.
4. Muytjens H. L., van der Ros-van de Repe, J. and van Druten H. A. M. *J. Clin. Microbiol.* 1984; 20. 684-686.

CHROMOGENIC LISTERIA AGAR

Code: CM1080

A medium for isolation, enumeration and presumptive identification of Listeria species and Listeria monocytogenes from food samples.

Formula	***gm/litre***
Peptone	18.5
Yeast extract	4.0
Sodium chloride	9.5
Sodium pyruvate	2.0
Lithium chloride	15.0
Maltose	4.0
X-glucoside chromogenic mix	0.2
Agar	14.0
Final pH 7.2 ± 0.2	

CHROMOGENIC LISTERIA SELECTIVE SUPPLEMENT

Code: SR0227

Vial contents (each vial is sufficient for 500 ml of medium)	***per vial***	***per litre***
Nalidixic acid	13.0 mg	26.0 mg
Polymyxin B	5.0 mg	10.0 mg
Ceftazidime	3.0 mg	6.0 mg
Amphotericin	5.0 mg	10.0 mg

CHROMOGENIC LISTERIA DIFFERENTIAL SUPPLEMENT

Code: SR0228

Vial contents (each vial is sufficient for 500 ml of medium)	***per vial***	***per litre***
Lecithin solution	20.0 ml	40.0 ml

Directions
Suspend 33.6 grams of Chromogenic Listeria Agar (CM1080) in 480 ml of distilled water. Mix well and sterilize by autoclaving at 121°C for 15 minutes. Cool the medium to 46°C and add one vial of Chromogenic Listeria Selective Supplement, reconstituted as directed and one vial of Chromogenic Listeria Differential Supplement. Mix well and pour into sterile Petri dishes.

Description
OCLA is a modification of the formulation described by Ottaviani and Agosti[2]. As in the original formulation, the medium is designed to identify *Listeria* spp. based on their utilisation of a chromogenic substrate. However, in this modification the pathogenic *Listeria* spp. are then further differentiated by the detection of lecithinase (phosphotidlycholine phospholipase C - PCPLC) activity, rather than phosphotidlyinositol phospholipase C (PIPLC) activity. Both enzymes, PCPLC and PIPLC, are required for virulence, although detection of one is sufficient for identification of pathogenicity. *Listeria monocytogenes* is the most common pathogenic Listeria spp. and has been shown to be pathogenic to both man and animals. Some *L. ivanovii* strains also possess lecithinase activity and although *Listeria ivanovii* are primarily pathogenic to animals, there are strains which have been shown to cause infection in humans[3]. Studies have shown this medium to be superior to PALCAM or Oxford medium for the isolation of *Listeria monocytogenes*[4].

OCLA uses the chromogen X-glucoside for presumptive identification of *Listeria* spp. This chromogen is cleaved by β-glucosidase which is common to all *Listeria* species. Other organisms that possess this enzyme, such as enterococci, are inhibited by the selective agents within the medium: lithium chloride, polymyxin B and nalidixic acid whilst amphotericin inhibits the growth of any yeasts and moulds present in the sample.

Listeria monocytogenes and pathogenic *Listeria ivanovii* are then further differentiated by their ability to produce the phospholipase enzyme lecithinase. This enzyme hydrolyses the lecithin in the medium, producing an opaque white halo around the colony.

Technique
OCLA can be used following a variety of enrichment procedures i.e. ISO, NMKL, BAM etc. The following is a suggested protocol using Oxoid Novel Enrichment Broth-Listeria (ONE Broth). This method has been validated by AFNOR and been shown to give equivalent results to ISO 11290-1:1997[1,5].

1. Add 25 g of food sample to 225 ml of ONE Broth (CM1066 & SR0234) and stomach for a minimum of 30 seconds to mix the sample.
2. Incubate the broth without agitation at 30ºC for 24 ± 2 hours.
3. Gently agitate the bag then, using a microbiological loop, remove 10 µl and inoculate onto an OCLA plate and incubate at 37ºC for 24 ± 2 hours. Examine the plate for blue colonies with and without opaque white halos.
4. When testing meat samples, incubate negative plates for a further 24 ± 2 hours and examine again.
5. Confirm presumptive colonies on the agar plate as Listeria monocytogenes or Listeria species by

appropriate methods e.g. Gram-stain, catalase, Oxoid O.B.I.S. mono ID0600M, Oxoid Listeria Latex Test Kit DR1126A, Microbact Listeria 12L MB1128A.

Appearance:
Dehydrated medium: straw coloured, free-flowing powder.
Prepared medium: translucent white gel.

Storage conditions and Shelf life
The dehydrated medium should be stored at 10-30°C and used before the expiry date on the label.
Prepared medium may be stored for up to 2 weeks at 2-8°C.

Quality control

Positive controls:	***Expected results***
Listeria monocytogenes ATCC® 7644*	Good growth: blue/green plus halo
Listeria innocua ATCC® 33090*	Good growth: blue/green no halo
Negative contol:	
Enterococcus feacalis ATCC® 29212*	Inhibited

*This organism is available as a Culti-Loop®

References

1. Oxoid Folio No. 1059.
2. Ottaviani F., Ottaviani M. and Agosti M. (1997) Quimper Froid Symposium Proceedings, P6 A.D.R.I.A. Quimper (F) 16-18 June
3. Cummins A. J., Fielding A. K. and McLauchlin J. (1994) Listeria ivanovii infection in a patient with AIDS. *Journal of Infection* 28, p89-91
4. Data on file at Oxoid.
5. ISO 11290-1:1997 Horizontal method for the detection and enumeration of *Listeria monocytogenes* Part 1: Detection Method.

CHROMOGENIC SALMONELLA AGAR BASE

Code: CM1007

Salmonella Chromogenic Medium is a selective and differential agar base for the identification of Salmonella species from other organisms in the family Enterobacteriaceae.

Formula	***gm/litre***
Special Peptone	10.0
Chromogenic mix	28.0
Agar	12.0
pH 7.2 ± 0.2	

SALMONELLA SELECTIVE SUPPLEMENT

Code: SR0194

Vial contents (each vial is sufficient for 500 ml of medium)	***per vial***	***per litre***
Cefsulodin	6.0 mg	12.0 mg
Novobiocin	2.5 mg	5.0 mg

Directions
Suspend 25 g in 500 ml of distilled water and add the contents of one vial of Salmonella Selective Supplement reconstituted as directed. Mix well and bring to the boil with frequent agitation. **DO NOT AUTOCLAVE. DO NOT HOLD AT BOILING TEMPERATURE**. Cool to 50°C, mix well and pour into sterile Petri dishes.

Description

Salmonella Chromogenic Medium is designed to identify *Salmonella* species based on their utilisation of one chromogenic substrate. Their inability to utilise another chromogenic substrate, that most other members of the family Enterobacteriaceae can utilise, enables rapid and reliable identification of *Salmonella* species.

There are in excess of 2000 known species of *Salmonella*, some of which differ from the typical rod-shaped, Gram-negative motile bacterium. In the U.S. alone there are between 800,000 and 4 million reported cases of salmonellosis per year resulting in 500 deaths. Infections due to *Salmonella* are of particular concern in the very young, the elderly and in the severely immunosuppressed where salmonellosis is recognised as an AIDS-defining condition. With incidence showing a continued rise, infections due to *Salmonella* remain a principal health issue[1]. Because of the widespread occurrence there is a need for the rapid detection and identification of *Salmonella* in food and water to aid in the prevention and control of outbreaks[2].

Traditionally, media used to differentiate *Salmonella* species from other members of the family Enterobacteriaceae depend upon the ability of *Salmonella* species to produce hydrogen sulphide coupled with their inability to ferment lactose[2,3]. These are, however, essentially inadequate methods, with a significant number of the 2000 plus species not exhibiting these characteristics. In recent times chromogenic media have been developed for the rapid and more reliable identification of *Salmonella*.

Salmonella Chromogenic Agar Base CM1007 combines two different chromogens for the detection of *Salmonella* spp., 5-Bromo-6-Chloro-3-Indolyl caprylate (Magenta-caprylate) and 5-Bromo-4-Chloro-3-Indolyl β-D-galactopyranoside (X-gal). X-gal is a substrate for the enzyme β-D-galactosidase. Hydrolysis of the chromogen, Mag-caprylate, by lactose negative *Salmonella* species results in magenta colonies.

The medium contains bile salts to inhibit the growth of Gram-positive organisms and the addition of the Salmonella Selective Supplement SR0194 is recommended to increase the selectivity of the medium. This uses novobiocin to inhibit *Proteus* growth and cefsulodin to inhibit growth of Pseudomonads.

Technique

Inoculate the plates with a food or clinical sample to produce single colonies. A Salmonella enrichment broth may be used prior to streaking out, e.g. Rappaport-Vassiliadis Enrichment Broth CM0669, Selenite Broth CM0395 & LP0121 or Tetrathionate Broth Base CM0029. Incubate for 18-24 hours at 37°C. Examine the plates for coloured colonies.

On Salmonella Chromogenic Medium CM1007 & SR0194, typical colonies will be coloured as follows:

Species Colour	***Colony Diameter***	***Colony Morphology***	***Colony***
Salmonella spp.	Magenta	1.0 mm	Raised, smooth
Salmonella typhi	Magenta	1.0 mm	Raised, smooth
Salmonella paratyphi	Magenta	1.0 mm	Raised, smooth
Salmonella arizonae	Magenta/blue*	1.5 mm	Raised, smooth
Salmonella gallinarum	Magenta	0.75 mm	Raised, smooth
Salmonella indiana	Blue*	1.0 mm	Raised, smooth
Escherichia coli	Blue	1.0 mm	Raised, smooth
Enterobacter spp.	Blue	1.5 mm	Raised, smooth
Klebsiella spp.	Blue	3.0 mm	Raised, mucoid
Citrobacter spp.	Blue	1.5 mm	Raised, mucoid
Proteus spp.	No growth/straw	0.25 mm	-
Pseudomonas spp.	No growth	-	-
Shigella sonnei	Blue	4.0 mm	Undulate
Shigella dysenteriae	Magenta	1.0 mm	Raised

*Colour of colonies is a presumptive identification as it is dependent on enzyme activity. Some strains of *Salmonella arizonae* and *Salmonella indiana* can appear as blue colonies. In addition, some *Shigella* spp. can appear as magenta colonies. Further confirmatory tests are required.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label. Salmonella Selective Supplement should be stored at 2-8°C.

Store the prepared medium for up to 6 weeks at 2-8°C.

Appearence
Dehydrated medium: Straw coloured, free flowing powder.

Prepared medium: White coloured gel.

Quality control

Positive controls:	***Expected result***
Salmonella enteritidis ATCC® 13076*	Good growth, purple colonies
Salmonella poona NCTC 4840*	Good growth, purple colonies
Negative controls:	
Escherichia coli ATCC® 25922*	Growth, blue colonies
Pseudomonas aeruginosa ATCC® 27853*	Inhibited

*This organism is available as a Culti-Loop®

References
1. Gaillot O. *et al*. (1999) *J. Clin. Microbiol.* 37: 762-765.
2. Rambach A. (1990) *Appl. Environ. Microbiol.* 56: 301-303.
3. Gruenewald R. (1991) *J. Clin. Microbiol.* 29: 2354-2356.

CHROMOGENIC TBX – see TBX MEDIUM

CHROMOGENIC URINARY TRACT INFECTION (UTI) MEDIUM

Code: CM0949

A chromogenic medium for the presumptive identification and differentiation of the main micro-organisms that cause urinary tract infections (UTIs).

Formula	***gm/litre***
Peptone	15.0
Chromogenic mix	26.3
Agar	15.0
Final pH 6.8 ± 0.2	

Directions
Suspend 56.3 g of Chromogenic UTI Medium in 1 litre of distilled water, mix well and sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C and mix well before pouring plates.

Description
Chromogenic UTI Medium contains two specific chromogenic substrates which are cleaved by enzymes produced by *Enterococcus* spp., *Escherichia coli* and coliforms. In addition, it contains phenylalanine and tryptophan which provide an indication of tryptophan deaminase activity. It is based on electrolyte deficient CLED Medium which provides a valuable non-inhibitory diagnostic agar for plate culture of other urinary organisms, whilst preventing the swarming of *Proteus* spp.

One chromogen, X-Gluc, is targeted towards β-glucosidase, and allows the specific detection of enterococci through the formation of blue colonies.

The other chromogen, Red-Gal, is cleaved by the enzyme β-galactosidase which is produced by *Escherichia coli*, resulting in pink colonies. Any uncertainty in identification may be resolved by removing suspect *Escherichia coli* colonies from the plate and performing an indole test using DMACA reagent.

Cleavage of both chromogens occurs in the presence of coliforms, resulting in purple colonies.

The medium also contains tryptophan which acts as an indicator of tryptophan deaminase activity, resulting in colonies of *Proteus*, *Morganella* and *Providencia* spp. appearing brown.

Table of typical colour reactions

Organism	β-*galactosidase*	β-*galactosidase*	*Tryptophan deaminase TDA*	*Colony colour*
Enterococci		+		Blue
Escherichia coli	+			Pink
Coliforms	+	+		
Proteus, Morganella, Providencia			+	Brown
Pseudomonas				Fluoresce
Staphylococcus				Normal pigmentation

It should be noted that organisms with atypical enzyme patterns may give anomalous reactions. For example, in a trial[1], over 45% of *Enterobacter cloacae* were shown to lack β-glucosidase, resulting in pink colonies which were indistinguishable from *Escherichia coli*. In such cases, an indole test can be performed using DMACA indole (do not use Kovac's as the colour of the *Escherichia coli* colonies may interfere with the red colour of a positive indole test). The reagent should not be applied directly to the plate, but the test should be performed on filter paper. This test will distinguish between *Escherichia coli* and *Enterobacter*, and also between *Proteus mirabilis* and other species.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared plates of medium at 2-8°C.

Appearance

Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Pale buff coloured gel.

Quality control

Positive controls:	***Expected results***
Escherichia coli ATCC® 25922*	Good growth; pink coloured colonies
Enterobacter aerogenes ATCC® 13048*	Good growth; purple coloured colonies
Enterococcus faecalis ATCC® 29212*	Good growth; blue-green coloured colonies
Proteus vulgaris ATCC® 13315*	Good growth; straw coloured colonies; brown halo
Staphylococcus aureus ATCC® 25923*	Good growth; white colonies
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

Precautions

Wear dust mask when handling the dehydrated product. Avoid contact with eyes.

References

1. Data on file.

CHROMOGENIC UTI MEDIUM (CLEAR)

Code: CM1050

A chromogenic medium for the presumptive identification and differentiation of all the main micro-organisms that cause urinary tract infections (UTIs).

Formula	***gm/litre***
Peptone	15.0
Chromogenic mix	13.0
Agar	15.0
Final pH 7.0 ± 0.2	

Directions
Suspend 43 g of Chromogenic UTI Medium in 1 litre of distilled water. Mix well and sterilise by autoclaving at 121°C for 15 minutes. Cool the medium to 50°C and pour into sterile Petri dishes.

Description
Chromogenic UTI Medium (Clear) is a differential agar which provides presumptive identification of the main pathogens which cause infection of the urinary tract. This medium uses the same chromogenic substrates as the existing opaque Chromogenc UTI Medium (CM0949) but has a clear background to make multiple sample testing easier.

Chromogenic UTI Medium (Clear) contains two specific chromogenic substrates which are cleaved by enzymes produced by *Enterococcus* spp., *Escherichia coli* and coliforms. In addition, it contains tryptophan which indicates tryptophan deaminase activity (TDA), indicating the presence of *Proteus* spp. It is based on Cystine Lactose Electrolyte Deficient (CLED) Medium which provides a valuable non-inhibitory diagnostic agar for plate culture of other urinary organisms, whilst preventing the swarming of *Proteus* spp.

The chromogen, X-glucoside, is targeted towards β-glucosidase enzyme activity, and allows the specific detection of enterococci through the formation of blue colonies.

The other chromogen, Red-Galactoside, is cleaved by the enzyme β-galactosidase which is produced by *Escherichia coli*, resulting in pink colonies. Any uncertainty in identification may be resolved by removing suspect colonies from the plate and performing an indole test using Microbact Reagent spot indole DMACA (Oxoid order code MB1448).

Cleavage of both the chromogens by members of the coliform group, results in purple colonies.

The medium also contains tryptophan which acts as an indicator of tryptophan deaminase activity (TDA), resulting in haloes around the colonies of *Proteus*, *Morganella* and *Providencia* spp.

Organism	***β-galactosidase***	***β-glucosidase***	***TDA***	***Colony colour***
Enterococci	-	+	-	Blue
E. coli	+	-	-	Pink
Coliforms	+	+	-	Purple
Proteus/ Morganella & *Providencia* spp.	-	-	+	Brown
Pseudomonads	-	-	-	Fluoresce
Staphylococci	-	-	-	Normal pigmentation

It should be noted that organisms with atyptical enzyme patterns may give anomalous reactions. For example, in a trial, over 45% of *Enterobacter cloacae* were shown to lack β-glucosidase, resulting in pink colonies which were indistinguishable from *Escherichia coli*[1]. In such cases an indole test can be performed using DMACA indole (do not use Kovac's as the colour of the *Escherichia coli* colonies may interfere with the red colour of a positive indole test). The test should be performed on filter paper, not directly on the plate. This test will distinguish between *Escherichia coli* and *Enterobacter* spp., and also between *Proteus* mirabilis and other species.

Appearance
Dehydrated Chromogenic UTI Medium (Clear) is a free-flowing straw coloured powder.

The prepared medium is a straw coloured, transparent agar.

Precautions
Chromogenic UTI Medium (Clear) should only be used for *in vitro* diagnostic purposes. Do not use beyond the stated expiry date, or if the product shows any sign of deterioration.

Storage conditions and Shelf life
The dehydrated medium should be stored at 10-30°C and used before the expiry date on the label. Prepared medium may be stored, out of direct light, for up to 2 weeks at 2-8°C.

Quality control

Positive controls:	***Expected results***
Escherichia coli ATCC® 25922*	Good growth; pink coloured colonies
Enterobacter aerogenes ATCC® 13048*	Good growth; purple coloured colonies
Enterococcus faecalis ATCC® 29212*	Good growth; blue-green coloured colonies
Proteus vulgaris ATCC® 13315*	Good growth; straw coloured colonies; brown halo
Staphylococcus aureus ATCC® 25923*	Good growth; white colonies
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

Reference
Data on file at Oxoid.

CIN AGAR – see YERSINIA SELECTIVE AGAR

CLARK & LUBBS MEDIUM – see MRVP MEDIUM

CLAUSEN MEDIUM
DITHIONITE-THIOGLYCOLLATE (HS-T) BROTH

Code: CM0353

The Nordic Pharmacopoeia Board have recommended this medium, containing neutralising compounds and supplementary minerals, for sterility testing.

Formula	***gm/litre***
Tryptone	15.0
Yeast extract	6.0
Soya peptone	3.0
Glucose	6.0
Sodium chloride	2.5
Dipotassium hydrogen phosphate	2.0
Sodium citrate	1.0
L-cystine	0.5
L-asparagine	1.25
Sodium dithionite	0.4
Sodium thioglycollate	0.5
Lecithin	0.3
Magnesium sulphate .7H_2O	0.4
Calcium chloride .2H_2O	0.004
Cobalt sulphate .7H_2O	0.001
Cupric sulphate .5H_2O	0.001
Ferrous sulphate .7H_2O	0.001
Zinc sulphate .7H_2O	0.001
Manganese chloride .4H_2O	0.002
Resazurin	0.001
Agar	0.75
pH 7.1 ± 0.2	

Directions
Suspend 40 g in a solution composed of Tween 80 (polyethylene sorbitan mono-oleate) 3 g: glycerol 5 g and distilled water 1 litre. Bring to the boil to dissolve completely.

Distribute into tubes or bottles and sterilise by autoclaving at 121°C for 15 minutes.

THE MEDIUM MUST NOT BE RE-STERILISED.

Description
Dithionite-thioglycollate (HS-T) Broth was developed by Clausen in Oslo University and has been recommended for sterility testing by the Nordic Pharmacopoeia Board. The problems of sterility testing by selecting random samples is recognised by the Board and they refer to the process as the Microbial-Contamination Test. The standard microbial-contamination test is designed solely to establish that the number of non-sterile units, if any, in a batch is below a certain level.

The following description of the Standard Microbial-Contamination Test has been abridged from the detailed description published as an addendum in the Nordiska Farmakopenämnden.

The tests must be performed with all precautions taken to prevent laboratory contamination occurring more than once in every 100 tests. The use of laminar air-flow cabinets is recommended. Tests are to be made by qualified and experienced staff and the efficiency of the methods used must be checked at regular intervals.

A random sample of sufficient quantity to be representative of the whole bulk, should be examined.

Two methods of detecting non-sterile units may be used in the microbial-contamination test.

Membrane Filter Method
The test substance is dissolved or suspended in 200 ml of 0.1% w/v sterile (pH 7.0-7.2) Peptone water CM0009 and immediately filtered through one or more membrane filters (average pore diameter 0.45 m or less).

Each filter is then washed three times, by passing 100 ml volumes of peptone solution through the membrane.

After filtration the membranes are transferred to tubes of media, containing at least 15 ml of Clausen Medium and tubes of Tryptone Soya Broth (soybean-casein digest medium) CM0129. If only one filter is used, this is divided into two and the two halves placed in separate tubes.

Tubes of Clausen Medium are incubated for at least 14 days at 30-32°C.

Tubes of Tryptone Soya Broth (soybean-casein digest medium) are incubated for at least 14 days at 20-25°C.

Dilution Method
From each sample 1.0 ml of material or suspension is transferred to each of at least 10 tubes containing a minimum quantity of 15 ml of Clausen Medium.

One half of the number of tubes is incubated at 30-32°C for at least 14 days and the other half at 20-25°C for the same time.

If the medium becomes turbid on incubation, sub-cultures must be taken as soon as possible. Sub-cultures must also be taken after normal incubation and observed for a further period of 14 days.

Assessment of the Results
The standard microbial contamination test is passed if growth is not observed in any of the tubes. If growth is observed, the test may be repeated with twice the number of samples. The test is then passed if no growth is observed in any of these tubes. Growth is diagnosed by the appearance of turbidity in fluid or semi-fluid media, by the formation of colonies on solid media, or by microscopy of culture samples.

Controls
Both methods of testing must be controlled for microbial inhibitors by adding a small inoculum of organisms (approximately 10 colony-forming bacteria) either to the tubes prepared in Method II or to peptone diluent, prior to filtration, in Method I.

If no growth occurs in the tubes containing the test organisms then the test must be repeated with the growth-inhibitory effect inactivated.

The test organisms recommended are:

Staphylococcus epidermidis
Clostridium sporogenes
Rhodotorula rubra

They are maintained on agar slants or deep agar stabs and the test inoculum is prepared from 24 hour cultures grown in Clausen Medium at 30-32°C. The *Rhodotorula rubra* inoculum is prepared from a 48 hour culture grown in the same broth at 20-25°C.

Dithionite-Thioglycollate Broth was formulated by Clausen as a highly nutritious medium containing reducing agents and essential metals for the recovery of anaerobic spore-bearing organisms. It also contains lecithin and Tween 80 to overcome the effects of cationic agents which may show powerful bacteriostatic effects *in vitro*.

The broth should be prepared as directed and transferred to tubes or bottles in sufficient volumes (at least 15 ml) for the standard microbial-contamination test and rapidly cooled to 20°C after sterilisation.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Prepared medium: The medium can be stored in a cool place (above 4°C) away from light, for a maximum period of one month.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw/yellow coloured gel.

Quality control

Positive control:	***Expected results***
Staphylococcus epidermidis ATCC® 14990*	Turbid growth
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

Precautions
The medium is yellowish in colour and almost clear. It turns pale-pink under aerobic conditions. The upper third only of the medium should be pink by the time it is to be used.

References

1. Clausen O. G., Aasgaard N. B. and Solberg O. (1973) *Ann. Microbiol.* (Inst. Pasteur) 124 B. 205.
2. Christensen E. A., Kristensen H. and Jensen K. M. (1969) *Arch. Pharm. Chem.* 76. 625.
3. Clausen O. G. (1973) 'A study of the growth-promoting properties of fluid and solid microbial-contamination test media on small numbers of micro-organisms.' *Pharmaceutica Acta Helvetiae* 48. 541-548.
4. Clausen O. G. (1973) 'An examination of the Bactericidal and Fungicidal Effects of Cetylpyridinium Chloride, separately and in combinations embodying EDTA and Benzyl Alcohol'. *Die. Pharm. Ind.* 35. Nr. 12. 869-874.
5. Mohamed A. and Abdou F. (1974) 'Comparative Study of Seven Media for Sterility Testing'. *Jnl of Pharma*. *Sci.* Vol. 63. No.1 Jan.
6. Mohamed A. and Abdou F. (1974) 'Sterilitatstest III Vergleichsuntersuchungen von 3 Medien zum Nachweis von Bakterien'. *Pharm. Ind.* 36. Nr. 5. 337-334.

CLED MEDIUM

Code: CM0301

Recommended for diagnostic urinary bacteriology. The medium supports the growth of all urinary potential pathogens giving good colonial differentiation and clear diagnostic characteristics.

Formula	***gm/litre***
Peptone	4.0
'Lab-Lemco' powder	3.0
Tryptone	4.0
Lactose	10.0
L-cystine	0.128
Bromothymol blue	0.02
Agar	15.0

Directions
Suspend 36.2 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Mix well before pouring.

Description
A dehydrated Cystine-Lactose-Electrolyte Deficient (CLED) medium made to the formula described by Mackey and Sandys[1] as a modification for urinary bacteriology of the Electrolyte Deficient Medium developed by Sandys[2].

This medium is recommended for urinary bacteriology, supporting the growth of all urinary pathogens and giving good colonial differentiations and clear diagnostic characteristics. The presence of important contaminants such as diphtheroids, lactobacilli and micrococci is also clearly elicited, giving an indication of the degree of contamination.

In the laboratory CLED Medium provides a valuable non-inhibitory diagnostic agar for plate culture of urinary organisms. It is electrolyte deficient to prevent the swarming of *Proteus* species.

The medium has been used successfully in the Dip-inoculum Transport Medium technique (Mackey and Sandys[1,3]).

A variant of this technique has been described by Guttman and Naylor[4] who employed media-coated slides.

These techniques overcome false bacteriological results associated with delay in the transport of the specimens of urine to the laboratory and permit a clinically accurate routine differential viable count. They are, therefore, suitable for both general practitioner and hospital work including the screening of ante-natal specimens for symptomless bacteriuria.

For full details, the original papers should be consulted.

Growth Characteristics on CLED Medium (18 hours incubation)

Escherichia coli	yellow, opaque colonies with a slightly deeper coloured centre about 1.25 mm diam. Non-lactose – fermenting strains – blue colonies
***Klebsiella* species**	extremely mucoid colonies varying in colour from yellow to whitish-blue
***Proteus* species**	translucent blue colonies usually smaller than *E. coli*.
***Salmonella* species**	flat blue colonies
Pseudomonas aeruginosa	green colonies with typical matt surface and rough periphery
Enterococcus faecalis	yellow colonies about 0.5 mm diameter
Stapylococcus aureus	deep yellow colonies about 0.75 mm diameter, uniform in colour
Coagulase negative staphylococci	pale yellow or white, more opaque than *E. faecalis*, often with paler periphery
Corynebacteria	very small grey colonies
Lactobacilli	similar to corynebacteria but with a rougher surface

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance
Dehydrated medium: Pale green coloured, free-flowing powder.

Prepared medium: Blue/green coloured gel.

Quality control

Positive controls:	***Expected results***
Proteus mirabilis ATCC® 10975	Good growth; blue colonies; no swarming
Staphylococcus aureus ATCC® 25923*	Good growth; yellow colonies
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

References
1. Mackey J. P. and Sand G. H. (1966) *B.M.J.* 1. 1173.
2. Sandys G. H. (1960) *J. Med. Lab. Techn.* 17. 224.
3. Mackey J. P. and Sandys G. H. (1965) *B.M.J.* 2. 1286-1288.
4. Guttman D. and Naylor G. R. E. (1967) *B.M.J.* 2. 343-345.

CLED MEDIUM (WITH ANDRADE INDICATOR)

Code: CM0423

A modification of the CLED Medium of Mackey and Sandys[1] containing Andrade Indicator to enhance the differentiation of colony characteristics.

Formula	***gm/litre***
Peptone	4.0
'Lab-Lemco' powder	3.0
Tryptone	4.0
Lactose	10.0
L-cystine	0.128
Bromothymol blue	0.02
Andrade Indicator	0.1
Agar	15.0
pH 7.5 ± 0.2	

Directions
Suspend 36.2 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Mix well before pouring.

Description
The formula for this medium is similar to that for CLED Medium, CM0301, but with the addition of acid fuchsin which enhances the colonial appearance and aids in identification of the organisms. The colour of the medium differs from that of the standard medium at various pH levels.

Bevis[2] listed the pH and colour as follows:

pH	***Colour of Medium***
7.4	Deep blue
7.0	Blue-grey
6.8	Pale slate grey
6.6	Pinkish grey
6.4	Bright red with slight smokey tint
6.0	Bright red

The medium should not be incubated for longer than 24 hours since, if lactose-fermenters predominate, the whole of the medium may turn pink, masking the presence of non-lactose-fermenters.

Colonial Characteristics
Escherichia coli – Bright pink semi-translucent colonies with a surrounding pink halo in the medium.
Proteus mirabilis – Blue-green translucent colonies.
Klebsiella aerogenes – Grey-green mucoid colonies.
Aeromonas anitratus – Small, grey-green, translucent colonies.
Staphylococcus aureus – Smooth, entire, opaque; bright golden yellow colonies. Lactose-fermenting.
Staphylococcus albus – Smooth, entire, opaque porcelain white or very pale pink colonies.
Enterococcus faecalis – Similar to *Staphylococcus aureus* but smaller and a much deeper orange yellow colour.
Streptococcus pyogenes – Small opaque grey-green colonies.

Furniss *et al*.[3] describe the use of CLED Medium with Andrade's Indicator for rapidly distinguishing vibrios into halophilic and non-halophilic groups. Non-halophilic vibrios grow; halophilic vibrios do not.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared plates of medium at 2-8°C.

Appearance
Dehydrated medium: Pale green coloured, free-flowing powder.

Prepared medium: Blue-green coloured gel.

Quality control

Positive controls:	***Expected results***
Proteus vulgaris ATCC® 8427*	Good growth; blue-green translucent colonies
Staphylococcus aureus ATCC® 25923*	Good growth; yellow-orange colonies with pink halo
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

Precautions
Note the incubation time limit of 24 hours if lactose-fermenting colonies present.
Shigella species may not grow on electrolyte-deficient medium.

References
1. Mackey J. P. and Sandys G. H. (1966) *B.M.J.* 1. 1173.
2. Bevis T. D. (1968) *J. Med. Lab. Technol.* 25. 38-41.
3. Furniss A. L., Lee J. V. and Donovan T. J. (1978) *P.H.L.S. Monograph series*, London, H.M.S.O., 11.

CLOSTRIDIUM DIFFICILE AGAR BASE

Code: CM0601

For the isolation of Cl. difficile when used with Culture Media Supplements SR0096 or SR0173.

Formula	***gm/litre***
Proteose peptone	40.0
Disodium hydrogen phosphate	5.0
Potassium dihydrogen phosphate	1.0
Magnesium sulphate	0.1
Sodium chloride	2.0
Fructose	6.0
Agar	15.0
pH 7.4 ± 0.2	

CLOSTRIDIUM DIFFICILE SELECTIVE SUPPLEMENT

Code: SR0096

Vial contents	***per vial***	***per litre***
D-cycloserine	125 mg	250 mg
Cefoxitin	4 mg	8 mg

Directions
Suspend 34.5 g in 500 ml of distilled water and bring gently to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Allow to cool to 50°C and add aseptically the contents of 1 vial of Oxoid Clostridium Difficile Supplement rehydrated with 2 ml sterile distilled water, together with 7% (v/v) Defibrinated Horse Blood SR0050. Sheep Blood SR0051 may be used in place of Horse Blood SR0050 but some strains of the organism will show a slightly reduced growth recovery. Mix well and pour into sterile Petri dishes.

Description
Clostridium difficile was first isolated in 1935 by Hall and O'Toole[1] who proposed the name 'difficile' because it was very difficult to isolate. In 1940 Snyder[2] isolated *Clostridium difficile* from infants aged 10 weeks to 1 year. No further isolations were reported until 1960, when the organism was cultured by McBee[3] from the intestinal contents of a seal, and in 1962 Smith and King[4] reported its presence in human infections.

Toxicogenic isolates of *Clostridium difficile* have been demonstrated to be a major cause of antibiotic-associated ileo-caecitis in laboratory animals[5] and pseudomembranous colitis in man[6,7]. Keighley[8] found *Clostridium difficile* was associated with colitis and diarrhoea without pseudomembranous changes after antibiotic therapy following gastrointestinal operations.

Hafiz and Oakley[9] devised a medium for the selective isolation of *Clostridium difficile* based on the observation that the organism has a high tolerance to cresol, which it produces during its growth, and used Reinforced Clostridial Medium CM0151 plus 0.2% phenol or p-cresol.

George *et al*.[10] in a study of selective media for the routine isolation of *Clostridium difficile* from faecal specimens found this medium was inhibitory compared with growth on blood agar. They recommended the use of a fructose-containing nutrient medium plus egg yolk, with D-cycloserine and cefoxitin as selective agents for the isolation of *Clostridium difficile*.

The combination of Oxoid Clostridium difficile Agar Base plus the Culture Media Supplement SR0096 is based on the formulation proposed by George *et al*.[10]

The selective agents D-cycloserine (500 µg/ml) and cefoxitin (16 µg/ml) inhibit growth of the majority of Enterobacteriaceae, as well as *Strep. faecalis*, staphylococci, Gram-negative non-sporing anaerobic bacilli and *Clostridia* species (except *Clostridium difficile*) which may be found in large numbers in faecal samples.

Levett[11], noting reports[12,13] that some strains of *Clostridium difficile* had low minimum inhibitory concentrations to both cycloserine and cefoxitin, reduced the antibiotic concentrations to 125 µg per ml cycloserine and 4µg per ml cefoxitin and combined this with alcohol shock[14] to compensate for the reduction in selectivity. *Clostridium difficile* was isolated from all of the 33 faecal specimens plated on to CCFA Medium containing cycloserine and cefoxitin at 250 µg per ml and 8 µg per ml respectively, but from only 25/33 specimens plated onto medium containing 500 µg per ml cycloserine and 16 µg per ml cefoxitin. The specimen should be treated with alcohol before inoculation (see technique).

It can be expected that medium containing the lower concentration of antibiotics will yield a greater growth of contaminating organisms if antibiotics are used alone, but Levett reported that there was no difference in the growth of contaminating organisms on plates containing either concentration of antibiotics following alcohol shock treatment of the specimen.

Phillips and Rogers[15] have described a simple modification to the medium in which the ability of *Clostridium difficile* to produce p-cresol from p-hydroxyphenyl acetic acid is used for the rapid presumptive identification by gas chromatographic detection of the p-cresol.

Addition of 7% horse blood to the agar base increases the recovery of *Clostridium difficile* and produces larger colonies compared with Egg Yolk Emulsion used by George *et al*.[10]

Technique

1. Lightly inoculate the medium with the faecal sample spreading part of the original inoculum in order to obtain well separated colonies.
2. Incubate plates at 35°C for 18-24 hours in a conventional anaerobic gas jar. The use of the Oxoid Anaerobic Jar HP0011 with an Anaerobic Gas Generating Kit BR0038 is strongly recommended. Alternatively use AnaeroGen AN0025 or AN0035. AnaeroGen does not require the addition of water or a catalyst.
3. Colonies of *Clostridium difficile* after 48 hours incubation are 4-6 mm diameter irregular, raised opaque, grey-white.

Technique for Alcohol Shock Treatment

1. Mix equal parts of industrial methylated spirit or absolute alcohol and the faecal specimen.
2. Homogenise using a vortex mixer.
3. Leave at room temperature for 1 hour.
4. Inoculate onto Clostridium Difficile Selective Agar and incubate anaerobically.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C no longer than 5-7 days.

Appearance

Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured coloured gel.

Quality control

Positive control:	***Expected results***
Clostridium difficile NCTC 11204	Good growth; grey-white coloured colonies
Negative controls:	
Staphylococcus aureus ATCC® 25923*	No growth
Escherichia coli ATCC® 25922*	No growth

Precautions

Colonies of *Clostridium difficile* from faecal cultures are smaller when egg yolk is used in place of horse blood.

The Oxoid formula does not contain the neutral red indicator proposed by George *et al*.[10] because it is designed for use with horse blood. On this medium the typical colour of the colony of *Clostridium difficile* will not appear, however there will be a fluorescent reaction.

Typical Gram stain morphology of *Clostridium difficile* may not be evident in colonies picked from this medium because of the antibiotics present. Sub-culture to blood agar to obtain characteristic morphology[10].

CLOSTRIDIUM DIFFICILE MOXALACTAM NORFLOXACIN (CDMN) SELECTIVE SUPPLEMENT

Code: SR0173

Vial contents	***per vial***	***per litre***
Cysteine hydrochloride	250.0	500.0
Norfloxacin	6.0	12.0
Moxalactam	16.0	32.0

Directions

Aseptically add 2 ml of sterile distilled water to a vial and mix gently to dissolve the supplement completely. Avoid frothing. Add to 500 ml of Clostridium difficile Agar Base, prepared as directed and cooled to 50°C. Add 7% v/v of Defibrinated Horse Blood SR0050. Mix well and pour into Petri dishes.

Description

Clostridium difficile CDMN medium is an alternative selective medium based on a formula described by Aspinall *et al*.[16] for the isolation of *Clostridium difficile* from faeces. It has been found to be significantly more productive than CCFA medium. Inclusion of cysteine hydrochloride speeds the growth rate of *Clostridium difficile*. CDMN medium was reported to isolate 20% more *Clostridium difficile* strains than CCFA and the use of norfloxacin and moxalactam as selective agents reduces the number of contaminating micro-organisms by 30% when compared to CCFA[16].

Pre-treatment of specimens with alcohol is not necessary with this medium but its use will further enhance selectivity. See *Clostridium difficile* Selective Supplement SR0096 for the technique.

Storage conditions and Shelf life

CDMN supplement SR0173 should be stored at 2-8°C in the dark.

Positive control:	***Expected results***
Clostridium difficile NCTC 11204	Good growth; grey-white coloured colonies
Negative controls:	
Escherichia coli ATCC® 25922*	No growth
Clostridium perfringens ATCC® 13124*	No growth

*This organism is available as a Culti-Loop®

References

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CNA AGAR – see COLUMBIA CNA AGAR

CN AGAR – see PSUDOMONAS AGAR (CN FORMULATION)

COBA SELECTIVE MEDIUM

A selective supplement for the isolation of Streptococcus species.

COLUMBIA BLOOD AGAR BASE

Code: CM0331

Formula	*gm/litre*
Special peptone	23.0
Starch	1.0
Sodium chloride	5.0
Agar	10.0
pH 7.3 ± 0.2	

Directions
Add 39 g to 1 litre of distilled water. Boil to dissolve and sterilise by autoclaving at 121°C for 15 minutes.

STREPTOCOCCUS SELECTIVE SUPPLEMENT (COBA)

Code: SR0126

Vial contents (each vial is sufficient for 500 ml of medium)	*per vial*	*per litre*
Colistin sulphate	5.0 mg	10.0 mg
Oxolinic acid	2.5 mg	5.0 mg

Directions
Reconstitute one vial as directed, aseptically add the contents to 500 ml of sterile Columbia Blood Agar Base containing 5% Defibrinated Horse Blood SR0050 cooled to approximately 50°C. Mix gently and pour into sterile Petri dishes.

Description
Streptococcus Selective Supplement is based on the formulation of Petts (COBA Medium)[1] and is recommended for the selective isolation of streptococci of medical and veterinary importance.

COBA Medium possesses advantages over other media described for selective isolation of streptococci.

Agents previously recommended for inhibition of Gram-positive organisms can be shown to have severe effects on streptococci even at subminimal inhibitory concentrations. The antibiotics gentamicin[2,3,4,5,6], amikacin[7], fucidic acid[8], neomycin[9] and cotrimoxazole[10] have all been shown to have adverse effects as have the long established inhibitors crystal violet and sodium azide. Both colistin and oxolinic acid are thermostable and can, if necessary, be stored without refrigeration.

Streptococci are commonly isolated from the upper respiratory tract. They are also often isolated from burns and other sites where frequently there is an abundance of competing organisms. In order to isolate streptococci, especially when present in small numbers, it is necessary to inhibit the competing flora without any adverse effect by the selective agents upon the *Streptococcus* species. The selective agents colistin sulphate (10 mg/ml) and oxolinic acid (5 mg/ml) have been found to have no inhibitory effect on *Streptococcus* species although amongst Group D organisms *Enterococcus faecalis* colonies are somewhat smaller. The combination of these two selective agents results in total inhibition of Gram-negative organisms and almost all non-streptococcal Gram-positive organisms. A very few staphylococci and coryneform organisms may grow with reduced colony size. The haemolytic reactions on media containing blood are clearly defined, and the colonial size and growth recovery of streptococcal groups A, B, C, D and G and *Streptococcus pneumoniae* are comparable to that on a nonselective medium. The selective agents can also be used with Islam's Medium (GBS Agar CM0755) for the isolation of Group B streptococci without loss of pigmentation occurring.

Technique

1. Prepare the medium from Columbia Blood Agar Base, Streptocccus Selective Supplement and Defibrinated Horse Blood SR0050, according to the directions.
2. Inoculate the plates in the normal way and incubate at 35°C overnight in an atmosphere enriched with 5% carbon dioxide or anaerobically.*
3. Confirm that the colonies are streptococci by microscopy, biochemical or serological tests. The Oxoid Streptococcus Grouping Kit DR0585 or Dryspot DR0400 recommended for this purpose.

*Improved haemolytic reactions are achieved by anaerobic incubation. Gram-positive anaerobic cocci (*Peptostreptococcus* and *Peptococcus* species) would be selectively isolated under these conditions[1].

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance

Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Opaque red coloured gel.

Quality control

Positive controls:	***Expected results***
Streptococcus pyogenes ATCC® 19615	Good growth; gas production
Negative control:	
Staphylococcus aureus ATCC® 25923	Inhibited

*This organism is available as a Culti-Loop®

References

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COLD FILTERABLE TRYPTONE SOYA BROTH (TSB) (SOYBEAN CASEIN DIGEST MEDIUM USP)

Code: CM1065

A gamma-irradiated, cold filterable Tryptone Soya Broth suitable for microbiological Media Fill Trials (MFT) for the pharmaceutical industry.

Formula	***gm/litre***
Pancreatic digest of casein	17.0
Papaic digest of soybean meal	3.0
Sodium chloride	5.0
Di-potassium hydrogen phosphate	2.5
Glucose	2.5
pH 7.3 ± 0.2	

Directions
Cold Filterable TSB should be used at a working dilution of 30 g in 1 litre of distilled water (3% w/v). The medium may be made at this concentration and then sterilised by filtration or by autoclaving at 121°C for 15 minutes.

Description
Tryptone Soya Broth is highly nutritious, general purpose medium which can support the growth of a wide range of bacteria, yeasts and moulds when incubated under the appropriate conditions[1].

The formulation of Cold Filterable TSB conforms to that stated in the *European Pharmacopoeia* 4th Edition 2002[2], the *British Pharmacopoeia* 2003[3], the *US Pharmacopoeia* 27 NF22 2004[4] and the *Japanese Pharmacopoeia* XIV 2001[5]. Each component of this medium has been specially screened and selected to give a highly filterable solution.

Packs of Cold Filterable TSB have been given a sterilising dose of gamma-irradiation (minimum 25 KGy) validated as a lethal dose for all yeasts, moulds and bacteria including bacterial spores and mycoplasmas.

Aqueous liquid products that are required to be sterile but cannot be terminally sterilised due to the heat-sensitive nature of one or more component, may be produced by filtering-sterilising the dissolved solution and maintaining sterility by filling and closing the product under aseptic conditions.

The purpose of MFT is to provide a measure of the likelihood of microbiological contamination occurring in a particular aseptic process. Cold Filterable TSB can be used as a substitute for filter-sterilised drug products and is processed in a manner identical to that in which the product would be processed, i.e. filtering, filling and closing. The medium is then incubated and the number of contaminated units is scored versus those that are un-contaminated. A decision following pre-determined guidelines can be made based on the proportion of contaminated units and the identity of the micro-organisms recovered[6].

For solid presentations where a sterile end product is required, aseptic production processes can be monitored by adding medium to a suitable placebo. The placebo chosen should allow the aseptic process to be simulated exactly and the pre-sterilised TSB is added downstream of the processing[6].

Technique
'Sterile for use' liquid drugs often contain heat-sensitive components which means that terminal sterilisation by autoclaving is not an option. Sterilisation by filtering (for soluble liquids) followed by filling under aseptic conditions is the method for preparation of these types of drug. The purpose of MFT is to provide a measure of the likelihood of microbiological contamination arising in a particular aseptic process.

Typically, the composition of a liquid injectable drug means that that a very large volume can be filtered before blocking of that filter occurs. Due to the biological nature of TSB, filters will block sooner which will mean that the medium will have to be heated or filters changed during a MFT. Oxoid pre-screen and select the raw materials that go into Cold Filterable TSB so that every batch of product will have a high V_{cap} value. V_{cap} is the theoretical maximum volumetric throughput for the filter under test. With this information the maximum filterable volume of TSB may be calculated before starting a MFT.

At Oxoid a filter management system is used with test filters to determine V_{cap} values for each batch of Cold Filterable TSB. The final filterable volume of TSB will depend on the membrane type, pore size and area of the process filter used. Each batch of Oxoid Cold Filterable TSB will have a minimum V_{cap} of 2,800 litres/m^2 for the three filter types tested (0.2 µm pore size).

Typical V_{cap} values for Oxoid Cold filterable TSB:

Filter membrane	***V_{cap} (ml) 47 mm disc (area 14 cm²)***	***V_{cap} (litres/m²)***
Polyvinylidene fluoride (PVDF)	4,909	3,506
Polyethersulfone (PES)	6,700	4,786
Nylon (NR)	4,561	3,258

V_{cap} is the extrapolation to a "flow = zero" point; the time to this point may be very long. Therefore V_{cap} is good for comparative analysis but is not practical for MFT where time for a process is limited. A more useful value is V90 which is calculated as 68% of V_{cap} and is the point at which flow has decayed to 10% of the initial rate. Contact your filter manufacturer for guidance.

N.B. Cold Filterable TSB should not be used to validate the suitability of the chosen filtration system for its ability in providing a sterile drug product. The components of TSB will be quite different to those found in an aqueous drug formulation and validation for this purpose should be carried out on the drug preparation itself.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium in the dark at room temperature.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured solution.

Quality control

Positive controls:	***Expected results***
Staphylococcus aureus ATCC® 6538*	Turbid growth
Pseudomonas aeruginosa ATCC® 9027*	Turbid growth
Bacillus subtilis ATCC® 6633*	Flocculent/surface growth
Aspergillus niger ATCC® 16404*	White mycelia, black spores or no spores
Candida albicans ATCC® 10231*	Flocculent/surface growth
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

References

1. *Oxoid Manual* 8th Edition, 1998, pp. 2-208.
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COLUMBIA BLOOD AGAR BASE

Code: CM0331

A multi-purpose medium suitable for the cultivation of fastidious organisms.

Formula	***gm/litre***
Special peptone	23.0
Starch	1.0
Sodium chloride	5.0
Agar	10.0
pH 7.3 ± 0.2	

Directions
Add 39 g to 1 litre of distilled water. Boil to dissolve the medium completely. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C and add 5% sterile defibrinated blood.

Description
Traditionally, blood agar bases have been either casein hydrolysate or meat infusion media. The advantage of the first lies in the rapid production of large colonies, and of the second in clearly defined zones of haemolysis and good colonial differentiation.

Columbia Agar Base (Ellner *et al*.[1]) combines the virtues of both these types of media to give an improved all-round performance.

This new base has shown versatility and superior performance in several applications.

BRUCELLA
To prepare a selective medium add Brucella Selective Supplement SR0083 to 500 ml of sterile, molten Columbia Blood Agar Base, containing 5-10% v/v inactivated horse serum and 1% w/v dextrose[2,3].

CAMPYLOBACTER AND HELICOBACTER
To prepare a selective medium add:

Campylobacter Selective Supplement (Skirrow) SR0069[4]

or Campylobacter Selective Supplement (Butzler) SR0085[5,6]

or Modified Butzler (ISO) Selective Supplement SR0214

or Campylobacter Selective Supplement (Blaser-Wang) SR0098[7,8,9]

or Helicobacter pylori Supplement SR0147 to 500 ml of sterile, molten Columbia Agar Base containing Campylobacter Growth Supplement SR0084 or SR0232[10,11] and 5-7% v/v horse or sheep blood (SR0048, SR0050 or SR0051).

Egg Yolk Emulsion Agar made using Oxoid Columbia Agar Base and Egg Yolk Emulsion SR0047 has been shown to be a satisfactory isolation medium for *Helicobacter pylori*[12].

GRAM-POSITIVE COCCI
see Staph/Strep Selective Supplement SR0070

see Modified CNA Selective Supplement SR0176

see Streptococcus Selective Supplement SR0126

GARDNERELLA
see Gardnerella vaginalis Selective Supplement SR0119

OTHER APPLICATIONS
Elek Test
Columbia Agar Base with added sterile serum provides an efficient *Corynebacterium diphtheriae* virulence test medium. After following the established technique, lines of toxin-antitoxin precipitation are clearly visible in 48 hours.

Nagler Test
The addition of 5 ml Fildes Extract SR0046 and 10 ml of Egg Yolk Emulsion SR0047 to 100 ml of sterile, molten Columbia Blood Agar Base will provide a diagnostic medium for *Clostridium perfringens*, when used with *Clostridium perfringens* antitoxin (Nagler reaction) and neomycin (100-125 m/ml)[13].

Reversed CAMP Test for *Clostridium perfringens*
The reversed CAMP test[14] is a highly sensitive and specific test for *Clostridium perfringens* which may be used as an alternative to the Nagler test.

Technique
Inoculate the culture suspected to be *Clostridium perfringens* in a straight line across a plate of Columbia sheep blood agar. Streak an overnight (or older) culture of *Streptococcus agalactiae* known to produce the CAMP factor at right angles to the first inoculation taking care that the lines do not touch. Incubate anaerobically at 35-37°C for 18-24 hours.

A positive reverse CAMP test is indicated by the formation of an ‘arrowhead’ of haemolysis between the lines of the *Clostridium perfringens* and *Streptococcus agalactiae* growth.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared plates of medium at 2-8°C.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.
Prepared medium: Straw coloured gel.

Quality control

Positive controls:	***Expected results***
Columbia Agar	
Staphylococcus aureus ATCC® 25923*	Good growth; cream coloured colonies
Streptococcus pyogenes ATCC® 19615*	Good growth; pale straw coloured colonies
Negative control:	
Uninoculated medium	No change
Brucella Medium	
Positive control:	
†*Brucella abortus* ATCC® 4315	Good growth
Negative control:	
Escherichia coli ATCC® 25922*	Inhibited
Campylobacter Media	
Positive control:	
Campylobacter jejuni ATCC® 33291*	Good growth; grey-brown coloured colonies
Negative control:	
Escherichia coli ATCC® 25922*	Inhibited
Helicobacter pylori Medium	
Positive control:	
Helicobacter pylori ATCC® 43526	Good growth; colourless colonies
Negative control:	
Candida albicans ATCC® 10231*	Inhibited or no growth
Staph./Strep. Medium	
Positive controls:	
Staphylococcus aureus ATCC® 25923*	Good growth
Streptococcus pyogenes ATCC® 19615*	Good growth; colourless/white colonies; β-haemolysis
Negative control:	
Escherichia coli ATCC® 25922*	Inhibited
Streptococcus Selective Medium	
Positive control:	
Streptococcus pyogenes ATCC® 19615*	Good growth; colourless/white colonies; β-haemolysis
Negative control:	
Staphylococcus aureus ATCC® 25923*	No growth
Gardnerella Selective Medium	
Positive control:	
Gardnerella vaginalis ATCC® 14018*	Good growth; grey/white colonies
Negative control:	
Proteus mirabilis ATCC® 29906*	Inhibited

*This organism is available as a Culti-Loop®

Precautions
† *Brucella* cultures are highly infective and must be handled under properly protected conditions. Incubate in 5-10% carbon dioxide atmosphere for 24-48 hours.
Campylobacter species are best grown at 42°C (except *Campylobacter fetus* subsp. *fetus*) in a micro-aerophilic atmosphere (Oxoid Campylobacter Gas Generating Kit BR0056 or BR0060 or CampyGen CN0025/CN0035).

Staph./Strep. supplemented plates should be incubated aerobically at 35°C for 18 hours. Incubation in carbon dioxide-enriched air will cause inhibition of staphylococcal growth[15].

Strep. supplemented plates may be incubated aerobically or anaerobically at 35°C for 18 hours.

Prepared plates of both supplemented media should be used within 18 hours of preparation for maximum selectivity. *Gardnerella* supplemented plates should be incubated at 35°C for 48 hours in an atmosphere containing 7% carbon dioxide.

Carry out confirmatory tests on all colonies from horse blood medium and on beta-haemolytic colonies from human or rabbit blood medium.

Incubate plates of Clostridium E-Y Agar anaerobically at 35°C for 18 hours, look for lecithinase activity (pearly layer) and for proteolysis. Lecithinase activity is inhibited in the presence of specific anti-toxin.

References

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COLUMBIA CNA AGAR

A selective medium for staphylococci and streptococci

COLUMBIA BLOOD AGAR BASE

Code: CM0331

Formula	*gm/litre*
Special peptone	23.0
Starch	1.0
Sodium chloride	5.0
Agar	10.0
pH 7.3 ± 0.2	

Directions

Add 39 g to 1 litre of distilled water. Boil to dissolve and sterilise by autoclaving at 121°C for 15 minutes.

STAPH/STREP SELECTIVE SUPPLEMENT

Code: SR0070

Vial contents (each vial is sufficient for 500 ml of medium)	***per vial***	***per litre***
Nalidixic acid	7.5 mg	15.0 mg
Colistin sulphate	5.0 mg	10.0 mg

Directions
Reconstitute one vial as directed aseptically add the contents to 500 ml of Columbia Agar Base cooled to approximately 50°C. Mix well before pouring into sterile Petri dishes.

Description
A selective medium for *Staphylococci* and *Streptococci* of the type described by Ellner[1] and subsequently named Columbia CNA Agar can be made by adding Oxoid Staph/Strep supplement to Columbia Agar Blood Base.Columbia CNA Agar can thus be prepared quickly and conveniently as and when required. Because the antibiotics contained in the supplement are freezedried they always show optimal activity at the time of use.

The supplemented Columbia Agar is inhibitory to *Staphylococcus albus* and *Micrococcus* species as well as Gram-positive and Gram-negative rods. It suppresses growth of *Proteus*, *Klebsiella* and *Pseudomonas* species while permitting unrestricted growth of *Staphylococcus aureus*, haemolytic streptococci and enterococci.

Phenylethyl alcohol medium by comparison frequently permits growth of Proteus and Klebsiella species as well as showing a marked attenuation of the growth of Gram-positive cocci.

Staph/Strep Supplement enables important Gram-positive cocci to be recognised more readily and isolated easily from the mixed bacterial populations contained in many clinical specimens and foods.

Technique
The medium is inoculated in the normal way and incubated aerobically at 35°C for 18 hours.

DO NOT INCUBATE IN CO_2.

Storage conditions and Shelf life
Store the dehydrated medium below 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Opaque red coloured gel.

Quality control

Positive controls:	***Expected results***
Staphylococcus aureus ATCC® 25923	Good growth:
Streptococcus pyogenes ATCC® 19615	Good growth; colourless/white colonies, β-haemolysis
Negative control:	
Escherichia coli ATCC® 25922	Inhibited

*This organism is available as a Culti-Loop®

Precautions
Incubation in a CO_2-enriched atmosphere will cause inhibition of staphylococcal growth[2]. If it is necessary to incubate plates in such an atmosphere then Staph/Strep Supplement should not be used.

All suspected staphylococcal and streptococcal colonies should be further investigated to confirm their identity. The Staphylase Test DR0595 and the Streptococcal Grouping Kit DR0585 are useful for these purposes.

References
1. Ellner P. D., Stoessel C. J., Drakeford E. and Vasi F. (1966) *Tech. Bul. Reg. Med. Technol.* 36. No. 3.
2. Morton C. E. G. and Holt H. A. (1989) *Med. Lab. Sci.* 46. 72-73.

COOKED MEAT MEDIUM

Code: CM0081

An excellent medium for the primary growth and maintenance of aerobic and anaerobic organisms.

Formula	***gm/litre***
Heart muscle	454.0
Peptone	10.0
'Lab-Lemco' powder	10.0
Sodium chloride	5.0
Glucose	2.0
pH 7.2 ± 0.2	

Directions
Suspend 10 g in 100 ml of distilled water (or 1 g amounts in 10 ml volumes of water in tubes). Allow to stand for 15 minutes until the meat particles are thoroughly wetted. Sterilise by autoclaving at 121°C for 15 minutes. Do not cool the bottles rapidly because ebullition will expel the meat particles from the containers.

Description
Cooked Meat Medium prepared from heart tissue is a well established medium for the cultivation of anaerobic and aerobic organisms[1].

It has the ability to initiate bacterial growth from very small inocula and to maintain the viability of cultures over long periods of time. Mixed cultures of bacteria survive in Cooked Meat Medium without displacing the slower growing organisms. The products of growth do not rapidly destroy the inoculated organisms and therefore it is an excellent medium for the storage of aerobic and anaerobic bacteria.

The addition of glucose to the formulation allows rapid, heavy growth of anaerobic bacteria in a short time and leads to a more rapid identification of important anaerobes. The improved growth also enhances GLC identification of anaerobic bacteria.

The improved clarity of the supernatant broth permits earlier detection of growth especially when combined with the increased growth of most organisms. Slower growing isolates will yield detectable growth within 45 hours incubation.

Technique

Anaerobic Culture
It is preferable to use freshly reconstituted and sterile medium which is inoculated as soon as it has cooled to approximately 35°C. Tubes which are not used on the day of preparation should be placed in a boiling water bath or steamer for about 15 minutes to remove dissolved oxygen. They should be allowed to cool without agitation and then inoculated.

Inoculation should be made near the bottom of the tube in the meat particles.

Clostridia may be divided into two main groups by their action on the medium.

(i) Saccharolytic Organisms
There is rapid production of acid and gas but no digestion of the meat. Cultures may have a slightly sour smell, with reddened protein.

(ii) Proteolytic Organisms
Proteolysis causes decomposition of the meat with the formation of foul-smelling sulphur compounds and blackening. However, some saccharolytic strains also produce Hydrogen sulphide which will cause blackening but to a lesser degree.

Aerobic Culture
The tube of medium is incubated with the cap loose and no seal is required. Aerobes grow at the top whilst more anaerobic species grow deeper in the medium.

Incubation

Aerobic organisms
Incubate for up to 7 days at 35°C with loosened caps. Examine daily for turbidity, gas or changes in the meat particles.

Anaerobic organisms
Use freshly reduced medium and incubate for up to 21 days at 35°C. Examine daily for changes in the medium. Make films and subculture at intervals.

Maintenance of stock cultures
Hold at room temperature after the initial incubation at 35°C. Subculture every 4-6 months.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at room temperature, in the dark with tightened caps for up to 6 months.

Appearence
Dehydrated medium: Brown granules.

Prepared medium: Dark straw with brown granules.

Quality Control

Positive control:	***Expected results:***
Clostridium histolyticum ATCC® 19401	Turbid growth; proteolysis
Clostridium perfringens ATCC® 13124*	Turbid growth; saccharolysis, proteolysis
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-loop®

Precautions
The excellent recovery properties of Cooked Meat Medium mean that mixed cultures commonly result from sample inoculation.

Blackening of the medium will not take place if the pH is acid.

Carbohydrate fermentation may inhibit proteolysis.

Reference
1. Robertson M. (1916) *J. Path. Bact.* 20. 327-349.

CORN MEAL AGAR

Code: CM0103

A recommended medium for chlamydospore production by Candida albicans and for the maintenance of fungal stock cultures.

Formula	***gm/litre***
Corn Meal Extract (from 50 g whole maize)	2.0
Agar	15.0
pH 6.0 ± 0.2	

Directions
Suspend 17 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

Description
Corn Meal Agar is a well established mycological medium which is a suitable substrate for chlamydospore production by *Candida albicans* and the maintenance of fungal stock cultures.

When grown on this medium, microscopic examination of *Candida albicans* shows the characteristic chlamydospore production which is an accepted criterion for the identification of this species. Prospero and Reyes[1] investigated the use of corn meal agar, soil extract agar, and purified polysaccharide medium for the morphological identification of *Candida albicans*. Out of 290 yeast colonies isolated on Sabouraud agar, corn meal agar stimulated the production of chlamydospores in 149 colonies (51%), soil extract agar in 103 (36%) and purified polysaccharide medium in 94 (32%).

The addition of 'Tween 80' (e.g. 1%) to Corn Meal Agar greatly enhances the development of chlamydospores on the medium[2,3,4,5,6].

Mackenzie[7] found that all 163 isolates of *Candida albicans* obtained from laboratories in the United Kingdom produced chlamydospores on Oxoid Corn Meal Agar but Dawson[8] using only 27 isolates of *Candida albicans*, found that Oxoid Czapek Dox Agar CM0097 and rice infusion agar were slightly superior for chlamydospore production.

Corn meal agar is a nutritionally impoverished medium and so may be employed for the maintenance of stock cultures of fungi, especially the black-pigmented varieties.

The addition of glucose (0.2 g% w/v) to Corn Meal Agar will enhance the chromogenesis of some species of *Trichophyton* e.g. *Trichophyton rubrum*[9].

Technique

A single Petri dish containing Corn Meal Agar may be used to identify four or five different colonies of *Candida* grown on Sabouraud Dextrose Agar CM0041. Using a straight wire, pick a colony off the surface of the latter medium and make a deep cut in the Corn Meal Agar (i.e. a horizontal furrow). Repeat for each colony. Place a flamed sterile coverslip over the line of inoculum. After incubation for 24 to 48 hours at 22°C, the streaks are examined microscopically, through the cover slip, using a low power objective. Along such streaks, *Candida albicans* produces mycelium-bearing ball-like clusters of budding cells and the characteristic thick-walled round chlamydospores[9].

The addition of 0.001g % w/v Trypan blue to Corn Meal Agar provides a contrasting background for the observation of characteristic morphological features of yeast cultures[10].

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance

Dehydrated medium: Off white coloured, free-flowing powder.

Prepared medium: Light straw coloured gel.

Quality control

Positive control:	***Expected results***
Chlamydospore Production	
Candida albicans ATCC® 10231*	Good growth; white colonies and chlamydospores
Negative control:	
Candida krusei ATCC® 6258	Good growth; white/cream colonies, no chlamydospores

*This organism is available as a Culti-Loop®

Precautions

Glucose supplemented Corn Meal Agar should not be used for chlamydospore production.

Corn Meal Agar with 'Tween 80' (or other wetting agents) will allow *Candida stellatoides* and *Candida tropicalis* to produce chlamydospores.

Some *Candida* strains lose their ability to produce chlamydospores after repeated sub-culturing.

References

1. Prospero Magdalene T. and Reyes A. C. (1955) *Acta Mel. Phillipina* 12(2). 69-74.
2. Rosenthal S. A. and Furnari D. (1958) *J. Invest. Derm.* 31. 251-253.
3. Kelly J. P. and Funigiello (1959) *J. Lab. Clin. Med.* 53. 807-809.
4. Walker L. and Huppert M. (1959) *Am. J. Clin. Path.* 31. 551-558.
5. Walker L., Huppert M. and Woods A. (1960) *Am. J. Clin. Path.* 33. 190-194.
6. Gordon M. A. and Little G. N. (1962-63) *sabouraudia* 2. 171-175.
7. Mackenzie D. W. R. (1962) *J. Clin. Path.* 15(6). 563-565.
8. Dawson Christine O. (1962) *sabouraudia* 1(4). 214-219.
9. Conant N. F., Smith D. T., Baker R. D., Callaway J. L. and Martin D. S. (1971) *Manual of Clinical Mycology.* 3rd edn. W. B. Saunders, Philadelphia, USA.
10. Washington J. A. (1981) *Laboratory Procedures in Clinical Microbiology.* Springer-Verlag, New York, USA.

CR-SMAC – see CEFIXIME RHAMNOSE SORBITOL MacCONKEY AGAR

CROSSLEY MILK MEDIUM

Code: CM0213

This medium is suitable for use where Litmus Milk was previously specified.

Formula	***gm/litre***
Skim milk powder	100.0
Peptone	10.0
Bromocresol purple	0.1
pH 6.8 ± 0.2	

Directions
Cream 110 g of the powder with a little distilled water and gradually dilute to 1 litre with continuous mixing. Tube in 10 ml quantities and autoclave at 121°C for 5 minutes.

Description
A simple medium originally described by Crossley[1] for the routine examination of canned food samples for anaerobic bacteria.

This medium was evolved as the result of comparative trials carried out by Crossley with several standard media. It is capable of giving rapid growth without the use of special anaerobic apparatus, yet the bacteria detected may be provisionally identified by their reactions upon the medium.

Crossley milk medium is recommended, in the second edition of Tanner's *The Microbiology of Foods*[2], for the examination of meat, meat products, and canned foods for sporing anaerobes.

Technique
The following method of examination is suggested: Inoculate 10 ml of Oxoid Crossley Milk Medium with 1.5-2.0 g of the sample. Incubate for 3 to 4 days at 37°C and examine for the following striking and characteristic reactions.

	Reaction	***Organism indicated***
1.	Neutral or alkaline pH (purple colour), gas production,soft curd followed by rapid digestion of casein, often to clear brown liquid, formation of black sediment accompanied by typical foul odour.	*Cl. putrificum* *Cl. sporogenes* *Cl. flabelliferum* *Cl oedematiens* *Cl. histolyticum*
2.	No initial change of pH, formation of soft curd within 2-3 days, slight gas formation. Complete digestion later with alkaline reaction, no odour.	*Cl. centrosporogenes*
3.	Slight acidity (pale yellow colour), formation of soft curd, and whey. Slight gas production.	*Cl. sphenoides*
4.	Acid (bright yellow colour), formation of firm clot and gas. Bleaching of the indicator may sometimes occur.	*Cl. butyricum*
5.	(a) Acid, formation of 'stormy' clot. (b) Acid and 'stormy' clot but with less gas and cloudy whey.	(a) *Cl. perfingens* (b) Usually *Cl. tertium*
6.	Strong alkaline pH, with peptonisation commencing at the surface and spreading downwards. Digestion not complete, blackening, no odour, no gas production.	*B. subtilis* *B. vulgaris*
7.	Acid and clot, or slightly acid only. Peptonisation in some cases.	*B. cereus* *B. coagulans* *B. silvaticus* and various cocci (more detailed tests required)

Crossley[1] modified his medium, by the addition of 20% (w/v) of autoclaved meat or fish paste, so that it was suitable for the examination of vegetable and dairy products.

The medium recommended by Jepsen[3] in *Meat Hygiene* published by the World Health Organization, is Crossley Milk Medium modified by the addition of 20% (w/v) of cooked fish; it is suitable for the examination of meat products for clostridia, and gives diagnostic reactions essentially similar to those outlined above. Riemann[4] modified Crossley Milk Medium by the addition of 0.08% of cysteine hydrochloride, before autoclaving, or by the addition of 1 ml of a sterile 10% sodium thioglycollate solution just before use.

Any of the above additions may be used to supplement Oxoid Crossley Milk Medium.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance

Dehydrated Medium: Pale blue coloured, free-flowing powder.

Prepared medium: Pale blue coloured solution.

Quality control

Positive controls:	***Expected results***
Clostridium sporogenes ATCC® 19404*	Purple colour, gas production, soft curd (see table)
Bacillus cereus ATCC® 10876*	Yellow colour, acid and clot (see table)
Salmonella poona NCTC® 4840*	Blue colour, alkaline reaction (see table)
Negative control:	
Uninoculated media	No change

*This organism is available as a Culti-Loop®

References

1. Crossley E. L. (1941) *J. Soc. Chem. Ind.* 60. 131-136.
2. Tanner F. W. (1944) *The Microbiology of Foods* 2nd edn., Garrard Press, London, pp.893, 1001-1002.
3. Jepsen A. and Albertsen V. E. *et al*. (1957) *Meat Hygiene*, World Health Organization, Geneva, pp.424-426, 439.
4. Riemann H. (1959) Personal communication.

CT-SMAC – see CEFIXIME RHAMNOSE SORBITOL MacCONKEY AGAR

CZAPEK DOX AGAR (MODIFIED)

Code: CM0097

A solid defined medium for the cultivation of those fungi and bacteria which are able to utilise sodium nitrate as the sole source of nitrogen. The acidity of the medium may be increased for the cultivation of acidophilic organisms such as yeasts.

Formula	***gm/litre***
Sodium nitrate	2.0
Potassium chloride	0.5
Magnesium glycerophosphate	0.5
Ferrous sulphate	0.01
Potassium sulphate	0.35
Sucrose	30.0
Agar	12.0
pH 6.8 ± 0.2	

Directions

Suspend 45.4 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at

121°C for 15 minutes. Mix well before pouring. If required, adjust the reaction to pH 3.5 ± 0.2 by adding 10 ml of Lactic Acid 10% SR0021 per litre after sterilisation.

Description

Czapek Dox Agar (Modified) is a medium containing sodium nitrate as the sole source of nitrogen, it is one of the most useful solid media for the general cultivation of fungi.

In the Oxoid medium magnesium glycerophosphate and potassium sulphate replace the magnesium sulphate and potassium phosphate of the original. This modification prevents the precipitation of magnesium phosphate. The medium is also a highly satisfactory substrate for chlamydospore production by *Candida albicans*[1].

Dawson[1] employed Oxoid Czapek Dox Agar (Modified) in her technique for the identification of *Candida albicans* by chlamydospore formation in primary culture, using swabs taken from the mouth and from the vagina. Identification was usually possible within 24 hours. The Oxoid medium showed good chlamydospore production whereas the original formulation did not. After 24 hours incubation 23 out of 27 *Candida albicans* strains had formed chlamydospores on Oxoid Czapek Dox Agar (Modified), 21 on rice infusion agar, 10 on Oxoid Corn Meal Agar and 10 on a corn meal agar made in the laboratory. After 48 hours 25 strains had formed chlamydospores on both the Oxoid medium and the rice agar, 24 on Oxoid Corn Meal Agar and 20 on the laboratory medium. Dawson concluded that the Oxoid Czapek Dox medium and the rice infusion agar were the most satisfactory media. None of 14 strains of unidentified yeasts formed chlamydospores on any medium.

Smith[2] cited the following recommendations for the use of Czapek Dox Agar for taxonomic studies: by Thom and Church[3] for *Aspergillus*; by Thom[4] and by Raper and Thom[5] for *Penicillium*; and by Wakesman[6] for actinomycetes.

Technique

General Cultivation

To avoid excessive condensation cool the molten medium to 50°C before pouring approximately 12 ml into each 9 cm diameter Petri dish. Store the poured plates in an inverted position and inoculate using a needle or wire, with the plate still inverted in order to avoid scattering stray fungal spores over the surface of the medium. Time and temperature of incubation vary considerably according to the species being cultivated. As a general guide, incubate for 1-2 weeks at 25°C. Most *Penicillium* species have an optimum growth temperature between 20° and 25°C, whilst many *Aspergillus* species grow best at about 30°C. However, different fungi grow over a wide range of temperatures; *Aspergillus fumigatus* grows well at 50°C (Smith[2]) and *Cladosporium herbarum* will grow on meat at –6°C[7,8].

Identification of *Candida albicans*[1].

1. Using an inoculating needle (previously flamed, cooled and rubbed against the swab) cut across and through the medium in a Czapek Dox Agar plate to the base of the Petri dish. With the same needle, raise the medium along the whole of one side of the cut – so that the inoculum is spread between the agar and the base of the dish.
2. Incubate the inoculated plates for 24 hours at 28°C.
3. Using a low-power objective, microscopically examine the unopened plates for chlamydospores through the base of each dish. Alternatively, remove the tops of the dishes, and examine through the top of the medium.
4. If no chlamydospores are seen, incubate for a further 24 hours and re-examine.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared agar plates at 2-8°C.

Appearance

Dehydrated medium: White coloured, free-flowing powder.

Prepared medium: Off white coloured gel.

Quality control

Positive controls:	***Expected results***
Aspergillus niger ATCC® 9642*	White/yellow mycelium, black spores
Candida albicans ATCC® 10231*	Good growth; cream coloured colonies
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

References
1. Dawson Christine O. (1962) *Saboutaudia* 1. 214-219.
2. Smith G. (1960) *An Introduction to Industrial Mycoloogy* 5th edn., Edward Arnold Ltd., London.
3. Thom C. and Church M. B. (1926) *The Aspergilli* Williams and Wilkins Co. Baltimore.
4. Thom C. (1930) *The Aspergilli* Williams and Wilkins Co. Baltimore.
5. Raper K. B. and Thom C. (1949) *Manual of the Penicillia* Williams and Wilkins Co. Baltimore.
6. Wakesman S. A. (1931) *Principles of soil Microbiology* Bailliere Tindall and Cox, London.
7. Brooks F. T. and Kidd M. N. (1921) *Specia. Report No 6, Food Invest. Board*, DSIR, London.
8. Brooks F. T. and Handsford C. G. (1922) *Trans. Brit. Mycol. Soc*. 8. 113-142.

CZAPEK DOX LIQUID MEDIUM (MODIFIED)

Code: CM0095

A defined fluid medium for the cultivation of those fungi and bacteria which are able to utilise sodium nitrate as the sole source of nitrogen.

Formula	***gm/litre***
Sodium nitrate	2.0
Potassium chloride	0.5
Magnesium glycerophosphate	0.5
Ferrous sulphate	0.01
Potassium sulphate	0.35
Sucrose	30.0
pH 6.8 ± 0.2	

Directions
Add 33.4 g to 1 litre of distilled water. Mix well and distribute into final containers. Sterilise by autoclaving at 121°C for 15 minutes.

Description
A defined fluid medium for the cultivation of fungi and bacteria capable of utilising sodium nitrate as the sole source of nitrogen.

Storage conditions and Shelf life
Store dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared agar plates at 2-8°C.

Store tubes of broth at 15-25°C.

Appearance
Dehydrated Medium: White coloured, free-flowing powder.

Prepared medium: Colourless solution.

Quality control

Positive controls:	***Expected results***
Aspergillus niger ATCC® 9642*	Good growth; white mycelium with black spores.
Candida albicans ATCC® 10231*	Suspended growth
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

References
1. Dawson Christine O. (1962) *Saboutaudia* 1. 214-219.
2. Smith G. (1960) '*An Introduction to Industrial Mycology*' 5th ed., Edward Arnold Ltd., London.
3. Thom C. and Church M. B. (1926) '*The Aspergilli*' Williams and Wilkins Co., Baltimore.
4. Thom C. (1930) '*The penicillia*' Williams and Wilkins Co., Baltimore.
5. Raper K. B. and Thom C. (1949) '*Manual of the Penicillia*' Williams and Wilkins Co., Baltimore.

6. Wakesman S. A. (1931) '*Principles of soil Microbiology*' Bailliere Tindall and Cox, London.
7. Brooks F. T. and Kidd M. N. (1921) *Specia. Report No.6, Food Invest. Board*, DSIR, London.
8. Brooks F. T. and Handsford C. G. (1922) *Trans. Brit. Mycol. Soc.* 8. 113-142.

DCA AGAR – see DESOXYCHOLATE AGAR

DCA HYNES – see DESOXYCHOLATE AGAR (HYNES)

DCLS AGAR

Code: CM0393

A modified DCA containing sucrose to improve the accuracy of recognition of pathogenic enterobacteriaceae.

Formula	***gm/litre***
Special peptone	10.0
Sodium citrate	10.5
Sodium thiosulphate	5.0
Lactose	5.0
Sucrose	5.0
Sodium desoxycholate	2.5
Neutral red	0.03
Agar	12.0
pH 7.2 ± 0.2	

Directions
Suspend 50 g in 1 litre of distilled water. Bring to the boil to dissolve the medium completely. Cool to 50°C and pour into sterile Petri dishes. DO NOT AUTOCLAVE.

Description
DCLS Agar is a modified form of Desoxycholate Citrate Agar[1] which includes sucrose in its formulation. The addition of this fermentable carbohydrate increases the usefulness of the medium because non-pathogenic sucrose-fermenting organisms may be recognised by their red colonies, e.g. some *Proteus*, *Enterobacter* and *Klebsiella* species.

DCLS Agar reduces the number of false-positive sub-cultures when picking colonies and therefore improves the efficiency of isolation.

The Special peptone, used in DCLS Agar, includes the nucleic acid factors, vitamins and carbon compounds of meat extract, as well as a rich variety of polypeptides. It has improved the growth of shigellae and salmonellae, but it should be noted that *Shigella sonnei* may exhibit a translucent, pink colony which should not be confused with the red *Escherichia coli* colony.

The selectivity of DCLS Agar is similar to Desoxycholate Citrate Agar and it will grow *Vibrio* species, as well as salmonellae and shigellae, whilst inhibiting the growth of *Escherichia coli*.

DCLS Agar may be inoculated directly from the specimen, or inoculated after enrichment through Selenite Broth CM0395 and LP0121, Muller-Kauffmann Tetrathionate Broth CM0343 or Tetrathionate Broth CM0029. The plates should be incubated overnight (18-24 hours) at 35°C and examined for the presence of pale, translucent or colourless colonies. Sub-cultures can be made into confirmatory media such as Kligler Iron Agar CM0033 or Triple Sugar Iron Agar CM0277 or picked for transfer to nutrient broth for subsequent motility tests and serological agglutinations.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared agar plates at 2-8°C.

Appearance
Dehydrated medium: Straw/pink coloured, free-flowing powder.

Prepared medium: Red coloured gel.

Quality control

Positive controls:	***Expected results***
Lactose/sucrose-fermenters	
Escherichia coli ATCC® 25922*	Reduced growth; pink coloured colonies
Non-lactose/sucrose-fermenters	
Salmonella typhimurium ATCC® 14028*	Good growth; translucent colonies
Negative control:	
Staphylococcus aureus ATCC® 25923*	No growth

*This organism is available as a Culti-Loop®

Precautions
Boil the medium for the minimal period of time to get the agar into solution. Overheating reduces the agar gel strength and increases the degree of inhibition. It is therefore important not to hold the molten medium at 50°C for more than the short time required to distribute it into dishes.

Reference
1. Leifson E. (1935) *J. Path. Bact.* 40. 581-599.

DE MAN ROGASA SHARPE AGAR – see MRS AGAR

DEMI FRASER – see HALF FRASER

DERMASEL AGAR BASE

Code: CM0539

A selective medium for dermatophyte fungi recommended for the examination of hair, skin scrapings, nails, etc.

Formula	***gm/litre***
Mycological peptone	10.0
Glucose	20.0
Agar	14.5
pH 6.9 ± 0.2	

DERMASEL SELECTIVE SUPPLEMENT

Code: SR0075

Vial contents	***per vial***	***per litre***
Cycloheximide	200 mg	400 mg
Chloramphenicol	25 mg	50 mg

Directions
Suspend 44.5 g in 1 litre of distilled water and heat gently to dissolve completely. Add the contents of 1 vial of Dermasel Selective Supplement, reconstituted as directed, to each 500 ml of medium to give a level of cycloheximide 0.4 g/l and chloramphenicol 0.05 g/l. Mix gently and sterilise by autoclaving at 121°C for 10 minutes. Avoid overheating at any time.

Description

Oxoid Dermasel Agar is used for the primary isolation and identification of dermatophyte fungi from hair, nails or skin scrapings.

Emmons[1] suggested that media for growth of dermatophytes should have a pH of 6.8-7.0 rather than pH 5.6 as is often recommended. A near neutral pH is better for the growth of some fungi and the acid pH used to suppress bacterial contaminants can be replaced by antibiotics.

The addition to the medium of Dermasel Selective Supplement to give a level of cycloheximide 0.4 g/l and chloramphenicol 0.05 g/l renders the medium selective for dermatophytes, inhibiting the growth of saprophytic fungi, yeasts and bacterial skin flora[2].

The chloramphenicol and cycloheximide supplement reduces the potential risk to health from these antibiotics. To include them in the powder mix could allow them to be scattered as dust whilst weighing the medium. It also ensures a fixed, accurate dose of antibiotic that has been protected from degradation on storage.

The addition of cycloheximide and an anti-bacterial agent has been reported to improve considerably the isolation of dermatophytes, especially when the inoculum, such as horse hair was heavily contaminated[3,4]. The presence of staphylococci, which may grow in the absence of the antibiotic has been shown to prevent the *in vitro* growth of *Trichophyton rubrum*[5].

The presence of cycloheximide in the medium inhibits the growth of *Trichosporon cutaneum*, *Candida parasilosis*, *Candida krusei*, *Aspergillus*, *Penicillium*, *Fusarium* and *Cephalosporium* species which have been associated with diseased nails[6,7].

The incorporation of griseofulvin at a level of 20 µg/ml into one of paired tubes of selective media has been recommended as an additional aid in the diagnosis of dermatophytosis[8]. The absence of growth on the medium containing griseofulvin provides presumptive identification of a dermatophyte fungus.

Dermatophyte fungi cultured on Oxoid Dermasel Agar show characteristic colonial morphology with typical pigmentation. Macroconidia and microconidia are typical for the species when studied microscopically.

Technique

Oxoid Dermasel Agar may be prepared as slopes in test tubes with loose caps to ensure adequate aeration, or in vented Petri dishes.

Small, pin-head sized samples of the test material are stabbed into the surface of the agar. A number of samples may be inoculated onto the same surface.

The medium is incubated at 22-30°C and examined at regular intervals for two to four weeks.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared agar plates at 2-8°C.

Appearance

Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured gel.

Quality control

Positive controls:	***Expected results***
Trichophyton rubrum ATCC® 28191	White mycelium, buff spores
Candida albicans ATCC® 10231*	Good growth; cream coloured colonies
Negative controls:	
Aspergillus niger ATCC® 16404*	Inhibited or no growth
Escherichia coli ATCC® 25922*	No growth

*This organism is available as a Culti-Loop®

Precautions

This medium should not be used if agents causing systemic mycoses are being sought[9]. If such agents are suspected, e.g. cryptococcus, histoplasma etc., either Dermasel Agar Base without antibiotic supplement must be used in parallel or Brain Heart Infusion Agar CM0375.

If the fungal agent sought is suspected to be nutritionally fastidious, the use of Brain Heart Infusion Agar is particularly helpful.

Dermasel Selective Supplement SR0075 contains a toxic concentration of cycloheximide. Note the precautions to be taken under HAZARDS.

References

1. Emmons C. W., Binford C. H. and Utz J. P. (1963) *Medical Mycology.* Henry Kimpton.
2. Georg L. K., Ajello L. and Papageorge C. (1954) *J. Lab. Clin. Med.* 44. 422.
3. Quaife R. A. (1968) *J. Med. Lab. Technol.* 25. 227-232.
4. Merz W. G., Berger C. L. and Silva-Huntar M. (1970) *Arch. Derm.* 102. 545-547.
5. Silva M., Kesten B. M. and Benham R. W. (1955) *J. Invest. Derm.* 25. 311-328.
6. Zaias N. (1966) *Sabouraudia* 5. 99-103.
7. Rosenthal S. A., Stritzler R. and Villafane J. (1968) *Arch. Derm.* 97. 685-687.
8. Blank H. and Rewbell G. (1965) *Arch. Derm.* 92. 319-322.
9. McDonough E. S., Georg L. K., Ajello L. and Brinkman S. (1960) *Mycopath. et Mycol. Appl.* 13. 113-115.

DESOXYCHOLATE AGAR

Code: CM0163

A differential medium for the enumeration of coliforms in dairy products. It may be employed as a non-selective medium for the isolation of enteric pathogens.

Formula	***gm/litre***
Peptone	10.0
Lactose	10.0
Sodium desoxycholate	1.0
Sodium chloride	5.0
Dipotassium hydrogen phosphate	2.0
Ferric citrate	1.0
Sodium citrate	1.0
Neutral red	0.03
Agar	15.0
pH 7.1 ± 0.2	

Directions

Suspend 45 g in 1 litre of distilled water. Bring to the boil over gauze and flame to dissolve the medium completely. Agitate to prevent charring.

THIS MEDIUM IS HEAT SENSITIVE. AVOID EXCESSIVE OR PROLONGED HEATING DURING RECONSTITUTION. DO NOT AUTOCLAVE OR REMELT.

Description

Desoxycholate Agar is a differential medium for the direct count of coliforms in dairy products (American Public Health Association[1]). It may also be employed for the isolation of enteric pathogens from rectal swabs, faeces, or other specimens.

The medium may be used in a ‘pour-plate’ technique or as a surface inoculated medium. A thin layer of uninoculated desoxycholate agar poured over the surface of a gelled ‘pour-plate’ assists subsequent counting.

Technique

Enumeration of Coliforms in Milk and Cream (APHA[1])

1. Pipette 1-4 ml of the sample (or decimal dilution of the sample) into a sterile Petri dish.
2. Cool freshly prepared Desoxycholate Agar to 42-44°C and add 10-20 ml to each dish.
3. Mix the contents of the dishes by gentle tilting and rotation.
4. Allow the plates to solidify and pour on an overlay of 3-4 ml of uninoculated Desoxycholate Agar.
5. When the overlay has set, invert the plates and incubate them for 18-24 hours at 35°C.
6. Count all dark red colonies measuring at least 0.5 mm in diameter, and calculate the number of coliform colonies per millilitre or gram of original sample.

Isolation of Enterobacteriaceae
It is advisable to use Desoxycholate Agar in parallel with other plating media for this purpose.

Lightly inoculate a Desoxycholate Agar plate with faeces, rectal swab, or enrichment culture. Incubate for 18-24 hours at 35°C and examine. Non-lactose-fermenters of enteric origin form colourless colonies. Non-lactose-fermenters which are not of enteric origin are generally inhibited by the sodium desoxycholate in the medium. Identify suspect colonies in the usual manner.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared agar plates at 2-8°C.

Appearance
Dehydrated medium: Straw pink coloured, free-flowing powder.

Prepared medium: Pink coloured gel.

Quality control

Positive controls:	***Expected results***
Lactose-Fermenters	
Escherichia coli ATCC® 25922*	Good growth; pink coloured colonies with bile precipitation
Non-Lactose-Fermenters	
Shigella sonnei ATCC® 25931	Good growth; straw coloured colonies
Negative control:	
Staphylococcus aureus ATCC® 25923*	Inhibited

*This organism is available as a Culti-Loop®

Precautions
As with all desoxycholate media, this medium is heat sensitive. Observe the precautions stated under Directions.

Reference
1. American Public Health Association (1978) *Standard Methods for the Examination of Dairy Products* 14th edn. APHA Inc., New York, pp. 58-59.

DESOXYCHOLATE CITRATE AGAR

Code: CM0035

A modification of Leifson's medium for the isolation of intestinal pathogens.

Formula	***gm/litre***
'Lab-Lemco' powder	5.0
Peptone	5.0
Lactose	10.0
Sodium citrate	5.0
Sodium thiosulphate	5.0
Ferric citrate	1.0
Sodium desoxycholate	2.5
Neutral red	0.025
Agar	15.0
pH 7.0 ± 0.2	

Directions
Suspend 48.5 g in 1 litre of distilled water. With frequent agitation bring to the boil over a gauze and flame to dissolve completely. Mix well and pour plates immediately. Dry the agar surface before use.

THIS MEDIUM IS HEAT SENSITIVE. AVOID EXCESSIVE OR PROLONGED HEATING DURING RECONSTITUTION. DO NOT AUTOCLAVE, OR REMELT.

Description
An Oxoid modification of Leifson medium[1], for the isolation and maximum recovery of intestinal pathogens. It is less selective and inhibiting than Desoxycholate Citrate Agar (Hynes) CM0227 but colonial characteristics are similar on the two media.

Desoxycholate Citrate Agar provides an opaque background against which one may more easily discern the clearing produced by alkali-producing pathogens.

The use of a less selective medium for direct sampling of faeces and a more selective medium for post-enrichment sampling, would be advantageous. Similarly, the less inhibitory medium is often preferable when shigellae are being sought as well as salmonellae[2].

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared agar plates at 2-8°C.

Appearance
Dehydrated medium: Straw/pink coloured, free-flowing powder.

Prepared medium: Pink coloured gel.

Quality control

Positive controls:	***Expected results***
Salmonella typhimurium ATCC® 14028*	Good growth; straw coloured colonies
Shigella sonnei ATCC® 25931*	Good growth; straw coloured colonies
Salmonella poona NCTC 4840*	Good growth; straw coloured colonies
Negative control:	
Enterococcus faecalis ATCC® 29212*	No growth

*This organism is available as a Culti-Loop®

Precautions
Observe the precautions about overheating shown under Directions.

The medium is best used freshly prepared.

Stock cultures of *Shigella* species may become predominantly in the R-phase when sub-cultured away from DCA media. Such cultures are difficult to use for control purposes without first heavily streaking the cultures on DCA plates and picking off the few S-phase colonies, i.e. the macro-colonies on the agar surface, for further sub-culture.

When making biochemical tests on colonies picked from the surface of DCA plates, purity sub-cultures should be carried out because the colony may be contaminated with *Escherichia coli* present as micro-colonies.

References
1. Leifson E. (1935) *J. Path. Bact.* 40. 581-599.
2. Fricker C. R. (1987) *J. Appl. Bact.* 63. 99-116.

DESOXYCHOLATE CITRATE AGAR (HYNES)

Code: CM0227

A selective medium for the isolation of Salmonella and Shigella organisms.

Formula	***gm/litre***
'Lab-Lemco' powder	5.0
Peptone	5.0
Lactose	10.0
Sodium citrate	8.5
Sodium thiosulphate	5.4
Ferric ammonium citrate	1.0
Sodium desoxycholate	5.0
Neutral red	0.02
Agar	12.0
pH 7.3 ± 0.2	

Directions
Suspend 52 g in 1 litre of distilled water. Bring to the boil over gauze and flame, to dissolve completely. Agitate to prevent charring. Cool to approximately 50°C and pour into sterile Petri dishes. Dry the agar surface before use.

THIS MEDIUM IS HEAT SENSITIVE: AVOID EXCESSIVE OR PROLONGED HEATING DURING RECONSTITUTION. DO NOT AUTOCLAVE OR REMELT.

Description
An improved medium, based on the Hynes[1] modification of Leifson medium for the isolation of salmonellae and shigellae.

The improvement gives larger and more numerous colonies of *Shigella* species which can easily be picked off and emulsified in saline for slide agglutination tests.

Desoxycholate Citrate Agar (Hynes) is more selective than Desoxycholate Citrate Agar CM0035. In particular, Desoxycholate Citrate Agar (Hynes) is more inhibitory to coliforms and *Proteus* species.

Technique
Inoculate the medium heavily with faeces or rectal swabs, spreading part of the original inoculum in order to obtain well separated colonies on some portion of the plate. Incubate for 18-24 hours at 35°C. If organisms are late developers or if no non-lactose-fermenters are observed, incubate for a further 24 hours.

Colonies may be picked directly off the medium for serological and biochemical tests. It should be noted that *Escherichia coli* survives on the medium even though it does not usually grow – therefore colonial purity should be established by sub-culture onto a differential but less inhibitory medium, e.g. MacConkey Agar CM0007.

Colonial Characteristics
(Following incubation at 35°C)

The medium is clear and pale pink. Lactose-fermenting organisms produce pink colonies and may be surrounded by a zone of precipitated desoxycholic acid, which is due to acid production. The colonies of non-lactose-fermenters are colourless, and due to their alkaline reaction they are surrounded by a clear orange-yellow zone of medium.

Escherichia coli – most strains are inhibited, but the few strains which grow produce pink umbilicated colonies 1-2 mm in diameter which may be surrounded by a zone of precipitation.

Shigella sonnei – the colonies grow from 1 mm diameter after 18 hours incubation to 2 mm after 38 hours; they are smooth and initially colourless, becoming pale pink on further incubation due to late lactose fermentation.

Shigella flexneri – colonies are colourless and similar in appearance to those of *Shigella sonnei*, but often with a narrow plane periphery round a central dome.

Salmonella paratyphi B – from 1 mm diameter after 18 hours incubation to 2-4 mm on the second day, when they are slightly opaque, dome-shaped, with a central black dot.

Salmonella typhi – 0.25-1 mm in diameter after 18 hours and pale pink, a day later they are flat, conical, 2mm in diameter, colourless and slightly opaque, often with a central grey dot.

Other *Salmonella* colonies – similar to those of *Salmonella paratyphi B*. Non-pathogenic non-lactose-fermenters, such as *Proteus* and *Pseudomonas* species, grow on the medium and may produce colonies which closely simulate those of the salmonellae or shigellae. *Proteus* colonies are often glossy (more translucent than those of the pathogens), with a large central black dot and a ‘fishy’ odour.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared agar plates at 2-8°C.

Appearance
Dehydrated medium: Straw/pink coloured, free-flowing powder.

Prepared medium: Pink coloured gel.

Quality control

Positive controls:	***Expected results***
Salmonella typhimurium ATCC® 14028*	Good growth; straw coloured colonies with black centres
Shigella sonnei ATCC® 25931*	Good growth; straw coloured colonies
Salmonella poona NCTC® 4840*	Good growth; straw coloured colonies with black centres
Negative control:	
Enterococcus faecalis ATCC® 29212*	No growth

*This organism is available as a Culti-Loop®

Precautions

Observe the precautions about overheating shown under Directions.

The medium is best used freshly prepared.

Stock cultures of *Shigella* species may become predominant in the R-phase when sub-cultured away from DCA media. Such cultures are difficult to use for control purposes without first heavily streaking the cultures on DCA plates and picking off the few S-phase colonies, i.e. the macro-colonies on the agar surface, for further sub-culture.

When making biochemical tests on colonies picked from the surface of DCA plates, purity sub-cultures should be carried out because the colony may be contaminated with *Escherichia coli* present as micro-colonies.

Reference

1. Hynes M. (1942) *J. Path. Bact.* 54. 193-207.

DEXTROSE TRYPTONE AGAR

Code: CM0075

For the detection and enumeration of 'flat-sour' thermophiles and mesophiles in food products. Acid-producing organisms such as 'flat-sour' thermophiles form yellow colonies surrounded by a yellow zone.

Formula	***gm/litre***
Tryptone	10.0
Dextrose	5.0
Bromocresol purple 1	0.04
Agar	12.0
pH 6.9 ± 0.2	

Directions

Suspend 27 g in 1 litre of distilled water and bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

Description

A bacteriologically controlled medium for the detection and enumeration of thermophilic and mesophilic organisms in food products, etc.

Dextrose Tryptone Agar evolved as the result of several years research by Williams[1] is most suitable for the cultivation and enumeration of the thermophilic bacteria causing 'flat-sour' spoilage of canned food. Its use for routine cultural purposes is recommended by Cameron[2] and the Association of Official Analytical Chemists[3]. Dextrose Tryptone Agar is also recommended by:

1. Tanner[4] for the examination of canned food, sugar, and starch for thermophilic bacteria of the *Bacillus stearothermophilus* type (i.e. 'flat-sour' spoilage bacteria).

2. The American Public Health Association[5] for the enumeration of mesophilic and thermophilic aerobic bacteria in sweetening agents used in frozen dairy foods.

3. The National Canners Association[6] for determination of the total plate and 'flat-sour' count of thermophilic bacteria spores in ingredients, such as sugar and starch.

4. The American Public Health Association[7] for the enumeration of mesophilic organisms and 'flat-sour'

spores in sugars, starches and other complex carbohydrates; and for the enumeration of 'flat-sour' thermophiles in cereals and cereal products, dehydrated fruits and vegetables, and spices.

5. Baumgartner and Hersom[8] for the examination of low and medium-acid canned food (above pH 4.5) for 'flat-sour' thermophiles, mesophilic aerobes, and facultative anaerobes.

Bashford[9] reported that the addition of 0.5-1% of meat extract greatly improves the medium.

Townsend *et al*. (National Canners Association[10]) showed that some batches of bromocresol purple are more inhibitory than others but this variability is overcome in the Oxoid medium by stringent biological control.

Technique

The instructions given below are included only as an indication of the mode of use of Dextrose Tryptone Agar, and will vary according to the original sample and the exact purpose of the investigation. For more exact details of technique it is advisable to consult one of the standard manuals mentioned in the references.

Enumeration of Mesophiles – into each of 5 Petri dishes, pipette dilutions of the sample to be tested. Cover and mix the inoculum with sterile Dextrose Tryptone Agar and incubate for 72 hours at 32°C. Count the total number of colonies, with separate totals for acid-producing (yellow halo) and non-acid-producing colonies.

Enumeration of 'flat-sour' Thermophiles – inoculate as above and incubate for 48 hours at 55°C. 'Flat-sour' colonies (e.g. *Bacillus stearothermophilus*) are typically round, 2-5 mm in diameter, with an opaque centre, and surrounded by a yellow zone in contrast with the purple medium.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared agar plates at 2-8°C.

Appearance

Dehydrated medium: Mauve coloured, free-flowing powder.

Prepared medium: Purple coloured gel.

Quality control

Positive control:	***Expected results***
Bacillus stearothermophilus NCIB 8919/ATCC® 12976	Good growth; yellow colonies and media
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

Precautions

Incubation at 55°C must be carried out under humid conditions e.g. wrapped dishes or in a high humidity environment.

References

1. Williams O. B. (1936) *Food Res*. 1(3). 217-221.
2. Cameron E. J. (1936) *J. Assoc. Official Agr. Chem.* 19. 433-438.
3. Association of Official Analytical Chemists (1978) *Bacteriological Analytical Manual* 5th edn. AOAC Washington DC.
4. Tanner F. W. (1944) *The Microbiology of Foods* 2nd edn., Garrard Press, Champaers pp.762-763; 1127-1128.
5. American Public Health Association (1972) *Standard Methods for the Examination of Dairy Products*. 13th edn. APHA. Washington DC.
6. National Canners Association (1968) *Laboratory Manual for Food Canners and Processors*. Vol.1. p.13.
7. American Public Health Association (1992) *Compendium of Methods for the Microbiological Examination of Foods*. APHA. Washington DC.
8. Baumgartner J. G. and Hersom A. C. (1956) *Canned Foods* 4th edn., Churchill Ltd., London, pp.229-230 and 247.
9. Bashford T. E. (1948) Personal Communication.
10. National Canners Association (1954) *A Laboratory Manual for the Canning Industry* 1st edn., National Canners Association, Washington.

DEXTROSE TRYPTONE BROTH

Code: CM0073

A liquid medium for the bacteriological examination of canned foods etc. Acid-producing organisms such as 'flat-sour' thermophiles change the colour of the medium from purple to yellow.

Formula	***gm/litre***
Tryptone	10.0
Dextrose	5.0
Bromocresol purple	0.04
pH 6.9 ± 0.2	

Directions
Add 15 g to 1 litre of distilled water. Mix well and distribute into final containers. Sterilise by autoclaving at 121°C for 15 minutes.

Description
Dextrose Tryptone Broth is widely recommended for the aerobic cultivation and detection of many different organisms causing spoilage in canned foods and other products.

The American Public Health Association[1] and Baumgartner and Hersom[2] recommended this formulation for the bacteriological examination of low and medium-acid canned foods (pH 4.5 and above). Both methods include inoculation of 10 ml amounts of the broth with one or two grams of the food product. For food products in this pH range, the suggested procedure is aerobic cultivation in Dextrose Tryptone Broth in parallel with anaerobic cultivation in other media; Liver Broth CM0077 is most suitable for this purpose. Duplicate sets of tubes are incubated at 35°C and at 55°C. Organisms which produce acid from dextrose, such as *Bacillus stearothermophilus* and other 'flat-sour' organisms, are detected by the colour change of the medium from purple to yellow.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared broth below 25°C.

Appearance
Dehydrated medium: Pale green coloured, free-flowing powder.

Prepared medium: Purple coloured solution.

Quality control

Positive control:	***Expected results***
Bacillus stearothermophilus ATCC® 12976	Turbid growth; acid
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

References

1. American Public Health Association (1976) *Compendium of Methods for the Microbiological Examination of Foods*. APHA Washington DC.
2. Baumgartner J. G. and Hersom A. C. (1956) *Canned Foods* 4th ed., Churchill Ltd. London, pp.229-230 and 247.

DIAGNOSTIC SENSITIVITY TEST AGAR (DST AGAR)

Code: CM0261

A susceptibility test agar for antimicrobial testing.

Formula	***gm/litre***
Proteose peptone	10.0
Veal infusion solids	10.0
Glucose	2.0
Sodium chloride	3.0
Disodium phosphate	2.0
Sodium acetate	1.0
Adenine sulphate	0.01
Guanine hydrochloride	0.01
Uracil	0.01
Xanthine	0.01
Aneurine	0.00002
Agar	12.0
pH 7.4 + 0.2	

Directions

Add 40 g to 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

For blood agar, cool the base to 50°C and add 7% of Defibrinated Horse Blood SR0050. Mix with gentle rotation and pour into Petri dishes (12 ml for a 9 cm dish) or other containers.

RECONSTITUTION AND MIXING SHOULD BE PERFORMED IN A FLASK AT LEAST 2.5 TIMES THE VOLUME OF MEDIUM TO ENSURE ADEQUATE AERATION OF THE BLOOD.

Description

Diagnostic Sensitivity Test Agar was developed in Oxoid as a dual-purpose medium which would satisfy both diagnostic and susceptibility requirements.

The diagnostic role was supported by the nutritional amino-acid base with glucose to encourage early growth. The inclusion of the buffers (disodium phosphate and sodium acetate) helped prevent excessive movements of pH values which could result from utilisation of glucose or amino-acids. Such pH movements would interfere with haemolytic reactions[1] and the MIC values of pH-susceptible antimicrobials[2].

Long before the mechanisms of folate antagonism had been discovered, the addition of the bases adenine, guanine, uracil and xanthine were shown to improve the performance of the medium as an antimicrobial test medium.

Aneurine, added as a general purpose vitamin, improved the growth of several organisms especially staphylococci.

The agar used in the formulation has been specially processed to allow unimpeded diffusion of antimicrobials from discs[3].

DSTA is now primarily used for susceptibility tests and its role in diagnostic microbiology, i.e. the primary isolation of organisms from clinical samples, has diminished.

An essential requirement for satisfactory antimicrobial susceptibility media is that the reactive levels of thymidine and thymine must be sufficiently reduced to avoid antagonism of trimethoprim and sulphonamides[4].

DSTA meets this requirement and in the presence of lysed horse blood (or defibrinated horse blood if the plates are stored long enough to allow some lysis of the erythrocytes) the level of thymidine will be further reduced. This is caused by the action of the enzyme thymidine phosphorylase which is released from lysed horse erythrocytes[5]. Thymidine is an essential growth factor for thymidine-dependent organisms and they will not grow in its absence or they will grow poorly in media containing reduced levels[6]. It is important that users of DSTA are aware of this limitation of thymidine which now exists in the medium and the effect it will have on a small proportion of organisms.

Details of the function of the medium and the methodology used for antimicrobial susceptibility tests are discussed in the Section ‘Susceptibility Testing’.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared agar plates at 2-8°C.

Appearance
Dehydrated medium: Light straw coloured, free-flowing powder.

Prepared medium: Straw coloured gel.

Quality control

Positive controls:	***Expected results***
With blood	
Streptococcus pneumoniae ATCC® 6303*	Good growth; grey/green mucoid colonies with alpha-haemolysis
Staphylococcus aureus ATCC® 25923*	Good growth; white/grey coloured colonies
Without blood	
Enterococcus feacalis ATCC® 29212*	Good growth; straw colonies
Pseudomonas aeruginosa ATCC® 27853*	Good growth; straw colonies with green pigmentation
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

Precautions
Diagnostic Sensitivity Test Agar has reduced thymidine activity and this will affect its performance as a primary isolation medium.

References

1. Expert Committee on Antibiotics (1961) World Health Organization Technical Report Series No. 210. WHO, Geneva.
2. Bechtle R. M. and Scherr G. H. (1958) *Antibiotics and Chemotherapy* 8(12). 599-606.
3. Marshall J. H. and Kelsey J. C. (1960) *J. Hyg., Camb.* 58. 367-372.
4. Ferone R., Bushby S. R. M., Burchall J. J., Moore W. D. and Smith D. (1975) *Antimicrob. Agents Chemotherap*. 7. 91-98.
5. Ferguson R. W. and Weissfeld A. S. (1984) *J. Clin. Microbiol.* 19. 85-86.
6. Stokes E. J. and Ridgway G. L. (1980) *Clinical Bacteriology* 5th edn. Arnold. London. p.54.

DICHLORAN-GLYCEROL (DG18) AGAR BASE

Code: CM0729

A selective low water activity (a_w) medium for xerophilic moulds from dried and semi-dried foods.

Formula gm/litre	***gm/litre***
Peptone	5.0
Glucose	10.0
Potassium dihydrogen phosphate	1.0
Magnesium sulphate	0.5
Dichloran	0.002
Agar	15.0
Final pH 5.6 ± 0.2	

CHLORAMPHENICOL SELECTIVE SUPPLEMENT

Code: SR0078

Vial contents	*per vial*	*per litre*
Chloramphenicol	50 mg	100 mg

Directions
Suspend 15.75 g in 500 ml of distilled water and heat to dissolve completely. Add 110 g of Glycerol (Analytical Reagent grade). Rehydrate 1 vial of Chloramphenicol Supplement as directed and add to the DG18 Agar Base. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C, mix well and pour into sterile Petri dishes.

Description
Dichloran-Glycerol (DG18) Agar Base is based on the formulation described by Hocking and Pitt[1] and is recommended for the enumeration and isolation of xerophilic moulds from dried and semi-dried foods. Examples of these are dried fruits, spices, confectionery, cereals, nuts and dried meat and fish products.

The medium formulation contains glycerol at 16% (w/w) which lowers the water activity (a_w) from 0.999 to 0.95. Glycerol was chosen because of advantages it showed over sodium chloride and sugars which have traditionally been used to formulate media of reduced a_w[1]. The medium also contains dichloran which inhibits spreading of mucoraceous fungi and restricts the colony size of other genera. This restrictive characteristic makes the medium especially suitable for enumeration because it allows unobscured growth of organisms that ordinarily form small colonies.

A modification to the formula has been described in which the addition of Triton-X to DG18 agar increases the inhibition of vigorously-spreading fungi[2].

In a comparative study carried out between DG18 and DRBC (a medium of higher a_w), greater recovery of xerophilic moulds was achieved on the DG18 medium[1]. In this study it was found that two of the fungi commonly isolated from dried foods in high numbers, *Aspergillus penicilloides* and *Wallemia sebi*, grow very poorly or not at all on DRBC.

Technique
1. Prepare the DG18 medium as directed using CM0729, SR0078 and glycerol.
2. Process the food sample in a Seward 'Stomacher' adding 40 g to 200 ml of 0.1% peptone water. For powdered products shake periodically for 30 minutes with 0.1% peptone water.
3. Dilute the sample 1:10 in 0.1% peptone water.
4. Surface plate 0.1 ml of the prepared sample per plate.
5. Incubate at 25°C and examine after 4, 5 and 6 days.
6. Report as number of xerophilic colonies per gram of food.

Further experience with this medium has shown it to be a good general purpose medium. In a collaborative exercise in Holland the DG18 medium gave the best results for yeasts and moulds isolated from foodstuffs[2].

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared plates at 2-8°C.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured gel.

Quality control

Positive controls:	***Expected results***
Saccharomyces cerevisiae ATCC® 9763*	Good growth; cream coloured colonies
Aspergillus niger ATCC® 9642*	White/Yellow mycelium, black spores
Negative controls:	
Escherichia coli ATCC® 25922*	No growth
Bacillus subtilis ATCC® 6633*	No growth

*This organism is available as a Culti-Loop®

Precautions
The dichloran compound used in this medium is Botran® 2,6-Dichloro-4-Nitro-Analine (CAS: 99-30-9).

References

1. Hocking A. D. and Pitt J. I. (1980) *J. Appl. & Env. Microbiol.* 39. 488-492.
2. Beuchat L. R. and Hwang C. A. (1996) *Int. J. Food Microbiol.* 29. 161-166.
3. Beckers H. J., Boer E., van Eikelenboom C., Hartog B. J., Kuik D., Mol N., Nooitgedagt A. J., Northolt M. O. and Samson R. A. (1982) *Intern. Stand. Org. Document* ISO/TC34/SC9/N151.

DICHLORAN ROSE-BENGAL CHLORAMPHENICOL AGAR

Code: CM0727

DRBC Agar is a selective medium for yeasts and moulds associated with food spoilage.

Formula	***gm/litre***
Peptone	5.0
Glucose	10.0
Potassium dihydrogen phosphate	1.0
Magnesium sulphate	0.5
Dichloran	0.002
Rose-Bengal	0.025
Agar	15.0
pH 5.6 ± 0.2	

CHLORAMPHENICOL SELECTIVE SUPPLEMENT

Code: SR0078

Vial contents	***per vial***	***per litre***
Chloramphenicol	50 mg	100 mg

Directions
Suspend 15.75 g in 500 ml of distilled water and heat to dissolve completely. Rehydrate[1] vial of Chloramphenicol Supplement SR0078 as directed and add to the DRBC Agar Base. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C, mix well and pour into sterile Petri dishes.

Description
Dichloran Rose-Bengal Chloramphenicol Medium (DRBC) is based on the formulation described by King *et al*.[1,2], and is recommended as a selective medium for the isolation and enumeration of yeasts and moulds that are of significance in food spoilage.

DRBC is a modification of Rose-Bengal Chloramphenicol Medium[3] and differs as follows: pH is lowered to 5.6, the Rose-Bengal content is reduced by 50% and Dichloran is added.

The cumulative effect of these modifications is to further inhibit bacterial growth, inhibit spreading moulds such as *Rhizopus* and *Mucor* and make the medium capable of supporting the growth of those species that cannot be isolated on Rose-Bengal Chloramphenicol Agar or acidified Potato Dextrose Agar[1]. The inhibition of spreading moulds and the general restriction of colony size results in improved enumeration and detection of mycotoxigenic moulds and other species of significance in food spoilage[6].

In a collaborative exercise in the UK between nine laboratories, in which mould and yeast counts were made on different samples of food and feed, DRBC came out best of the five different media tested[7].

Rose-Bengal Chloramphenicol Agar should be used in addition where it is necessary to gain an overall impression of the fungal flora, including spreading types, when the use of DRBC Agar alone would inhibit these.

The reduced pH of DRBC Agar increases the inhibition of yeasts by Rose-Bengal[1] and the use of Rose-Bengal Chloramphenicol Agar (pH of 7.2) in parallel should be considered where it is necessary to enumerate yeasts in the presence of moulds.

Technique

1. Prepare the DRBC Medium as directed.
2. Add 40 ml of the food sample to 200 ml of 0.1% peptone water and process in a Seward 'Stomacher' for 30 seconds[4] or alternatively weigh into 0.1% peptone water and leave for 30 minutes shaking periodically[5].
3. Inoculate 0.1ml of the prepared sample on the medium surface.
4. Incubate the plates at 25°C and examine after 3, 4 and 5 days.
5. Report as number of colonies per gram of food.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared plates of medium at 2-8°C.

Appearance

Dehydrated medium: Pink coloured, free-flowing powder.

Prepared medium: Pink coloured gel.

Quality control

Positive controls:	***Expected results***
Aspergillus niger ATCC® 9642*	White/Yellow mycelium, black spores
Saccharomyces cerevisiae ATCC® 9763*	Good growth; pink coloured colonies
Negative controls:	
Escherichia coli ATCC® 25922*	No growth
Bacillus subtilis ATCC® 6633*	No growth

*This organism is available as a Culti-Loop®

Precautions

ROSE-BENGAL PHOTO-OXIDISES TO FORM TOXIC COMPOUNDS. STORE PLATES OF THE MEDIUM IN THE DARK AND AVOID EXPOSURE TO LIGHT[8].

Some strains of fungi may be inhibited on this medium.

The dichloran compound used in this medium is Botran® 2,6-Dichloro-4-Nitro-Analine (CAS: 99-30-9).

References

1. King D. A. Jr., Hocking A. D. and Pitt J. I. (1979) *J. Appl. & Environ. Microbiol.* 37. 959-964.
2. Pitt J. I. (1984) Personal Communication.
3. Jarvis B. (1973) *J. Appl. Bact.* 36. 723-727.
4. Sharp A. N. and Jackson A. K. (1972) *J. Appl. Bact.* 24. 175-178.
5. Sharf J. M. (ed) (1966) 2nd edn. *American Public Health Association*, New York.
6. Thomson G. F. (1984) *Food Microbiol.* 1. 223-227.
7. Seiler D. A. L. (1985) *Int. J. Food Techn.* 2. 123-131.
8. Kramer C. L. and Pady S. M. (1961) *Trans. Kan. Acad. Sci.* 64. 110-116.

DNASE AGAR

Code: CM0321

For the detection of microbial deoxyribonuclease enzymes, particularly from staphylococci.

Formula	***gm/litre***
Tryptose	20.0
Deoxyribonucleic acid	2.0
Sodium chloride	5.0
Agar	12.0
pH 7.3 ± 0.2	

Directions

Suspend 39 g in 1 litre of distilled water and bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

Description

Weckman & Catlin[1] suggested that DNase activity could be used to identify pathogenic staphylococci after they had established a close correlation with coagulase production. Jeffries *et al*.[2] incorporated DNA in the agar medium to provide a simple method of detecting DNase activity. Organisms are streaked onto the surface of the agar medium and incubated. The growth on the surface of the agar is then flooded with 1N hydrochloric acid. Polymerised DNA precipitates in the presence of 1N HCl and makes the medium opaque. If the organisms produce DNase enzymes, in sufficient quantity to hydrolyse the DNA, then clear zones are seen around the colonies.

Good correlation was shown between DNase production and coagulase activity when testing *Staphylococcus aureus* strains from clinical samples[2,3,4]. Both *Staphylococcus aureus* and *Staphylococcus epidermis* produce extracellular DNase[5,6,7] but *Staphylococcus aureus* produces greater quantities[1,7].

A modification of the medium is to add mannitol (1% w/v) and phenol red or bromothymol blue (0.0025% w/v) as an indicator of mannitol fermentation[9]. The pH reaction around the colonies must be read before the plate is flooded with acid.

The DNase reaction helps in the differentiation and identification of non-pigmented *Serratia marcescens*[8] (positive DNase reaction) from *Klebsiella-Enterobacter* (negative DNase reaction).

Normal HCl is bactericidal and the organisms cannot be recovered from the surface of the agar after flooding. The incorporation of dyes into the medium which can distinguish hydrolysis of DNA is a further modification which avoids the use of acid. Toluidine blue[8] and methyl green[10] form coloured complexes with polymerised DNA; these colours change as the DNA is hydrolysed.

It should be noted that toluidine blue inhibits Gram positive organisms and it is used to detect DNase production by the Enterobacteriaceae. It has been used with ampicillin (30 mg/litre) to demonstrate DNase production by *Aeromonas hydrophila* from faeces[11].

Technique

Inoculate the plates by spotting the organism onto the surface of the agar so that a thick plaque of growth is evident after 18 hours incubation.

Examine plates for colour changes in or around the colonies if mannitol/indicator or dyes have been added to the medium. In the absence of dyes, flood the plates with 1N HCl and allow them to stand on the bench (lids uppermost) for a few minutes. Look for zones of clearing around the colonies.

Appearance of colonies with media modifications

1. Mannitol/pH indicator:	
Yellow, with yellow zones	Mannitol +
Same colour as medium	Mannitol –
2. Toluidine blue:	
Pink zones in blue medium	DNase +
No zones	DNase -
3. Methyl green:	
Almost colourless zones	DNase +
No zones	DNase -
4. Acid flood:	
Well defined clear zones	DNase +
No clear zones	DNase -

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared plates of medium at 2-8°C.

Appearance

Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured gel.

Quality control

Positive control:	***Expected results***
Staphylococcus aureus ATCC® 25923*	Good growth; clear zones
Negative control:	
Staphylococcus epidermidis ATCC® 12228*	Good growth; no zones

*This organism is available as a Culti-Loop®

Precautions

The DNase reaction for staphylococci is an indication of pathogenicity, it cannot be used as the sole criterion for identification.

Small zones of clearing may be caused by other enzymes or organic acid production[7].

Other organisms than staphylococci, *Serratia* and aeromonads can produce DNases.

Once the hydrochloric acid has been applied to the medium the plate must be read within a few minutes and further testing cannot be carried out by re-incubation.

The methyl green must be purified by extraction with chloroform[10].

Toluidine blue varies in performance according to source.

Merck Toluidine blue 1273 is satisfactory. Note that this dye cannot be used for Gram positive organisms.

References

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DSTA AGAR – see DIAGNOSTIC SENSITIVITY TEST AGAR

EC BROTH

Code: CM0853

A selective broth for the growth of coliforms and Escherichia coli from food and environmental samples.

Formula	***gm/litre***
Tryptone	20
Lactose	5.0
Bile Salts No. 3	1.5
Di-potassium phosphate	4.0
Mono-potassium phosphate	1.5
Sodium chloride	5.0
pH 6.9 ± 0.2	

Directions

Dissolve 18.5 g of EC Broth in 500 ml of distilled water. Dispense into final containers holding inverted Durham tubes. Sterilise by autoclaving at 121°C for 15 minutes. Gas production from lactose fermentation is indicated by using inverted Durham's tubes.

Description

EC Broth is a selective medium for the differentiation of faecal coliforms and the confirmatory test for *Escherichia coli* from food and environmental samples[3,4]. It is also suitable for the enumeration of presumptive *Escherichia coli* in milk and milk products using the most probable number technique[2].

Escherichia coli is a common Gram-negative micro-organism which may be present in food and water samples. The presence of *Escherichia coli* cells may be indicative of faecal contamination, although their ingestion does not necessarily have adverse effects on health. Nevertheless, certain strains of *Escherichia coli* are responsible for diarrhoeal disease and can also lead to more serious forms of illness.

The presence of *Escherichia coli* in water is also regarded as a specific indicator of faecal pollution, but lack of rapid identification methods means that the non-specific group – faecal coliforms – is also used as the indicator of such pollution. In this regard, it is important to differentiate between coliforms of faecal origin and those from other sources.

The identification criterion for *Escherichia coli* and faecal coliforms is the fermentation of lactose to acid and gas within 24-48 hours at 44-45.5°C, dependent upon which method and/or material is used:

Examination of shellfish[4]	44.5°C
Milk and milk products[2]	44.0°C
Water and wastewater[3]	44.5°C
Other foodstuffs[4]	45.5°C

Technique

The formulation includes a buffered lactose broth and bile salts No. 3. The presence of bile salts inhibits the growth of spore formers and enterococci, but allows the growth of *Escherichia coli* and coliforms. Gas production from lactose fermentation is indicated by using inverted Durham's tubes.

1. Milk and Milk Products/Presumptive *Escherichia coli*[2] (ISO)
   Tubes containing inverted Durham tubes with single and double strength Lauryl Tryptose Broth (CM0451) are inoculated with samples as described in the standard method. Incubate at 37°C for 24-48 hours and examine for gas production. Inoculate tubes showing gas production into a new series of tubes containing 10 ml of EC Broth containing Durham's tubes and incubate at 44°C for 24-48 hours. Transfer an inoculum from tubes giving rise to gas formation to tubes containing Tryptone Water (CM0087) and incubate for 24-48 hours at 44°C. Examine for indole production and calculate Most Probable Number of presumptive *Escherichia coli.* For details please refer to the ISO Standard Method.
2. Faecal Coliform Test 3 (APHA) For differentiation of faecal coliforms inoculate EC Broth fermentation tubes containing inverted Durham's tubes and incubate in a water bath at 44.5°C ± 2°C for 24 hours. Gas production and growth within 24 hours or less is considered a positive faecal coliform reaction. For details please refer to the APHA Standard Method.
3. Confirmatory Test for *Escherichia coli*[4] (FDA/BAM)
   From each gassing Lauryl Tryptose Broth (CM0451) tube, transfer a loopful of suspension to EC Broth tubes, containing inverted Durham's tubes. Incubate at 45.5°C and examine for gas production after 24 and 48 hours. Gassing tubes are streaked onto EMB Agar (CM0069) and incubated at 35°C for 18-24 hours. Transfer[2] suspicious colonies from each plate onto PCA (CM0325), incubate 18-24 hours at 35°C and confirm with morphological and biochemical test. For details please refer to the FDA/BAM Standard Methods.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C for up to 4 weeks.

Appearance

Dehydrated medium: White free-flowing powder.

Prepared medium: Straw coloured solution.

Quality control

Positive control:	***Expected results***
Escherichia coli ATCC® 25922*	Turbid growth; gas production
Negative control:	
Bacillus subtilis ATCC® 6633*	No growth

*This organism is available as a Culti-Loop®

References

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3. American Public Health Association (1998) *Standard Methods for the Examination of Water and Wastewater.* 20th edn. APHA Inc. Washington DC.
4. 'Association of Official Analytical Chemists' F.D.A. *Bacteriological Analytical Manual* 8th Edition (1995) AOAC, Arlington Va.
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EC BROTH with MUG

Code: CM0979

A selective broth for the growth of coliforms and Escherichia coli from food and environmental samples.

Formula	***gm/litre***
Tryptone	20.0
Lactose	5.0
Bile Salts No. 3	1.5
Di-potassium phosphate	4.0
Mono-potassium phosphate	1.5
Sodium chloride	5.0
4-methylumbelliferyl-β-D-glucuronide (MUG)	0.05
pH 6.9 ± 0.2	

Directions

Suspend 37 g of EC Broth with MUG in 1 litre of distilled water. Mix well to dissolve and distribute into final containers containing inverted Durham tubes. Sterilise by autoclaving at 121°C for 15 minutes.

Description

Escherichia coli is a common Gram-negative micro-organism which may be present in food, water and environmental samples. Owing to its importance as an indicator of feacal contamination, continuing efforts have been put into developing rapid and specific methods for its identification.

The finding of a highly specific enzyme of *Escherichia Coli*, beta-glucuronidase, has offered a range of oppertunities for the improvement of culture media for the identification of this organism. Numerous studies have confirmed a beta-glucuronidase-positive reaction in the middle to upper 90% range of *Escherichia coli* isolated from a variety of sources[1]. The highly sensitive substrate 4-methylumbellifery-β-D-gluuronide (MUG) is cleaved by the enzyme and the hydrolisis product 4-methylumbelliferone shows a visible green/blue flourence under long-wave ultra-violet light. (366nm).

EC Broth with MUG contains 4-methyl-β-D glucuronide which is cleaved by the enzyme glucuronidase (GUD) produced by *Escherichia coli* resulting in a fluorescent end-product. This medium is recommended by FDA/BAM[2] for the confirmation of *Escherichia coli* from shellfish meats.

For other details regarding the formulation of EC broth with MUG please refer to EC broth CM0853

Technique

Perform the MPN presumptive and confirmed test for faecal coliforms in shellfish. Transfer one loopful from the positive cultures into tubes of EC broth with MUG. Incubate at 44.5°C for 22-26 hours. Examine the tubes growth and gas production and for fluorescence (using long wave (365nm) UV light).

For full details of the method please refer to the FDA/BAM standard methods.

Storage Conditions and Shelf Life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance

Dehydrated Medium: Straw-free flowing powder

Prepared medium: Straw coloured solution

Quality Control

Positive Control:	***Expected Results***
Escherichia coli ATCC® 25922*	Turbid growth, fluorescence and gas production
Negative control:	
Bacillus subtilis ATCC® 6633*	Inhibited, negative fluorescence

*This organism is available as a Culti-Loop®.

Precautions
Ensure that the glass tubes used for the preparation of the medium do not auto fluoresce and take the relevant safety precautions when using a UV lamp.

Some pathogenic strains, typically *Escherichia coli* O157:H7 are usually glucuronidase negative and therefore will not give a positive result in this test.

Other enteric organisms e.g. some *Shigella* species are also glucuronidase positive.

References
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2. "Association of Official Analytical Chemists" F.D.A. *Bacteriological Analytical Manual* 8th Edition (1995) AOAC, Arlington Va.

EC BROTH (REDUCED BILE SALTS)

Code: CM0990

A selective enrichment broth for the growth of Escherichia coli O157, from food and environmental samples.

Formula	***gm/litre***
Tryptone	20.0
Lactose	5.0
Bile Salts No.3	1.12
Di-potassium phosphate	4.0
Mono-potassium phosphate	1.5
Sodium chloride	5.0
pH 6.9 ± 0.2	

NOVOBIOCIN SUPPLEMENT

Code: SR0181

Vial contents:	***per vial***	***per litre***
Novobiocin	10 mg	20 mg

Directions
Dissolve 18.3 g in 500 ml of distilled water. Sterilise by autoclaving at 121°C for 15 minutes. Cool the EC Broth (Reduced Bile Salts) to 50°C and aseptically add the contents of 1 vial of Novobiocin Supplement, reconsituted as directed. Dispense into final sterile containers.

Description
Novobiocin is an antibiotic which is active against Gram-positive bacteria such as *Staphylococcus aureus* (including methicillin-resistant strains) and other staphylococci. Some Gram-negative organisms including *Haemophilus influenzae*, *Neisseria* spp. and some strains of *Proteus* spp. are also susceptible; however most Enterobacteriaceae are resistant.

Novobiocin is primarily bacteriostatic, but in higher concentrations it may be bactericidal against more sensitive species. It principally inhibits synthesis of DNA, although RNA synthesis is also inhibited to a lesser extent. Its action on DNA is specifically related to the selective and competitive inhibition of the binding of ATP to gyrase B substrates. The gyrase enzyme forms negative supercoils from closed circular double-

stranded DNA, and is inhibited by novobiocin at low concentrations. However, novobiocin-resistant *Escherichia coli* cells contain a DNA gyrase which is unaffected by the drug. Novobiocin also inhibits protein and cell wall synthesis.

EC Broth (Reduced Bile Salts) can be used for the enrichment of *Escherichia coli* O157 directly from food and environmental samples, especially for use with frozen food samples, where the bacterium may be sub-lethally damaged[1]. Novobiocin may be added to provide a more selective primary enrichment medium.

The formulation includes a buffered lactose broth and reduced levels of Bile Salts No. 3 based on a recommendation of Szabo *et al.*[2]. The presence of the lower level of bile salts inhibits the growth of spore formers and enterococci, but allows the growth of *Escherichia coli* O157 at 37°C.

When using the reduced temperature of 37°C suggested by Okrend, supplementation with Novobiocin Supplement at a final concentration of 20 mg per litre improves reduction in background flora with no reduction in growth of *Escherichia coli* O157[3].

Technique

One method of use is as follows (based on Okrend[3]):

Prepare a 1 in 10 dilution of the sample in EC Broth (Reduced Bile Salts), supplemented with novobiocin as directed. Blend in a Stomacher™ for 2 minutes. Incubate for either 6 hours at 37°C on a rotary shaker at 100 rpm or static for 24 hours at 35°C.

After incubation, dilute the shaken culture to 10-2 and the overnight broth culture to 10-6. Streak onto appropriate selective agar e.g. Sorbitol MacConkey Agar (SMAC) CM0813 or SMAC with BCIG CM0981. Cefixime Tellurite Selective Supplement SR0172 may be added to increase selectivity of either medium.

Incubate at 37°C for 24 hours. *Escherichia coli* O157 colonies should look clear and colourless. Confirm suspected *Escherichia coli* O157 with the *Escherichia coli* O157 Latex Test DR0620 or Dryspot *Escherichia coli* O157 DR0120. Please refer to the reference for full method.

EC Broth (Reduced Bile Salts) and Novobiocin Supplement is a USDA/FSIS[4] and AIFST[1] recommended enrichment medium for the detection of *Escherichia coli* O157:H7 and O157:NM (NonMotile) from meat and poultry products.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Novobiocin Supplement should be stored in the dark at 2-8°C. When stored as directed, the supplement remains stable until the expiry date printed on the label.

Appearence

Dehydrated medium: White coloured, free flowing powder.

Prepared medium: Straw coloured solution.

Quality control

Positive control:	***Expected result***
Escherichia coli O157 (Non-toxigenic strain) NCTC 12900*	Turbid growth
Negative control:	
Staphylococcus aureus ATCC® 25923*	No growth

*This organism is available as a Culti-Loop®

References

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EDEL-KAMPELMACHER MEDIUM – see BRILLIANT GREEN AGAR (MODIFIED)

EDWARDS MEDIUM (MODIFIED)

Code: CM0027

A selective medium for the rapid isolation of Streptococcus agalactiae and other streptococci involved in bovine mastitis.

Formula	***gm/litre***
'Lab-Lemco' powder	10.0
Peptone	10.0
Aesculin	1.0
Sodium chloride	5.0
Crystal violet	0.0013
Thallous sulphate	0.33
Agar	15.0
pH 7.4 ± 0.2	

POISON – Contains Thallium Salt.

Directions

Suspend 41 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 115°C for 20 minutes. Cool to 50°C, add 5-7% of sterile bovine or sheep blood, mix well and pour plates.

Description

A selective medium for the rapid isolation of *Streptococcus agalactiae* and other streptococci involved in bovine mastitis.

Crystal violet or gentian violet and thallium salts have long been used in selective media for streptococci. Haxthausen[1] employed a selective crystal violet medium for the isolation of skin streptococci. Bryan[2] using gentian violet blood agar, found that the growth of saprophytic milk bacteria was prevented whilst that of streptococci was unaffected. Edwards[3] employed a crystal violet aesculin blood agar for the cultural diagnosis of bovine mastitis, whilst McKenzie[4] used a medium containing thallium acetate for the same purpose.

Hauge *et al*.[5] described a composite medium containing all the components of modified Edwards Medium.

Aesculin differentiates the negative *Streptococcus agalactiae* (blue colonies) from aesculin-positive Group D streptococci (black colonies).

Technique

Inoculate the surface of the medium with centrifuged deposits from milk samples and incubate at 35°C.

Look for pale blue colonies which should then be sub-cultured for further identification tests.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared plates at 2-8°C.

Appearance

Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured gel with blue hue.

Quality control

Positive controls:	***Expected results***
Streptococcus agalactiae ATCC® 13813*	Good growth; blue coloured colonies with no fermentation
Enterococcus faecalis ATCC® 29212*	Good growth; blue coloured colonies with fermentation
Negative controls:	
Escherichia coli ATCC® 25922*	No growth
Staphylococcus epidermidis ATCC® 12228*	No growth

*This organism is available as a Culti-Loop®

References

1. Haxsthausen H. (1927) *Ann. Derm. Syph*. 8. 201.
2. Bryan C. S. (1932) *Am. J. Pub. Hlth*. 22. 749.
3. Edwards S. J. (1933) *J. Comp. Path. Therap.* 46. 211-217.
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EE BROTH

Code: CM0317

An enrichment medium for Enterobacteriaceae in the bacteriological examination of foods.

Formula	***gm/litre***
Peptone	10.0
Glucose	5.0
Disodium hydrogen phosphate anhyd.	6.45
Potassium dihydrogen phosphate	2.0
Ox Bile purified	20.0
Brilliant green	0.0135
pH 7.2 ± 0.2	

Directions

Add 43.5 g to 1 litre of distilled water. Distribute 100 ml quantities in 250 ml flasks and heat at 100°C for 30 minutes only. Cool rapidly in cold running water. This medium is heat sensitive. DO NOT AUTOCLAVE.

Description

EE Broth (Buffered glucose – Brilliant Green-bile broth) is recommended as an enrichment medium for Enterobateriaceae in the bacteriological examination of foods[1] and animal feed stuffs[2]. This medium is more inhibitory to non-Enterobacteriaceae than other non-selective media, e.g. Mannitol broth[3] or Lactose broth[4] by virtue of the presence of brilliant green and bile salts in the preparation.

The enumeration of Enterobacteriaceae is of great importance in monitoring the sanitary quality of food and drugs but the reliability of the methods used depends upon resuscitation of damaged cells. Such weakened cells may arise from exposure to dehydration, low pH and other unfavourable conditions[5].

Incubation for 2 hours in well-aerated Tryptone Soya Broth CM0129 at 25°C should precede enrichment in EE Broth. This procedure is recommended for dried foods[6], animal feeds[7] and semi-preserved foods[8]. Occasionally, with a particular dry product, a longer incubation period is necessary but never over eight hours of resuscitation.

Oxoid EE Broth was formulated to overcome the unsatisfactory effects of inhibition on small numbers of Enterobacteriaceae cells due to bile salt variations. The inclusion of purified ox bile eliminated these problems and a preliminary assay can be used to check growth by inoculating approximately one viable cell per medium unit[9,10].

For the bacteriological evaluation of processed foods the entire Enterobacteriaceae group can be used as indicator organisms[10]. This will overcome the discrepancies that can arise when lactose-negative, anaerogenic lactose-positive or late lactose-fermenting Enterobacteria are present but are missed by the

standard 'coli-aerogenes' tests. To overcome these problems lactose media have been replaced by those containing glucose. Mossel *et al*.[1] cited several examples in the literature which referred to various foods contaminated with salmonellae, although results for coliforms were negative. A later example quoted by Mossel[9] involved an outbreak of diarrhoea caused by French mould-fermented soft cheese contaminated by *Escherichia coli* serotype O124. This organism is lactose-negative and therefore was not detected in coliform tests but only recognised when the commodity was tested for Enterobacteriaceae since it fermented glucose rapidly.

EE Broth should be used as an enrichment broth in conjunction with Violet Red Bile Glucose Agar CM0485. When specific organisms, rather than Enterobacteriaceae in general, are required sub-cultures must be made onto lactose differential media, e.g. Desoxycholate Citrate Agar CM0035, Brilliant Green Agar CM0329, or MacConkey Agar CM0007 for the detection of lactose-negative or delayed organisms.

Sample size should not be less than 10 g to yield the organisms being sought.

Technique

1. Resuscitate debilitated cells by incubating 1:10 dilutions of the food samples under investigation in Tryptone Soya Broth CM0129 at 25°C for 2-8 hours. The fluid layer should not be much deeper than one centimetre. Shake the flask to disperse the contents alternately in clockwise and anti-clockwise directions for 30 seconds on three successive occasions.
2. After the period of time necessary for resuscitation, ten-fold volumes of EE Broth are added to the resuscitated suspensions.
3. Shake to disperse as above. For large samples it is desirable to add the resuscitation medium containing the product under examination, to equal volumes of double strength EE Broth.
4. Incubate at 44°C for 18 hours for thermotrophic bacteria; 32°C for 24/48 hours for mesophilic bacteria; 4°C for 10 days for psychrotrophic bacteria depending on the groups of Enterobacteriaceae sought.
5. Examine the tubes of broth and look for turbidity with some change of colour towards yellowish-green for presumptive evidence of Enterobacteriaceae.
6. Sub-cultures can be made on to Violet Red Bile Glucose Agar CM0485 or onto lactose-containing media for confirmation of LF or NLF status. Further tests must be made to confirm the identity of the isolate.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared plates of medium at 2-8°C.

Appearance

Dehydrated medium: Pale green coloured, free-flowing powder.

Prepared medium: Green coloured solution.

Quality control

Positive controls:	***Expected results***
Salmonella typhimurium ATCC® 14028*	Turbid growth
Escherichia coli ATCC® 25922*	Turbid growth
Negative control:	
Staphylococcus aureus ATCC® 6538*	No growth

*This organism is available as a Culti-Loop®

Precautions

Avoid overheating the medium, especially the double-strength broth.

References

1. Mossel. D. A. A., Vissar M. and Cornellisen A. M. R. (1963) *J. Appl. Bact.* 26(3). 444-452.
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ENDO AGAR BASE

Code: CM0479

A modified medium requiring the addition of basic fuchsin to form Endo Agar.

Formula	***gm/litre***
Peptone	10.0
Lactose	10.0
Dipotassium phosphate	3.5
Sodium sulphite	2.5
Agar	10.0
pH 7.5 ± 0.2	

Directions

Suspend 36 g in 1 litre of distilled water. Add 4 ml (or as directed by the supplier) of a 10% w/v alcoholic solution of basic fuchsin BR0050 (95% Ethyl Alcohol). Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Mix well before pouring.

BASIC FUCHSIN IS A POTENTIAL CARCINOGEN AND CARE SHOULD BE TAKEN TO AVOID INHALATION OF THE POWDERED DYE AND CONTAMINATION OF THE SKIN.

Plates should be stored in the dark to preserve their pale pink colour.

Description

Endo Agar is a long established medium which was originally devised for the isolation of the typhoid bacillus. More reliable media for this purpose have since been evolved, and the medium is now used for the differentiation of lactose-fermenting and non-lactose-fermenting intestinal organisms, particularly during confirmation of the presumptive test for coliforms. Production of both acid and aldehyde by lactose-fermenting organisms, such as *Escherichia coli*, gives rise to the characteristic red coloration of the colony and the surrounding medium.

Technique

For the confirmation of presumptive tests with liquid media, sub-culture tubes showing gas, or acid and gas formation, onto an Endo Agar plate. Incubate for 24 hours at 35°C.

Lactose-fermenting coliforms (e.g. *Escherichia coli*) give rise to deep red colonies which colour the surrounding medium and possess a golden metallic sheen.

Non-lactose-fermenters form colourless translucent colonies, against the pink to colourless medium.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared plates at 2-8°C away from light.

Appearance

Dehydrated medium: straw coloured, free-flowing powder.

Prepared medium: straw coloured gel.

Quality control

Positive controls:	***Expected results***
Escherichia coli ATCC® 25922*	Good growth; red colonies, red medium, golden sheen
Enterobacter aerogenes ATCC® 13048*	Good growth; pink/red coloured colonies no sheen
Negative control:	
Staphylococcus aureus ATCC® 25923*	Inhibited

*This organism is available as a Culti-Loop®

Precautions

Weigh out the basic fuchsin (BR0050) in a fume cupboard and avoid inhalation of the powder or contamination of the skin.

Keep the prepared medium away from light to avoid photo-oxidation.

Endo Agar is quoted by the American Public Health Association as a 'Standard Methods' medium for use in water[1] and dairy products[2]. Windle Taylor[3] recommended the medium for the isolation and differentiation of coli-aerogenes bacteria from water.

References

1. American Public Health Association (1998) *Standard Methods for the Examination of Water and Wastewater.* 20th edn. APHA Inc. Washington DC.
2. American Public Health Association (1978) *Standard Methods for the Examination of Dairy Products*. 14th edn. APHA Inc. Washington DC.
3. Windle Taylor E. (1958) *The Examination of Waters and Water Supplies* 7th edn., Churchill Ltd., London, pp.417. 440-441, 780-781.

ENTEROCOCCUS AGAR – see SLANETZ AND BARTLEY MEDIUM

EOSIN METHYLENE BLUE AGAR (MODIFIED) LEVINE

Code: CM0069

An isolation medium for the differentiation of the Enterobacteriaceae.

Formula	***gm/litre***
Peptone	10.0
Lactose	10.0
Dipotassium hydrogen phosphate	2.0
Eosin Y	0.4
Methylene blue	0.065
Agar	15.0
pH 6.8 ± 0.2	

Directions

Suspend 37.5 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 60°C and shake the medium in order to oxidise the methylene blue (i.e. restore its blue colour) and to suspend the precipitate which is an essential part of the medium.

Description

This versatile medium, modified by Levine[1,2], is used for the differentiation of *Escherichia coli* and *Enterobacteria aerogenes*, for the rapid identification of *Candida albicans*, and for the identification of coagulase-positive staphylococci.

The medium is prepared to the formula specified by the APHA[3,4,5,6] for the detection and differentiation of the coliform group of organisms[7,8].

Weld[9,10] proposed the use of Levine eosin methylene blue agar, with added chlortetracycline hydrochloride for the rapid identification of *Candida albicans* in clinical materials. A positive identification of *Candida albicans*

could be made after 24 to 48 hours incubation at 37°C in 10% carbon dioxide from faeces, oral and vaginal secretions, and nail or skin scrapings. Vogel and Moses[11] confirmed the reliability of Weld's method for the relatively rapid identification of *Candida albicans* in sputum. They found that use of eosin methylene blue agar was just as reliable as more conventional methods for the identification of this organism in sputum. In addition, the medium provided a means for the identification of several Gram-negative genera. Doupagne[12] also investigated the use of the Levine medium under tropical conditions.

Haley and Stonerod[13] found that Weld's method was variable so that Walker and Huppert[14] advocated the use of corn meal agar and a rapid fermentation test in addition to the Levine medium. Using the combined rapid technique they were able to obtain results within 48 to 72 hours.

Subsequent to the findings of Vogel and Moses[11], Menolasino *et al*.[15] used Levine eosin methylene blue agar for the identification of coagulase-positive staphylococci which grew as characteristic colourless, pin-point colonies. The Levine medium was more efficient than tellurite glycine agar and showed good correlation with the plasma coagulase test.

Colonial Characteristics

Escherichia coli – isolated colonies, 2-3 mm diameter, with little tendency to confluent growth, exhibiting a greenish metallic sheen by reflected light and dark purple centres by transmitted light.

Enterobacter aerogenes – 4-6 mm diameter, raised and mucoid colonies, tending to become confluent, metallic sheen usually absent, grey-brown centres by transmitted light.

Non-lactose-fermenting intestinal pathogens – translucent and colourless.

Candida albicans – after 24 to 48 hours at 35°C in 10% carbon dioxide 'spidery' or 'feathery' colonies. Other *Candida* species produce smooth yeast-like colonies. Since a typical appearance is variable it is advisable to use a combined method such as that of Walker and Huppert[14].

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared plates at 2-8°C away from light.

Appearance

Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Light straw to straw coloured gel

Quality control

Positive controls:	***Expected results***
Escherichia coli ATCC® 25922*	Good growth; purple coloured colonies with green metallic sheen
Enterobacter aerogenes ATCC® 13048*	Good growth; purple mucoid colonies
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

Precautions

Further tests are required to confirm the presumptive identity of organisms isolated on this medium. Some strains of *Salmonella* and *Shigella* species will not grow in the presence of eosin and methylene blue. Store the medium away from light to prevent photo-oxidation.

References

1. Levine M. (1918) *J. Infect. Dis*. 23. 43-47.
2. Levine M. (1921) *Bacteria Fermenting Lactose and the Significance in Water Analysis* Bull. 62. Iowa State College Engr. Exp. Station.
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12. Doupagne P. (1960) *Ann. Soc. Belge de Med. Trop.* 40(6). 893-897.
13. Haley L. D. and Stonerod M. H. (1955) *Am. J. Med. Tech.* 21. 304-308.
14. Walker Leila and Huppert M. (1959) *Am. J. Clin. Path.* 31. 551-558.
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FRASER BROTH

Code: CM0895

A secondary selective diagnostic enrichment medium for the isolation of Listeria spp. from food and environmental specimens.

Formula	***gm/litre***
Proteose peptone	5.0
Tryptone	5.0
'Lab-Lemco' powder	5.0
Yeast extract	5.0
Sodium chloride	20.0
Di-sodium hydrogen phosphate	12.0
Potassium dihydrogen phosphate	1.35
Aesculin	1.0
Lithium chloride	3.0
pH 7.2 ± 0.2	

FRASER SUPPLEMENT

Code: SR0156

Vial contents (each vial is sufficient to supplement 500 ml of medium)	***per vial***	***per litre***
Ferric ammonium citrate	0.25 g	0.5 g
Nalidixic acid	10.0 mg	20.0 mg
Acriflavine hydrochloride	12.5 mg	25.0 mg

Directions
Suspend 28.7 g in 500 ml of distilled water. Sterilise by autoclaving at 121°C for 15 minutes, bring to the boil to dissolve completely. Cool to 50°C and aseptically add the contents of one vial of Fraser Selective Supplement reconstituted as directed. Mix well and distribute into sterile containers.

Description
Fraser Medium is a modification of the USDA-FSIS (United States Department of Agriculture-Food Safety Inspection Service) UVM secondary enrichment broth and is based on the formula described by Fraser and Sperber[1]. It contains ferric ammonium citrate and lithium chloride. Blackening of the medium is presumptive evidence of the presence of *Listeria*. Contrary to early indications, cultures which do not blacken cannot be assumed to be *Listeria*-free. All Fraser Broth enrichment cultures should be sub-cultured to plating medium.

The medium is intended for the isolation of *Listeria* spp. from food and environmental samples when used as the secondary enrichment medium in the USDA-FSIS methodology for *Listeria* isolation.

It is generally accepted that the USDA-FSIS two stage enrichment method employing UVM primary and secondary enrichment broths is the most suitable for the examination of meat products. Fraser Broth has proven to be remarkably accurate in detecting *Listeria* spp. in food and environmental samples[1,2].

All *Listeria* spp. hydrolyse aesculin to aesculetin. Aesculetin reacts with ferric ions which results in blackening. Another possible advantage to the addition of ferric ammonium citrate is that it has been shown that ferric ions enhance the growth of *Listeria monocytogenes*[3].

Lithium chloride is included in the medium to inhibit the growth of enterococci which can also hydrolyse aesculin.

Care must be taken when using Fraser Broth with DNA probe methodology because the high salt content of the medium may have an inhibitory effect on detection[4].

Technique

1. Inoculate 10 ml of Fraser Broth with 0.1 ml of the primary enrichment broth (i.e. FDA or UVM I enrichment broth) which has been incubated for 20-24 hours.
2. Incubate at 35°C for 26 ± 2 hours in air.
3. Compare each inoculated tube to an inoculated control against a white background. Tubes that darken or turn black should be sub-cultured onto Oxford Medium or PALCAM Medium. Tubes that retain the original yellow colour should also be inoculated on plating media and confirmed as free from *Listeria* spp. before discarding.

It should be emphasised that the incubation period should be controlled. Fraser Medium should be incubated for 26 ± 2 hours to ensure at least 24 hours incubation period to permit the development of the black colour.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the selective supplement in the dark at 2-8°C and use before the expiry date on the label.

The prepared medium may be stored for up to 2 weeks at 2-8°C.

Appearance

Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured solution.

Quality control

Positive control:	***Expected result***
Listeria monocytogenes ATCC® 7466*	Blackening
Negative control:	
Pseudomonas aeruginosa ATCC® 27853*	No blackening

*This organism is available as a Culti-Loop®

References

1. Fraser J. A. and Sperber W. H. (1988) *J. Food Protect.* 51. No.10. 762-765.
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3. Cowart R. E. and Foster B. G. (1985) *J. Infect. Dis.* 151. 721-730.
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HALF FRASER SUPPLEMENT

Code: SR0166

A selective supplement for the isolation of Listeria spp. from food and environmental samples when used with Fraser Broth.

Vial contents (each vial is sufficient to supplement 225 ml of medium)	***per vial***	***per litre***
Ferric ammonium citrate	112.50 mg	500 mg
Nalidixic acid	2.25 mg	10.0 mg
Acriflavine hydrochloride	2.8125 mg	12.5 mg

Directions

Suspend 14.35 g of Fraser Broth in 225 ml of distilled water. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C and aseptically add the contents of one vial of Half Fraser Selective Supplement as directed and mixed gently to dissolve. Mix well and distribute into final containers.

GBS MEDIUM – see GROUP B STREPTOCOCCI MEDIUM

GC MEDIUM – see NEW YORK CITY MEDIUM AND OR THAYER MARTIN MEDIUM

GARDNERELLA VAGINALIS SELECTIVE MEDIUM

A selective supplement for the isolation of Gardenerella vaginalis

COLUMBIA BLOOD AGAR BASE

Code: CM0331

Formula	***gm/litre***
Special peptone	23.0
Starch	1.0
Sodium chloride	5.0
Agar	10.0
pH 7.3 ± 0.2	

Directions
Add 39 g to 1 litre of distilled water. Boil to dissolve and sterilise by autoclaving at 121°C for 15 minutes.

GARDNERELLA VAGINALIS SELECTIVE SUPPLEMENT

Code: SR0119

Vial contents (each vial is sufficient for 500 ml of medium)	***per vial***	***per litre***
Gentamicin sulphate	2.0 mg	4.0 mg
Nalidixic acid	15.0 mg	30.0 mg
Amphotericin B	1.0 mg	2.0 mg

Directions
Reconstitute one vial as directed, aseptically add the contents to 450 ml of sterile Columbia Blood Agar Base cooled to approximately 50°C, and supplement with 50 ml of sterile human, rabbit or horse blood. Mix well and pour into sterile Petri dishes. For the double layer technique hold the medium in a water bath at 50°C.

Description
Gardnerella Vaginalis Selective Supplement, is based on the formulation of Ison *et al*.[1] and is recommended for the selective isolation of *Gardenerella vaginalis* from the vaginal discharge of patients with symptoms of Non-specific Vaginitis (NSV). The symptoms of this mild condition prior to the isolation of the aetiological agent(s) are:

1. The absence of recognised pathogens.
2. Foul smelling discharge.
3. pH greater than 4.5.
4. Release of 'fish' odour on the addition of potassium hydroxide (10%) to the discharge.
5. The presence of 'clue' cells in prepared wet mounts (these are epithelial cells with a characteristic stippled or granular appearance caused by Gram variable bacilli adhering to the cell surface).

Several media and techniques have been described for the isolation of *Gardenerella vaginalis*. Gardnerella Vaginalis Selective Medium can be used for the surface inoculation technique or the double layer technique[2].

With added human blood or rabbit blood[3], a betahaemolytic reaction is exhibited by *Gardenerella vaginalis*. This can be used as a preliminary diagnosis feature[1]. The addition of 'Tween 80' (0.02% v/v) to the medium containing human blood has been found to give enhanced beta-haemolytic zones[4,5].

Gardenerella vaginalis is a Gram variable, small, pleomorphic bacillus which forms 0.25-0.5 mm diameter colonies producing beta-haemolysis on medium containing human blood.

Technique

Surface Inoculation Method (Isolation)

1. Prepare the selective medium from Columbia Blood Agar Base, Gardnerella Vaginalis Selective Supplement and defibrinated Horse Blood SR0050, according to the directions. To demonstrate the characteristic haemolysis substitute horse blood with human or rabbit blood when preparing the medium.
2. Using a swab inoculate the vaginal discharge the medium.
3. Incubate, at 35°C for 48 hours in an atmosphere containing 7% carbon dioxide[6].
4. Carry out confirmatory tests on all colonies from medium containing horse blood and on betahaemolytic colonies from medium containing human blood or rabbit blood.

Double Layer Method (Isolation and Presumptive identification)

1. Prepare two lots of selective medium from Columbia Blood Agar Base, Gardnerella Vaginalis Selective Supplement and sterile human blood according to the directions.
2. Use one lot to prepare base medium plates and place the second lot in a water bath at 50°C.
3. Using the swab inoculate the vaginal discharge on to the surface of the prepared plates. Allow to dry at room temperature for half an hour.
4. Overlay with 5 ml of the selective medium at 50°C.
5. Allow the overlay medium to set.
6. Incubate at 35°C for 48 hours in an atmosphere containing 7% carbon dioxide.
7. Carry out confirmatory tests on isolates that show a beta-haemolytic zone. Use an inoculating wire to stab through the agar overlay to reach the colonies beneath.

The following tests have been compiled from the literature and personal communication.

Test or Substrate	*Test Result %*	*Positive*
Oxidase	Negative	0
Catalase	Negative	0
Haemolysis of:		
Human blood	Positive	967
Rabbit blood	Positive	96
Horse blood	Negative	some strains
Sheep blood	Negative	07
Hippurate hydrolysis	Positive	92
Starch hydrolysis	Positive	90
Metronidazole 50 µg	Susceptible	90
Trimethoprim 5 µg	Susceptible	100
Sulphonamide 1000 µg	Resistant	0

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared plates at 2-8°C.

Appearance

Dehydrated Medium: Straw coloured, free-flowing powder.

Prepared medium: Opaque red coloured gel.

Quality control

Positive controls:	***Expected results***
Gardenerella vaginalis ATCC® 14018	Good growth; grey/white colonies
Negative control:	
Proteus mirabilis ATCC® 29906	Inhibited

*This organism is available as a Culti-Loop®

References

1. Ison C. A., Dawson S. G., Hilton J., Csonka G. W. and Easmon C. S. F. (1982) *J. Clin. Path.* 35. 550-554.
2. Spiegel C. A., Eschenbach D., Schoenknech F. and Holmes K. K.(1980) *N. Engl. J. Med.* 303. 601-607.
3. King E. A. (1964) *'The Identification of Unusual Pathogenic Gram negative Bacteria' Center for Disease Control, Atlanta GA* (quoted in Reference 7).
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5. Totton P. A., Amsel R., Hale J., Piot P. and Holmes K. K. (1972) *J. Clin. Microbiol.* 15. 141-147.
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GIOLITTI-CANTONI BROTH

Code: CM0523

An anaerobic enrichment broth for Staphylococcus aureus.

Formula	***gm/litre***
Tryptone	10.0
'Lab-Lemco' powder	5.0
Yeast extract	5.0
Lithium chloride	5.0
Mannitol	20.0
Sodium chloride	5.0
Glycine	1.2
Sodium pyruvate	3.0
pH 6.9 ± 0.2	

Directions

Suspend 54.2 g in one litre of distilled water and heat gently to dissolve. Dispense 19 ml amounts into 20 mm x 200 mm test tubes and sterilise by autoclaving at 121°C for 15 minutes. Cool rapidly then aseptically add to each tube 0.3 ml of a sterile solution of Potassium tellurite 3.5% SR0030.

The medium requires the addition of a 3.5% solution of Potassium tellurite when there is a direct addition of 1 gram of the sample to 19 mls of broth. This level of Potassium tellurite is necessary to suppress the high numbers of contaminating organisms that could be expected.

The use of a diluted solution of Potassium tellurite is applicable when a 1 in 10 dilution of the food sample is carried out[1]. In such cases the SR0030 should first be diluted 1 in 10 with sterile distilled water.

The addition of 0.1% Tween 80 can be recommended in order to improve recovery of heat injured *Staphylococcus aureus* cells, e.g. from milk powder. 1 gram of Tween 80 should be added to 1 litre of prior to autoclaving[2].

Description

Oxoid Giolitti-Cantoni Broth, a tellurite-mannitol-glycine enrichment broth, based on the formulation of Giolitti and Cantoni[3] is used for the selection and enrichment of *Staphylococcus aureus* from foodstuffs. Mannitol and sodium pyruvate are growth stimulants for staphylococci and aid detection of the organism when present in small numbers only[4].

The growth of Gram-negative lactose-fermenting bacilli are inhibited by lithium chloride and Gram-positive bacilli are inhibited by Potassium tellurite in combination with glycine.

The creation of anaerobic conditions by overlaying with 2 cm of sterile paraffin wax inhibits the growth of micrococci.

Giolitti-Cantoni Broth is recommended for the detection of *Staphylococcus aureus* in dried baby milk and other weaning foods where the organism should be absent from 1 gram of test material[6].

The medium is suitable for the examination of meat and meat products[7]. For this purpose the concentration of the Potassium tellurite must be reduced to 0.35% and it is recommended that the weight of the test sample should be reduced to 0.1-0.01 gm.

Technique

The medium should be inoculated as soon as it has been cooled after autoclaving. If there is a delay in putting the medium to use it must be freed from dissolved air by immersion in free-flowing steam for 20 minutes.

Inoculate 1 gram of sample material and 1 ml aliquots of a series of suitable decimal dilutions into tubes containing 19 ml of Giolitti-Cantoni Broth. Two tubes are used for the sample material and for each of the dilutions. This increases the likelihood of detecting *Staphylococcus aureus* when it is present in very small numbers.

The medium is overlaid with 2 cm of molten sterile paraffin wax (melting temperature 42-44°C) and incubated for 48 hours at 35°C, examining daily. The result is considered negative for *Staphylococcus aureus* if no blackening of the medium is observed. If blackening does occur at the bottom of the tubes or general blackening of the medium, the broth is streaked on to a staphylococcal isolation medium, such as Baird-Parker Medium[8], and incubated at 35°C for 24-48 hours. The result is considered positive if black colonies, with a narrow white margin, surrounded by a zone of clearing, are seen.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance

Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured solution.

Quality control

Positive control:	***Expected results***
Staphylococcus aureus ATCC® 25923*	Turbid growth; blackening†
Negative control:	
Staphylococcus epidermidis ATCC® 12228*	Reduced growth; no blackening†

*This organism is available as a Culti-Loop®; †Inoculum dependent.

References

1. IDF International Standard 60A: 1978.
2. Chopin *et al.* (1985) *J. Food Prod.* 48 No.1. 21-27.
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4. Baird-Parker A. C. (1962) *J. Appl. Bact.* 25. 12.
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GBS AGAR BASE (ISLAM)

Code: CM0755

For the isolation and detection of Group B streptococci (GBS) in clinical specimens.

Formula	***gm/litre***
Proteose peptone	23.0
Soluble starch	5.0
Sodium dihydrogen phosphate	1.5
Di-sodium hydrogen phosphate	5.75
Agar	10.0
pH 7.5 ± 0.1	

Directions
Suspend 45.2 g in 1 litre of distilled water and bring to the boil to disolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C and aseptically add 50 ml of sterile inactivated Horse Serum‡. Mix well and pour into Petri dishes.

‡ Sterile Inactivated Horse Serum
Hold sterile Horse Serum (Oxoid SR0035) at 56°C for 30 minutes.

Description
GBS Agar Base is based on the formulation described by Islam[1]. The medium is designed to exploit the ability of most group B streptococci (GBS) to produce orange/red pigmented colonies when incubated under anaerobic conditions.

Group B streptococci are a recognised cause of serious neonatal infection acquired from the infected mother. A review[2] of national data over an 8 year period by the Public Health Laboratory Service showed that group B streptococci accounted for 29.5% of all reports of neonatal bacterial meningitis with organisms being isolated from CSF and blood.

Group B streptococci may also be isolated from adults infected in a variety of sites.

The pigment of group B streptococci has characteristics of a carotenoid[3] and was first noted by Lancefield in 1934 in nine of twenty-four strains grown anaerobically. Modifications of media[1,4,5] have improved the proportion of pigmented strains to about 97%. Noble *et al*.[6] reported that in their studies 99.5% of beta-haemolytic GBS strains produced pigment. GBS Agar also supports growth of other genital bacteria that cause perinatal infections[1], e.g. anaerobic streptococci, *Bacteroides* and *Clostridium* species.

Colonies of group B streptococci are 0.5-1 mm in diameter, round, entire and pigmented orange/red after 24-48 hours anaerobic incubation. Other organisms able to grow on this medium do not produce the orange/red pigment.

de la Rosa *et al*.[7] demonstrated the pigment-enhancing effect of trimethoprim/sulphonamides added to their medium. Work carried out in the Oxoid laboratories has shown that this pigment-enhancing effect can also be demonstrated around a sulphonamide disc placed on the inoculated plate. Standard discs of SF300 or SF500 can be used for this purpose. No inhibition of growth occurs and the enhanced pigment effect is clearly seen over a radius of 10-20 mm.

Technique
1. Swabs should be collected into Stuart's Transport Medium CM0111 and processed within 1/2- 2 hours of collection[8].
2. Inoculate the swab on to the surface of GBS Agar.
3. If desired, apply a disc containing 300 or 500 of Sulphafurazole on to an area of the plate where growth can be expected to be moderately profuse. These discs are available from Oxoid.
4. Incubate the plates anaerobically at 35°C for 24-48 hours.
5. Report all orange/red pigmented colonies as presumptive group B streptococci.
6. Identity can be confirmed using an Oxoid Streptococcal Grouping Kit DR0585 or Oxoid Dryspot Streptococcal Grouping Kit DR0400

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared plates at 2-8°C away from light.

Appearence
Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured gel.

Quality Control

Positive control:	***Expected results:***
Streptococcus agalactiae ATCC® 13813*	Good growth orange pigmented colonies
Negative control:	
Enterococcus faecalis ATCC® 29212*	Good growth non-pigmented colonies

*This organism is available as a Culti-loop®

Precautions
The medium must be at its correct pH value to ensure good pigmentation.

Some strains of Group B streptococci do not produce pigmented colonies.

Do not hold the molten medium any longer than necessary.

References

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GVPC MEDIUM – see LEGIONELLA GVPC SELECTIVE MEDIUM

GVPN MEDIUM – see LEGIONELLA GVPN SELECTIVE MEDIUM

HAEMOPHILUS TEST MEDIUM HTM BASE

Code: CM0898

A medium specifically formulated for the susceptibility testing of Haemophilus influenzae.

Formula	***gm/litre***
Mueller-Hinton Agar	38.0
Yeast extract (specifically selected for low antagonist levels)	5.0
pH 7.4 ± 0.2	

HTM SUPPLEMENT

Code: SR0158

Vial contents (each vial is sufficient for 500 ml of medium)	***per vial***	***per litre***
NAD	7.5 mg	15 mg
Haematin	7.5 mg	15 mg

Directions
Suspend 21.5 g of Haemophilus Test Medium Base in 500 ml of distilled water. Bring to the boil to dissolve. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C and aseptically add the contents of 1 vial of Haemophilus Test Medium Supplement, as directed. Mix well and pour into sterile Petri dishes.

Description

Haemophilus Test Medium (HTM) has been specifically formulated for the susceptibility testing of *Haemophilus influenzae*. The medium forms part of the recommended methods of the United States National Committee for Clinical Laboratory Standards (NCCLS)[1].

Haemophilus influenzae require complex media for growth. These complex media have aggravated the routine susceptibility testing of *Haemophilus influenzae* because of antagonism between some essential nutrients and certain antimicrobial agents. Difficulties in interpreting inhibition zones may also arise.

Oxoid Haemophilus Test Medium (HTM) is based on the formulation developed by Jorgensen *et al*.[2] which is now recommended by the United States NCCLS. The results achieved using HTM have been found to be highly reproducible[3,4]. Comparisons with Mueller-Hinton Chocolate Agar have shown an overall agreement of 99.6%[5].

The transparency of the medium allows zones of inhibition to be read easily through the bottom of the Petri dish. HTM contains low levels of antimicrobial antagonists, which allows testing of trimethoprim/sulphamethoxazole to be carried out with greater confidence.

Technique

1. Prepare the inoculum in Mueller-Hinton Broth (Oxoid CM0405) or 0.9% saline, to match the turbidity of 0.5 McFarland standard.
2. Using a swab saturated with the above inoculum suspension, inoculate the surface of a Haemophilus Test Medium Agar plate to give confluent growth.
3. Apply the antimicrobial discs on to the surface of the Haemophilus Test Medium plate.
4. Incubate the plates at 35°C in 5-7% carbon dioxide for 16-18 hours and measure the zones of inhibition.

NB: The CO_2 enriched atmosphere can best be achieved by using the Oxoid Gas Generating Kit BR0039 in conjunction with Oxoid Anaerobic Jar or alternatively use CO_2Gen CD0025.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared plates of medium at 2-8°C.

HTM Supplement as supplied should be stored in the dark below 0°C. When stored as directed the supplement is stable until the expiry date stated on the label.

Appearance

Dehydrated medium: Light straw coloured, free-flowing powder.

Prepared medium: Straw coloured gel.

Quality control

Positive control:	***Expected results***
Haemophilus influenzae ATCC® 49766*	Good growth; straw coloured colonies
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

References

1. NCCLS Documents M2-A7 Vol. 20. No.1. and M7-A5 Vol. 20. No 2.
2. Jorgensen J. H., Redding J. S., Maher L. A. and Howell A. W. (1987) *J. Clin. Micro.* 25. 2105-2113.
3. Doern G. V., Jorgensen J. H., Thornsberry C. and Snapper H. (1990). *Eur. J. Clin. Microbiol. Infect. Dis.* 9. 329-336.
4. Barry A. L., Jorgensen J. H. and Hardy D. J. (1991) *J. Antimic. Chem.* 27. 295-301.
5. Evans G., Marsik F., Thompson L. and Fowler J. (1990) *Abstracts of ASM Meeting* 1990 C-252.

HALF FRASER BROTH – see FRASER BROTH

HEART INFUSION BROTH

Code: CM1032

A highly nutritious liquid medium recommended for the cultivation of fastidious organisms.

Formula	*gm/litre*
Beef heart infusion solids	17.5
Proteose peptone	10.0
Glucose	2.0
Sodium chloride	5.0
Di-sodium phosphate	2.5
pH 7.4 ± 0.2	

Directions
Dissolve 37 g in 1 litre of distilled water. Mix well and distribute into final containers. Sterilise by autoclaving at 121ºC for 15 minutes.

Description
Heart Infusion Broth has been developed to give the same performance characteristics as Brain Heart Infusion (BHI) Broth (CM0225). However, as bovine brain is a specified risk material, the exclusion of it from the Heart Infusion Broth means that the regulatory requirements when using it are lower.

The medium has been developed to give equivalent performance to Brain Heart Infusion but the exclusion of calf brain infusion means that Heart Infusion Broth carries a lower regulatory burden.

Simple additions may be made to the medium to make it suitable for the cultivation of yeasts and moulds and for use in blood culture.

A highly nutritious infusion medium recommended for the cultivation of streptococci, pneumococci, meningococci and other fastidious organisms.

Technique
Heart Infusion Broth can be supplemented with yeast extract, haemin and menadione to improve the growth of *Bacteroides* spp.

A medium for blood culture can be prepared by adding 1 g of agar per litre of Heart Infusion. Ensure that the agar is uniformly distributed in the sterile broth before dispensing into bottles. More conveniently, add 1 Agar Tablet (CM0049) to each 100 ml of Heart Infusion and sterilise by autoclaving for 15 minutes at 121ºC. Cool to 60-70ºC and mix gently to ensure uniform distribution of the agar.

Supplements to improve the recovery of organisms from blood can be added before sterilisation or aseptically post-sterilisation. Co-enzyme 1 (NAD), penicillinase and p-amino benzoic acid are examples. The addition of anti-microbial agents such as cycloheximide and chloramphenicol also make this medium suitable for the cultivation of yeasts and moulds.

Storage conditions and Shelf life
The dehydrated medium should be stored at 10-30°C and used before the expiry date on the label.

Store the prepared medium in the dark below 20°C.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured liquid.

Quality control

Positive controls:	***Expected results***
Streptococcus pneumoniae ATCC® 6303*	Turbid growth
Candida albicans ATCC® 13048*	Turbid growth
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

Precautions
Heart Infusion Broth should only be used for *in vitro* diagnostic purposes.

References
1. Bridson E. Y. (Ed) (1998) *The Oxoid Manual*, 8th Edition, Oxoid Ltd, UK, pp.2-51.2-52.

HEKTOEN ENTERIC AGAR

Code: CM0419

A differential, selective medium for the isolation of Shigella and Salmonella species from enteric pathological specimens.

Formula	***gm/litre***
Proteose peptone	12.0
Yeast extract	3.0
Lactose	12.0
Sucrose	12.0
Salicin	2.0
Bile salts No. 3	9.0
Sodium chloride	5.0
Sodium thiosulphate	5.0
Ammonium ferric citrate	1.5
Acid fuchsin	0.1
Bromothymol blue	0.065
Agar	14.0
pH 7.5 + 0.2	

Directions
Suspend 76 g of the medium in 1 litre of distilled water and soak for 10 minutes. Heat gently and allow to boil for a few seconds to dissolve the agar. DO NOT AUTOCLAVE. Cool to 50°C and pour plates.

Description
Hektoen Enteric Agar was developed by King & Metzger[1]. The high peptone content offsets the inhibitory effect of bile salts on *Shigella* species in particular. The additional carbohydrates (sucrose and salicin) give better differentiation than lactose alone and the lower toxicity of the double indicator improves recovery. The increased lactose content helps early recognition of slow lactose-fermenting organisms. The thiosulphate and ferric citrate are present to detect H_2S-producing organisms.

Taylor & Schelhaut[2] found the medium to be of value in the differentiation of pathogenic organisms and for better growth of shigellae.

Hoben *et al*.[3] added novobiocin 15 mg/litre to improve the selectivity of the medium by inhibiting *Citrobacter* and *Proteus* species.

Hektoen Enteric Agar meets the requirements of the APHA[4].

Technique
Inoculate the medium with fresh faeces suspended in Ringers solution or inoculate directly with rectal swabs. Spread the inoculum to obtain well separated colonies. Incubate for 18-24 hours at 37°C. Further incubation will improve differentiation between shigellae and salmonellae.

Organism characteristics:

Shigella	Green, moist raised colonies
Salmonella	Blue-green colonies with or without black centres
Coliforms (rapid lactose/sucrose/ salicin-fermenters)	Salmon-pink to orange colonies surrounded by a zone of bile precipitation

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared plates at 2-8°C.

Appearance
Dehydrated medium: Pale green coloured, free-flowing powder.

Prepared medium: Green to dark green coloured gel.

Quality control

Positive controls:	***Expected result***
Salmonella typhimurium ATCC® 14028*	Good growth; blue-green coloured colonies with black centres
Shigella flexneri ATCC® 12022*	Good growth; green coloured colonies
Negative controls:	
Escherichia coli ATCC® 25922*	Inhibited or no growth
Enterococcus faecalis ATCC® 29212*	No growth

*This organism is available as a Culti-Loop®

Precautions
Do not overheat the medium or hold it molten for long periods. *Proteus* species may resemble salmonellae or shigellae.

Further testing must be carried out to confirm the presumptive identification of organisms isolated on this medium.

References
1. King S. and Metzger W. I. (1968) *Appl. Microbiol.* 16. 577-561.
2. Taylor W. I. and Schelhaut D. (1971) *Appl. Microbiol.* 21. 32-37.
3. Hoben D. A., Ashton D. H. A. and Peterson A. C. (1973) *Appl. Microbiol.* 21. 126-129.
4. American Public Health Association (1992) *Compendium of Methods for the Microbiological Examination of Foods* 3rd Edition. APHA Inc. Washington DC.

HELICOBACTER PYLORI SELECTIVE MEDIUM

A selective supplement for the isolation of Helicobacter pylori from clinical specimens.

COLUMBIA BLOOD AGAR BASE

Code: CM0331

Formula	***gm/litre***
Special peptone	23.0
Starch	1.0
Sodium chloride	5.0
Agar	10.0
pH 7.3 ± 0.2	

Directions
Add 39 g to 1 litre of distilled water. Boil to dissolve and sterilise by autoclaving at 121°C for 15 minutes.

HELICOBACTER PYLORI SELECTIVE SUPPLEMENT (DENT)

Code: SR0147

Vial contents (each vial is sufficient for 500 ml of medium)	***per vial***	***per litre***
Vancomycin	5.0 mg	10.0 mg
Trimethoprim	2.5 mg	5.0 mg
Cefsulodin	2.5 mg	5.0 mg
Amphotericin B	2.5 mg	5.0 mg

Directions
Reconstitute one vial as directed, aseptically add the contents to 500 ml of Columbia Blood Agar Base cooled to approximately 50°C. Add 35 ml of Laked Horse Blood SR0048 and mix well before pouring into sterile Petri dishes.

Description
Helicobacter pylori is associated with a number of gastric conditions, chiefly gastritis and peptic ulcers[1,2,3].

Helicobacter pylori Selective Supplement (Dent) was developed from Dent's selective medium described for the isolation of *Helicobacter pylori* from gastric biopsies[2]. This is a modification of Skirrow's medium[4] in which polymixin B is replaced by cefsulodin and amphotericin B is added to inhibit *Candida* species.

When used routinely in the laboratory for 100 gastric biopsies, Dent's medium achieved a higher isolation rate for *Helicobacter pylori* and lower contamination by other organisms when compared with Skirrow's medium and chocolate blood agar[2]. The provision of a good selective medium for *Helicobacter pylori* will help establish the role of this organism in the aetiology of gastric disease.

Technique
1. Prepare the medium as directed. The plates can be stored at 4°C for three weeks but it is essential that they are kept moist. This can be achieved simply by keeping the plates in a plastic bag.
2. Smear the specimen on to the medium. Note – the recovery of *Helicobacter pylori* from gastric biopsies is improved by direct cultivation as soon as possible after collection. If transportation is necessary, then place the biopsy against the neck of a small, sterile glass bottle containing 0.1 ml of sterile saline[2]. The biopsy should adhere to the glass but be protected from dehydration by water vapour.
3. Incubate at 35°C for three to five days under microaerophilic conditions. Use Campylobacter Gas Generating Kit BR0056 or BR0060 with an active catalyst BR0042. Alternatively use CampyGen CN025 or CN035. CampyGen does not require the addition of water or a catalyst.
4. Examine for the presence of discrete, translucent and non-coalescent colonies. Note that colonies of *Helicobacter pylori* do not resemble those of *Campylobacter* species.
5. Confirm the identity of the isolates with the following tests:

   Gram negative, curved or spiral bacillus. Growth at 35°C, no growth at 25°C, variable growth at 42°C. Urease positive, Catalase positive, Oxidase positive, Hippurate negative.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance
Dehydrated Medium: Straw coloured, free-flowing powder.

Prepared medium: Opaque red coloured gel.

Quality control

Positive controls:	***Expected results***
Helicobacter pylori ATCC® 43526	Good growth; colourless colonies
Negative control:	
Candida albicans ATCC® 10231	Inhibited or no growth

*This organism is available as a Culti-Loop®

References

1. Marshall B. K., Warren J. R., Blincow E. D., Phillips M., Goodwin C. S., Murray R., Blackbourne S. J. and Waters T. E. (1988) *The Lancet*, December 24/31, No 8626/8627.
2. Dent J. C. and McNulty C. A. M. (1988) *Eur. J. Clin. Microbiol. Infec. Dis.* 7. 555±568.
3. Buck G. E. (1988) *Laboratory Management*, 26, No.9.
4. Skirrow M. B. (1977) *BMJ*, 1. 9-11.

HOYLE MEDIUM BASE

Code: CM0083

A modification of Neill's medium for the isolation and differentiation of Corynebacterium diphtheriae types.

Formula	***gm/litre***
'Lab-Lemco' powder	10.0
Peptone	10.0
Sodium chloride	5.0
Agar	15.0
pH 7.8 ± 0.2	

Directions

Suspend 40 g in 1 litre of distilled water and bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 55°C and add 50 ml of Laked Horse Blood SR0048 and 10 ml of 3.5% Potassium Tellurite solution SR0030, mix, and pour plates.

Description

Hoyle medium is the well known modification[1] of Neill's medium for the cultural isolation and differentiation of *Corynebacterium diphtheriae* types. Hoyle medium does not exert the inhibitory effect manifested by Neill's on some mitis types, but gives very rapid growth with all types of *Corynebacterium diphtheriae*, so that diagnosis is possible after 18 hours' incubation.

Technique

This is a highly selective medium which is used in parallel with non-selective media such as blood agar (e.g. Blood Agar Base CM0055 with 10% of horse blood). Unlike non-selective media, Hoyle medium should be inoculated by rubbing the throat swab (or other material) over the entire surface – spreading with a platinum loop is not necessary.

Normally incubation for 18 hours at 35°C is sufficient but, when a negative result is obtained, incubation for up to 72 hours may be advisable. Gentian violet stained films made from colonies picked straight off the medium, are satisfactory for *Corynebacterium diphtheriae* morphology. For the demonstration of the characteristic morphology and staining reactions of *Corynebacterium diphtheriae* by Neisser's or Albert's method, it is preferable to utilise colonies from Loeffler medium. The toxigenicity of *Corynebacterium diphtheriae* strains may be determined by the Elek[2] method.

Colonial Characteristics

It is best to examine with a low-power microscope, the colonies being illuminated from above by daylight.

Type differentiation is good, and typical colonial appearances after 18 hours' incubation are as follows:

Corynebacterium diphtheriae type gravis – grey colonies, 1.5-2.5 mm diameter dull, matt surface. May be slightly umbonate and show indented margins. Colonies can be pushed along the surface of the medium.

Corynebacterium diphtheriae type mitis – grey colonies, 1.5-2.0 mm diameter with regular margins and shining surface. Variation in size common.

Corynebacterium diphtheriae type intermedius – grey colonies, 0.5-0.75 mm diameter with shining surface. Colonies are very uniform in size with darker centres.

Corynebacterium hofmannii – usually rounded, white or greyish, 0.5-0.75 mm diameter. Colonies may be up to 1 mm diameter, when they are black in more heavily inoculated areas and white when well isolated.

Corynebacterium xerosis – black shining colonies of variable size.

Streptococci – minute black or brownish-black colonies.

Other organisms may occasionally grow which resemble *Corynebacterium diphtheriae* type intermedius but are larger, while sporing anaerobes may produce brownish mucoid colonies.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared plates at 2-8°C.

Appearance
Dehydrated Medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured gel

Quality control

Positive controls:	***Expected results***
Corynebacterium diphtheriae biotype *gravis* ATCC® 19409	Good growth; dull grey/black colonies
Corynebacterium diphtheriae biotype *intermedius* ATCC® 14779	Good growth; shiny grey/black colonies
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

Precautions
It should be noted that not all corynebacteria produce the typical colonies described above – so in all cases it is advisable to use Hoyle medium in conjunction with the additional media and tests mentioned above.

References
1. Hoyle L. (1941) *Lancet.* i. 175-176.
2. Elek S. D. (1948) *Brit. Med. J.* 1. 493-496.

HIGH RESOLUTION (H.R.) MEDIUM

Code: CM0845

A chemically defined medium for fluconazole susceptibility testing.

Formula	***gm/litre***
Dextrose	19.98
Potassium dihydrogen phosphate	1.99
Ammonium sulphate	4.99
L-Glutamine	0.58
Magnesium sulphate (anhydrous)	0.99
Sodium chloride	0.20
Calcium chloride	0.20
L-Lysine monohydrochloride	0.073
Valine	0.047
L-Arginine monohydrochloride	0.042
L-Histidine	0.023
Methionine (DL)	0.0189
Tryptophane	0.020
Inositol	0.00397
Boric acid	0.00099
Calcium D-pantothenic acid	0.00079
Nicotinic acid	0.00079
Pyridoxine hydrochloride	0.00079
Aneurine hydrochloride	0.00079
Manganous sulphate	0.00079
Zinc sulphate	0.0014
4-Aminobenzoic acid	0.000395
Riboflavin BP/USP	0.000395
Ferric chloride	0.000395
Cupric sulphate	0.00012
Biotin crystalline	0.000004
Folic acid	0.000395
L-Isoleucine	0.052
Sodium molybdate	0.00047
Potassium iodide	0.00020
L-Leucine	0.052
Threonine	0.0476

Directions

PART A

Prepare a 2% w/v solution of Agar Technical LP0013 and buffer to pH 7.5 with 0.1 ml phosphate buffer. Sterilise at 115°C for 10 minutes.

PART B

Dissolve 29.34 g of High Resolution Medium in 900 ml of distilled water. Stir continuously and add 2 g of sodium bicarbonate (analar) and make up the volume to 1 litre with distilled water. Sterilise by filtration. The medium can be kept at 4°C for 2 weeks.

Aseptically mix equal volumes of molten High Resolution Medium Part A cooled to 60°C and High Resolution Medium Part B. Mix thoroughly and pour into sterile Petri dishes.

Description

High Resolution Medium is a chemically defined medium, specifically developed for the *in vitro* testing of fluconazole. The MIC values generated using the medium give sensible correlations with efficacy *in vitro* and with clinical outcome.

In a comparison of a disc diffusion method against a microdilution method correlation using HR medium was

good for a number of antibiotics including nystatin and amphotericin B but generally better for new triazoles such as fluconazole than for the other antifungals[6].

Technique

Preparation of the inocula
The preparation and standardisation of the inoculum varies with different fungi:

***CANDIDA* spp.**
1. Grow *Candida* spp overnight at 37°C in Sabouraud Liquid Medium CM0147.
2. Vortex mix and make a 1 in 100 dilution of each culture in normal saline and estimate the cell numbers using a haemocytometer.
3. Appropriately dilute each culture in normal saline to give the following cell densities:

Candida albicans	10^5/ml
Candida krusei	10^5/ml
Candida tropicalis	10^5/ml
Candida guillermondii	10^6/ml
Candida parapsilosis	10^6/ml
Candida pseudotropicalis	10^6/ml

Inoculation of the plates
1. Surface inoculate each series of plates using a multipoint inoculator which delivers 1 ml of each culture inoculum. It is possible to inoculate 36 isolates per 90 mm Petri dish. Inoculate control plates at both the beginning and end of the inoculation run.
2. Incubate the plates at 28°C for 48 hours.

Endpoint determination
1. Check the control plates to ensure that all the organisms have grown adequately.
2. Read all plates against a standard background and record, for each isolate, the lowest concentration of fluconazole that completely suppresses visible (to the naked eye) growth. This is the MIC value.

***DERMATOPHYTE* spp.**
1. Grow the dermatophytes on Sabouraud Dextrose Agar CM0041 at 28°C for 5-10 days.
2. Scrape off the mycelium from the agar surface using a scalpel and place in a bijou bottle containing 4 g of glass beads (approximately 2 mm diameter) plus 2 ml of 0.85% saline.
3. Vortex mix to evenly disperse.
4. Adjust the density of the suspension with 0.85% saline to give a 65% light transmission on an absorptiometer.
5. Inoculate the plates using a multipoint inoculator. It is possible to inoculate 20 isolates, evenly spaced, per 90 mm Petri dish.
6. Incubate at 28°C for 6 days.
7. Check the control plates to ensure that all isolates have grown adequately.
8. Determine the endpoint as for *Candida*.

Candida albicans ATCC® 76615 has an MIC value of 1.56 mg/ml against the antifungal agent fluconazole.

Storage conditions and Shelf life
High Resolution Medium should be stored tightly capped in the original container at 10-30°C away from bright light. When stored as directed the medium will remain stable until the expiry date printed on the label.

Please note shelf life of 2 years.

Appearance
Dehydrated medium: White coloured, free-flowing powder.

Prepared medium: Light straw coloured gel.

Quality Control

Positive control:	***Expected result***
Candida albicans ATCC® 10231*	Good growth
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

References

1. Hoeprich P. D. and Finn P. D. (1972) *J. Infect. Dis.* 126, 353-361.
2. Cook R. A., McIntyre K. A. and Galgiani J. N. (1990). *Antimicrob. Agents and Chemother*. 34, 1542-1545.
3. Pfaller M. A. *et al*. (1990) *Antimicrob. Agents Chemother*. 34, 1648-1654.
4. Pfaller M. A. *et al*. (1992) *Antimicrob. Agents and Chemother.* 36, 1805-1809.
5. Pfizer Ltd, private communication (1990).

IRON SULPHITE AGAR

Code: CM0079

A medium for the detection of thermophilic anaerobic organisms.

Formula	***gm/litre***
Tryptone	10.0
Sodium sulphite	0.5
Iron (III) citrate	0.5
Agar	12.0
pH 7.1 ± 0.2	

Directions

Suspend 23.0 g in 1 litre of distilled water and boil to dissolve completely. Sterilise by autoclaving for 15 minutes at 121°C. Mix well before pouring.

Description

This medium is a modification of Cameron Sulphite Agar developed by the National Canners Association of America[1].

It had been shown that the medium was improved by reducing the concentration of sodium sulphite. Beerens[2] showed that some strains of *Clostridium sporogenes* would not tolerate 0.1% sulphite. This was confirmed by Mossel *et al*.[3] who consequently used iron sulphite agar containing only 0.05% sulphite and obtained no apparent inhibition.

Technique

Iron Sulphite Agar is particularly suitable for the detection of thermophilic anaerobic organisms causing sulphide spoilage in food. The medium should be dispensed in 10 ml amounts in tubes for deepshake cultures, and inoculated whilst fluid at about 50°C. Allow to set and incubate at 55°C for thermophilic species. *Desulfotomaculum nigrificans*, the type species, produces distinct black spherical growths in the depth of the medium.

In the Attenborough and Scarr method[4], diluted samples of the sugar were filtered through membrane filters which were then rolled up and placed in tubes containing enough melted Iron Sulphite Agar (at approximately 50°C) to cover them. The medium was allowed to solidify and the tubes were incubated at 56°C. After 48 hours the number of black colonies on the membrane was counted. This membrane filter technique is quicker than the standard method but of comparable accuracy, and permits the examination of a much larger sample[5].

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium below 25°C.

Appearance

Dehydrated medium: Light straw coloured, free-flowing powder.

Prepared medium: Straw grey coloured gel.

Quality Control

Positive controls:	***Expected result***
Clostridium sporogenes ATCC® 19404*	Good growth; blackening
Desulfotomaculum nigrificans ATCC® 19858	Good growth; blackening
Negative control:	
Escherichia coli ATCC® 25922*	Good growth; no blackening

*This organism is available as a Culti-Loop®

Precautions
The blackening reaction is only presumptive evidence of clostridial growth. Confirmation tests must be carried out to identify the organism growing in the medium.

References
1. Tanner F. W. (1944) *The Microbiology of Foods* 2nd edn., Garrard Press, Illinois p.1127.
2. Beerens H. (1958) *DSIR, Proc. 2nd Internat. Symp. Food Microbiol.* 1957, HMSO, London, pp.235-245.
3. Mossel D. A. A., Golstein Brouwers G. W. M. V. and de Bruin A. S. (1959) *J. Path. Bact.* 78. 290-291.
4. Attenborough Sheila J. and Scarr M. Pamela (1957) *J. Appl. Bact.* 20. 460-466.
5. Bufton A. W. J. (1959) *J. Appl. Bact.* 22. 278-280.

ISLAM MEDIUM – see GROUP B STREPTOCOCCI MEDIUM

'ISO-SENSITEST AGAR'

Code: CM0471

A semi defined medium with stabilised mineral content for antimicrobial susceptibility testing.

Formula	***gm/litre***
Hydrolysed casein	11.0
Peptones	3.0
Glucose	2.0
Sodium chloride	3.0
Soluble starch	1.0
Disodium hydrogen phosphate	2.0
Sodium acetate	1.0
Magnesium glycerophosphate	0.2
Calcium gluconate	0.1
Cobaltous sulphate	0.001
Cupric sulphate	0.001
Zinc sulphate	0.001
Ferrous sulphate	0.001
Manganous chloride	0.002
Menadione	0.001
Cyanocobalamin	0.001
L-Cysteine hydrochloride	0.02
L-Tryptophan	0.02
Pyridoxine	0.003
Pantothenate	0.003
Nicotinamide	0.003
Biotin	0.0003
Thiamine	0.00004
Adenine	0.01
Guanine	0.01
Xanthine	0.01
Uracil	0.01
Agar	8.0
pH 7.4 ± 0.2	

Directions
Suspend 31.4 g in 1 litre of distilled water and bring to the boil to dissolve the agar. Sterilise by autoclaving at 121°C for 15 minutes.

Description
Oxoid 'Iso-Sensitest Agar' was developed specifically for antimicrobial susceptibility tests. Its formulation was carefully constructed to give a reproducible, semi-defined medium in which the undefined components were kept to a minimal level. However, it allows the growth of the great majority of micro-organisms without further supplementation.

It has been designed to overcome the objections made about Mueller-Hinton media by various workers[1,2,3,4,5,6,7].

These objections were:

1. Different MIC values in the broth and agar versions of the medium.
2. Agar versions showing antagonistic effects towards tetracycline.
3. High levels of sulphonamide and trimethoprim antagonists.
4. Poor reproducibility with different manufacturers' peptones.
5. Poor growth-supporting ability for streptococci and variable growth rates with Gram-positive organisms generally.

Some mutant strains which are totally dependent on thymine and thymidine for their growth will, however, not grow in Oxoid 'Iso-Sensitest Agar', which has these two compounds at very low levels, as they are the naturally-occurring antagonists of trimethoprim. Care must be taken to recognise these strains[8,9,10].

Oxoid 'Iso-Sensitest Agar', was developed from Oxoid 'Sensitest' Agar CM0409 which has been used successfully in several centres throughout the world[11,12,13].

The role of metal ions as antagonists to certain antibiotics has been described by many workers[14,15,16,17,18,19]. A stabilised mineral content in sensitivity test media is, therefore, important.

Reller, Schoenknecht, Kenny & Sherris[3] demonstrated the contribution of cations provided in media by ordinary agars. A considerable difference in mineral content between the agar and broth media was shown. Only by using an agar, which is specially processed to remove the free anions and cations, can a stabilised mineral content be obtained.

Oxoid 'Iso-Sensitest Agar' has stability in mineral content and the ability to produce optimum antimicrobial zones of inhibition.

The amino-nitrogen base of acid-hydrolysed casein and special peptones has been supplemented with defined growth factors. Careful preparation of the nutrients ensures that trimethoprim and sulphonamide antagonists are at very low levels.

THE ADDITION OF LYSED HORSE ERYTHROCYTES TO THE MEDIUM IS NOT REQUIRED WHEN CARRYING OUT ANTIMICROBIAL SUSCEPTIBILITY TESTS WITH THESE COMPOUNDS.

SUPPLEMENTAL NUTRIENTS FOR NUTRITIONALLY DEPENDENT ORGANISMS
Some pathogenic organisms may be nutritionally dependent because of intrinsic demands for special growth factors, e.g. *Streptococci*, *Neisseria*, *Haemophilus*, *Campylobacter* etc. Other organisms may exhibit mutant characteristics, e.g. dwarf *Staphylococcus aureus*, thymidine-dependent *Escherichia coli*.

Supplemental nutrients can be added to 'Iso-Sensitest Agar' to obtain or improve growth of these organisms[20].

Nutrient	***Micro-organism***
Laked Blood (5% v/v)	*Neisseria*, *Streptococcus*
Fildes Peptic Digest of Blood (5% v/v)	*Haemophilus*
Menadione (0.5 mg/ml), Thiamine (2 mg/ml)	Dwarf colonies of *Staph aureus* and coliform organisms
Pyridoxine hydrochloride (1 mg/ml)	Symbiotic streptococci

Certain supplements interfere with antimicrobial activity and tests must be made to measure their effect.

Nutrient	***Antimicrobial***
Thymidine	Trimethoprim
Blood	Sulphonamides, trimethoprim, aminoglycosides
CO_2	Aminoglycosides, erythromycin, lincomycin, tetracyline, novobiocin
Cysteine and other SH compounds	Aminoglycosides
Vitox/Isovitalex	Sulphonamides, trimethoprim

'Iso-Sensitest Agar' with the addition of 10% of horse blood has been recommended as a suitable medium when testing susceptibility of *Helicobacter pylori*[21]. Although Mueller-Hinton Agar performed nearly as well, 'Iso-Sensitest Agar' was preferred because its contents are better defined.

Iso-sensitest Agar is recommended by the BSAC for use within their Disc Diffusion Method for Antimicrobial Susceptibility Testing[22].

Storage conditions and Shelf Life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C and use as freshly as possible.

Appearance

Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured gel.

Quality control

Positive controls:	***Expected results***
Escherichia coli ATCC® 25922*	Good growth; pale straw coloured colonies
Staphylococcus aureus ATCC® 25923*	Good growth; cream coloured colonies
Pseudomonas aeruginosa ATCC® 27853*	Good growth; straw coloured colonies
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

Note: Please refer to relevant standards for further quality control testing.

References

1. Ericsson H. M. and Sherris J. C. (1971) *Acta. Pathol. Microbiol. Scand. Suppl.* 217. 1-90.
2. Garrod L. P. and Waterworth P. M. (1971) *J. Clin. Path.* 24. 779-789.
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16. Zimelis V. M. and Jackson G. G. (1973) *J. Infect. Dis.* 127. 663-669.
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18. Brenner V. C. and Sherris J. C. (1972) *Antimicrob. Agents Chemother.* 1. 116-122.
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20. Acar J. F. (1980) *Antibiotics in Laboratory Medicine*, Lorian V. (Ed.) Williams and Wilkens, Baltimore, USA, 48-51.
21. Hartzen S. H., Andersen L. P., Bremmelgaard A. *et al.* (1997) *Antimicrob. Ag. and Chemother.* 41. 2634-2639.
22. British Society for Antimicrobial Chemotherapy (2002) *Disc Diffusion Method for Antimicrobial Susceptibility Testing.* Version 2.1.1. BSAC.

'ISO-SENSITEST BROTH'

Code: CM0473

A semi defined medium designed for antimicrobial susceptibility testing.

Formula	***gm/litre***
Hydrolysed casein	11.0
Peptones	3.0
Glucose	2.0
Sodium chloride	3.0
Soluble starch	1.0
Disodium hydrogen phosphate	2.0
Sodium acetate	1.0
Magnesium glycerophosphate	0.2
Calcium gluconate	0.1
Cobaltous sulphate	0.001
Cupric sulphate	0.001
Zinc sulphate	0.001
Ferrous sulphate	0.001
Manganous chloride	0.002
Menadione	0.001
Cyanocobalamin	0.001
L-Cysteine hydrochloride	0.02
L-Tryptophan	0.02
Pyridoxine	0.003
Pantothenate	0.003
Nicotinamide	0.003
Biotin	0.0003
Thiamine	0.00004
Adenine	0.01
Guanine	0.01
Xanthine	0.01
Uracil	0.01
pH 7.4 ± 0.2	

Directions
Add 23.4 g to 1 litre of distilled water. Mix well and distribute into tubes or bottles. Sterilise by autoclaving at 121°C for 15 minutes.

Description
Oxoid Iso-Sensitest Broth has been produced in parallel with Iso-Sensitest Agar CM0471. The broth has an identical nutrient formulation without the specially purified agar. Where studies on antimicrobial susceptibilities are to be made in both broth and agar, this will be found to be of particular value.

Details of the function of the medium and the methodology used for antimicrobial susceptibility tests are discussed in the Section 'Susceptibility Testing'.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Pale straw coloured solution.

Quality control

Positive controls:	***Expected results***
Streptococcus pneumoniae ATCC® 49619*	Turbid growth
(with blood)	
Staphylococcus aureus ATCC® 25923*	Turbid growth
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

References
See Iso-Sensitest Agar CM0471.

KANAMYCIN AESCULIN AZIDE AGAR BASE

Code: CM0591

A selective medium for the isolation of enterococci in foodstuffs.

Formula	***gm/litre***
Tryptone	20.0
Yeast extract	5.0
Sodium chloride	5.0
Sodium citrate	1.0
Aesculin	1.0
Ferric ammonium citrate	0.5
Sodium azide	0.15
Agar	10.0
Final pH 7.0 ± 0.2	

KANAMYCIN SULPHATE SELECTIVE SUPPLEMENT

Code: SR0092

Vial contents	***per vial***	***per litre***
Kanamycin sulphate	10 mg	20 mg

Directions
Suspend 21.3 g in 500 ml of distilled water. Add one vial of Kanamycin Supplement reconstituted as directed. Bring to the boil. Dispense into final containers and sterilise by autoclaving at 121°C for 15 minutes.

Description
Kanamycin Aesculin Azide Agar Base replaces KAA Agar CM0481. This change has been made to follow the Oxoid Health & Safety rules that antibiotics should not be present in powdered culture media where they can be inhaled or contaminate surfaces.

Kanamycin sulphate is added separately to 500 ml of reconstituted agar from freeze-dried vials (Kanamycin Supplement) containing the precise amount of antibiotic required.

The medium contains the selective inhibitory components kanamycin sulphate and sodium azide. It also contains an indicator system to detect the growth of aesculin-hydrolysing streptococci. These organisms produce black zones around the colonies from the formation of black iron phenolic compounds derived from aesculin-hydrolysis products and ferrous iron.

Kanamycin Aesculin Azide Agar was designed by Mossel *et al*.[1,2] to detect enterococci in foodstuffs. Round, white or grey colonies about 2 mm in diameter, surrounded by black zones of at least 1 cm diameter are considered to be enterococci (presumptive). Incubation is carried out aerobically at 35°C or 42°C ± 0.3°C for 18-24 hours. The higher incubation temperature increases the selectivity of the medium.

This medium was used by Mossel *et al*.[3] in the Dip Slide technique for bacteriological monitoring of foods. Kanamycin-Aesculin-Azide Agar has been used successfully for the isolation of glycopeptide-resistant enterococci from clinical specimens and foods[4,5].

Technique
Inoculation method for samples: spread 0.1 ml of sample dilutions over the whole of a pre-dried 9 cm diameter plate.

The following procedure for testing foodstuffs is adapted from Mossel, Harrewijn and Elzebroek[6].

1. Prepare tubes of sterilised Tryptone Water CM0087 in 9 ml volumes. Chill to 0-5°C by storing in a refrigerator for 18 hours prior to use.
2. Add 1 g or 1 ml of the thoroughly mixed food sample to a tube containing 9 ml of pre-chilled diluent (10^{-1} dilution). Shake well for 30 seconds. Sample 1 ml of the contents, within 30 seconds after mixing, into a fresh tube of diluent. Continue the process using fresh sterile pipettes until a dilution is reached which will produce 100 colonies per 1 ml. Store the decimal dilutions in the refrigerator and examine within 3 hours of their preparation.
3. Streak onto plates of Kanamycin Aesculin Azide Agar and incubate for 16-24 hours at 35°C ± 1°C. Consider the result positive for enterococci when colonies surrounded by black haloes are grown.
4. Confirmatory tests may be carried out, e.g. catalase test, utilisation of glucose, growth at 45°C ± 1°C, chain-forming Gram-positive cocci.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.
Store the prepared media at 2-8°C.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw/green coloured gel with blue tint.

Quality control

Positive controls:	***Expected results***
Enterococcus faecalis ATCC® 19434*	Good growth; black colonies; aesculin hydrolysis
Streptococcus bovis ATCC® 27960*	Good growth; straw coloured colonies
Negative controls:	
Bacillus subtilis ATCC® 6633*	No growth
Escherichia coli ATCC® 25922*	Inhibited

*This organism is available as a Culti-Loop®

Precautions
Observe the hazard precautions regarding sodium azide when disposing of the medium.

There is no universal medium which will isolate all strains of enterococci[7].

Unless a presumptive count is acceptable all isolates should have their identity confirmed with further tests.

References

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6. Mossel D. A. A., Harrewijn G. A. and Elzebroek B. J. M. (1973) UNICEF Geneva.
7. Reuter G. (1985) *Inter. J. Food Microbiol.* 2. 103-114.

KARMALI SELECTIVE MEDIUM

CAMPYLOBACTER AGAR BASE (KARMALI)

Code: CM0935

A blood free selective medium for the isolation of Campylobacter jejuni and Campylobacter coli when incubated at 42°C.

Formula	***gm/litre***
Columbia Agar Base	39.0
Activated charcoal	4.0
Haemin	0.032
Final pH 7.4 ± 0.2	

CAMPYLOBACTER SELECTIVE SUPPLEMENT (KARMALI)

Code: SR0167

Vial contents (each vial is sufficient for 500 ml of medium)	***per vial***	***per litre***
Sodium pyruvate	50 mg	100 mg
Cefoperazone	16 mg	32 mg
Vancomycin	10 mg	20 mg
Cycloheximide	50 mg	100 mg

MODIFIED KARMALI SELECTIVE SUPPLEMENT

Code: SR0205

Vial contents: (each vial is sufficient for 500 ml of medium)	***per vial***	***per litre***
Sodium pyruvate	50.0 mg	100.0 mg
Cefoperazone	16.0 mg	32.0 mg
Vancomycin	10.0 mg	20.0 mg
Amphotericin B	5.0 mg	10.0 mg

Directions

Add 21.5 g of Campylobacter Agar Base (Karmali) to 500 ml of distilled water and bring to the boil to dissolve. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C. Aseptically add 1 vial of Campylobacter Selective Supplement (Karmali) or Modified Karmali Selective Supplement, reconstituted as directed. Mix well and pour into sterile Petri dishes.

Description

Campylobacter Medium (Karmali) is based on the formulation described by Karmali *et al*.[1] and is recommended for the isolation of *Campylobacter jejuni* and Campylobacter coli from clinical specimens.

The original Campylobacter Blood Free medium in the Oxoid product range contains sodium pyruvate in the agar base. Campylobacter Medium (Karmali) incorporates this ingredient into the selective supplement. The original medium also contains sodium desoxycholate for the inhibition of Gram positive organisms, whereas with Campylobacter Medium (Karmali) suppression of Gram positives is achieved by the inclusion of vancomycin. Modified Karmali Selective Supplement SR0205 offers a cycloheximide free alternative by utilising amphotericin B as an antifungal agent[3].

Campylobacter jejuni strains produce grey, moist, flat spreading colonies after 42 hour incubation at 42°C.

If plates are first examined after 24 hours incubation, read them immediately and quickly return them to a reduced oxygen atmosphere to ensure continued viability of the more oxygen-sensitive strains.

At 42°C selectivity is increased and growth is faster but non-thermophilic strains will not grow e.g. *Campylobacter fetus* subsp. *fetus*.

Colonies tend to swarm when initially isolated from clinical specimens.

Technique

1. Prepare Campylobacter Selective Medium (Karmali) plates as described in the directions for use.
2. Emulsify approximately 0.5 g of the specimen in 5 ml of sterile 0.1% peptone water to form a 1:10 dilution.
3. Inoculate onto selective medium with cotton-tipped swabs so that single isolated colonies are formed.
4. Incubate the plates in an atmosphere consisting of approximately 5-6% oxygen, 10% carbon dioxide and 84-85% nitrogen for 48 hours at 42°C. This can best be achieved by using the Oxoid Gas Generating Kit for Campylobacters BR0056 in conjunction with the Oxoid Anaerobic Jar and an active catalyst BR0042. For jars of smaller capacity (2.5 litres) use the Oxoid Gas Generating Kit for Campylobacters BR0060. Alternatively use CampyGen CN0025 or CN0035. CampyGen does not require the addition of water or a catalyst.
5. Examine the plates and confirm the typical colonies as *Campylobacter jejuni* or *Campylobacter coli*. A simple schema for differentiating *Campylobacter* species is described by Skirrow and Benjamin[2].

Storage conditions and Shelf life

Campylobacter Agar Base (Karmali) should be stored tightly capped in the original container in a cool, dry place away from bright light. When stored at 10-30°C the medium will remain stable until the expiry date printed on the label.

Appearance

Dehydrated medium: Black, free-flowing powder.

Prepared medium: Black gel.

Quality control

Positive control:	***Expected results***
Campylobacter jejuni ATCC® 33291*	Good growth; grey coloured colonies
Negative control:	
Escherichia coli ATCC® 25922*	Inhibited

*This organism is available as a Culti-Loop®

Precautions

Campylobacter Selective Supplement (Karmali) contains cycloheximide which is toxic.

Note the precautions to be taken under HAZARDOUS Products section of the Manual.

References

1. Karmali M. A., Simor A. E., Roscoe M., Fleming P. C., Smith S. S. and Lane J. (1986) *J. Clin. Micro.* 23. 456-459.
2. Skirrow M. B. and Benjamin J. (1980) *J. Clin. Path*. 33. 1122.
3. Data on file at Oxoid.

KAUFFMANN MEDIUM – see BRILLIANT GREEN AGAR

KF STREPTOCOCCUS AGAR

Code: CM0701

A selective medium for the isolation and enumeration of group D streptococci.

Formula	***gm/litre***
Proteose peptone	10.0
Yeast extract	10.0
Sodium chloride	5.0
Sodium glycerophosphate	10.0
Maltose	20.0
Lactose	1.0
Sodium azide	0.4
Bromocresol purple	0.015
Agar	20.0
pH 7.2 ± 0.2	

Directions

Suspend 76.4 g in 1 litre of distilled water. Bring to the boil with frequent agitation. Boil for 5 minutes.‡

Cool to 50°C and add aseptically 1 ml of sterile aqueous 1% solution of 2,3,5-Triphenyltetrazolium chloride to each 100 ml of medium. Pour into sterile Petri dishes when using the membrane filtration method or hold at 45°C when using the pour-plate method.

‡ Note: The medium can be autoclaved at 121°C for 10 minutes if total selectivity is required.

Description

KF Streptococcus Agar is based on the formulation described by Kenner *et al*.[1] and is recommended[2] for the detection and enumeration of enterococci in faeces, milk, water and other materials by the pour-plate or membrane filtration method. The presence of enterococci in the material under test is indicative of faecal pollution by man or animals.

KF Streptococcus Agar Medium is selective for the following Group D and Group Q species.

Enterococcus Group

Enterococcus faecalis	Group D
Enterococcus subsp. *liquefaciens*	Group D
Enterococcus faecalis subsp. *zymogenes*	Group D
Enterococcus faecium	Group D
Enterococcus bovis	Group D
Enterococcus equinus	Group D
Streptococcus avium	Group Q

Streptococcus avium (Group Q) has been included in the 'enterococci' group as it has very similar biochemical and antigenic characteristics to the Group D species and also occurs in warm-blooded animals.

The detection of enterococci may provide more specific information about the source of pollution because certain enterococci are host specific. For example, a predominance of *Enterococcus bovis* and *Enterococcus equinus* would indicate pollution due to animal excrement.

The detection of *Enterococcus bovis* and *Enterococcus equinus* species has been found to be associated with pollution involving meat processing plants, dairy wastes and feedlot and farmland run-off.

The detection of these enterococcal species is indicative of recent contamination as the organisms survive for only a short period outside their natural habitat. The coliform/enterococci ratios may also provide information on possible sources of pollution[2].

Caution must be observed when assessing the quality of marine recreational waters, particularly in tropical climates, because a high incidence of false-positive presumptive counts for enterococci may occur. Anaerobic incubation of tropical marine water samples is recommended[3].

Colonies of enterococci on the membrane filter or agar plate are red or pink with a variation in diameter from 0.3-2 mm. It is recommended that counting should be done with the aid of a low power (10-15 magnification) binocular wide field dissecting microscope or equivalent optical device.

Technique

Membrane Filtration Technique

1. Prepare the KF Streptococcus Agar Medium as directed.
2. Filter samples through a sterile membrane to give 20-100 colonies on the membrane surface. Use volumes of 100, 10, 1, 0.1 or 0.01 ml, depending on the degree of pollution present.
3. Transfer the membrane filter directly to agar medium in the Petri dishes, avoiding the formation of air bubbles.
4. Invert the plates and incubate at 35°C for 48 hours.
5. Count all red or pink colonies with the aid of a low power (10-15 magnification) binocular wide field dissecting microscope.
6. Calculate the number of enterococci and report as faecal streptococci per 100 ml.
7. Confirm the colonies as enterococci.

Plate Count Method

1. Prepare the KF Streptococcus Agar Medium as directed.
2. Prepare dilutions to give a count of 30-300 colonies. For most potable water samples plates suitable for counting will be obtained by inoculating 1 ml and 0.1 ml of undiluted sample and 1 ml of sample diluted 1:100.
3. Measure the selected volume of sample into a Petri dish.
4. Pour 15 ml of liquified medium into each plate.
5. Thoroughly mix the medium and sample to give a uniform dispersion of the organisms.
6. Solidify the agar as rapidly as possible after pouring
7. Incubate the plates in an inverted position at 35°C for 48 hours.
8. Count all red to pink surface and subsurface colonies.
9. Calculate the numbers of enterococci and report as faecal streptococci per 100 ml.
10. Confirm the colonies as enterococci[2]. Use an inoculating wire to stab through the agar to reach the colonies.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared plates at 2-8°C.

Appearance

Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Purple coloured gel.

Quality control

Positive control:	***Expected results***
Enterococcus faecalis ATCC® 29212*	Good growth; red coloured colonies with yellow haloes
Negative control:	
Escherichia coli ATCC® 25922*	Inhibited

*This organism is available as a Culti-Loop®

Precautions

Observe the HAZARD precautions regarding sodium azide when disposing of the medium. The pH of the medium should not fall below 7.0 as it may become inhibitory towards enterococci[1]. KF Streptococcus Agar is not specific for the presumptive identification of Group D streptococci. Further tests must be made to confirm the identity of the organisms isolated.

References

1. Kennor G. A., Clark H. F. and Kabler P. W. (1961) *J. Appl. Microbiol.* 9. 15-20.
2. American Public Health Association (1981) *Standard Methods for the Examination of Water and Wastewater*, 15th edn. APHA Inc. Washington DC.
3. Fujioka R. S., Ueno A. A. and Narikawa O. T. (1984) *Technical Report number* 168, Water Resources Research Center, University of Hawaii at Manoa, Honolulu.

KLIGLER IRON AGAR

Code: CM0033

A medium for the identification of Enterobacteriaceae, based on double sugar fermentation and hydrogen sulphide production.

Formula	***gm/litre***
'Lab-Lemco' powder	3.0
Yeast extract	3.0
Peptone	20.0
Sodium chloride	5.0
Lactose	10.0
Glucose	1.0
Ferric citrate	0.3
Sodium thiosulphate	0.3
Phenol red	0.05
Agar	12.0
pH 7.4 ± 0.2	

Directions
Suspend 55 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Mix well and distribute into containers. Sterilise by autoclaving at 121°C for 15 minutes. Allow to set as slopes with 1 inch butts.

Description
A differential medium for the identification of Enterobacteriaceae on a basis of double sugar fermentation and hydrogen sulphide production. Oxoid Kligler Iron Agar based on the original medium[1,2,3] combines the principles of Russell[4], double sugar agar, with ferric citrate as an indicator to detect hydrogen sulphide production. The medium is recommended for the identification of colonies picked off from plating media such as MacConkey Agar, Bismuth Sulphite Agar, or Desoxycholate Citrate Agar, etc.

Technique
Smear the surface of a Kligler Iron Agar slope and stab the butt with a colony picked off one of the solid media.

There are three reactions to record when interpreting a KIA tube.

1. Carbohydrate utilisation:

(i) slant reaction	***(ii) butt reaction***
acidity: yellow colour	acidity: yellow colour
alkalinity: red colour	alkalinity: red colour

2. Gas production:

aerogenic	***anaerogenic***
bubbles or splitting of agar	no gas production

3. H_2S production:
Blackening in whole or part of butt.

Record the slant reaction/the butt reaction/gas production/H_2S production; in that order.

Red slant/yellow butt – glucose only fermented

Yellow slant/yellow butt – glucose + lactose fermented

Red slant/red butt – neither glucose nor lactose fermented.

Reactions after 18-24 hours at 35°C.

Organism	***Slope***	***Butt***	***Gas***	***H_2S***
Shigella sonnei	Red	Yellow	-	-
Shigella dysenteriae	Red	Yellow	-	-
Salmonella typhi	Red	Yellow	-	+
Salmonella species	Red	Yellow	+	+
Enterobacter species	Red	Yellow	+	-
Klebsiella species	Yellow	Yellow	+	-
Escherichia coli	Yellow	Yellow	+	-
Proteus mirabilis	Red	Yellow	-	+
Morganella species	Red	Yellow	V	-
Citrobacter freundii	Yellow	Yellow	+	+
Yersinia species	Red	Yellow	V	-

V = variable, + = positive, – = negative.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label

Store the prepared medium at 2-8°C.

Appearance
Dehydrated medium: Straw-orange coloured, free-flowing powder.

Prepared medium: Red coloured gel.

Quality control

Positive control:	***Expected results***			
	Slope	***Butt***	***Gas***	***H_2S***
Citrobacter freundii ATCC® 8090*	Yellow	Yellow	+	+
Shigella sonnei ATCC® 25931*	Red	Yellow	-	-
Alcaligenes feacalis ATCC® 19018	Red	Red	-	-
Negative control:				
Uninoculated medium		No change		

*This organism is available as a Culti-Loop®

Precautions
It is essential that Kligler Iron Agar is examined and reported at 18-24 hours. Early or late readings will give false results.

KIA will grow both oxidative and fermentative organisms. Confusion will result if care is not taken to distinguish between the two groups.

Always use a straight wire to inoculate the butt, to avoid splitting the agar with a loop.

Pure cultures are essential to avoid misinterpretation.

Do not use screw-capped tubes or bottles for KIA medium. It is essential that air is freely available to growth on the slant.

References
1. Kligler I. J. (1917) *Am. J. Pub. Hlth* 7. 1042-1044.
2. Kligler I. J. (1918) *J. Exper. Med.* 28. 319-322.
3. Bailey Sadie F. and Lacey G. R. (1927) *J. Bact.* 13. 182-189.
4. Russell F. F. (1911) *J. Med. Res.* 25. 217-229.

'LAB-LEMCO' AGAR

Code: CM0017

A nutrient agar for general bacteriological use, it may be used as the basis for selective, differential, or enriched media.

Formula	***gm/litre***
'Lab-Lemco' powder	3.0
Peptone	5.0
Agar	15.0
pH 7.4 ± 0.2	

Directions
Suspend 23 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

Description
'Lab-Lemco' Agar is a general purpose medium used for the examination of water and dairy products[1,2].

It is used as a nutrient meat extract agar for general bacteriology and for the preservation of stock cultures. The absence of sodium chloride in the formulation prevents *Proteus mirabilis* forming 'swarming' colonies.

When used as a basal medium, other substances can be added to produce selective, differential or enriched media.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared plates at 2-8°C.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured gel.

Quality control

Positive controls:	***Expected results***
Staphylococcus aureus ATCC® 25923*	Good growth; cream coloured colonies
Escherichia coli ATCC® 25922*	Good growth; straw coloured colonies
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

Precautions
'Lab-Lemco' Agar is slightly more alkaline than the medium used in the APHA publications (pH 6.8).

If blood is to be added to the agar then it is necessary to add sodium chloride (0.5% w/v) to prevent haemolysis.

References

1. American Public Health Association (1980) *Standard Methods for the Examination of Water and Wastewater.* 15th edn. APHA Inc. Washington DC.
2. American Public Health Association (1978) *Standard Methods for the Examination of Dairy Products*. 14th edn. APHA Inc. Washington DC.

'LAB-LEMCO' BROTH

Code: CM0015

A nutrient broth for general bacteriological use, and for the examination of water, sewage, and dairy products by American standard methods.

Formula	***gm/litre***
'Lab-Lemco' powder	3.0
Peptone	5.0
pH 7.4 ± 0.2	

Directions
Dissolve 8 g in 1 litre of distilled water and distribute into final containers. Sterilise by autoclaving at 121°C for 15 minutes.

Description
'Lab-Lemco' Broth is a general purpose liquid medium used for the examination of water and dairy products[1,2].

It is used as a nutrient meat extract broth for general bacteriology.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at room temperature (18-22°C).

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured solution.

Quality control

Positive controls:	***Expected results***
Staphylococcus aureus ATCC® 25923*	Turbid growth
Escherichia coli ATCC® 25922*	Turbid growth
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

Precautions
'Lab-Lemco' Broth is slightly more alkaline than the medium used in the APHA publications (pH 6.8).

References

1. American Public Health Association (1980) *Standard Methods for the Examination of Water and Wastewater.* 15th edn. APHA Inc. Washington DC.
2. American Public Health Association (1978) *Standard Methods for the Examination of Dairy Products.* 14th edn. APHA Inc. Washington DC.
3. American Public Health Association (2001) *Compendium of Methods for the Microbiological Examination of Foods 4th Edn.*

LACTOSE BROTH

Code: CM0137

A liquid medium for use in the performance or confirmation of the Presumptive Test for coliforms in water, milk, etc. as specified by the American Public Health Association.

Formula	***gm/litre***
'Lab-Lemco' powder	3.0
Peptone	5.0
Lactose	5.0
pH 6.9 ± 0.2	

Directions

Dissolve 13 g in 1 litre of distilled water and distribute into containers with fermentation tubes (Durham).

Sterilise by autoclaving at 121°C for 15 minutes.

Description

Lactose broth is recommended for use in the presumptive identification of coliform organisms in milk, water and foods as specified by the American Public Health Association[1,2,3].

Tubes of Lactose Broth are inoculated with dilutions of the samples and incubated at 35°C. Examination for gas formation is carried out after 24 and 48 hours incubation. This presumptive evidence of coliform organisms must be confirmed by further tests.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at room temperature (18-22°C).

Appearance

Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured solution.

Quality control

Positive control:	***Expected results***
Escherichia coli ATCC® 8739*	Turbid growth; acid and gas production
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

Precautions

Ensure that the fermentation tubes are free from air bubbles before inoculation.

Large water samples may require double-strength lactose broth to reduce the final volumes. Do not overheat double-strength broth or inhibitory products will be produced.

References

1. American Public Health Association (1978) *Standard Methods for the Examination of Dairy Products.* 14th edn. APHA Washington DC.
2. American Public Health Association (1980) *Standard Methods for the Examination of Water and Wastewater*. 15th edn. APHA Inc. Washington DC.
3. American Public Health Association (1998) *Compendium of Methods for the Microbiological Examination of Foods* 3rd Edition. APHA Inc. Washington DC.

LAURYL TRYPTOSE BROTH (LAURYL SULPHATE BROTH)

Code: CM0451

A medium for the detection of coliform organisms in water and waste water, according to the formula of the American Public Health Association.

Formula	***gm/litre***
Tryptose	20.0
Lactose	5.0
Sodium chloride	5.0
Dipotassium hydrogen phosphate	2.75
Potassium dihydrogen phosphate	2.75
Sodium lauryl sulphate	0.1
pH 6.8 ± 0.2	

Directions
Dissolve 35.6 g in 1 litre of distilled water and distribute into containers with fermentation tubes (Durham). Sterilise by autoclaving at 121°C for 15 minutes.

Description
Lauryl Tryptose Broth provides a selective medium which is used for the detection of coliform organisms in water, dairy products and other foods. The APHA[1] recommend that Lauryl Tryptose Broth should be used for the Mean Probable Number Presumptive Test of coliforms in waters, effluent or sewage as a confirmatory test of lactose fermentation with gas production for milk samples (APHA[2]) and for the detection of coliforms in foods (APHA[3]).

Surface active agents have long been used as the inhibitory ingredients in selective media. MacConkey[4] introduced bile salts for this purpose and later Albus and Holm[3] working with lactobacilli found that sodium ricinoleate exerted a selective action. The development of synthetic wetting agents opened up new fields of investigation. Mallmann and Darby[6], after comparative tests with a large number of these compounds, showed that sodium lauryl sulphate gave the best results in selective media for the coliform group.

Lauryl Tryptose Broth was designed to promote a rich growth and copious gas production from small inocula of coliform organisms. Aerobic sporing bacteria are completely inhibited. The advantage in using this product is that, in addition to giving the fermentation reaction typical of MacConkey Broth, it can also be directly tested for the presence of indole. Unlike MacConkey Broth, the medium contains no indicator, but this can be added (if required) after incubation.

Technique
For details of the APHA standard methods please consult the references below.

Lauryl Tryptose Broth is recommended for the detection and enumeration of coliform organisms in water and milk products, especially in the control of ice-cream manufacture and in dairy hygiene. A suggested procedure (Dyett[7]) is as follows:

Inoculate samples of ice-cream into tubes of Lauryl Tryptose Broth in the manner normally employed in the MacConkey test. Examine the tubes after overnight incubation at 35°C and, if no gas is visible, examine again at the end of 48 hours' incubation. For every tube showing fermentation ('primary fermentation') two further tubes of Lauryl Tryptose Broth are inoculated from a tube of the primary fermenting broth, and these are incubated at 35°C and 44°C respectively. It is advisable that the tube to be incubated at 44°C be warmed up in a water bath at this temperature before inoculation.

If the 44°C incubated broth ferments after seven hours, test for indole production with either Ehrlich or Kovac's reagent. Due to the lauryl sulphate present, shaking the reagent culture mixture forms a persistent emulsion which interferes with the test. This may be avoided by shaking with ether, which separates rapidly, and then adding Kovac's reagent to the layer without shaking. If fermentation has not occurred after seven hours, leave the tube overnight at 44°C and test the following day. A positive indole reaction in a broth that has produced gas at 44°C indicates the presence of *Escherichia coli*. The tube at 35°C is incubated for 24 hours. If no fermentation occurs, the primary fermentation is assumed to be due to organisms other than coliforms. False positives are not uncommon in the primary fermentation tubes, due to fermentation of the sucrose in the added ice-cream by organisms other than coliforms.

After the two tubes of Lauryl Tryptose Broth have been inoculated for secondary fermentation, test the original primary fermentation tube (which was inoculated directly with ice-cream) for indole production.

A positive reaction suggests the presence of *Escherichia coli* and confirmation will be obtained later with the secondary fermentation from the 44°C bath. A negative indole reaction in the primary fermentation tube indicates the absence of *Escherichia coli*.

MUG Reagent BR0071 – The addition of 4-methylumbelliferyl-β-D-glucuronide (MUG) BR0071 to this medium will enhance the detection of *Escherichia coli.*

The use of MUG in a Most Probable Number (MPN) technique for enumeration of presumptive *Escherichia coli* in milk and milk products has been specified in a standard procedure[8]. For further information about MUG see MUG Reagent BR0071 under Biological Reagents.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at room temperature.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured solution.

Quality control

Positive control:	***Expected result at 35°C***
Escherichia coli ATCC® 25922*	Turbid growth; gas
Negative control:	
Staphylococcus aureus ATCC® 25923*	Inhibited or no growth

*This organism is available as a Culti-Loop®

Precautions
If stored at 2-8°C the broth will become cloudy or form a precipitate. This should clear at room temperature but gas formation is the criterion of growth not turbidity.

References

1. American Public Health Association (1980) *Standard Methods for the Examination of Water and Wastewater.* 15th edn. APHA Inc. Washington DC.
2. American Public Health Association (1978) *Standard Methods for the Examination of Dairy Products.* 14th edn. APHA Inc. Washington DC.
3. American Public Health Association (1976) *Compendium of Methods for the Microbiological Examination of Foods.* APHA Inc. Washington DC.
4. MacConkey A. T. (1938) *J. Hyg.* 8. 322-334.
5. Albus W. R. and Holm G. E. (1926) *J. Bact.* 12. 13-18.
6. Mallmann W. L. and Darby C. W. (1941) *Am. J. Pub. Hlth* 31. 127-134.
7. Dyett E. J. (1957) *Lab. Prac.* 6(6). 327-328.
8. ISO Standard 11866-2 *Milk and Milk Products – Enumeration of presumptive* Escherichia coli *– part 2: Most probable number technique using 4-methylumbelliferyl-β-D-glucuronide*.

LAURYL TRYPTOSE BROTH with MUG

Code: CM0980

Formula	***gm/litre***
Tryptose	20.0
Lactose	5.0
Di-potassium hydrogen phosphate	2.75
Potassium dihydrogen phosphate	2.75
Sodium chloride	5.0
Sodium lauryl sulphate	0.1
4-methylumbelliferyl-β-D-glucuronide (MUG)	0.05
pH 6.8 ± 0.2	

Directions
Suspend 35.65 g of Lauryl Tryptose Broth with MUG in 1 litre of distilled water. Warm to 40°C. Mix well to dissolve and distribute into final containers holding inverted Durham tubes. (Please be aware of the special glassware requirements when using ultra-violet light). Sterilise by autoclaving at 121°C for 15 minutes.

Description
Escherichia coli is a common Gram-negative micro-organism which may be present in food, water and environmental samples. Owing to its importance as an indicator of feacal contamination, continuing efforts have been put into developing rapid and specific methods for its identification.

The finding of a highly specific enzyme of *Escherichia Coli*, beta-glucuronidase, has offered a range of oppertunities for the improvement of culture media for the identification of this organism. Numerous studies have confirmed a beta-glucuronidase-positive reaction in the middle to upper 90% range of *Escherichia coli* isolated from a variety of sources[1]. The highly sensitive substrate 4-methylumbellifery-β-D-gluuronide (MUG) is cleaved by the enzyme and the hydrolisis product 4-methylumbelliferone shows a visible green/blue flourence under long-wave ultra-violet light. (366 nm).

Lauryl Tryptose Broth provides a selective medium which is used for the detection of coliform organisms in water, dairy products and other foods and with the addition of MUG is a Standard Methods medium recommended by FDA/BAM[2].

Lauryl Tryptose Broth was designed to promote a rich growth and copious gas production from small inocula of coliform organisms. Aerobic sporing bacteria are completely inhibited. The advantage in using this product is that in addition to giving the fermentation reaction typical of MacConkey Broth it can also be directly tested for the presence of indole. Unlike MacConkey Broth, the medium contains no indicator, but this can be added (if required) after incubation.

The addition of 4-methylumbelliferyl-β-D-glucuronide (MUG) BR0071 to this medium will enhance the detection of *Escherichia coli*.

The use of MUG in a Most Probable Number (MPN) technique for enumeration of presumptive *Escherichia coli* in milk and milk products has been specified in a standard ISO procedure. For further information about MUG see MUG Reagent BR0071 under Biological Reagents.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.
Store the prepared medium at room temperature 18-22°C.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.
Prepared medium: Straw coloured solution.

Quality Control

Positive control:	***Expected result at 35°C***
Escherichia coli ATCC® 25922*	Turbid growth, gas, flourescence
Negative control:	
Staphylococcus aureus ATCC® 25923*	Inhibited or no growth, no gas, no flourescence

*This organism is available as a Culti-Loop®

References
1. Frampton, E. W. and Restaino, L. (1993) *J. Appl. Bact*. 74, 223-233.
2. 'Association of Official Analytical Chemists' F.D.A. *Bacteriological Analytical Manual* 8th Edition (1995) AOAC, Arlington Va.

MODIFIED LAURYL SULPHATE TRYPTOSE BROTH WITH MUG AND ADDED TRYPTOPHAN

Code: CM0967

A modified Lauryl Tryptose Broth, incorporating 4-methylumbelliferyl-β-D-glucuronide (MUG) allowing the enumeration of presumptive Escherichia coli as well as other coliforms, using the Most Probable Number (MPN) method.

Formula	***gm/litre***
Tryptose	20.0
Lactose	5.0
Dipotassium hydrogen phosphate	2.75
Potassium dihydrogen phosphate	2.75
Sodium chloride	5.0
Sodium lauryl sulphate	0.1
4-methylumbelliferyl-β-D-glucuronide (MUG)	0.1
Tryptophan	1.0
pH 6.8 ± 0.2	

Directions
Dissolve 36.7 g of Modified Lauryl Sulphate Tryptose Broth with MUG in 1 litre of distilled water.

Dispense into final containers containing Durham tubes.

Sterilise by autoclaving at 121°C for 15 minutes.

Description
Oxoid Modified Lauryl Sulphate Tryptose Broth with MUG is formulated to allow use of the MPN technique for coliforms and also the enumeration of presumptive *Escherichia coli* by means of a culture technique involving a liquid medium containing MUG.

The formulation contains 4-methylumbelliferyl-β-D-glucuronide (MUG), which is cleaved by the enzyme β-glucuronide to release 4-methylumbelliferone. This blue-green fluorophore exhibits blue-green fluorescence visible when viewed under long wave ultra-violet (366 nm). The inclusion of tryptophan acts as a substrate for indole production. Both reactions are characteristic of *Escherichia coli* and can therefore be used to identify presumptive *Escherichia coli.*

Coliform organisms will ferment lactose to produce gas. This production of gas can be taken as positive for the presence of coliforms.

Technique
Prepare a sufficient number of dilutions of original sample to ensure tubes for the final dilution will yield a negative result. Inoculate each dilution into tubes of Modified Lauryl Sulphate Tryptose Broth with MUG containing inverted Durham tubes. For the MPN technique, inoculate each dilution in triplicate.

Incubate the tubes at 30°C for 24-48 hours. Examine the tubes for growth turbidity, gas production, fluorescence and formation of indole. Read as follows:

1. Tubes showing fluorescence gas and formation of indole indicate presumptive *Escherichia coli*.

2. Tubes showing gas formation indicate coliforms.

The MPN index can be determined from the numbers of positive tubes of selected dilutions by means of an MPB table, and a calculation of the MPN of presumptive *Escherichia coli* or coliforms per gram or per millilitre of the original sample carried out.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured solution.

Quality control

Positive control:	***Expected results***
Escherichia coli ATCC® 25922*	Turbid growth; gas and fluorescence
Negative controls:	
Staphylococcus aureus ATCC® 25923*	Inhibited; no gas or fluorescence
Enterobacter aerogenes ATCC® 13048*	Turbid growth; with or without gas, no fluorescence

*This organism is available as a Culti-Loop®

References

1. IDF-170L (1994) Milie & Milie products. Enumeration of presumptive *Escherichia coli*.
2. ISO-11866-2: (1997) (E).

LEGIONELLA GVPC SELECTIVE MEDIUM

A selective medium for the isolation of Legionella species from environmental samples.

LEGIONELLA CYE AGAR BASE

Code: CM0655

Formula	***gm/litre***
Activated charcoal	2.0
Yeast extract	10
Agar	13.0

LEGIONELLA (GVPC) SELECTIVE SUPPLEMENT

Code: SR0152

Vial contents (each vial is sufficient to supplement 500 ml of BCYE Medium)	***per vial***	***per litre***
Glycine (Ammonia free)	1.5 g	3.0 g
Vancomycin hydrochloride	0.5 mg	1.0 mg
Polymixin B sulphate	39600 IU	79200 IU
Cycloheximide	40.0 mg	80.0 mg

Directions

Reconstitute one vial as directed. Add the contents to 500 ml of sterile BCYEα Medium (prepared using Legionella CYE Agar Base to which one vial of Legionella BCYEα Growth Supplement SR0110 has been added), cooled to 50-55°C. Mix gently and pour into sterile Petri dishes.

Description

Legionella pneumophila was first isolated by infecting guinea-pigs and fertile hens' eggs in 1977[1]. Examination of water samples for the presence of legionellae has frequently been undertaken using direct immunofluorescence methods[2]. However, isolation is still the most widely accepted method of demonstrating the presence of Legionellae and is of particular value in epidemiological studies.

Currently, a buffered charcoal yeast extract medium (BCYE) is generally considered to be the medium of choice[3], to which various antimicrobials have been added to make it selective.

GVPC Selective Supplement is based on the formulation described by Dennis *et al*[4]. This selective formulation has been reported to be the most efficient *in vitro* method for the isolation of *Legionella pneumophila* when used in conjunction with acid or heat pretreatments. Cycloheximide is included because it has a greater activity against fungi than anisomycin which is almost exclusively active against yeasts. Fungi commonly occur more frequently than yeasts in environmental waters examined for Legionella. This

formulation is to be specified by the BSI (British Standard) for the detection and enumeration of legionellae in water and related materials[5].

The antimicrobials Glycine, Vancomycin and Polymixin will collectively inhibit most Gram-positive and Gram-negative bacterial growth. Cycloheximide will suppress the growth of fungi.

Technique
For each sample, three plates should be inoculated[3,5]: one after pretreatment with heat, one after pretreatment with acid and one that has received neither pretreatment.

Heat pretreatment
1. Take 10 ml of concentrated sample[3] and place in a water bath at 50°C for 30 minutes.

Acid pretreatment
1. Take 10 ml of concentrated sample[3] and centrifuge in sealed buckets at 2,500 rpm for 20 minutes.
2. Decant the supernatant to leave approximately 1 ml of fluid.
3. Add 9 ml of HCl-KCl buffer (see below) and resuspend by gentle shaking. Leave to stand for 5 minutes and inoculate without further delay.

HCl-KCl Buffer

3.9 ml of 1.2 M HCl
25 ml of 0.2 M KCl
Adjust to pH 2.2 using 1M KOH

No pretreatment
1. Take 10 ml of concentrated sample[3] and do not pretreat.

Directions
1. Spread 0.1 ml of each portion, described above, onto plates of GVPC Selective Medium using a sterile spreader.
2. Incubate the plates at 36 + 1°C and examine on days 3, 5, 7 and 10.
3. Suspect colonies should be verified as presumptive Legionella by the following procedure: Select several colonies of each type and subculture on to a pair of plates, one of Buffered Charcoal Yeast Extract Agar and one of Buffered Charcoal Yeast Extract Agar containing Legionella BCYE Growth Supplement SR0110. Regard as presumptive Legionella all colonies which grow on Buffered Charcoal Yeast Extract Agar containing the supplement but which fail to grow on medium not containing the supplement.
4. Further confirmation of identity can be obtained by subculturing colonies taken from the primary culture plates on to Blood Agar. Isolates that fail to grow on Blood Agar and are small, poorly staining Gram-negative rods are presumptively identified as *Legionella* species.
5. Each presumptive Legionella colony should be confirmed by serology.

Legionella pneumophila colonies are white-grey-blue in appearance and up to 2 mm in diameter with a ground-glass appearance. Colonies of all Legionella spp. exhibit the same general appearance but they may differ in colour. Colours include brown, lime green, deep red and blue-purple.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the selective supplement in the dark at 2-8°C and use before the expiry date on the label.

Appearance
Dehydrated Medium: Black free flowing powder

Prepared medium: Black coloured gel.

Quality Control
The medium may be tested for performance using stable typical control cultures of organisms other than *Legionellae*. However, when testing Legionellae, 'wild' strains must be used because Legionellae easily become adapted to growth under laboratory conditions and will grow on media that would not support the primary isolation of 'wild' strains[5]. 'Wild' strains can be defined as strains which have been subcultured no more than twice following primary isolation. GVPC Medium should not be tested using *Legionella pneumophila* strains that may have become laboratory acclimatised.

Incubation at 35-37°C + 0.5°C for 5 days in air or in air enriched with 2.5% CO_2[5] in conditions of high humidity.

References

1. McDade J. E. *et al.* (1977) *N. Engl. J. Med.* 297. 1197-1203.
2. Fliermans C. B. *et al.* (1981) *Appl. Environ. Microbiol.* 41. 9-16.
3. Dennis P. J. L. (1988) *Isolation of legionellae from environmental specimens.* In Harrison T. G. and Taylor A. G. (eds). *A Laboratory Manual for Legionella*, John Wiley & Sons Limited, Chichester.
4. Dennis P. J. L., Bartlett C. L. R. and Wright A. E. (1984) *Comparison of isolation methods for Legionella spp.* In Thornsbury C. *et al.* (eds). *Legionella: Proceedings of the 2nd International Symposium*. Washington D. C. *Am. Soc. Microbiol.* pp 294-296.
5. *BSI Document. Determination of Legionellae in water and related materials. Method for their detection and enumeration*. July 1989 DRAFT DOCUMENT. 89/53406.

LEVINE MEDIUM – see EOSINE METHYLENE BLUE AGAR

LISTERIA SELECTIVE AGAR (OXFORD FORMULATION)

Code: CM0856

A selective and diagnostic medium for the detection of Listeria monocytogenes, when prepared from Listeria Selective Agar Base and Listeria Selective Supplement SR0140 or Modified Listeria Selective Supplement (Oxford) SR0206

Formula	***gm/litre***
Columbia Blood Agar Base	39.0
Aesculin	1.0
Ferric ammonium citrate	0.5
Lithium chloride	15.0
pH 7.0 ± 0.2	

LISTERIA SELECTIVE SUPPLEMENT (OXFORD FORMULATION)

Code: SR0140

Vial contents (each vial is sufficient for 500 ml of medium)	***per vial***	***per litre***
Cycloheximide	200 mg	400 mg
Colistin sulphate	10.0 mg	20.0 mg
Acriflavine	2.5 mg	5.0 mg
Cefotetan	1.0 mg	2.0 mg
Fosfomycin	5.0 mg	10.0 mg

MODIFIED LISTERIA SELECTIVE SUPPLEMENT (OXFORD)

Code: SR0206

Vial contents (each vial is sufficient for 500 ml of medium)	***per vial***	***per litre***
Amphotericin B	5.0 mg	10.0 mg
Colistin sulphate	10.0 mg	20.0 mg
Acriflavine	2.5 mg	5.0 mg
Cefotetan	1.0 mg	2.0 mg
Fosfomycin	5.0 mg	10.0 mg

Directions
Suspend 27.75 of the Listeria Selective Agar Base (Oxford Formulation) in 500 ml of distilled water. Bring gently to the boil to dissolve. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C and aseptically add the contents of one vial of Listeria Selective Supplement (Oxford Formulation) or Modified Listeria Selective Supplement (Oxford) SR0206, SR0140 reconstituted with 5 ml of 70% ethanol. Mix well and pour into sterile Petri dishes.

Description
Foodborne infection by *Listeria monocytogenes* has prompted increased concern for detecting this organism in foods, in the environment and in pathological specimens from both human and animal subjects.

Most infections in adult humans are symptomless and result in intestinal, vaginal and cervical carriage. Infection during pregnancy may cause abortion, premature delivery and neonatal infection. The possibility of listeriosis should be considered in any woman with unexplained recurrent miscarriage, premature labour or foetal death. The organism should be sought in blood cultures and genital-tract swabs[1].

The most common clinical manifestation in both adults and neonates is meningitis. Widely disseminated infection, abscesses, sub-acute bacterial endocarditis and opportunistic infections in immunosuppressed patients occur less frequently.

Birds, fish and other animals are all susceptible to infection with *Listeria*. It is of particular importance in domestic farm animals. In the Federal Republic of Germany reporting of listeriosis in animals is compulsory and meat inspection law in the same country requires examination for *Listeria* because of its significance in meat hygiene.

Listeria monocytogenes is very widespread in the environment. Isolation has been reported from milk[2,3], cheese[4], sewage and riverwater[5], and silage[6]. Because *Listeria* is so widespread sources of infections are numerous. Uncooked vegetable foods have been implicated; an episode associated with consumption of coleslaw[7] was linked with cabbage from a farm using sewage fertiliser. In outbreaks caused by dairy products, cattle with mastitis may be the source of the organism. Of great importance to veterinarians is the considerable increase amongst sheep of infection manifesting as abortion or encephalitis due largely to changing practices in silage manufacture[8].

The ability to isolate the organism has been impeded in the past by lack of an effective selective medium, as *Listeria monocytogenes* can be easily and completely overgrown by competing flora.

Listeria Selective Medium (Oxford Formulation) is based on the formulation described by Curtis *et al*.[9] and is recommended for the detection of *Listeria monocytogenes* from clinical and food specimens.

The medium utilises
(i) the selective inhibitory components lithium chloride, acriflavine, colistin sulphate, cefotetan, cycloheximide or amphotericin B and fosfomycin,
(ii) the indicator system aesculin and ferrous iron for the isolation or differentiation of *Listeria monocytogenes*.

Listeria monocytogenes hydrolyses aesculin, producing black zones around the colonies due to the formation of black iron phenolic compounds derived from the aglucon. Gram-negative bacteria are completely inhibited. Most unwanted gram- positive species are suppressed, but some strains of enterococci grow poorly and exhibit a weak aesculin reaction, usually after 40 hours incubation. Some staphylococci may grow as aesculin-negative colonies.

Typical *Listeria monocytogenes* colonies are almost always visible after 24 hours, but incubation should be continued for a further 24 hours to detect slow-growing strains.

Techniques for isolation vary with the author and the material under examination[10,11]. For all specimens selective enrichment and cold enrichment have been shown to increase isolation rates significantly[12,13,14]. The efficacy of Listeria Selective Medium (Oxford Formulation) has been confirmed for various foods[15,16] following the methodology and using selective enrichment media described in the literature[16,17,18,19].

Oxford agar is a specified plating medium in the FDA/BAM isolation procedure[20] and in the standardised testing methods of other national and international bodies[21].

Oxford agar base was used by Al-Zoreki and Sandine as the basal medium for their ASLM agar which incorporates ceftazidime, moxalactam and cycloheximide as selective agents[22].

Technique

Faecal and Biological Specimens
The sample is homogenised in 0.1% Peptone Water CM0009 (1 part to 9 parts peptone water).

Direct Surface Plate Method

1. Inoculate 0.1 ml of the homogenised specimen onto the Listeria Selective Medium plates.
2. Incubate at 35°C for up to 48 hours.
3. Examine for typical colonies of Listeria after 24 and 48 hours incubation.

Selective Enrichment Method

1. Add the homogenised specimen to the selective enrichment broth and incubate at 30°C for up to 7 days.
2. Inoculate 0.1 ml of the selective enrichment broth, after 24 hours, 48 hours and 7 days, onto the Listeria Selective Medium plates.
3. Incubate the plates at 35°C for up to 48 hours.
4. Examine for typical colonies of Listeria after 24 and 48 hours incubation.

Food and Environmental Samples

Techniques for isolation vary with the author, material and authorities. For detection of *Listeria monocytogenes* when present in small numbers, the test samples must be inoculated into an enrichment broth to allow multiplication before isolation and identification. Depending on the type of sample under test, an appropriate method and selective enrichment broth should be chosen prior to inoculation onto the Listeria Selective Medium plates.

1. Inoculate 0.1 ml of the selective enrichment broth onto the Listeria Selective Medium plates.
2. Incubate at 35°C for up to 48 hours.
3. Examine for typical colonies after 24 and 48 hours incubation.

Colonies presumptively identified as *Listeria monocytogenes* must be confirmed by biochemical and serological testing[23].

Note

Differences in susceptibility of *Listeria monocytogenes*, *Listeria seeligeri* and *Listeria ivanovii* to β-lactam antibiotics and fosfomycin have been observed dependent on whether incubation is at 30°C or 35-37°C[24].

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared plates at 2-8°C in the dark.

Appearance

Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Pale green coloured gel.

Quality Control

Positive control:	***Expected result***
Listeria monocytogenes ATCC® 7644*	Good growth; brown coloured colonies with aesculin hydrolysis.
Negative control:	
Entercoccus feacalis ATCC® 29212*	No growth

*This organism is available as a Culti-Loop®

Precautions

Listeria monocytogenes is in ACDP Group 2 i.e. 'might be a hazard to laboratory workers' and should be handled in a suitable environment only. It is also recommended that pregnant staff should be excluded from working with known cultures of *Listeria*.

Listeria media containing acriflavine should be protected from light because photo-oxidation makes it inhibitory to *Listeria*.

Supplement SR0140 used in this medium contains a toxic concentration of cycloheximide. Note the precautions to be taken under HAZARDS.

References

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LISTERIA ENRICHMENT BROTH BASE

Code: CM0862

Formula	***gm/litre***
Tryptone soya broth	30.0
Yeast extract	6.0
pH 7.3 ± 0.2	

LISTERIA SELECTIVE ENRICHMENT SUPPLEMENT

Code: SR0141

Vial contents (each vial is sufficient for 500 ml of medium)	***per vial***	***per litre***
Nalidixic acid	20.0 mg	40.0 mg
Cycloheximide	25.0 mg	50.0 mg
Acriflavine hydrochloride	7.5 mg	15.0 mg

Directions
Suspend 18 g in 500 ml of distilled water. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C and aseptically add the contents of one vial of Listeria Selective Enrichment Supplement SR0141, reconstituted with 2 ml of sterile distilled water. Mix well and distribute into sterile containers in volumes as required.

LISTERIA SELECTIVE ENRICHMENT SUPPLEMENT

(modified with 10 mg/litre of Acriflavine)

Code: SR0149

Vial contents (each vial is sufficient for 2,250 mls of medium)	***per vial***	***per litre***
Nalidixic acid	90.0 mg	40.0 mg
Cycloheximide	112.5 mg	50.0 mg
Acriflavine hydrochloride	22.5 mg	10.0 mg

Directions

Aseptically add 10 ml of sterile distilled water to one vial and invert gently to dissolve. Aseptically add the vial contents to 2.25 litres of Listeria Enrichment Broth Base, cooled to 50°C.

Description

Listeria Selective Enrichment Medium is based on the formulation described by Lovett *et al*.[1] and is recommended for the selective enrichment of Listeria species from food. The enrichment procedure has been shown to recover an inoculum of less than 10 cfu/ml from raw milk.

In order to achieve a higher isolation rate it is recommended that the enrichment broth is subcultured onto Listeria Selective Agar plates after 1, 2 and 7 days. Agello *et al*.[2], have shown that extending the incubation period to 7 days allows better recovery of environmentally stressed listeria from milk and milk products.

Technique

1. Add 25 g or 25 ml samples to 225 ml of Listeria Selective Enrichment Broth. Homogenise if required.

2. Incubate at 30°C for 7 days.

3. Subculture from the Listeria Selective Enrichment Broth onto Listeria Selective Agar plates (see Note) after 1, 2 and 7 days by:

(i) Direct plating onto Listeria Selective Agar plates.

(ii) Adding 1 ml of the Listeria Selective Enrichment Broth to 9 ml of 0.5% KOH, vortex mixing, and plating onto Listeria Selective Agar plates.

Note:

Suitable Listeria Selective Media are:

1. Listeria Selective Medium (Oxford formulation) (CM0856 and SR0140).

2. PALCAM Medium (CM0877 and SR0150).

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance

Dehydrated Medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured solution.

Quality control

Positive controls:	***Expected results***
Listeria monocytogenes ATCC® 7644*	Turbid growth
Negative control:	
Enterococcus feacalis ATCC® 29212*	Inhibited

*This organism is available as a Culti-Loop®

Precautions

Note the precautions stated under Listeria Selective Medium (Oxford) CM0856 and SR0140. Broth cultures are more dangerous than colonies on agar plates.

Store prepared medium away from light. Acriflavine can photo-oxidise to form inhibitory compounds against *Listeria*.

Supplement SR0141 used in this medium contains a toxic concentration of cycloheximide. Note the precautions to be taken under HAZARDS.

References

1. Lovett J., Francis D. W. and Hunt J. M. (1987) *Journal of Food Protection* 50. 188-192.
2. Agello G., Hayes P. and Feeley J. (1986) *Abstracts of the Annual Meeting, ASM, Washington DC* p5.

LISTERIA ENRICHMENT BROTH BASE (UVM FORMULATION)

Code: CM0863

A two step selective enrichment medium for USDA-FSIS method.

Formula	***gm/litre***
Proteose peptone	5.0
Tryptone	5.0
'Lab-Lemco' powder	5.0
Yeast extract	5.0
Sodium chloride	20.0
Disodium hydrogen phosphate	12.0
Potassium dihydrogen phosphate	1.35
Aesculin	1.0
pH 7.4 ± 0.2	

LISTERIA PRIMARY SELECTIVE ENRICHMENT SUPPLEMENT (UVM I)

Code: SR0142

Vial contents (each vial is sufficient for 500 ml of medium)	***per vial***	***per litre***
Nalidixic acid	10.0 mg	20 mg
Acriflavine hydrochloride	6.0 mg	12 mg

LISTERIA PRIMARY SELECTIVE ENRICHMENT SUPPLEMENT (UVM II)

Code: SR0143

Vial contents (each vial is sufficient for 500 ml of medium)	***per vial***	***per litre***
Nalidixic acid	10 mg	20 mg
Acriflavine hydrochloride	12.5 mg	25 mg

Directions

Suspend 27.2 g in 500 ml of distilled water. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C.

To Prepare Listeria Primary Selective Enrichment Medium (UVM I)
Aseptically add the contents of 1 vial of Listeria Primary Selective Enrichment Supplement (UVM I) SR0142 reconsituted as directed. Mix well and distribute into sterile containers.

To Prepare Listeria Secondary Selective Enrichment Medium (UVM II)
Aseptically add the contents of 1 vial of Listeria Primary Selective Enrichment Supplement (UVM II) SR0143 reconsituted as directed. Mix well and distribute into sterile containers.

Description
The Listeria Selective Enrichment Media (UVM formulations) are based on the original formulation described by Donnelly and Baigent[1], and its subsequent modification[2] which reduced the nalidixic acid concentration in both the primary and secondary selective enrichment media and increased the concentration of acriflavine hydrochloride in the secondary selective enrichment medium.

This modification, and the two step selective enrichment method developed (USDA-FSIS method)[2], results in a higher detection rate of *Listeria monocytogenes* from meat products and has the added advantage of only taking 3-4 days.

UVM Broth has been recommended as a primary enrichment broth for recovery of heat-injured *Listeria monocytogenes*[3].

Care must be taken when using UVM broth with DNA probe methodology because the high salt content of the medium may have an inhibitory effect on detection[4].

Technique

Primary Enrichment

1. Add 25 g or 25 ml samples to 225 ml of Listeria Primary Selective Enrichment Medium (UVM I). Homogenise in a Stomacher for 2 minutes.
2. Incubate the prepared sample in the Stomacher bag at 30°C.
3. From this bag, carry out the following procedures: After 4 hours incubation, spread 0.2 ml on Listeria Selective Agar plates (see Note).

   After 24 hours incubation,

   (i) transfer 0.1 ml to 10 ml of Listeria Secondary Enrichment Medium (UVM II), and

   (ii) transfer 1 ml to 4.5 ml KOH solution. Vortex mix and within one minute sub-culture onto Listeria Selective Agar plates. For details of KOH preparation see below.

Secondary Enrichment

4. Incubate the inoculated Listeria Secondary Selective Enrichment Medium (UVM II) at 30°C. See 3(i).
5. After 24 hours incubation,

   (i) spread 0.2 ml onto Listeria Selective Agar plates,

   (ii) transfer 1 ml to 4.5 ml KOH solution. Vortex mix and within one minute sub-culture this mixture onto Listeria Selective Agar plates.

Preparation of KOH Solution

Dissolve 2.5 g of KOH and 20 g of NaCl in 1000 ml of distilled water. Sterilise by autoclaving at 121°C and ensure that the pH is above 12.0 before use.

Note

The Listeria Selective Agar recommended for use in the USDA method[2] is LPM plating medium[3]. However, Oxoid laboratory studies[5] have shown that comparable results can be achieved with Listeria Selective Medium (Oxford formulation) CM0856 and SR0140.

Updated USDA methodology[6] has replaced LPM medium with Modified Oxford Medium (MOX). There is no longer a requirement to treat enrichment culture with potassium hydroxide before plating.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance

Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured solution.

Quality control

Positive control:	***Expected results***
Listeria monocytogenes ATCC® 7644*	Turbid growth
Negative control:	
Enterococcus faecalis ATCC® 29212*	No change

*This organism is available as a Culti-Loop®

Precautions

Note the precautions stated under Listeria Selective Medium (Oxford) CM0856 and SR0140.

Broth cultures are more dangerous than colonies on agar plates.

Store prepared medium away from light. Acriflavine can photo-oxidise to form inhibitory compounds against listeriae.

References
1. Donnelly C. W. and Baigent G. J. (1986) *Appl. Environ. Microbiol.* 52. 689-695.
2. McClain D. and Lee W. H. (1988) *Assoc. Off. Anal. Chem.* 71. 660-664.
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4. Partis L., Newton L., Marby J. and Wells R. J. (1994) *Appl. Environ. Microbiol.* 60. 1693-1694.
5. Sawhney D. R. and Dodds L. (1988) *Internal project report.* Oxoid R&D Laboratory.
6. McLain D. and Lee W. H. (1989) *FSIS Method for the isolation and identification of Listeria monocytogenes from processed meat and poultry products. Laboratory Communications number 57*.

LISTERIA SELECTIVE AGAR PALCAM – see PALCAM AGAR

LIVER BROTH

Code: CM0077

A liquid medium, containing liver particles, for the examination of foods for saccharolytic or putrefactive mesophilic and thermophilic anaerobes.

Formula	***gm/litre***
Infusion from fresh liver	23.0
Peptone	10.0
Potassium phosphate	1.0
Extracted liver tissue	30.0
pH 6.8 ± 0.2	

Directions
Suspend 64 g in 1 litre of distilled water and soak for 15 minutes, with occasional stirring. Distribute into 18mm diameter tubes to a depth of 50 mm so that the bottom of the tube is filled with liver particles. Agitate frequently during distribution to keep the liver tissue in suspension. Sterilise by autoclaving for 20 minutes at 115°C. Inoculate when cool and then aseptically seal with a layer of sterile 2% Oxoid Agar No. 3 (LP0013) solution.

Description
A liver infusion medium, containing liver particles, for the examination of foods for saccharolytic or putrefactive mesophilic and thermophilic anaerobes. Also recommended for the maintenance of aerobes and anaerobes in pure culture.

Technique
Gillespie in communication with Scarr[1] recommended Liver Broth for the examination of canners' sugar for hydrogen swells caused by thermophilic anaerobes (*Clostridium thermosaccharolyticum*). A 20% w/v solution of the sugar is steamed for 30 minutes to destroy vegetative forms and inoculated into Liver Broth sealed with agar. The standard proposed was a maximum of 1 positive tube in six – with 20 ml inocula incubated for 72 hours at 56°C.

This medium should be made up only when required for use. Storage of the reconstituted medium is not recommended because air may be absorbed and the re-steaming necessary for the restoration of anaerobic conditions darkens the medium. Liver broth is not an homogeneous medium; consisting of a layer of liver particles and a cloudy supernatant. Growth produces an obvious increase in turbidity and some organisms (e.g. *Clostridium thermosaccharolyticum*) also produce gas which often pushes the agar plug towards the top of the tube. Some organisms also digest the solid liver tissue.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance
Dehydrated medium: Brown powder/granules.

Prepared medium: Dark brown solution/particles.

Quality control

Positive control:	***Expected result***
Clostridium thermosaccharolyticum ATCC® 7956	Good growth; with gas
Negative control:	
Uninoculated medium	No change

Precautions
Note the comments on storage and reheating.

Reference
1. Scarr M. Pamela (1958) *DSIR, Proc. 2nd Internat. Symp. Food Microbiol*. 1957, HMSO London, pp.29-33.

LYSINE DECARBOXYLASE BROTH (TAYLOR MODIFICATION)

Code: CM0308 (Tablets)

To detect lysine decarboxylase production by salmonellae and some other Enterobacteriaceae.

Formula	***gm/litre***
Yeast extract	3.0
Glucose	1.0
L-lysine	5.0
Bromocresol purple	0.016
pH 6.1 ± 0.2	

Directions
Add 1 tablet to 5 ml of distilled water in an appropriate bottle. Sterilise by autoclaving at 121°C for 15 minutes.

Description
Lysine Decarboxylase Broth is a diagnostic medium which distinguishes salmonellae (and some other Enterobacteriaceae) by a distinct biochemical reaction.

Taylor's modification of the medium[1] shows an improved performance over the formulation described by Falkow[2]. This is achieved by omitting peptone from the medium, thus eliminating false positives caused by *Citrobacter freundii* and its paracolon types. These organisms utilise peptone as a nitrogen source, produce an alkaline reaction masking the absence of lysine decarboxylase.

Taylor's modification shares the advantages of Falkow's formulation over that of Moller[3] in that it does not require anaerobic culture and low pH and it is relatively easy to control.

During the initial stages of incubation, fermentation of glucose by the organism, with acid production results in a colour change to yellow. On further incubation, if lysine is decarboxylated to cadaverine, there will be an alkaline reaction. The colour then changes to purple (positive). If the colour remains yellow, the reaction is negative.

Decarboxylase reactions of various members of the Enterobacteriaceae on lysine

Organism	***Lysine Decarboxylation***
Salmonella species	+
S. paratyphi A	–
Shigella species	–
Escherichia coli (including late-lactose variants Alcalescens-Dispar) V	V
Citrobacter species (including the Bethesda-Ballerup group)	–
Providencia species	–
Proteus species	–
Serratia species	V
Klebsiella species	V
Enterobacter species	V

Technique
The medium is inoculated with the organism and incubated for 24 hours at 35°C.

Results after 24 hours:

Purple colour – positive reaction.

Yellow colour – negative reaction.

Storage conditions and Shelf life
Store the tablets at 2-8°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance
Dehydrated Medium: blue/grey tablets.

Prepared medium: blue/grey solution.

Quality Control

Positive controls:	***Expected results***
Salmonella typhimurium ATCC® 14028*	Turbid growth, purple medium
Negative control:	
Citrobacter freundii ATCC® 8090*	Turbid growth, yellow medium

*This organism is available as a Culti-Loop®

Precautions
Use light inocula for the tubes of lysine medium.

Note the negative reaction of *Salmonella paratyphi A*.

Do not read the test after less than 24 hours incubation. Some organisms may require prolonged incubation for up to 4 days.

References
1. Taylor W. I. (1961) *Appl. Microbiol.* 9. 487-490.
2. Falkow S. (1958) *Amer. J. Clin. Path.* 29. 598-600.
3. Moller V. (1955) *Acta Path. Microbiol. Scand.* 36. 158-172.

LYSINE IRON AGAR

Code: CM0381

A diagnostic medium for salmonellae including Salmonella arizonae.

Formula	***gm/litre***
Bacteriological peptone	5.0
Yeast extract	3.0
Glucose	1.0
L-lysine	10.0
Ferric ammonium citrate	0.5
Sodium thiosulphate	0.04
Bromocresol purple	0.02
Agar	14.5
pH 6.7 ± 0.2	

Directions

Suspend 34 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Dispense into tubes and sterilise by autoclaving at 121°C for 15 minutes. Cool the tubes in an inclined position to form slants with deep butts.

Description

Lysine Iron Agar is a differential medium which detects salmonellae (including lactose-fermenting *Salmonella arizonae*) by lysine decarboxylase activity and H_2S production. Edwards & Fife[1] developed the medium to detect lactose-fermenting salmonellae which will produce pink colonies on lactose-containing media e.g. DCA and BGA. In the usual examination for enteric pathogens these organisms would be overlooked. Further, many of these cultures, when transferred to Triple Sugar Iron (TSI) Agar slants, produced acid conditions in the medium so quickly that the expected positive reaction for hydrogen sulphide was suppressed. Since *Salmonella arizonae* strains which ferment lactose rapidly are found occasionally in outbreaks of food infection, it is important to determine their occurrence.

The only recognised groups of Enterobacteriaceae which regularly decarboxylate lysine rapidly and which produce large amounts of hydrogen sulphide, are the salmonellae[2,3].

Lysine Iron Agar is therefore a sensitive medium for the detection of lactose-fermenting and non-lactose-fermenting salmonellae.

Technique

The medium is tubed, sterilised and slanted so that a short slant and deep butt are formed. It is inoculated with a straight needle by stabbing to the base of the butt and streaking the slant. The caps of the tubes must be replaced loosely so that aerobic conditions prevail on the slant. Incubate at 35°C overnight.

Cultures which rapidly produce lysine decarboxylase cause an alkaline reaction (purple colour) throughout the medium. Those organisms that do not decarboxylate lysine produce an alkaline slant and an acid butt (yellow colour).

Cultures which produce hydrogen sulphide cause an intense blackening in the medium.

Due to deamination of the lysine, *Proteus* and *Providencia* cultures produce a red slant over an acid butt.

Reactions

Cultures	***Slant***	***Butt***	***H_2S***
Salmonella	Alkaline	Alkaline	+
Proteus	Red	Acid	–
Providencia	Red	Acid	–
Citrobacter	Alkaline	Acid	+
Escherichia	Alkaline	Acid or neutral	–
Shigella	Alkaline	Acid	–
Klebsiella	Alkaline	Alkaline	–

Thatcher & Clark[4] described a procedure for the isolation of salmonellae from foods in which suspect colonies from selective agar plates were purified and then inoculated into Lysine Iron Agar and Triple Sugar Iron Agar. Using this combination of media a greater discrimination can be made between the coliform organisms, e.g. *Escherichia* and *Shigella*.

Timms[5] described the techniques of isolation and identification of salmonellae infection in turkeys, using Lysine Iron Agar.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.
Store the prepared medium at 2-8°C.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.
Prepared medium: Purple coloured gel.

Quality control

Positive controls:	***Expected results***
Lysine decarboxylation	
Enterobacter aerogenes ATCC® 13048*	Slant: Alkaline. Butt: Alkaline. H_2S: Negative
Deamination	
Proteus mirabilis ATCC® 29906*	Slant: Red. Butt: Acid. H_2S: Positive
Negative control:	
Enterobacter cloacae ATCC® 23355*	Slant: Alkaline. Butt: Acid. H_2S: Negative

*This organism is available as a Culti-Loop®

Precautions
Salmonella paratyphi A does not produce lysine decarboxylase and therefore will give an alkaline slant and an acid butt.
H_2S-producing *Proteus* species do not blacken this medium[6].

References

1. Edwards P. R. and Fife Mary A. (1961) *Appl. Microbiol.* 9. 478-480.
2. Moeller V. (1954) *Acta. Pathol. Microbiol. Scand.* 355. 259-277.
3. Ewing W. H., Davis B. R. and Edwards P. R. (1960) *Pub. Hlth Labs.* 18. 77-83.
4. Thatcher F. S. and Clark D. S. (1968) University of Toronto Press, p.100.
5. Timms L. (1971) *Med. Lab. Tachn.* 28. 150-156.
6. Finegold S. M. & Martin W. J. (1982) *Bailey & Scott's Diagnostic Microbiology.* 6th edn. C. V. Mosby. St. Louis. p.63l.

LYSINE MEDIUM

Code: CM0191

A synthetic medium for the isolation and enumeration of wild yeasts encountered in brewing. On this medium, pitching yeasts are suppressed.

Formula	***gm/litre***
Glucose	44.5
Potassium dihydrogen phosphate	1.78
Magnesium sulphate	0.89
Calcium chloride fused	0.178
Sodium chloride	0.089
Adenine	0.00178
DL-methionine	0.000891
L-histidine	0.000891
DL-tryptophane	0.000891
Boric acid	0.0000089
Zinc sulphate	0.0000356
Ammonium molybdate	0.0000178
Manganese sulphate	0.0000356
Ferrous sulphate	0.0002225
Lysine	1.0
Inositol	0.02
Calcium pantothenate	0.002
Aneurine	0.0004
Pyridoxine	0.0004
p-aminobenzoic acid	0.0002
Nicotinic acid	0.0004
Riboflavin	0.0002
Biotin	0.000002
Folic acid	0.000001
Agar	17.8
pH (see directions)	

Directions
Suspend 6.6 g in 100 ml distilled water containing 1.0 ml Potassium lactate SR0037. Bring to the boil to dissolve completely. Agitate frequently to prevent superheating. Cool to 50°C and add 0.1 ml of lactic acid 10% SR0021 to adjust to pH 4.8 ± 0.2. Dispense into Petri dishes and remove surface moisture by drying at 37°C.

Description
A complex medium, originally described by Morris & Eddy[1] for the isolation and enumeration of wild yeasts in pitching yeast. Walters & Thiselton[2] examined 180 species of yeasts in a liquid synthetic medium containing lysine as the sole nitrogen source. They found that no normal cerevisiae or *Carlsbergensis* strains utilised lysine whereas many other yeasts, including wild yeasts, did so. They kept their stock cultures on malt extract agar slopes or on malt extract chalk agar in the case of *Brettanomyces* species. Later, Morris & Eddy[1] described a solid lysine medium for the isolation and enumeration of wild yeasts in pitching yeast. Oxoid Lysine Agar is made to their published formula.

Technique
Wash and centrifuge the sample of pitching yeast three times with distilled water. Remove 0.2 ml of a suspension containing approximately 107 cells per ml and spread with a bent platinum wire, over the surface of a Lysine Medium plate. Incubate at 25°C and examine daily for evidence of growth. Count the number of colonies which develop, and express the degree of contamination as the number of wild yeast cells per million cells of the original inoculum.

The number of cells in the inoculum is important as it has been shown by Morris & Eddy that small numbers of cells (approximately 100 to 1,000) still grow to a limited extent on the medium. Where the number of

brewing yeast cells exceeds approximately 10,000, a count of the colonies developing provides a direct measure of the contamination by wild yeasts[3].

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance
Dehydrated medium: White coloured, free-flowing powder.

Prepared medium: Light straw coloured gel.

Quality control

Positive control:	***Expected results***
Pichia fermentans ATCC® 10651	Good growth; white colonies
Negative control:	
Saccharomyces (carlsbergensis) uvarum ATCC® 2700	Slight background film

Precautions
The pitching yeast may grow as a slight background film with the 'wild' yeast appearing as colonies on the film.

References
1. Morris E. O. and Eddy A. A. (1957) *J. Inst. Brew.* 63(1). 34-35.
2. Walters L. S. and Thiselton M. R. (1953) *J. Inst. Brew.* 59. 401.
3. Fowell R. R. (1965) *J. Appl. Bact.* 28. 373-383.

M17 AGAR

Code: CM0785

For improved growth of lactic streptococci and their bacteriophages and selective enumeration of Streptococcus thermophilus from yogurt.

Formula	***gm/litre***
Tryptone	5.0
Soya peptone	5.0
'Lab-Lemco' powder	5.0
Yeast extract	2.5
Ascorbic acid	0.5
Magnesium sulphate	0.25
Di-sodium-glycerophosphate	19.0
Agar	11.0
pH 6.9 ± 0.2	

Directions
Suspend 48.25 g in 950 ml of distilled water and bring gently to the boil. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C and add 50 ml of sterile lactose solution (10% w/v).

Lactose solution 10% (w/v)
Dissolve 10 g of Lactose Code LP0070 in 100 ml of distilled water. Sterilise by autoclaving at 121°C for 15 minutes or by membrane filtration through a 0.2 µm membrane.

Description
M17 Agar is based on the formulation described by Terzaghi and Sandine[1] and is recommended as an improved medium for the growth and enumeration of lactic streptococci and their bacteriophages.

Because it supports better host growth it allows the demonstration of phenomena commonly associated with other bacterial virus systems but not previously reported for lactic streptococcal phages and makes possible detailed studies of plaque morphology and lysogeny.

Lactic streptococci are nutritionally fastidious and require complex media for optimal growth[2,3]. Their homofermentative acid-producing nature requires that the medium is well buffered so that the culture pH is maintained above 5.7 during active growth. This maintenance of the pH is important as lower pH can result in injury and reduced recovery of Lactic streptococci.

M17 Agar contains di-sodium-glycerophosphate which has sufficient buffering capacity to maintain the pH above 5.7 of actively growing cultures even after 24 hours at 30°C. This buffering agent also allows the addition of calcium without a precipitation complex being formed. The calcium-containing medium is used for the assay of bacteriophages of Lactic streptococci[1].

Shankar and Davies[4] reported that M17 Agar was suitable for the isolation and enumeration of *Streptococcus thermophilus* from yogurt as the high concentration of di-sodium-glycerophosphate resulted in suppression of *Lactobacillus bulgaricus*. M17 Agar has been recommended[5,6] by the International Dairy Federation for the selective enumeration of *Streptococcus thermophilus* from yogurt.

M17 Agar is also suitable for growing and maintaining starter cultures for cheese and yogurt manufacture as it has little deleterious effect on their subsequent acid-producing ability in milk at either 30°C or 22°C[1].

One further useful property of this agar is its ability to detect streptococcal mutants which are unable to ferment lactose[1]. These mutant Lac-strains form much smaller colonies than the parent lactose-fermenting strain.

Technique

Bacteriophage assay.

Microbiologists wishing to assay phage activity should consult the paper of Terzaghi and Sandine[1] for a comprehensive description of the method.

For the enumeration of *Streptococcus thermophilus* in yogurt.

1. Mix or blend the yogurt sample to obtain a uniform homogenicity.
2. Weigh 10 ± 0.1 g of the test sample into a 200 ml round bottom centrifuge tube made of strengthened glass, or the container of the mechanical mixer.
3. Add sterile 0.1% (w/v) peptone solution* to the test sample until the mass of the test sample and diluent is 50 g.
4. Prepare a suitable series of decimal dilutions of the yogurt suspension in 9 ml volumes of sterile 0.1% (w/v) peptone solution.
5. (i) Inoculate duplicate plates of M17 Agar with a loopful from each dilution and spread to obtain single colonies.
   (ii) Add duplicate 1 ml aliquots of the dilution into a Petri dish and prepare pour plates with 14 ml of sterile M17 Agar cooled to 43°C ± 1°C.
6. Incubate at 35°C for 48 hours.
7. Examine the plates after 24 and 48 hours incubation. *Streptococcus thermophilus* colonies are visible after 18-24 hours and after 48 hours incubation form colonies of 1-2 mm in diameter. *Lactobacillus bulgaricus* do not grow or produce very restricted colonies.
8. Carry out the counts on pour plates and express the results as the number of colony-forming units per gram of sample.

0.1% peptone water[6] can be prepared as follows:*

LP0042 Tryptone	0.5 g
LP0049 Peptone	0.5 g
Distilled water	1 litre

Sterilise by autoclaving at 121°C for 15 minutes.

Confirmation

Colonies isolated from milk products that are suspected to be *Streptococcus thermophilus* can be confirmed by the Gram stain (Gram-positive cocci) and catalase test (negative).

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured gel.

Quality control

Positive control:	***Expected result***
Streptococcus thermophilus ATCC® 14485	Good growth; white colonies
Negative control:	
Lactobacillus bulgaricus ATCC® 11842	No growth

*This organism is available as a Culti-Loop®

References

1. Terzaghi B. E. and Sandine W. E. (1975) *Applied Microbiology* 29. 807-813.
2. Anderson A. W. and Elliker P. R. (1953) *J. Dairy Science* 36. 161-167.
3. Reiter B. and Oram J. D. (1962) *J. Dairy Res.* 29. 63-77.
4. Shankar P. A. and Davies F. L. (1977) *J. Soc. Dairy Technology* 30. 28-30.
5. International Dairy Federation (1981) *Joint IDF/ISO/AOAC Group* E44.
6. International Organization for Standardization (1985) ISO/DIS 7889.

M17 BROTH

Code: CM0817

For improved growth of lactic streptococci and their bacteriophages.

Formula	***gm/litre***
Tryptone	5.0
Soya peptone	5.0
'Lab-Lemco' powder	5.0
Yeast extract	2.5
Ascorbic acid	0.5
Magnesium sulphate	0.25
Di-sodium-glycerophosphate	19.0
pH 6.9 ± 0.2	

Directions
Suspend 37.25 g in 950 ml of distilled water and bring gently to the boil. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C and aseptically add 50 ml of sterile lactose solution (10% w/v).

Lactose solution 10% (w/v)
Dissolve 10 g of Lactose LP0070 in 100 ml of distilled water. Sterilise by autoclaving at 121°C for 15 minutes or by membrane filtration through a 0.2 µm membrane.

Description
M17 Broth has been produced in parallel with M17 Agar CM0785. Its use in conjunction with M17 Agar in bacteriophage assays has been described by Terzaghi and Sandine[1]. These workers also suggest that M17 Broth would be a suitable medium for the maintenance of starter cultures because of its considerable buffering capacity and the little effect it has on the subsequent acid-producing ability of these cultures.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured solution.

Positive control:	***Expected results***
Streptococcus thermophilus ATCC® 14485	Turbid growth
Negative control:	
Lactobacillus bulgaricus ATCC® 11842	No growth

References

1. Terzaghi B. E. and Sandine W. E. (1975) *Applied Microbiology* 29. 807-813.
2. Anderson A. W. and Elliker P. R. (1953) *J. Dairy Science* 36. 161-167.
3. Reiter B. and Oram J. D. (1962) *J. Dairy Res.* 29. 63-77.
4. Shankar P. A. and Davies F. L. (1977) *J. Soc. Dairy Technology* 30. 28-30.
5. International Dairy Federation (1981) *Joint IDF/ISO/AOAC Group E44*.
6. International Organization for Standardization (1985) *ISO/DIS* 7889.

MacCONKEY AGAR

Code: CM0007

A differential medium for the isolation of coliforms and intestinal pathogens in water, dairy products and biological specimens.

Formula	***gm/litre***
Peptone	20.0
Lactose	10.0
Bile salts	5.0
Sodium chloride	5.0
Neutral red	0.075
Agar	12.0
pH 7.4 ± 0.2	

Directions

Suspend 52 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Dry the surface of the gel before inoculation.

Description

A differential medium for the detection, isolation and enumeration of coliforms and intestinal pathogens in water, dairy products and biological specimens. MacConkey Agar corresponds to the medium recommended by the World Health Organization[1], the Dept. of Health[2] and by Windle Taylor[3] for the bacteriological examination of water.

Although principally used for coliforms, this medium may also be employed for the differentiation of other enteric bacteria (including pathogens) and is suitable for the differentiation of *Pasteurella* species[4].

Technique

Pathological specimens

Due to its ability to support the growth of pathogenic Gram-positive cocci (e.g. staphylococci and enterococci) as well as Enterobacteriaceae, MacConkey Agar is particularly recommended for the cultivation of pathogens which may be present in a variety of specimens such as urine, faeces and wound swabs. Whilst it is selective it does not suppress a mixed bacterial flora to the same extent as other inhibitory media (including other MacConkey agars). It provides a number of other diagnostic indications in addition to bile tolerance, such as colony morphology and chromogenesis. MacConkey Agar should be used in parallel with other selective indicator media such as Desoxycholate Citrate Agar, Bismuth Sulphite Agar, Brilliant Green Agar and Brilliant Green Bile (2%) Broth, and a non-selective medium such as Blood Agar.

Water Examination[2,3]

The medium may be used for the direct count of coli-aerogenes bacteria, using pour-plates prepared from known volumes of the water sample, but a more exact role for the medium is for the differentiation of organisms producing acid and gas in MacConkey Broth at 35°C: all positive broth tubes are plated on MacConkey Agar, the plates are incubated for 24 hours at 35°C and examined for typical colonies (see below). Colonies composed of Gram-negative non-sporing rods are sub-cultured for further identification.

The presence of enterococci in azide or tellurite media may be confirmed by sub-culture on MacConkey Agar. See below for colonial morphology.

Yersinia and _Pasteurella_ differentiation
MacConkey Agar can be used to differentiate *Yersinia* species from *Pasteurella* species[4]. *Yersinia pestis*, *Yersinia pseudotuberculosis* and *Yersinia enterocolitica* will show growth on MacConkey Agar after 24 hours incubation at 35°C[5].

Pasteurella species (including *Pasteurella multocida*) will not grow on MacConkey Agar.

Pectinolytic Organisms (Stewart[6])
Stewart used Oxoid MacConkey Agar as the basis of a selective-diagnostic medium for pectinolytic organisms, in order to isolate soft-rot *Erwinia* species from specimens containing other Enterobacteriaceae. MacConkey Agar-calcium chloride plates (5.2 g powder, 0.4g $CaCl_2$, 75 ml distilled water) overlaid with a pectate-EDTA layer (0.1% EDTA containing 2% sodium polypectate) are inoculated and incubated for 48 hours at 25°C. Lactose fermenting *Erwinia* produce red colonies in shallow pits formed by pectate liquefaction.

Colonial Characteristics
After 24 hours at 35-37°C typical colonies are as follows:

Organism	***Colour***	***Remarks***
Escherichia coli	red	non-mucoid
Aerobacter aerogenes	pink	mucoid
Enterococcus species	red	minute, round
Staphylococci	pale pink	opaque
Pseudomonas aeruginosa	green-brown	fluorescent growth

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared plates of medium at 2-8°C.

Appearance
Dehydrated medium: Straw pink coloured, free-flowing powder.

Prepared medium: Dark red coloured gel.

Quality control

Positive controls:	***Expected results***
Enterococcus faecalis ATCC® 29212*	Good growth; red coloured colonies
Staphylococcus aureus ATCC® 25923*	Good growth; pale pink coloured colonies
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

Precautions
The colonial characteristics described give presumptive identification only of the isolated organisms. It is necessary to sub-culture and carry out confirmation tests for final identification.

To enhance the pigment of suspected *Staphylococcus aureus*, hold the plates on the bench at ambient temperature for 12-18 hours.

References

1. World Health Organization (1963) *International Standards for Drinking Water* 2nd ed. WHO, Geneva.
2. Environment Agency (2002) *The Microbiology of Drinking Water 2002*. Methods for examination of Waters and Associated Materials.
3. Windle Taylor E. (1958) *The examination of Waters and Water Supplies* 7th ed., Churchill Ltd., London.
4. Hoogendijk J. L. (1962) Antonie van Leeuwenhoek *J. Microbiol. Serol.* 28(3) 315-320.
5. Wilson G. S. and Miles A. A. (1964) *Topley and Wilson's Principles of Bacteriology and Immunity* 5th ed., Edward Arnold Ltd., London. vol. 2.
6. Stewart D. J. (1962) *Nature* 195(4845), 1023.

MacCONKEY AGAR (WITHOUT SALT CM7b)

Code: CM0507

A differential medium on which swarming of Proteus species is suppressed. Recommended for urine examination.

Formula	***gm/litre***
Peptone	20.0
Lactose	10.0
Bile salts	5.0
Neutral red	0.075
Agar	12.0
pH 7.4 ± 0.2	

Directions
Suspend 47 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Mix well before pouring. Dry the surface of the gel before inoculation.

Description
This medium has the same formulation as MacConkey Agar CM0007 except that it does not contain added salt and therefore provides a 'low electrolyte medium' on which most *Proteus* species do not spread. For this reason the medium has found particular favour for use in the examination of urine so that overgrowth of other organisms is prevented.

Colonial Characteristics
After 24 hours at 35-37°C typical colonies are as follows:

Organism	***Colour***	***Remarks***
Escherichia coli	red	non-mucoid
Aerobacter aerogenes	pink	mucoid
Enterococcus species	red	minute, round
Staphylococcus species	pale pink	opaque
Pseudomonas aeruginosa	green-brown	fluorescent growth

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared plates of medium at 2-8°C.

Appearance
Dehydrated medium: Straw-pink coloured powder.

Prepared medium: Dark red coloured gel.

Quality control

Positive controls:	***Expected results***
Enterococcus faecalis ATCC® 29212*	Good growth; red coloured colonies
Staphylococcus aureus ATCC® 25923*	Good growth; pale pink coloured colonies
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

Precautions
The colonial characteristics described give presumptive identification only of the isolated organisms. It is necessary to sub-culture and carry out confirmation tests for final identification.

To enhance the pigment of suspected *Staphylococcus aureus*, hold the plates on the bench at ambient temperature for 12-18 hours.

References
See MacConkey Agar CM0007.

MacCONKEY AGAR No. 2

Code: CM0109

A modification of MacConkey Agar No. 3 containing Oxoid Bile Salts No. 2 for the recognition of enterococci.

Formula	***gm/litre***
Peptone	20.0
Lactose	10.0
Bile salts No. 2	1.5
Sodium chloride	5.0
Neutral red	0.05
Crystal violet	0.001
Agar	15.0
pH 7.2 ± 0.2	

Directions

Suspend 51.5 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

Description

MacConkey Agar No. 2 is a modification of the original MacConkey solid medium and is especially useful for the recognition of enterococci, in the presence of coliforms and non-lactose-fermenters from water, sewage, food products, etc.

On this medium enterococci appear as small intensely red colonies with a pale periphery about 1 mm in diameter. These organisms are frequently sought as an index of faecal pollution. Non-lactose-fermenters are colourless. Bile tolerant Gram positive cocci, such as staphylococci and non-faecal streptococci, are completely inhibited.

McGeachie & Kennedy[1] employed Oxoid MacConkey Agar No. 2 in a simplified method for counting the bacteria in urine. Using a bacteriological loop (delivering a known volume) they streaked well mixed uncentrifuged urine directly on to a blood agar and a MacConkey Agar plate – and spread the urine in a 1 cm wide strip across one edge of the plate using 20 strokes. With a second sterile loop they spread a 1 cm wide portion to form a second strip at right angles to the first. This was repeated to give a square pattern of four 1 cm wide strips around the edge of the plate. After incubation, growth was noted as +, + +, + + +, or + + + + depending on whether 1, 2, 3 or 4 sides of the square showed colonies. The approximate estimate obtained agreed well with a more complicated pour-plate method and the simplified method was recommended for routine use.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared plates at 2-8°C.

Appearance

Dehydrated medium: Pink coloured, free-flowing powder.

Prepared medium: Dark red coloured gel.

Quality control

Positive control:	***Expected results***
Enterococcus faecalis ATCC® 29212*	Good growth; pink coloured colonies with red centres
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

Reference

1. McGeachie J. and Kennedy A. C. (1963) *J. Clin. Path.* 16. 32-38.

MacCONKEY AGAR No. 3

Code: CM0115

A selective medium giving excellent differentiation between coliforms and non-lactose-fermenters with inhibition of Gram-positive micrococci.

Formula	***gm/litre***
Peptone	20.0
Lactose	10.0
Bile salts No. 3	1.5
Sodium chloride	5.0
Neutral red	0.03
Crystal violet	0.001
Agar	15.0
pH 7.1 ± 0.2	

Directions
Suspend 51.5 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

Description
A more selective modification of MacConkey medium which is suitable for the detection and enumeration of coliform organisms and also for the detection and isolation of *Salmonella* and *Shigella* species occurring in pathological and food specimens. Due to the inclusion of a specially prepared fraction of bile salts in addition to crystal violet, the medium gives improved differentiation between coliforms and non-lactose-fermenting organisms whilst Gram-positive cocci are completely inhibited.

This formulation corresponds with that recommended by the American Public Health Association[1] for the direct plating of water samples for coliform bacilli, for the examination of food samples for food poisoning organisms[2] and for the isolation of *Salmonella* and *Shigella* species in cheese[3].

Amongst other examples of the use of Oxoid MacConkey Agar No. 3 are: the count of coli-aerogenes bacteria in poultry faecal specimens[4]; the count of coli-aerogenes bacteria in cattle and sheep faeces[5]; the count of coli-aerogenes and non-lactose-fermenting organisms in poultry carcasses[6]; bacterial counts on irradiated canned minced chicken[7]; the recognition of coli-aerogenes bacteria during investigations on the genus *Aeromonas*[8].

Anderson *et al.*[9] added 10 mg/ml of kanamycin to MacConkey Agar to isolate epidemic strains of *Citrobacter diversus* which were causing neonatal meningitis.

The addition of 100 mg of 4-methylumbelliferyl-β-D-glucuronide to one litre of MacConkey Agar detects the enzyme β-glucuronidase[10]. The cleaved 4-methylumbelliferyl moiety is fluorescent at 366 nm. Thus colonies of *Escherichia coli* can be detected rapidly in mixed cultures by examining the plate under a UV lamp after overnight incubation at 35°C. However, it should be remembered that other organisms may also be β-glucuronidase positive.

Technique
After inoculation the plates are usually incubated for 18 to 24 hours at 35°C and for a further 24 hours if non-lactose-fermenting organisms are sought and have not appeared. Lower incubation temperatures may sometimes be used for more psychrophilic species. After 18 hours at 35°C, coliforms produce intense violet-red colonies whilst non-lactose-fermenters are colourless.

Storage conditions and Shelf life
The dehydrated medium should be stored at 10-30°C and used before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance
Dehydrated medium: Straw-pink coloured, free-flowing powder.

Prepared medium: Dark red coloured gel.

Quality control

Positive controls:	***Expected results***
Escherichia coli ATCC® 25922*	Good growth; red colonies with bile precipitation
Shigella sonnei ATCC® 25931*	Good growth; straw coloured colonies
Negative control:	
Enterococcus faecalis ATCC® 29212*	Inhibited

*This organism is available as a Culti-Loop®

Precautions

Prolonged incubation may lead to confusing results. Do not incubate beyond 48 hours.

Test the medium with a laboratory stock strain of *Shigella* species which is in the R-phase. R-phase shigellae should grow satisfactorily on MacConkey Agar.

References

1. American Public Health Association (1998) *Standard Methods for the Examination of Water and Wastewater*. 20th edn. APHA Inc. Washington DC.
2. American Public Health Association (1976) *Compendium of Methods for the Microbiological Examination of Foods*. APHA Inc. Washington DC.
3. American Public Health Association (1978) *Standard Methods for the Examination of Dairy Products*. 14th Edn. APHA Inc. Washington DC.
4. Barnes Ella M. and Goldberg H. S. (1962) *J. Appl. Bact.* 25(1). 94-106.
5. Medrek T. F. and Barnes Ella M. (1962) *J. Appl Bact.* 25(2). 159-168.
6. Barnes Ella M. and Shrimpton D. H. (1957) *J. Appl. Bact.* 20(2). 273-285.
7. Thornley Margaret J. (1957) *J. Appl. Bact.* 20(2). 273-285.
8. Eddy B. P. (1960) *J. Appl. Bact.* 23(2). 216-249.
9. Anderson R. L., Graham D. R. and Dixon R. E. (1981) *J. Clin. Microbiol.* 14. 161-164.
10. Trepeta A. W. and Edburg S. C. (1984) *J. Clin. Microbiol.* 19. 172-174.
11. Maddocks J. L. and Greenan M. J. (1975) *J. Clin. Pathol.* 28. 686-687.

MacCONKEY BROTH

Code: CM0005

A differential medium containing neutral red for the detection of coliform organisms in water and milk examination.

Formula	***gm/litre***
Peptone	20.0
Lactose	10.0
Bile salts	5.0
Sodium chloride	5.0
Neutral red	0.075
pH 7.4 ± 0.2	

Directions

To prepare single strength broth, add 40 g to 1 litre of distilled water. Mix well and distribute into containers fitted with fermentation (Durham) tubes. Sterilise by autoclaving at 121°C for 15 minutes.

Description

For the past fifty years, MacConkey Broth has been the standard medium for the primary isolation of coliform bacteria, and has been recommended for this purpose by the Public Health Laboratory Service Water Committee[7] and the World Health Organization[1]. The Oxoid product conforms to their specification for water testing and also to the formulation specified by the Dept. of Health[2] for milk grading.

The advantages of MacConkey Broth in the presumptive coliform test are the low proportion of false positive reactions (PHLS Water Subcommittee[3]) and the fact that most strains of *Escherichia coli* produce a positive reaction within 24 hours[4]. Disadvantages, due to variability of the peptone and bile salts contained in the original medium, have been overcome by large scale production, pooling of batches and careful quality control – including titrimetric standardisation of the bile salts by a method described by Burman[5].

The neutral red is pre-tested for the absence of toxic substances before inclusion in the Oxoid medium. Childs & Allen[6] have shown that some samples of neutral red were inhibitory. For those who prefer, this medium is also available with bromocresol purple as the indicator – for details of this alternative medium and the presumptive coliform test see MacConkey Broth Purple CM0505 (CM5a).

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance
Dehydrated medium: Straw-pink coloured, free-flowing powder.

Prepared medium: Dark red coloured solution.

Quality control

Positive control:	***Expected results***
Escherichia coli ATCC® 8739*	Turbid growth; acid and gas production
Negative control:	
Staphylococcus aureus ATCC® 25923*	Turbid growth; no acid or gas production

*This organism is available as a Culti-Loop®

Precautions
The neutral red indicator is carefully selected for this formulation and therefore shows no inhibitory effect. However, the more sensitive reaction of bromocresol purple in MacConkey Broth Purple CM0505 (CM5a) is often preferred.

References

1. World Health Organization (1963) *International Standards for Drinking Water* 2nd ed., WHO, Geneva.
2. Dept. of Health (1937) Memo 139/Foods, HMSO, London.
3. Public Health Laboratory Service Water Subcommitee (1953) *J. Hyg. Camb.* 51. 268-277.
4. Windle Taylor E. (1958) *The Examination of Waters and Water Supplies* 7th ed., Churchill Ltd., London.
5. Burman N. P. (1955) *Proc. Soc. Water Treat. Exam.* 4. 10-20 and discussion 20-26.
6. Childs Eileen and Allen L. A. (1953) *J. Hyg. Camb.* 51. 468-477.
7. Dept. of Health and Social Security (1969) *4th impression, HMSO London*.

MACCONKEY BROTH (PURPLE)

Code: CM0505 (Powder CM5a)

Code: CM0506 (Tablets CM6a)

A differential medium containing BCP for the detection of coliform organisms in water and milk examination.

Formula	***gm/litre***
Peptone	20.0
Lactose	10.0
Bile salts	5.0
Sodium chloride	5.0
Bromocresol purple	0.01
pH 7.4 ± 0.2	

Directions

Powder
To prepare single strength broth add 40 g to 1 litre of distilled water. Distribute into containers fitted with fermentation (Durham) tubes. Sterilise by autoclaving at 121°C for 15 minutes.

Tablets
Add 1 tablet to 10 ml of distilled water. Insert a fermentation (Durham) tube and sterilise by autoclaving at 121°C for 15 minutes.

Description

MacConkey Broth has long been used as a presumptive medium for the detection of the coli-aerogenes organisms. In the original medium, litmus was employed as the indicator of acid production but, in later publications, MacConkey suggested neutral red as a more satisfactory alternative. Childs & Allen[1] showed that some samples of neutral red exerted an inhibitory effect on the growth of *Escherichia coli* in this medium.

Bromocresol purple is less inhibitory, and the colour change from purple to yellow provides a more sensitive and definite indication of acid formation; therefore this indicator is used in Oxoid MacConkey Broth (Purple), which corresponds to the alternative formulations recommended in 'The Bacteriological Examination of Water Supplies'[2] and 'International Standards for Drinking Water'[3].

Technique

The presumptive coliform examination consists of the inoculation of measured volumes of water into tubes of MacConkey Broth (Purple) which are incubated at 35°C for 48 hours. Choice of volumes for inoculation will depend on the bacteriological grade of the water being tested; for 'medium' waters the Public Health Laboratory Service Water Committee (1961) recommend one 50 ml, five 10 ml and five 1 ml quantities of water – 50 ml and 10 ml amounts being added to their own volume of double-strength MacConkey Broth while the 1 ml amounts are each added to 5 ml of single-strength MacConkey Broth. Acid formation is indicated by a yellow colouration of the broth, and gas formation is indicated by an amount of gas at least sufficient to fill the concavity at the top of the Durham tube. From the number of tubes showing the presence of acid and gas, the most probable number of (presumed) coliform bacteria present in 100 ml of the original water may be estimated by reference to probability tables; these tables based on McCrady's computations, are included in Report No. 71: 'The Bacteriological Examination of Water Supplies'[2] and in many other publications dealing with this subject. For the differential coliform test, each MacConkey tube showing acid and gas is then subcultured into a fresh tube of MacConkey Broth and incubated at 44°C. Formation of gas within 48 hours is practically specific for *Escherichia coli* and indicative of faecal pollution of the original water sample.

MacConkey Broth (Purple) is also suitable for the bacteriological examination of milk, as described by Davis[4]. This method, which is basically similar to that used for the examination of water, consisting in the inoculation of suitable dilutions of the milk into tubes of this medium followed by incubation and inspection, was originally recommended by the Dept. of Health, London[5].

The addition of 4-methylumbelliferyl-β-D-glucuronide (MUG) BR0071 to this medium will enhance the detection of Escherichia coli. See MUG Reagent BR0071 under Biochemical Reagents for further details.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at room temperature.

Appearance

Dehydrated Medium: Green coloured, free-flowing powder.

Prepared medium: Purple coloured solution.

Quality Control

Positive controls:	***Expected results***
Escherichia coli ATCC® 25922*	Turbid growth; acid and gas production
Negative control:	
Psudomonas aeruginosa ATCC® 9027*	Surface growth; no acid or gas production

*This organism is available as a Culti-Loop®

References

1. Childs Eileen and Allen L. A. (1953) *J. Hyg. Camb*. 51(4). 468-477.
2. Departments of the Environment, Health, Social Security and Public Health Laboratory Service (1982) *The Bacteriological Examination of Drinking Water Supplies. Report No. 71.* HMSO London.
3. World Health Organization (1963) *'International Standards for Drinking Water' 2nd ed.,* WHO, Geneva.
4. Davis J. G. (1959) *'Milk Testing' 2nd ed*., Dairy Industries Ltd., London.
5. Dept. of Health (1937) *Memo. 139/Foods*, HMSO, London.

MALT EXTRACT AGAR

Code: CM0059

A medium for the detection, isolation and enumeration of yeasts and moulds. Bacteria may be suppressed by the addition of lactic acid.

Formula	***gm/litre***
Malt extract	30.0
Mycological peptone	5.0
Agar	15.0
pH 5.4 ± 0.2	

Directions
Suspend 50 g in 1 litre of distilled water and boil to dissolve. Sterilise by autoclaving at 115°C for 10 minutes.

If it is desired to adjust the medium to pH 3.5, cool to 55°C and add approximately 2-3 ml of 10% Lactic Acid SR0021 to 100 ml Malt Extract Agar. Once acidified with lactic acid, the medium should not be re-heated.

Description
This medium, similar to the one described by Galloway & Burgess[1], is recommended for the detection, isolation and enumeration of yeasts and moulds. For mycological counts it may be desirable to prepare the more acid medium in order to suppress bacterial growth.

Also see Wort Agar CM0247.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.
Store the prepared medium at 2-8°C.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Amber coloured gel.

Quality control

Positive controls:	***Expected result***
Aspergillus niger ATCC® 9642*	White mycelium, black spores
Candida albicans ATCC® 10231*	Good growth; cream coloured colonies
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

Precautions
Avoid overheating as the acid pH will soften the agar in the presence of heat.

Reference
1. Galloway L. D. and Burgess R. (1952) *Applied Mycology and Bacteriology* 3rd ed., Leonard Hill, London. pp.54 and 57.

MALT EXTRACT BROTH

Code: CM0057

A liquid medium recommended for the cultivation of moulds and yeasts, especially during tests for sterility.

Formula	***gm/litre***
Malt extract	17.0
Mycological peptone	3.0
pH 5.4 ± 0.2	

Directions
Add 20 g to 1 litre of distilled water. Mix well, distribute into final containers and sterilise by autoclaving at 115°C for 10 minutes. This liquid medium is recommended for the cultivation of moulds and yeasts, during tests for sterility, etc.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Amber coloured solution.

Quality control

Positive controls:	***Expected result***
Aspergillus niger ATCC® 9642*	Surface growth; white mycelium, black spores
Candida albicans ATCC® 10231*	Turbid growth
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

Reference
1. Galloway L. D. and Burgess R. (1952) *Applied Mycology and Bacteriology* 3rd ed., Leonard Hill, London, pp.54 and 57.

MANNITOL SALT AGAR

Code: CM0085

A selective medium for the isolation of presumptive pathogenic staphylococci. Most other bacteria are inhibited, with the exception of a few halophilic species.

Formula	***gm/litre***
'Lab-Lemco' powder	1.0
Peptone	10.0
Mannitol	10.0
Sodium chloride	75.0
Phenol red	0.025
Agar	15.0
pH 7.5 ± 0.2	

Directions
Suspend 111 g in 1 litre of distilled water and bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

Description
A selective medium prepared according to the recommendations of Chapman[1] for the isolation of

presumptive pathogenic staphylococci. Most other bacteria are inhibited by the high salt concentration with the exception of some halophilic marine organisms. Presumptive coagulase-positive staphylococci produce colonies surrounded by bright yellow zones whilst non-pathogenic staphylococci produce colonies with reddish purple zones.

Mannitol Salt Agar is recommended for the detection and enumeration of coagulase-positive staphylococci in milk[2], in food[3] and other specimens[4].

Oxoid Mannitol Salt Agar has been used for the examination of meat or fish[5,6,7,8,9].

The addition of 5% v/v Egg Yolk Emulsion SR0047 to Mannitol Salt Agar enables the lipase activity of staphylococci to be detected as well as mannitol fermentation[10]. The high concentration of salt in the medium clears the egg yolk emulsion and lipase production is detected as a yellow opaque zone around colonies of staphylococci which produce this enzyme.

Technique
Heavily inoculate the Mannitol Salt Agar plate and incubate for 36 hours at 35°C or for 3 days at 32°C – the latter is recommended by the APHA[3].

Presumptive coagulase-positive staphylococci produce colonies with bright yellow zones whilst coagulase-negative staphylococci are surrounded by a red or purple zone. Pick off suspect colonies and sub-culture in a medium not containing an excess of salt (e.g. Nutrient Broth No. 2 CM0067) to avoid interference with coagulase or other diagnostic tests.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared plates at 2-8°C.

Appearance
Dehydrated Medium: Straw-pink coloured, free-flowing powder.

Prepared medium: Red coloured gel.

Quality control

Positive controls:	***Expected results***
Staphylococcus aureus ATCC® 25923*	Good growth; yellow colonies with yellow halo
Staphylococcus epidermidis ATCC® 12228*	Good growth; pink colonies with pink medium
Negative control:	
Escherichia coli ATCC® 8739*	No growth

*This organism is available as a Culti-Loop®

Precautions
A few strains of *Staphylococcus aureus* may exhibit a delayed fermentation of mannitol. Negative plates should be re-incubated overnight before discarding.

Presumptive *Staphylococcus aureus* must be confirmed with a coagulase test (Staphylase Test DR0595 or Staphytect Plus DR0100).

References

1. Chapman G. H. (1945) *J. Bact*. 50. 201-203.
2. Davis J. G. (1959) *Milk Testing* 2nd edn., Dairy Industries Ltd., London.
3. American Public Health Association (1966) *Recommended Methods for the Microbiological Examination of Foods* 2nd edn., APHA Inc., New York.
4. Silverton R. E. and Anderson M. J. (1961) *Handbook of Medical Laboratory Formulae* Butterworths, London.
5. Barnes Ella M. and Shrimpton D. H. (1957) *J. Appl. Bact.* 20. 273-285.
6. Thornley Margeret J. (1957) *J. Appl. Bact.* 20. 286-298.
7. Bain Nora, Hodgkiss W. and Shewan J. M. (1958) *DSIR, Proc. 2nd Internat. Symp. Food Microbiol.*, 1957, HMSO, London, pp.103-116.
8. Spencer R. (1961) *J. Appl. Bact.* 24. 4-11.
9. Eddy B. P. and Ingram M. (1962) *J. Appl. Bact.* 25. 237-247.
10. Gunn B. A., Dunkelberg W. E. and Creitz J. R. (1972) *Am. J. Clin. Path.* 57. 236-238.

MANNITOL SELENITE BROTH BASE

(See Selenite Broth Base)

Code: CM0399

A modification of Selenite F Broth especially recommended for the enrichment of salmonellae.

Formula	***gm/litre***
Bacteriological peptone	5.0
Mannitol	4.0
Sodium phosphate	10.0
pH 7.1 ± 0.2	

Directions

Add 19 g to 1 litre of distilled water to which 4 g of sodium biselenite LP0121 has been added.

Warm to dissolve, mix well and fill out into containers. Sterilise in a boiling water bath, or in free flowing steam, for 10 minutes. **DO NOT AUTOCLAVE.**

To minimise any possible risk of teratogenicity to laboratory workers, the sodium biselenite must be added to this medium separately.

Description

This medium is similar to the modification of Leifson[1] enrichment medium described by Hobbs & Allison[2] for the isolation of *Salmonella typhi* and *Salmonella paratyphi B*. Liefson[1] suggested that it is best to tube the medium to a depth of 2 inches (50 mm) or more.

Hobbs & Allison[2] compared two sets of selenite media, one containing lactose and the other mannitol. Of 38 positive stools *Salmonella typhi* was sub-cultured from both media in 32 instances, from the mannitol selenite alone in 5 instances and from the lactose selenite alone once. Comparisons showed that the mannitol selenite broth was superior to three other liquid media in its selective value for *Salmonella typhi* and that it was as good as tetrathionate for the isolation of *Salmonella paratyphi B*.

Technique

Sub-cultures from this selective, enrichment broth can be made to any combination of greater and lesser inhibitory selective agars for the Enterobacteriaceae.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C away from light.

Appearance

Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Light straw coloured solution.

Quality control

Positive control:	***Expected results***
Salmonella typhimurium ATCC® 14028*	Good growth
Negative control:	
Escherichia coli ATCC® 25922*	

*This organism is available as a Culti-Loop®

Precautions

Observe the precautionary comments made about sodium biselenite in Selenite Broth Base CM0395.

Discard the prepared medium if large amounts of reduced selenite can be seen as a red precipitate in the bottom of the bottle.

Do not incubate longer than 24 hours because the inhibitory effect of selenite is reduced after 6-12 hours incubation.

Mannitol fermentation by salmonella helps correct the alkaline pH swing which can occur during incubation.

Take sub-cultures of broth from the upper third of the broth column, which should be at least 5 cm in depth.

References

1. Leifson E. (1936) *Am. J. Hyg.* 24(2). 423-432.

2. Hobbs Betty C. and Allison V. D. (1945) *Mon. Bull. Min. Hlth Pub. Hlth Lab. Serv.* 4. 12-19.

MAXIMUM RECOVERY DILUENT (PEPTONE SALT BROTH)

Code: CM0733

A protective and isotonic diluent for maximal recovery of micro-organisms (ISO/DIS 6649).

Formula	***gm/litre***
Peptone	1.0
Sodium chloride	8.5
pH 7.0 ± 0.2	

Directions
Dissolve 9.5 g in 1 litre of distilled water. Dispense into the final containers and sterilise by autoclaving at 121°C for 15 minutes.

Description
Maximum Recovery Diluent combines the protective effect of peptone in the diluting solution[1] with the osmotic support of physiological saline[1,2].

The low concentration of peptone does not cause multiplication of the organisms within 1-2 hours of dilution of the sample.

The isotonic strength of the diluent ensures recovery of organisms from various sources which may be vulnerable in distilled water or aqueous suspensions.

Technique
1. Prepare the medium according to the directions. For the 150 method[3] distribute the medium into 90 ml volumes or into 9 ml volumes.
2. Put 10 g of the test sample into a sterile blender jar or sterile plastic bag.
3. Add 90 ml of sterile Maximum Recovery Diluent.
4. Operate the blender according to its speed for sufficient time to give a total number of 15,000-20,000 revolutions. Alternatively operate a peristaltic type blender (Stomacher) for 2 minutes.
5. Within 15 minutes transfer 1 ml of the macerate to 9 ml of sterile diluent and mix well (10-1 dilution).
6. Prepare additional decimal dilutions in the same way.
7. Aseptically transfer 1 ml of each dilution of the initial suspension in duplicate to the centre of a dish.
8. Prepare pour plates with the medium of choice.
9. Allow the agar to solidify and incubate.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Colourless clear solution.

Quality control
Use a positive test sample divided between new and previous lot/batch of diluent. Carry out duplicate tests as described in Technique and look for equivalent yield of organisms between the diluent batches.

References
1. Straker R. P. and Stokes J. L. (1957) *Appl. Microbiol.* 5. 21-25.
2. Patterson J. W. and Cassells J. A. (1963) *J. Appl. Bact.* 26. 493-497.
3. ISO/DIS 6649. *Meat and Meat Products – Detection and Enumeration of Clostridium perfringens*.

MEMBRANE CLOSTRIDIUM PERFRINGENS (m-CP) MEDIUM

Code: CM0992

A medium for rapid isolation and presumptive identification of Clostridium perfringens from water samples.

Formula	***gm/litre***
Tryptose	30.0
Yeast extract	20.0
Sucrose	5.0
L-cysteine hydrochloride	1.0
Magnesium sulphate $7H_2O$	0.1
Agar	15.0
Bromocresol purple	0.04
pH 7.6 ± 0.2	

m-CP SELECTIVE SUPPLEMENT

Code: SR0188

Vial contents (each vial is sufficient to supplement 500 ml of medium)	***per vial***	***per litre***
Polymyxin B sulphate	12.5 mg (105,000 IU)	25.0 mg (210,000 IU)
D-Cycloserine	200.0 mg	400.0 mg

Directions

Suspend 35.55 g of m-CP Agar Base in 500 ml of distilled water. Mix well and sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C and aseptically add the contents of one vial of m-CP Selective Supplement reconstituted as directed. Aseptically add the following sterile solutions dissolved in distilled water:

Component	***Solution Strength***	***Volume***
Phenolphthalein biphosphate tetrasodium salt	0.5%	10 ml
Ferric chloride hexahydrate	4.5%	1 ml
Indoxyl β-D-glucoside	0.75%*	4 ml

*equivalent to 30 mg in 4 ml

NB. Fresh solutions must be used. Mix well and pour into sterile Petri dishes.

To reconstitute m-CP Selective Supplement, aseptically add 2 ml of sterile distilled water to 1 vial of supplement. Mix gently to dissolve.

Description

Membrane Clostridium Perfringens (m-CP) Medium is a selective and chromogenic medium for the presumptive identification of *Clostridium perfringens* from water samples.

m-CP Medium was first described by Bisson and Cabelli[1] for the rapid quantitation of *Clostridium perfringens* from a variety of water samples (seawater, potable water and sewage). The medium was shown to give better recovery of *Clostridium perfringens* from water and sewage samples than the Bonde pour tube method[1].

m-CP Medium has now been recommended in European Council Directive 98/83/EC for testing the quality of water intended for human consumption[2].

In m-CP Medium lack of β-D-glucosidase activity (an enzyme involved in cellobiose fermentation), fermentation of sucrose and production of acid phosphatase are used to differentiate presumptive *Clostridium perfringens* colonies from other *Clostridium* spp.

Lack of β-D-glucosidase activity means that *Clostridium perfringens* does not cleave the chromogen, indoxyl β-D-glucoside, in the medium. Furthermore, as the organisms ferment the sucrose in the medium, reducing the pH, bromocresol purple changes from purple to yellow. This results in characteristic opaque yellow *Clostridium perfringens* colonies.

Most other *Clostridium* spp. will appear as either purple colonies, due to the lack of sucrose fermentation, or blue/green colonies where the organism is still cleaving indoxyl β-D-glucoside and also fermenting sucrose (see table).

Presumptive positive *Clostridium perfringens* colonies can be further tested for acid phosphatase activity by exposure to ammonium hydroxide vapour for 20-30 seconds. *Clostridium perfringens* colonies turn pink or red as phenolphthalein diphosphate is cleaved by acid phosphatase. No colour change will be seen with colonies of organisms that do not posses acid phosphatase. It is important this further test is carried out as there are a very small number of non-perfringens clostridia that produce yellow colonies. However, these colonies will remain yellow after exposure to ammonium hydroxide as they are acid phosphatase negative.

D-cycloserine, polymyxin B and incubation at 44°C inhibit the growth of background flora such as Gram-negative organisms and staphylococci.

Technique

Filter the water sample using a 0.45 mm cellulose acetate or cellulose nitrate filter, then place the filter onto the m-CP Medium. Incubate anaerobically for 21 ± 3 hours at 44 ± 1°C. Examine the plates for presumptive positive opaque yellow colonies that turn pink or red after exposure to ammonium hydroxide vapours for 20-30 seconds.

On m-CP Medium typical colonies will appear as follows:

Organism	***Typical Colony Colour***
Clostridium perfringens	**Opaque Yellow** Sucrose positive/Glucosidase negative then **pink/red** after exposure to NH_4OH
Other *Clostridium* spp.	**Blue/Green** Sucrose positve/Glucosidase positive (e.g. *Cl. baratii*, *Cl. paraputrificum*, *Cl. tertium*)
	Purple Sucrose negative/Glucosidase positive or negative (e.g. *Cl. biferentans*, *Cl. difficile*, *Cl. sporogenes*)
	Opaque Yellow Sucrose positive/Glucosidase negative remain **yellow** after exposure to NH_4OH

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

m-CP Selective Supplement SR0188 should be stored at 2-8°C.

Prepared medium may be stored for up to 5 days at 2-8°C in the dark.

Quality control

Positive control:	***Expected results***
Clostridium perfringens ATCC® 13124*	Yellow, then Pink/Red
Negative controls:	
Escherichia coli ATCC® 25922*	Inhibited
Clostridium sporogenes ATCC® 19404*	Purple

*This organism is available as a Culti-Loop®

References

1. Bisson J. W. and Cabelli V. J. (1979) *Applied and Environmental Microbiology*, Vol. 37, No. 1, pp.55-88.

2. E.U. (1998) 98/83/EC of Council of 3rd of November 1998 on the quality of water intended for human consumption. *Off. J. Eur. Commun*. L330, 32-54.

MEMBRANE ENDO AGAR LES

Code: MM0551

A membrane filtration medium requiring Basic Fuchsin for enumeration of coliform organisms in water, using a 2-stage enrichment technique.

Formula	***gm/litre***
Yeast extract	1.2
Tryptone	3.7
Peptone P	3.7
Tryptose	7.5
Lactose	9.4
Dipotassium phosphate	3.3
Monopotassium phosphate	1.0
Sodium chloride	3.7
Sodium desoxycholate	0.1
Sodium lauryl sulphate	0.05
Sodium sulphite	1.6
Agar	10.0
pH 7.2 ± 0.2	

Basic Fuchsin to be added at 0.8 gm/litre.

BASIC FUCHSIN

Code: BR0050

For each litre of medium use 8 ml of a 10% w/v solution of this dye dissolved in 50:50 ethanol:distilled water.

Directions

Suspend 45 grams in 1 litre of distilled water. Add 8 ml of a 10% w/v alcoholic solution of Basic Fuchsin. Heat gently with frequent agitation until the medium boils. DO NOT AUTOCLAVE. Cool to 45°C and dispense into 50-60 mm dishes in 4 ml volumes. For larger dishes use sufficient medium to give an equivalent depth (approx. 1.5 mm).

Plates should be protected from light and may be stored for up to two weeks in the refrigerator.

WARNING

Basic Fuchsin is a potential carcinogen and care must be taken to avoid inhalation of the powdered dye and contamination of the skin.

Description

M-Endo Agar LES is prepared according to the Lawrence Experimental Station formulation of McCarthy, Delaney and Grasso[1] and used for the enumeration of coliform organisms in water[2].

The value of the membrane filter technique for the enumeration of coliform organisms in water lies in its greater reliability and precision when compared with the MPN multiple tube test[3].

McCarthy, Delaney and Grasso[1] have recommended a two-stage process of enrichment to provide a non-toxic environment for maximal resuscitation of the coliforms.

Calabrese and Bissonnette4 found that supplementation of M-Endo medium with catalase and sodium pyruvate resulted in improved recovery of coliform bacteria from chlorinated sewage effluent.

Experiments carried out by Noble[5] indicated that sodium sulphite and Basic Fuchsin can be extremely detrimental to the stressed coliforms, reducing the total count.

Enrichment for a period of 2 hours ± 0.5 hours in single strength Lauryl Tryptose Broth CM0451 will give adequate resuscitation to the stressed coliform organisms and provide the best assessment of the quality of the drinking water.

Enrichment is usually not necessary for the examination of non-potable waters and sewage effluents.

Selection of the sample volume is governed by the expected bacterial density. An ideal quantity will result in growth of more than 50 coliform colonies and less than 200 colonies of all types.

All organisms which produce a colony with a golden-green metallic sheen within 24 hours incubation are considered members of the coliform group. The sheen may cover the entire colony or be restricted to the central area or the periphery. The recommended depth of M-Endo Agar LES in plates restricts the colony size and hence facilitates carrying out the colony count.

Technique

The water sample is filtered through a sterile membrane filter[6].

For the first stage of enrichment, place a sterile incubating pad in the upper half of a sterile Petri dish and pipette onto this 2 ml of Lauryl Tryptose Broth CM0451. Aseptically place the filter membrane on to the incubating pad and incubate, without inverting the dish, for 1-1.5 hours at 35°C in an atmosphere having 100% humidity. Place Petri dishes of M-Endo Agar LES in the incubator for the entire period so that they will be at the correct temperature when required for the second stage of enrichment. The first stage enrichment culture is removed from the incubator and the filter membrane is stripped aseptically from the incubating pad and transferred to the surface of the Petri dish of M-Endo Agar LES. It is important that complete contact is made between the membrane and the agar surface. The plate is inverted and incubated for 22-24 hours at 35°C.

Alternatively, the membrane filter incubating pad can be placed inside the lid of the Petri dish of M-Endo Agar LES and 2 ml of Lauryl Tryptose Broth CM0451 pipetted onto the pad. The filter membrane is placed face upwards on the pad and incubated for 1-1.5 hours at 35°C.

To carry out the second stage of enrichment, the first-stage enrichment is removed from the incubator and the filter membrane is stripped from the pad and placed face upwards on the surface of the M-Endo Agar LES medium. The incubating pad is left in the lid and the plates are incubated in the inverted position for 24 hours at 35°C.

If preferred the second stage only may be used. The prepared membrane filter is placed directly on the agar surface and incubated as described.

All the organisms which produce a colony with a golden-green metallic sheen within 24 hours incubation may be considered as presumptive coliforms.

Calculation of Coliform Density

Report the coliform density in terms of total coliform/100 ml. Compute the count using those membrane filters with 20-80 coliform colonies and not more than 200 of all types per membrane.

$$\text{Total Coliform colonies/100 ml} = \frac{\text{Coliform colonies} \times 100}{\text{ml of sample filtered}}$$

Storage conditions and Shelf life

Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium in the dark and at 2-8°C.

Precautions

Use care when handling basic fuchsin to avoid inhaling the powder and staining the skin.

References

1. McCarthy J. A., Delaney J. E., Grasso R. J. (1961) *'Measuring Coliforms in Water', Water and Sewage Works*, 108. 238-243.

2. American Public Health Association (1980) *Standard Methods for the Examination of Water and Wastewater.* 15th Edn, APHA Inc, Washington DC.

3. McCarthy J. A., Thomas H. A. J., Delaney J. E. (1958) '*Evaluation of the Reliability of Coliform Density Tests'.* AJPH, 48. 16-28.

4. Calabrese J. P. and Bissonnette G. M. (1990) *Appl. Env. Microbiol.* 56. 3558-3564.

5. Noble R. E. (1960) '*Reliability of MPN Indexes for Coliform organisms*'. JAWWA, 52. 803.

6. Departments of the Environment, Health & Social Security and PHLS (1982) *The Bacteriological Examination of Drinking Water Supplies. Report on Public Health and Medical Subjects* No. 71. HMSO. London.

MEMBRANE LACTOSE GLUCURONIDE AGAR (MLGA)

Code: CM1031

A medium for the differentiation and enumeration of Escherichia coli and other coliforms by a single membrane filtration technique.

Formula	***gm/litre***
Peptone	40.0
Yeast extract	6.0
Lactose	30.0
Phenol red	0.2
Sodium lauryl sulphate	1.0
Sodium pyruvate	0.5
Agar	10.0
X-Glucuronide (BCIG)	0.2
pH 7.4± 0.2	

Directions
Suspend 88 g in 1 litre of distilled water. Mix well and sterilise by autoclaving at 121°C for 15 minutes. Cool the medium to 50°C and pour into sterile Petri dishes.

Description
Tests for coliforms and *Escherichia coli* are the most important routine microbiological examinations carried out on drinking water. They provide the most sensitive means for detection of faecal contamination, for assessing the effectiveness of water treatment and disinfection, and for monitoring water quality in distribution[1].

Organisms are isolated on a membrane filter and placed onto MLGA. The medium contains lauryl sulphate to inhibit Gram-positive organisms. Identification of *Escherichia coli* and coliforms is facilitated through two biochemical reactions within the medium: Lactose fermentation is detected by the dye phenol red which gives yellow colonies when acid is produced.

The chromogenic substrate 5-bromo-4-chloro-3-indolyl-β-D-glucuronide (BCIG) is cleaved by the enzyme glucuronidase and produces a blue chromophore which builds up in the bacterial cells.

Coliforms are lactose-positive so colonies will be yellow; *Escherichia coli* is both lactose-positive and possesses glucuronidase so will appear as green colonies[1].

Technique
For the full methodology refer to The Environment Agency – Methods for Examination of Waters and Associated Material – 'The Microbiology of Drinking Water 2002, Part 4, Method B: The enumeration of coliforms and *Escherichia coli* by single membrane filtration technique'.

Filter the water sample to be analysed through a membrane filter (47 mm diameter, cellulose-based 0.45 µm nominal pore size). The volume and dilution of water filtered should be chosen to give the number of colonies to be counted on the membrane as 20-80. For treated waters 100 ml should be filtered. For polluted waters a smaller volume or a diluted sample should be used.

Place the membrane filter onto a MLGA plate ensuring that no air-bubbles are trapped under the membrane. Incubate the plates at 30°C for 4 hours, then at 37°C for 14 hours. For an early indication of results, plates may be examined for colonies at 12 hours but must be re-incubated for the full 18 hours.

Count all yellow and green colonies. The yellow colonies are presumptive non-*Escherichia coli* coliform bacteria and the green colonies are *Escherichia coli*. The combined count can be regarded as the number of coliform bacteria. Results are expressed in colony-forming units per volume of sample.

The specificity of the reactions within the medium means the likelihood of green colonies on MLGA being *Escherichia coli* is very high. Following suitable confirmation of performance within the laboratory, confirmation of green colonies may not be needed. If confirmation tests are required please refer to The Environment Agency document for the full methodology[1].

Storage conditions and Shelf life
The dehydrated medium should be stored at 10-30°C and used before the expiry date on the label.

Store the prepared medium may be stored for up to 1 week at 2-8°C.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Bright red coloured gel.

Quality control

Positive controls:	***Expected results***
Escherichia coli ATCC® 25922*	Good growth; green coloured colonies
Enterobacter aerogenes ATCC® 13048*	Good growth; yellow coloured colonies
Pseudomonas aeruginosa ATCC® 27853*	Good growth; pink coloured colonies
Negative control:	
Bacillus subtilis ATCC® 6633*	Inhibited

Reference
1. The Environment Agency – Methods for Examination of Waters and Associated Material – *The Microbiology of Drinking Water* 2002.

MEMBRANE LAURYL SULPHATE BROTH

Code: MM0615

A replacement medium for Membrane Enriched Teepol Broth for the enumeration of coliform organisms and Escherichia coli in water.

Formula	***gm/litre***
Peptone	39.0
Yeast extract	6.0
Lactose 30.0	30.0
Phenol red	0.2
Sodium lauryl sulphate	1.0
pH 7.4 ± 0.2	

Directions
Dissolve 76.2 g in 1 litre of distilled water. Distribute into final containers, e.g. 100 ml screw cap bottles. Sterilise by steaming for 30 minutes on three consecutive days or by autoclaving at 121°C for 15 minutes.

Description
In formulating Membrane Enriched Teepol Broth, Burman[1] substituted Teepol in place of bile salts in the membrane filtration test medium used to detect coliform organisms in water. The use of Teepol in place of bile salts had been previously recommended by Jameson and Emberley[2] and its value was confirmed by other workers (Jebb[3] and Windle-Taylor[4,5]). It is essential to use one standard grade of Teepol and Teepol 610 (BDH Ltd.) has been recommended.

Burman[6] showed that resuscitation media are not required with Membrane Enriched Teepol Broth if a preliminary incubation is carried out at a lower temperature. Thus non-chlorinated organisms benefit from 4 hours incubation at 30°C, but chlorinated organisms require 6 hours incubation at 25°C.

Membrane Lauryl Sulphate Broth is similar to Membrane Enriched Teepol Broth except that the selective agent Teepol 610 has been replaced by sodium lauryl sulphate.

In 1976 the production of Teepol 610 ceased and studies were carried out to identify a suitable alternative selective agent that could be incorporated into the basal medium. As a result of this work it was recommended[7] that Teepol 610 should be replaced by sodium lauryl sulphate (BDH No. 44244) at a concentration of 0.1% w/v.

Membrane Lauryl Sulphate Broth CM0615 has been recommended[7,8] as a standard medium for the enumeration of coliform organisms and *Escherichia coli* from water and sewage by the membrane filtration technique.

The Medium and method are fully described in The Bacteriological Examination of Water Supplies Report 719.

Technique
The coliform and *Escherichia coli* count are made on separate volumes of water. The volumes should be chosen so that the number of colonies to be counted on the membrane lies between 10 and 100. If the water is suspected to contain less than 100 coliform organisms per 100 ml, then a 100 ml sample should be filtered.

The water samples are filtered through a sterile membrane filter (Report 71[9]) and the membrane filter is placed face upwards on an absorbent pad previously saturated with Membrane Lauryl Sulphate Broth. The pad and membrane filter should be incubated in a vapour-tight container to prevent evaporation.

Membranes to be incubated at 44°C should be placed in watertight heavy containers and immersed in a closely controlled water-bath.

Burman recommends the following incubation periods and temperatures:

Unchlorinated waters

Coliform organisms	4 hours at 30°C followed by 14 hours at 35°C
Escherichia coli	4 hours at 30°C followed by 14 hours at 44°C

If rapid results are required, the membrane may be examined after a total incubation time of 12 hours. If no colonies are present, a nil count can be assumed. If small colonies of indeterminate colour are present, then the membranes must be returned to the incubator for the full period.

Presumptive coliform organisms
After incubation, yellow colonies from membranes incubated at 35°C should be sub-cultured to Lactose Peptone Water to confirm that they will produce gas at 35°C after 43 hours incubation.

Presumptive *Escherichia Coli*
Yellow colonies from membranes in 44°C should be sub-cultured to Lauryl Tryptose Mannitol Broth CM0831, incubated at 44°C to confirm gas production and indole production at this temperature after 24 hours incubation.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Quality Control

Positive control:
Escherichia coli ATCC® 25922
Negative control:
Bacillus subtilis ATCC® 6633

Precautions
Avoid overheating.

References

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MILK AGAR

Code: CM0021

A nutrient medium enriched with milk solids for the determination of the viable micro-flora of dairy and water samples.

Formula	***gm/litre***
Yeast extract	3.0
Peptone	5.0
Milk solids (equivalent to 10ml fresh milk)	1.0
Agar	15.0
pH 7.2 ± 0.2	

Directions
Suspend 24 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

Description
Oxoid Milk Agar is made to a formula corresponding to the official medium described in Dept. of Health Memo. 139/ Foods[1]. It is recommended for performing the plate count test on milks, rinse waters, milk products and ice cream, etc.

It also complies with the recommendations of EUROGLACE (EEC Ice cream Industries) submitted to the EEC Commission for the examination of ice cream[2].

Statutory tests for milk must be carried out exactly as described in the appropriate Statutory Instrument e.g. Statutory Instrument 1963, Food & Drugs, Milk and Dairies: The Milk (Special Designation) (Amendment) Regulations. Ditto. (1965).

Technique
The sample bottle is inverted 25 times and after flaming the mouth, some of the milk sample is discarded – while the remainder is re-shaken thoroughly and used for the preparation of decimal dilutions in 14 strength Ringer solution.

For milk, dilutions of 1/10, 1/100 and 1/1000 are prepared and 1 ml of each pipetted aseptically into separate Petri dishes. 10 ml of molten Milk Agar, cooled to 45°C, is then added to each dish and the contents mixed by a combination of rapid to-and-fro shaking and circular movements lasting 5-10 seconds. The recommended procedure is five to-and-fro movements followed by five circular movements in a clockwise direction, succeeded by five to-and-fro movements at right angles to the first set followed by five anti-clockwise circular movements.

No more than fifteen minutes should elapse between preparation of the dilutions and pouring the plates.

The plates are allowed to stand on the bench for about an hour and then transferred to the incubator, where they are incubated in an inverted position for 2 days at 35°C or 3 days at 30°C.

Appreciably higher counts may be obtained after incubation at 22°C and 30°C than at 35°C[3,4,5].

After incubation the colonies are counted, within four hours, and the result expressed as plate count per ml.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured gel.

Quality control

Positive control:	***Expected results***
Escherichia coli ATCC® 25922*	Good growth; straw coloured colonies
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

Precautions
Make sure that the procedures and media used in milk product testing comply with the National Regulations required for each country.

References
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3. Davis J. G. (1959) *Milk Testing* 2nd ed., Dairy Industries Ltd., London, pp.175-187.
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MILK PLATE COUNT AGAR
PLATE COUNT AGAR WITH ANTIBIOTIC FREE SKIM MILK

Code: CM0681

A medium for the enumeration of viable organisms in milk and dairy products.

Formula	***gm/litre***
Tryptone	5.0
Yeast extract	2.5
Glucose	1.0
Antibiotic free skim milk	1.0
Agar	10.0
pH 6.9 ± 0.1	

Directions
Suspend 19.5 g in 1 litre of distilled water. Bring to the boil with frequent agitation, mix and distribute into final containers. Sterilise by autoclaving at 121°C for 15 minutes.

Description
Plate Count Agar with Antibiotic Free Skim Milk is equivalent to the medium recommended by British Standards Institution[1] and International Organization for Standardization[2]. The medium is used for the enumeration of viable organisms in milk and dairy products.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared plates at 2-8°C.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured gel.

Quality control

Positive control:	***Expected results***
Escherichia coli ATCC® 11775*	Good growth; pale straw coloured colonies
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

Compare with previous lot/batch using pasteurised and raw milk samples, incubated at 32-35°C for 48 hours.

Precautions
Make sure that the procedures and media used in milk product testing comply with the National Regulations required for each country.

References
1. British Standards Institution BS4285 Section 1.2 1984.
2. International Organization for Standardization. *Draft International Standard ISO/DIS* 6610 : 1982.

MINERALS MODIFIED GLUTAMATE MEDIUM

MINERALS MODIFIED GLUTAMATE MEDIUM BASE + SODIUM GLUTAMATE LP0124

Code: CM0607

Formula (double strength)	*gm/litre*
Lactose	20.0
Sodium formate	0.5
L-cystine	0.04
L(-)aspartic acid	0.048
L(+)arginine	0.04
Thiamine	0.002
Nicotinic acid	0.002
Pantothenic acid	0.002
Magnesium sulphate $7H_2O$	0.200
Ferric ammonium citrate	0.020
Calcium chloride $2H_2O$	0.020
Dipotassium hydrogen phosphate	1.80
Bromocresol purple	0.020
pH 6.7 ± 0.1	

Directions

Double Strength Dissolve 5 g of ammonium chloride in 1 litre of distilled water. To this add 22.7 g of Minerals Modified Medium Base, and 12.7 g of Sodium glutamate LP0124. Mix to dissolve completely. Sterilise by autoclaving for 10 minutes at 116°C; alternatively heat to 100°C for 30 minutes on three successive days.

Single strength Dissolve 2.5 g of ammonium chloride in 1 litre of distilled water. To this add 11.4 g of Minerals Modified Medium Base, and 6.4 g of Sodium glutamate LP0124. Mix to dissolve completely. Sterilise by autoclaving for 10 minutes at 116°C; alternatively heat to 100°C for 30 minutes on three successive days.

Note

To improve the stability of the dehydrated medium on storage the sodium glutamate LP0124 is supplied separately and must be added to the basal medium CM0607.

The pH of the final medium is critical for optimum performance and the sterilised broth should be checked to confirm that it is at pH 6.7 before use.

Differences in heating procedures cause differences in final pH value. If necessary the heating procedure should be adjusted so that the final pH, after sterilisation, is 6.7[1].

Description

A chemically-defined medium based on glutamic acid was first advocated by Folpmers[2] for the enumeration of the coliform group of bacteria in water.

The Public Health Laboratory Service[3] carried out a trial and concluded that glutamic acid media containing glucose gave too many false positives in 48 hours. Gray[4] modified a glutamate medium containing lactose and later published a formulation for an improved Formate Lactose Glutamate Medium[5].

This latter medium was incorporated in another large trial carried out by the PHLS[6] in which three glutamate media were compared with Teepol Broth (Jameson & Emberly[7]) and MacConkey Broth. The results showed that Gray's improved formate lactose glutamate medium was superior to the other glutamate media on trial.

The report carried criticism of the mineral content of the medium and it was considered that it could be improved by modifying the amounts of minerals.

A co-operative investigation was carried out between the Metropolitan Water Board Laboratories and Oxoid Laboratories which resulted in a Minerals Modified Glutamate Medium CM0289.

The Oxoid Minerals Modified Glutamate Medium was used in further PHLS[6] trials and the results with the Oxoid medium confirmed the superior performance of glutamate media reported previously (PHLS[6]).

The superior performance of Minerals Modified Glutamate Medium over MacConkey Broth is due mainly to improved detection of *Escherichia coli*. The table (adapated from PHLS[8]) illustrates the results obtained in the trial.

The table shows that for chlorinated water, incubation for >18 hours is required for glutamate media to demonstrate their superiority.

The medium and method are fully described in The Microbiology of Drinking Water 2002[1].

More recently further trials showed Minerals Modified Glutamate Medium to be the medium of choice for the detection of *Escherichia coli i*n chlorinated waters, especially where the numbers of organisms concerned were small.

It was also found better than Lauryl Tryptose Lactose Broth for detection of small numbers of *Escherichia coli* in other water, although the latter medium gave quicker results (18-24 hours compared to the 48 hours required by Minerals Modified Glutamate Medium).

Papadakis[10] investigated the isolation of *Escherichia coli* from sea-water and found Minerals Modified Glutamate Medium to be better than MacConkey Broth formulations. However, to avoid high salt concentrations in the broth he recommended 1 ml only of sea-water to be added to 10 ml of single-strength MMG medium. Higher volumes of sea-water must be diluted out 1/10 with MMG medium.

Technique

The technique known as the Multiple Tube Method, Dilution Method or the Most Probable Number (MPN) method is used with Minerals Modified Glutamate Medium. A trial comparing membrane filtration and multiple tube methods showed glutamate medium to be unsatisfactory for use with membranes for enumerating coliform organisms in water[11].

With waters expected to be of good quality, the medium should be inoculated with one 50 ml volume and five 10 ml volumes. With waters of more doubtful quality, five 1 ml volumes should be used in addition to the 50 ml and 10 ml volumes. Dilutions of the 1 ml volumes may be required for polluted water and the 50 ml volume may be omitted.

The larger volumes of water (10 ml and 50 ml) are added to equal volumes of double-strength medium, whereas the 1 ml volumes (or dilutions of them) are added to 5 ml of single-strength medium.

The tubes are incubated at 35°C and examined after 18-24 hours. All those tubes showing acid (yellow colour in the medium) and gas in the inverted inner (Durham) tube should be regarded as ‘presumptive positive’ tubes, including those in which gas appears after tapping the tube. The tube may only have a bubble of gas after tapping. The remaining tubes should be re-incubated and examined after another 24 hours. Any further tubes becoming ‘positive’ should be treated as ‘presumptive positives’.

Each ‘presumptive positive’ tube should be sub-cultured to a tube of Brilliant Green Bile (2%) Broth CM0031 and incubated for 24 hours at 44°C.

At the same time a tube of 1% Tryptone Water CM0087, should be inoculated for the production of indole after 24 hours at 44°C.

The production of gas from lactose at 44°C and the production of indole at 44°C are accepted in the United Kingdom as evidence of *Escherichia coli*.

Samples of chlorinated water giving ‘presumptive positive’ tubes must be tested to exclude false positive results due to aerobic or anaerobic spore-bearing organisms that produce gas. Sub-cultures are made into Brilliant Green Bile (2%) Broth and incubated at 35°C for 48 hours. Production of gas within 48 hours can be taken as sufficient confirmation that coliform organisms are present. If the tubes are sub-cultured to MacConkey Agar CM0007 at the same time, the colonial morphology of the organisms can easily be obtained for further differential tests.

A further multi-laboratory trial has demonstrated the efficiency of Lauryl Tryptose Mannitol Broth as a single tube confirmatory test of *Escherichia coli*[12].

The Most Probable Number of organisms can be calculated from the tables in Appendix C of HMSO Report 711.

Comparison of Minerals Modified Glutamate Medium and MacConkey Broth by Number of Positive Tubes

	False positive reactions			***Coliform organisms***			***Escherichia coli***		
	18 hr	24 hr	48 hr	18 hr	24 hr	48 hr	18 hr	24 hr	48 hr
Unchlorinated samples									
MacConkey Broth	17	37	100	625	806	1060	467	528	582
Minerals Modified Glutamate Medium	2	20	97	557	858	1175	503	707	764
Chlorinated samples									
MacConkey Broth	4	19	49	125	216	315	77	121	128
Minerals Modified Glutamate Medium	0	1	37	59	223	395	39	144	203

Modified Direct Plate Method for counting *Escherichia coli* in food

A direct plate method (DPM) for the rapid enumeration of *Escherichia coli.* in foods has been described[13]. This method was modified by a resuscitation procedure using Minerals Modified Glutamate Agar[14]. In the modified method 15 g of agar per litre is added to Oxoid Minerals Modified Glutamate Broth. Using this resuscitation stage the authors have recovered damaged cells from frozen, dried, heat-processed or low pH foods.

Abbiss *et al*.[15] made a comparative assessment of the performance of Minerals Modified Glutamate Medium against three other enrichment broths in the enumeration of coliform organisms present in soft cheese, cooked meat and pâté. Minerals Modified Glutamate Medium was superior in sensitivity to Lauryl Sulphate Tryptose Broth, MacConkey Broth and Brilliant Green Bile Broth.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance

Dehydrated medium: Mauve coloured, free-flowing powder.

Prepared medium: Purple coloured solution.

Quality control

Positive control:	***Expected results***
Escherichia coli ATCC® 25922*	Turbid growth; acid and gas production
Negative control:	
Salmonella typhimurium ATCC® 14028*	Turbid growth; no acid or gas production

*This organism is available as a Culti-Loop®

Precautions

Presumptive positive tubes must be sub-cultured to Lauryl Tryptose Mannitol Broth CM0831 and incubated at 44°C to detect indole formation at this temperature before the identification of *Escherichia coli* can be made.

References

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5. Gray R. D. (1964) *J. Hyg. Camb.* 62. 495-508.
6. PHLS Standing Committee on Bacteriological Examination of Water Supplies (1968) *J. Hyg. Camb*. 66. 67-82.
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10. Papadakis J. A. (1982) 6th Workshop on Marine Pollution of the Mediterranean, Cannes.
11. PHLS Standing Committee on the Bacteriological Examination of Water Supplies (1972) *J. Hyg. Camb.* 70. 691-705.
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15. Abbiss J. S., Wilson J. M., Blood R. M. and Jarvis B. (1981) *J. Appl. Bact.* 51. 121-127.

MKTT-n – see MULLER-KAUFFMANN TETRATHIONATE-NOVOBIOCIN BROTH

MLCB AGAR

Code: CM0783

Mannitol Lysine Crystal Violet Brilliant Green Agar for the isolation of salmonellae (not Salmonella typhi or Salmonella paratyphi A).

Formula	***gm/litre***
Yeast extract	5.0
Peptone	10.0
'Lab-Lemco' powder	2.0
Sodium chloride	4.0
Mannitol	3.0
L-lysine hydrochloride	5.0
Sodium thiosulphate	4.0
Ferric ammonium citrate	1.0
Brilliant green	0.0125
Crystal violet	0.01
Agar	15.0
pH 6.8 ± 0.1	

Directions
Suspend 49.0 g in 1 litre of distilled water. Mix and bring gently to the boil with frequent agitation to dissolve the medium completely. Cool to 50°C and pour approximately 20 ml into sterile Petri dishes.

DO NOT AUTOCLAVE OR OVERHEAT.

Description
Mannitol Lysine Crystal Violet Brilliant Green Agar (MLCB Agar) is based on the formula of Inoue *et al*.[1] for the selective isolation of salmonellae from faeces and foods. Visual detection of very small numbers of hydrogen sulphide-producing strains is easy because of the distinctive colonial appearance.

The concentration of Mg++ appears to be critical for maximum growth of salmonellae on MLCB Agar. van Schothorst *et al*.[2] showed that Oxoid MLCB Agar did not inhibit any of the salmonellae species investigated.

Salmonellae serotypes that have a high incidence of H_2S-negative strains e.g. *Salmonella sendai*, *Salmonella berta*, *Salmonella pullorum* and *Salmonella senftenberg* may produce atypical pale colonies. MLCB Agar is not suitable for *Salmonella typhi* and *Salmonella paratyphi A* because of the inhibitory concentration of brilliant green.

The medium may be inoculated directly with the specimen or from an enrichment culture. Selectivity is relatively weak and its performance may be adversely affected by heavily contaminated specimens. Because of these limitations MLCB Agar should not be used alone.

MLCB Agar is specified as a plating medium following enrichment in Modified Semi-Solid Rappaport Vassiliadis (MSRV) CM0910 for isolation of *Salmonella* spp. from human faeces[3].

van Schothorst *et al*.[2] reported MLCB Agar to be excellent for the isolation of H_2S-positive salmonellae after enrichment in Rappaport-Vassiliadis (RV) Enrichment Broth CM0669. They found that the selectivity of MLCB Agar was substantially increased after RV Broth enrichment. They suggested Brilliant Green Agar and MLCB Agar should be used when examining heavily contaminated samples.

Salmonellae grow as large purple-black colonies due to hydrogen sulphide production. Mannitol is utilised by the organism and the resultant pH fall initiates lysine decarboxylation which controls further downward pH movement and promotes blackening.

MLCB Agar does not depend on lactose fermentation and is therefore recommended when investigating lactose-fermenting salmonellae (*Salmonella arizona*).

Atypical *Salmonella* strains that produce little or no hydrogen sulphide grow as mauve-grey colonies and may develop a central black 'bulls eye'.

To assist the detection of these atypical strains Brilliant Green Agar (modified) CM0329 or Bismuth Sulphite Agar CM0201 should also be used.

Gram-positive and most Gram-negative organisms are inhibited although some strains of *Citrobacter* spp. may grow sufficiently well to mimic the appearance of *Salmonella* spp. and some *Proteus* spp. may swarm.

Most contaminating organisms that are able to grow develop as small colourless colonies.

Technique

Dry the surface of the agar before use.

Inoculate the medium heavily with the specimen or enrichment culture and incubate for 18-24 hours at 35°C.

Examine for typical large purple-black colonies of H_2S positive *Salmonella*. Search carefully for H_2S negative strains that atypically grow as large mauve-grey colonies with a cratered centre. A proportion may show a black 'bulls eye'.

Pick all colonies presumed to be *Salmonella* spp. and confirm by biochemical and serological testing.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared plates of medium at 2-8°C.

Appearance

Dehydrated medium: Straw/green coloured, free-flowing powder.

Prepared medium: Purple coloured gel.

Quality control

Positive controls:	***Expected results***
Salmonella typhimurium ATCC® 14028*	Good growth; mauve coloured colonies with black centres
Salmonella poona NCTC 4840*	Good growth; mauve coloured colonies with black centres
Negative control:	
Escherichia coli ATCC® 25922*	Inhibited

*This organism is available as a Culti-Loop®

Precautions

The identity of colonies presumed from their appearance to be *Salmonella* spp. must be confirmed by biochemical and serological testing. In common with other enteric media care must be taken to ensure the purity of colonies taken for further testing as organisms that are inhibited from developing into colonies remain viable and may accidentally be picked on sub-culture.

References

1. Takao Inoue *et al*. (1968) Proceedings of the Japanese Society of Veterinary Science. Number 169. *Jap*. *J. Vet. Sci.* 30.
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MLGA – see MEMBRANE LACTOSE GLUCURONIDE AGAR

MLSB – see MEMBRANE LAURYL SULPHATE BROTH

MODIFIED BRILLIANT GREEN AGAR – see BRILLIANT GREEN AGAR (MODIFIED)

MODIFIED LAURYL TRYPTOSE BROTH WITH MUG AND ADDED TRYPTOPHAN – see LAURYL TRYPTOSE BROTH (MODIFIED) WITH MUG AND ADDED TRYPTOPHAN

MODIFIED NEW YORK CITY MEDIA – see NEW YORK CITY MEDIA

MODIFIED SEMI-SOLID RAPPAPORT VASSILIADIS (MSRV) MEDIUM BASE

Code: CM0910

A semi-solid medium for the detection of motile Salmonella spp. from food and environmental samples.

Formula	***gm/litre***
Tryptose	4.59
Casein hydrolysate	4.59
Sodium chloride	7.34
Potassium dihydrogen phosphate	1.47
Magnesium chloride (anhydrous)	10.93
Malachite green oxalate	0.037
Agar	2.7
pH 5.4 ± 0.2	

MSRV SELECTIVE SUPPLEMENT

Code: SR0161

Vial contents (each vial supplements 500 ml of medium)	***per vial***	***per litre***
Novobiocin	10 mg	20 mg

Directions

Suspend 15.8 g of MSRV Medium Base in 500 ml of distilled water. Bring to the boil with frequent agitation.

DO NOT AUTOCLAVE. Cool to 50°C and aseptically add the contents of 1 vial of MSRV Selective Supplement reconstituted as directed. Mix well and pour into sterile Petri dishes. Air dry at room temperature for at least one hour. (Plates may be air-dried overnight prior to storage at 2-8°C.)

Description

Modified Semi-solid Rappaport Vassiliadis (MSRV) Medium is based on the formulation described by De Smedt *et al.* which has been shown to detect more *Salmonella*-positive samples than the traditional enrichment procedures[1,2]. Further collaborative studies have confirmed these findings[3,4].

Motility enrichment on MSRV Medium has been designed as a simple, sensitive method for the isolation of salmonellae from food and environmental samples. The efficiency of the medium is based on the ability of salmonellae to migrate through the selective medium ahead of competing motile organisms, thus producing opaque halos of growth.

Further tests can be carried out directly from the migrated culture with the inoculum being taken from the edge of the growth. The Oxoid Salmonella Latex Test (FT0203) may be used for serological confirmation of *Salmonella* species.

The medium is not suitable for the detection of non-motile strains of *Salmonella* (incidence <0.1%)[5]. (Figures obtained from records of the Department of Enteric Pathogens, Central Public Health Laboratory, Colindale, London. Dr. B. Rowe, Personal Communication, 1988.)

Technique

1. Inoculate three drops (of 0.1 ml) of the pre-enrichment culture (after incubation for 16-20 hours) in separate spots on the surface of the MSRV Medium plates.
2. Incubate the plates in an upright position at 42°C for up to 24 hours. (Care should be taken not to exceed 24 hours.)
3. Examine the plates for motile bacteria which will be shown by a halo of growth originating from the inoculation spot.
4. Sub-cultures can be taken from the outside edge of the halo to confirm purity and for further biochemical and serological tests.

De Smedt[6] reported that if MSRV medium is contained in test tubes and incubation is carried out under anaerobic conditions, visible migration zones are produced in 6 hours enabling *Salmonella* in foods to be detected in 24 hours.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the selective supplement in the dark at 2-8°C and use before the expiry date on the label.

The prepared medium may be stored for up to 2 weeks at 2-8°C in the dark.

Appearance

Dehydrated medium: Green coloured, free-flowing powder.

Prepared medium: Blue coloured semi-solid gel.

Quality control

Positive controls:	***Expected results***
Salmonella poona NCTC 4840	Straw colonies at site of inoculation surrounded by halo of growth
Salmonella enteritidis ATCC® 13076*	Straw colonies at site of inoculation surrounded by halo of growth
Negative control:	
Citrobacter freundii ATCC® 8090*	Restricted or no growth

*This organism is available as a Culti-Loop®

Precautions

The basal medium is very hygroscopic. When handling the powder a face mask and gloves must be worn.

References

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MODIFIED TRYPTONE SOYA BROTH m-TSB – see TRYPTONE SOYA BROTH

MODIFIED WADOWSKY YEE MEDIUM (MWY) – see WADOWSKY YEE MEDIUM

MRD – see MAXIMUM RECOVERY DILUENT

MRS AGAR (DE MAN, ROGOSA, SHARPE)

Code: CM0361

A solidified version of MRS Broth for the culture of 'lactic acid bacteria'.

Formula	***gm/litre***
Peptone	10.0
'Lab-Lemco' powder	8.0
Yeast extract	4.0
Glucose	20.0
Sorbitan mono-oleate	1 ml
Dipotassium hydrogen phosphate	2.0
Sodium acetate $3H_2O$	5.0
Triammonium citrate	2.0
Magnesium sulphate $7H_2O$	0.2
Manganese sulphate $4H_2O$	0.05
Agar	10.0
pH 6.2 ± 0.2	

Directions

Suspend 62 g in 1 litre of distilled water. Boil to dissolve the medium completely. Dispense into tubes, bottles or flasks and sterilise by autoclaving at 121°C for 15 minutes.

Description

The MRS formulation was developed by de Man, Rogosa and Sharpe[1] to replace a variable product (tomato juice) and at the same time to provide a medium which would support good growth of lactobacilli in general, even those strains which showed poor growth in existing media.

MRS medium is superior to the tomato juice medium of Briggs[2] and the meat extract tomato juice medium of de Man. It gives more profuse growth of all strains of lactobacilli, especially the difficult and slow growing strains of *Lactobacillus brevis* and *Lactobacillus fermenti*.

MRS Agar and Broth were designed to encourage the growth of the 'lactic acid bacteria' which includes species of the following genera: *Lactobacillus*, *Streptococcus*, *Pediococcus* and *Leuconostoc*. All these species can produce lactic acid in considerable amounts. They are Gram +ve, catalase and oxidase –ve and are fastidious in their nutritional requirements. Growth is enhanced considerably by micro-aerobic conditions.

Generally the 'lactic acid bacteria' show delayed growth and smaller colony size than other micro-organisms. They may be overgrown in non-selective media, especially if incubation is required for 2-4 days.

Selection can be made by pH adjustment, thus lactobacilli will tolerate lower pH levels than streptococci (pH 5.0-6.5) with pediococci and leuconostocs growing best within this range. Inhibitors of the main groups of competitor microflora include thallous acetate, sodium acetate, sorbic acid, acetic acid, sodium nitrite, cycloheximide and polymyxin. These substances can be used at varying concentrations and combinations but inevitably a compromise has to be reached between selectivity and productivity of the organism sought[3].

MRS Agar with sorbic acid has been described[3,4]. This is MRS medium with its pH reduced to 5.7 and 0.14% w/v sorbic acid added (=0.2% w/v potassium sorbate).

An evaluation of media for selective enumeration of *Lactobacillus acidophilus* and *Bifidobacterium* species showed that minor adjustments to the basic formula of MRS Agar can readily be made to optimise its performance for determining the content of *Lactobacillus acidophilus* and *Bifidobacterium* spp. in the presence of other lactic acid bacteria which are present in yoghurt[5].

The lactobacilli are micro-aerophilic and generally require layer plates for aerobic cultivation on solid media. Submerged or surface colonies may be compact or feathery, and are small, opaque and white.

Technique
Products to be examined for lactobacilli content are macerated or diluted in a diluent such as quarter-strength Ringer solution, and further dilutions are made in MRS Broth.

1 ml volumes of the diluted samples are added to sterile dishes, and molten MRS Agar (45°C) is poured into the dish and mixed thoroughly.

When the medium has set, another layer of uninoculated MRS Agar is poured over the surface to produce a layer-plate.

Plates are incubated as described below. It is important that adequate moisture vapour is present in the atmosphere above the agar because drying of the plates during incubation will concentrate the selective factors on the surface and make the medium inhibitory. The presence of carbon dioxide stimulates growth and plates should be incubated in an atmosphere of 5% CO_2. MRS medium is selective for lactobacilli but some growth of leuconostocs and pediococci may occur.

Incubation method

42°C thermophilic:	2 days
35°C mesophilic:	2 days
30°C + 22°C mesophilic-psychrotrophic:	2+1 days
25°C psychrotrophic:	3 days

Incubation carried out under anaerobic or micro-aerophilic conditions

Select isolated colonies on the agar medium and stain a smear from each to identify the presumptive *Lactobacillus* colonies; pick these off into MRS Broth. An advantage of this broth is that any other micro-organisms, originally lying dormant in the selective agar, are not given the opportunity to multiply, as may occur in a non-selective broth. Incubate the broths at temperatures and times similar to those used for the MRS Agar; they can then be examined microscopically and further sub-cultured to MRS Agar for subsequent confirmation and identification of species.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared plates at 2-8°C.

Appearance
Dehydrated medium: Dark straw coloured powder.

Prepared medium: Amber coloured gel.

Quality control

Positive control:	***Expected result***
Lactobacillus gasseri ATCC® 19992*	Good growth; pale straw coloured colonies
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

References
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2. Briggs M. (1953) *J. Dairy Res.* 20. 36-40.
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MRS BROTH (DE MAN, ROGOSA, SHARPE)

Code: CM0359

A non-selective medium for profuse growth of 'lactic acid bacteria'.

Formula	***gm/litre***
Peptone	10.0
'Lab-Lemco' powder	8.0
Yeast extract	4.0
Glucose	20.0
Sorbitan mono-oleate	1 ml
Dipotassium hydrogen phosphate	2.0
Sodium acetate $3H_2O$	5.0
Triammonium citrate	2.0
Magnesium sulphate $7H_2O$	0.2
Manganese sulphate $4H_2O$	0.05
pH 6.2 ± 0.2	

Directions

Add 52 g to 1 litre of distilled water at approximately 60°C. Mix until completely dissolved. Dispense into final containers and sterilise by autoclaving at 121°C for 15 minutes.

Description

MRS Broth may be used for tests in the identification of lactobacilli, such as temperature dependence, growth in 4% NaCl, growth in 0.4% Teepol, etc. as recommended by Sharpe, Fryer and Smith[1].

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance

Dehydrated medium: Dark straw powder.

Prepared medium: Amber coloured solution.

Quality control

Positive control:	***Expected result***
Lactobacillus gasseri ATCC® 19992*	Turbid growth
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

Reference

1. Sharpe M. Elisabeth, Fryer T. F. and Smith D. G. (1966) *Identification of the Lactic Acid Bacteria* in *Identification Method for Microbiologists Part A* (Gibbs B. M. and Skinner F. A. eds.) London and New York, Academic Press. Pages 65-79.

MRVP MEDIUM (CLARK AND LUBS MEDIUM)

Code: CM0043

A medium recommended for the Methyl-red and Voges-Proskauer tests for the differentiation of the coli-aerogenes group.

Formula	***gm/litre***
Peptone	7.0
Glucose	5.0
Phosphate buffer	5.0
pH 6.9 ± 0.2	

Directions
Add 17 g to 1 litre of distilled water. Mix well, distribute into final containers and sterilise by autoclaving at 121°C for 15 minutes.

Description
This glucose-phosphate medium is recommended for the Methyl-red and Voges-Proskauer tests, for the differentiation of the coli-aerogenes group[1].

Smith[2] noted the low acid production of *Enterobacter aerogenes* cultures as compared with those of *Escherichia coli.* Clark & Lubs[3] employed methyl-red as a hydrogen-ion concentration indicator in order to differentiate glucose phosphate peptone water cultures of members of the coli-typhoid group. This test, now known as the Methyl-red test, distinguishes those organisms able to form large amounts of acid from glucose so that the pH falls below 4.4 and those organisms which cannot produce a low pH level.

The difference in pH value is visualised by adding methyl-red to the culture, (< pH 4.4 red: pH 5.0-5.8 orange: > pH 6.0 yellow).

Methyl-red reaction

Colour	***Organism***
Orange to red (MR positive)	*Escherichia coli*, *Citrobacter* spp. and others
Orange to yellow (MR negative)	*Enterobacter* spp., *Klebsiella pneumoniae* and others

Voges & Proskauer[4] described a red fluorescent coloration which appeared after the addition of potassium hydroxide to cultures of certain organisms in glucose medium. The coloration was shown to be due to the oxidation of the acetylmethyl-carbinol producing diacetyl which reacts with the peptone of the medium to give a red colour[5,6]. Durham[7] noted that *Enterobacter aerogenes* gave a positive reaction but that *Escherichia coli* produce no coloration, and it later became clear that there was a negative correlation between the Methyl-red and Voges-Proskauer tests[8,9] for lactose-fermenting coliform organisms.

Voges-Proskauer reaction

Red (Positive)	*Enterobacter* spp. and others
No colour (Negative)	*Escherichia coli* and others

Technique
Inoculate a 10 ml tube of MRVP Medium with two loopfuls of a pure, 4-6 hours old, Peptone Water CM0009 culture of the organism under test.

Incubate not less than 48 hours at 35°C for the MR test but more usually 3-5 days at 30°C. A heavy inoculum and 18-24 hours incubation at 35°C may give a rapid result[10]. A rapid VP test may be carried out from a heavy inoculum and incubation in a water bath at 35°C for 4-5 hours. Some organisms (*Hafnia alvei*) require incubation at 25°C to give a positive VP test.

After incubation, test one portion of the broth with 5 drops of 0.4% w/v methyl-red solution and read the colour on the surface of the medium immediately.

The second portion of the broth is used for the VP reaction by one of the following methods:

1. Add 3 ml of 5% w/v alcoholic α-naphthol solution and 3 ml of 40% w/v KOH solution (Barritt's method[12]).
2. Add a trace amount of creatine (2 drops of a 0.3% w/v solution) and 5 ml of 40% KOH solution (O'Meara's method[13]).

A bright pink or eosin red colour will appear after gentle shaking for 30 seconds. A pink colour is positive; no colour is negative.

Barry & Feeney[14] obtained rapid results by adding creatine to Barritt's reagents.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-6°C.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured solution.

Quality control

Positive control:	***Expected results***
Positive MR:	
Escherichia coli ATCC® 25922*	Turbid growth; red methyl red
Positive VP:	
Enterobacter cloacae ATCC® 23355*	Turbid growth; red Vogues-Proskauer
Negative control:	
Negative MR:	
Enterobacter cloacae ATCC® 23355*	Turbid growth; no colour change (MR)
Negative VP:	
Escherichia coli ATCC® 25922*	Turbid growth; no colour change (VP)

*This organism is available as a Culti-Loop®

Precautions

The MR-VP reactions are only part of the tests required to identify organisms.

Each laboratory should standardise on the inoculum density, volume of broth and the test container size.

MR tests require a minimum incubation of 48 hours before the pH indicator is added.

When using Barritt's reagents add α-naphthol first and KOH second; do not reverse this order.

Vaughn *et al*.[15] warned of false positive VP reactions if the completed tests are left standing for over an hour.

References

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2. Smith T. (1895) *Amer. J. Med. Sci.* 110. 283.
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15. Vaughn R., Mitchell N. B. and Levine M. (1939) *J. Amer. Water Works Assoc.* 31. 993.

MUELLER-HINTON AGAR

Code: CM0337

An antimicrobial susceptibility testing medium which may be used in internationally recognised standard procedures.

Formula†	***gm/litre***
Beef, dehydrated infusion from	300.0
Casein hydrolysate	17.5
Starch	1.5
Agar	17.0
pH 7.3 ± 0.2	

†Modified to meet performance standards.

Directions

Add 38 g to 1 litre of distilled water. Bring to the boil to dissolve the medium completely. Sterilise by autoclaving at 121°C for 15 minutes.

Description

Mueller-Hinton Agar was designed to be a reproducible culture medium for the isolation of pathogenic *Neisseria* species (Mueller & Hinton[1]). The inclusion of starch ensures that toxic factors found during growth will be absorbed and its presence is often essential to establish growth from very small inocula[2].

However, GC selective media (GC Agar Base CM0367 plus supplements SR0091/SR0095/ SR0101/SR0104/SR0105) have replaced Meuller-Hinton Agar for this purpose.

The major use of Mueller-Hinton Agar is for Antimicrobial Susceptibility Testing (AST). It has become the standard medium for the Bauer-Kirby method[3,4] and its performance is specified by the NCCLS[5,6].

Oxoid Mueller-Hinton Agar meets the requirements of WHO[7,8]. Criticisms have been made about variation in performance of Mueller-Hinton Agar between and with manufacturers' batches/lots of medium[9]. The causes of such variation are:

1. The effects of differences in concentration of divalent cations Mg++ and Ca++. These effects are shown as MIC variations with aminoglycosides against *Pseudomonas aeruginosa* and tetracycline against *staphylococci*[10,11,12].
2. Variation in thymine and thymidine content, which affect sulphonamide and trimethoprim MIC values[13,14].
3. Differences in the characteristics of the agar used in the medium, especially diffusion properties[15].

In the light of such criticisms the NCCLS called interested manufacturers together to discuss the standardisation and stabilisation of Mueller-Hinton Agar. Control methods were established whereby critical antimicrobial/organism combinations had to yield consistent zones of inhibition within 2 mm of the specified diameters in the standards[6].

The result of this cooperative effort is that Mueller-Hinton Agar is now a standard medium and declares on the label that it conforms to the NCCLS standard M6-A.

This lot meets the NCCLS standard M6-A for dehydrated Mueller-Hinton Agar.

For further details of antimicrobial susceptibility testing see relevant section.

Mueller-Hinton Agar supplemented with yeast, NAD and haematin is used specifically for the susceptibility testing of *Haemophilus influenzae*[16]. For further details see Haemophilus Test Medium (HTM), CM0989.

Mueller-Hinton Agar and Broth are used as the basis of solid and liquid media containing cefoperazone, trimethoprim, piperacillin and cycloheximide for selective isolation of *Arcobacter* spp. from meats[16].

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared plates at 2-8°C.

Appearance

Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured gel.

Quality control

Positive controls:	***Expected results***
Escherichia coli ATCC® 25922*	Good growth; pale straw coloured colonies
Pseudomonas aeruginosa ATCC® 27853*	Good growth; straw coloured colonies
Staphylococcus aureus ATCC® 25923*	Good growth; cream coloured colonies
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

Note: Please refer to relevant standards for further quality control testing.

Precautions

Incubation in a CO_2 enriched atmosphere is not recommended because of its pH effect on the medium. If it is imperative to use CO_2 then known control organisms should be included with the test plates to measure its effect.

Carbohydrates should not be added to Mueller-Hinton Agar because they may influence the rate of growth of the organism and the resulting pH of the medium.

The addition of lysed horse blood to the medium may further reduce the levels of thymidine and prevent the growth of thymidine-dependent organisms.

References

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2. Olsen A. M. and Scott W. J. (1946) *Nature* 157. 337.
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MUELLER-HINTON BROTH

Code: CM0405

An antimicrobial susceptibility testing medium which may be used in internationally recognised standard procedures.

Formula†	***gm/litre***
Beef, dehydrated infusion from	300.0
Casein hydrolysate	17.5
Starch	1.5
pH 7.3 ± 0.1	

†Modified to meet performance standards.

Directions

Place 21 g in 1 litre of distilled water mix to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Chill and adjust cation levels if necessary[1].

Description

Oxoid Mueller-Hinton Broth has been produced in parallel with Oxoid Mueller-Hinton Agar CM0337. Where studies on antibiotic susceptibilities are being made both in broth and agar, it will be found to be of particular value to have media of identical nutrient formulation.

Mueller-Hinton Broth is recommended for broth dilution MIC studies[1].

Oxoid Mueller-Hinton Broth will require supplementation with the divalent cations Mg++ and Ca++ after sterilisation[2]. The NCCLS recommend the following cation levels Ca++, 10-12.5 mg/litre; Mg++, 20-25 mg/litre.

Lysed horse blood or thymidine phosphorylase may be added to the broth to improve the MIC endpoints of sulphonamides and trimethoprim[3].

Mueller-Hinton Broth containing horse serum and agar added to create a semi-solid agar medium was used in microtitre plates in an agar dilution method for determining the MIC for *Helicobacter pylori* of a number of antibiotics. The method does not require prolonged incubation in carbon dioxide-enriched air and results are available in 48 hours compared to 3-4 days for agar diffusion testing on solid medium[4].

For further details of antimicrobial susceptibility testing see relevant section.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.
Store the prepared medium at 2-8°C.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.
Prepared medium: Light straw to straw coloured solution.

Quality control

Positive controls:	***Expected results***
Escherichia coli ATCC® 25922*	Turbid growth
Pseudomonas aeruginosa ATCC® 27853*	Turbid growth
Staphylococcus aureus ATCC® 25923*	Turbid growth
Enterococcus faecalis ATCC® 29212*	Turbid growth
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

Precautions
Monitor the performance of the broth routinely using the standard QC organisms. If the broth does not yield the expected MIC values, modify the volumes of Mg++ and Ca++ solutions until the MIC values approximate to those in Table 3 in reference[1].
If the thymidine content is lowered, after the addition of lysed horse blood or thymidine phosphorylase, the MIC values may be lower.

References

1. National Committee for Clinical Laboratory Standards (2000) Methods for Dilution Antimicrobial Susceptibility Tests for bacteria that grow Aerobically. *Approved Standard M7-A5. NCCLS*. Villanova, Pa.
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MULLER-KAUFFMANN TETRATHIONATE BROTH BASE

Code: CM0343

An improved enrichment medium for the isolation of salmonellae and the suppression of Proteus species.

Formula	***gm/litre***
Tryptone	7.0
Soya peptone	2.3
Sodium chloride	2.3
Calcium carbonate	25.0
Sodium thiosulphate	40.7
Ox bile	4.75
pH 8.0 ± 0.2	

Directions
Suspend 82 g in 1 litre of distilled water and bring to the boil. Cool below 45°C and add, just prior to use, 19 ml of iodine solution and 9.5 ml of a 0.1% brilliant green solution. Mix well and fill out into sterile tubes or flasks.

Iodine Solution

Iodine	20 g
Potassium iodide	25 g
Distilled water to	100 ml

Dissolve the potassium iodide in approximately 5 ml of distilled water, add the iodide and gently warm the solution to completely dissolve it. Make up the volume to 100 ml with distilled water.

Brilliant Green Solution

Brilliant Green (BDH or Chroma)	0.1 g
Distilled water	100 ml

Add the brilliant green to the distilled water and shake to dissolve the dye. Heat the solution to 100°C for 30 minutes and shake from time to time whilst cooling, to ensure that the dye has completely dissolved. Store in a brown glass bottle or away from light.

Description

Muller[1] developed this medium in 1923. It was later modified by Kauffmann[2,3] with the addition of brilliant green and ox bile to suppress commensal organisms and thus improve the isolation of salmonellae.

The brilliant green dye used in the medium has been shown to be critical and Chroma or BDH brands should be used. It is essential that the dye is added as directed because heating the brilliant green or attempting to incorporate it in the basal medium seriously impairs its selective action.

The addition of novobiocin at 40 mg per litre of broth was described by Jeffries[4] to suppress the growth of *Proteus* species.

Muller-Kauffmann Tetrathionate Broth should not be used if *Salmonella typhi* is suspected.

Muller-Kauffmann Tetrathionate Broth was used in a large-scale investigation between nine laboratories in eight different countries[5].

Incubation of Muller-Kauffmann Broth at 43°C was shown to be essential in this trial and the technique used for enrichment of the salmonellae is as follows:

Add approximately 10 g of sample to 100 ml of Muller-Kauffmann Broth. Shake vigorously and immediately place the flasks of medium in a 45°C water-bath for 15 minutes. Remove the flasks from the water-bath, without drying them, and place in an incubator or another water-bath at 43°C.

Sub-culture the broth after 18-24 hours and again after 48 hours. Take one loopful of broth from the edge of the surface of the fluid and inoculate either two Oxoid Brilliant Green Agar (Modified) CM0329 plates (9 cm diameter) without recharging the loop between plates, or one large plate (14 cm diameter).

Incubate the plates at 35°C for 18-24 hours.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance

Dehydrated medium: Off-white, free-flowing powder.

Prepared medium: Off-white opaque suspension.

Quality control

Positive control:	***Expected results***
Salmonella typhimurium ATCC® 14028*	Good growth
Negative control:	
Escherichia coli ATCC® 8739*	Inhibited or no growth

*This organism is available as a Culti-Loop®

Precautions

Do not autoclave the base broth.

Add the iodine solution and brilliant green just prior to use.

The medium is not suitable for the growth of *Salmonella typhi*, *Salmonella sendai*, *Salmonella pullorum* and *Salmonella gallinarum*.

References
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MULLER-KAUFFMANN TETRATHIONATE-NOVOBIOCIN BROTH (MKTT-n)

Code: CM1048

Oxoid Muller-Kauffmann Tetrathionate-Novobiocin Broth (MKTT-n) is a selective enrichment medium for the isolation of Salmonella when used with Novobiocin Selective Supplement (SR0181).

Formula	***gm/litre***
Meat extract	4.3
Enzymatic digest of casein	8.6
Sodium chloride	2.6
Calcium carbonate	38.7
Sodium thiosulphate (anhydrous)	30.5*
Ox bile	4.78
Brilliant green	0.0096
pH 8.2 ± 0.2	

*equivalent to 47.8 g of sodium thiosulphate pentahydrate

Directions
Suspend 89.5 g of MKTT-n in 1 litre of distilled water, mix well and bring to the boil. Cool to below 45°C. Immediately before use add 20 ml of iodine-iodide solution prepared by dissolving 25 g of potassium iodide in 10 ml of water, adding 20 g of iodine and then diluting to 100 ml with sterile water. Also add the contents of four vials of Oxoid Novobiocin Selective Supplement (SR0181) reconstituted as directed. Mix well and aseptically dispense into sterile containers.

After the sample has been incubated in BPW (ISO), transfer 1 ml of the broth to 10 ml of MKTT-n Broth and 0.1 ml into 10 ml of RVS Broth (CM0866). Incubate the MKTT-n Broth at 37°C ± 1°C for 24 hours ± 3 hours and the RVS Broth at 41.5°C ± 1°C, for 24 hours ± 3 hours. Sub-culture the incubated MKTT-n and RVS broths onto XLD Agar (CM0469) and a second agar medium of choice and incubate for a further 24 hours ± 3 hours at 37°C ± 1°C. Presumptive *Salmonella* should be confirmed using appropriate biochemical and serological techniques. For the complete method please refer to ISO 6579:2002[1].

Description
Oxoid MKTT-n was developed by Muller[2] and later modified by Kauffmann[3,4] with the addition of ox bile and brilliant green to improve selectivity. The addition of novobiocin at 40 mg per litre was later described by Jeffries[5] to improve inhibition of *Proteus* species.

Oxoid MKTT-n is a selective enrichment medium for the isolation of *Salmonella*; the formulation conforms to ISO 6579:2002[1].

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Prepared medium base may be kept for up to 2 weeks at room temperature, prepared media in its final form containing iodine/iodide solution + Novobiocin Selective Supplement SR0181 should be used immediately.

Appearence
Dehydrated medium: White, free-flowing powder.

Prepared medium: Pale green, opaque suspension which on standing gives a heavy deposit.

Quality control

Positive controls:	***Expected result***
Salmonella typhimurium ATCC® 14028*	Good growth†
Escherichia coli ATCC® 25922*	Inhibited
Enterococcus faecalis ATCC® 29212*	Inhibited
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

†CM1048 is an opaque solution therefore turbidity cannot be used as a growth indicator.

References

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2. Muller L. (1923) *C. R. Soc. Biol (Paris)* 89. 434-447.
3. Kauffmann F. (1930) *Z. f. Hyg.* 113. 148-152.
4. Kauffmann F. (1935) *Z. f. Hyg.* 117. 26-32.
5. Jeffries L. (1959) *J. Clin. Path.* 12. 568-571.

MWY – see WADOWSKY YEE MEDIUM (MODIFIED)

MYCOPLASMA BROTH BASE

Code: CM0403

A basic medium which, after enrichment with a supplement can be used in the isolation and cultivation of mycoplasmas from clinical specimens.

Formula	***gm/litre***
Bacteriological peptone	10.0
'Lab-Lemco' powder	10.0
Sodium chloride	5.0
Mineral supplement	0.5
pH 7.8 ± 0.2	

Directions

Dissolve 25.5 g in 1 litre of distilled water. Mix well and distribute in 80 ml volumes. Sterilise by autoclaving at 121°C for 15 minutes. Cool to approximately 50°C and add one vial of Mycoplasma Supplement SR0059 reconstituted as directed.

MYCOPLASMA SUPPLEMENT – G SELECTIVE SUPPLEMENT

Code: SR0059

POISON – CONTAINS THALLIUM SALT

Vial contents (each vial is sufficient for 80 ml of medium)	***per vial***	***per litre***
Horse serum	20.0 ml	200.0 ml
Yeast extract (25% w/v)	10.0 ml	100.0 ml
Thallous acetate	25.0 mg	250.0 mg
Penicillin	20,000 IU	200,000 IU

Description

Oxoid Mycoplasma Broth Base complements Oxoid Mycoplasma Agar Base CM0401.

It requires supplementation with yeast extract, serum and antibiotics, which are available as Mycoplasma Supplement-G SR0059.

Carbohydrate fermentation by mycoplasmas can be tested by incorporating 1% w/v carbohydrate and 0.005% w/v phenol red into the broth medium.

A *Ureaplasma* broth[1] can be prepared by adding to 95 ml broth medium (pH adjusted to 6.0):

Vial contents	
Horse Serum	4.0 ml
Urea	0.05 g
Phenol red	0.001 g
Penicillin	100,000 units

A similar medium was described by Taylor-Robinson *et al*.[2] where reference is made to the use of HEPES buffer to induce large colony-forming *Ureaplasma* strains and for the isolation and titration of viable mycoplasma by the metabolism of arginine or glucose and measuring the pH change in the medium.

A selective medium for *Mycoplasma pneumoniae* was described by Kraybill & Crawford[3].

Most strains of mycoplasmas are encouraged by growth in a biphasic medium in which a layer of Mycoplasma Broth Base covers a basal layer of Mycoplasma Agar Base CM0401. Both broth and agar layers should be supplemented with Mycoplasma Supplement-G SR0059. Inclusion of the supplement provides the necessary factors for growth and prevents overgrowth of slow growing contaminating organisms.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance

Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured solution.

Quality Control

Positive control:	***Expected result at 35°C***
Mycoplasma pneumoniae ATCC® 15531	Slightly turbid
Negative control:	
Escherichia coli ATCC® 25922*	Inhibited

*This organism is available as a Culti-Loop®

Precautions

Thallous acetate is toxic, observe the precautions stated under Hazards section.

Sub-culture the broth to agar as soon as any indicator added begins to change colour before the pH change destroys the organism.

References

1. Shepard M. C. and Lanceford C. D. (1970) *Appl. Microbiol.* 20. 539-543.
2. Taylor-Robinson D., Martin-Bourgon C., Watanable T. and Addey J. P. (1971) *J. Gen. Microbiol*. 68. 97-107.
3. Kraybill W. H. and Crawford Y. E. (1965) *Proc. Soc. Exp. Biol. Med.* 118. 965-967.

MYCOPLASMA AGAR BASE

Code: CM0401

A basic medium which, after enrichment with supplements, will support the growth of Mycoplasma species.

Formula	***gm/litre***
Bacteriological peptone	10.0
'Lab-Lemco' powder	10.0
Sodium chloride	5.0
Mineral supplement	0.5
Agar	10.0
pH 7.8 ± 0.2	

Directions
Add 35.5 g to 1 litre of distilled water. Boil to dissolve the agar and distribute in 80 ml volumes. Sterilise by autoclaving at 121°C for 15 minutes. Cool to approximately 50°C and add one vial of Mycoplasma Supplement SR0059 reconstituted as directed.

MYCOPLASMA SUPPLEMENT-G

Code: SR0059

POISON – CONTAINS THALLIUM SALT

Vial contents (each vial is sufficient for 80 ml of medium)	***per vial***	***per litre***
Horse serum	20.0 ml	200.0 ml
Yeast extract (25% w/v)	10.0 ml	100.0 ml
Thallous acetate	25.0 mg	250.0 mg
Penicillin	20,000 IU	200,000 IU

MYCOPLASMA SUPPLEMENT-P

Code: SR0060

POISON – CONTAINS THALLIUM SALT

Vial contents	
Horse serum	6.0 ml
Yeast extract (25% w/v)	3.0 ml
Thallous acetate	0.008 g
Glucose	0.3 g
Phenol red	0.0012 g
Methylene blue chloride	0.0003 g
Penicillin	12,000IU
Mycoplasma Broth Base CM403	0.145 g

For the preparation of bi-phasic *Mycoplasma pneumonia* medium only.

Directions
Prepare the sterile supplement by aseptically adding 20 ml sterile distilled water to the vial and mix gently. Add 1 ml of Oxoid Mycoplasma Base without supplements to each of ten small bottles. Sterilise by autoclaving at 121°C for 15 minutes. Allow to set. Aseptically add 2 ml of the reconstituted Supplement-P to each bottle containing agar.

Description
Oxoid Mycoplasma Agar Base was formulated as a basal medium to be enriched with any satisfactory supplementary factors used for the growth of mycoplasmas (PPLO).

Edward[1] stressed the importance of the absence of toxic factors to mycoplasmas in the basal medium. Lynn & Morton[2] paid special attention to the inhibitory factors which can be present in batches of agar. Oxoid Mycoplasma Agar Base contains selected constituents shown to be free from such inhibitory or toxic

substances. It also contains a special mineral supplement which improves the growth and colony characteristics of mycoplasmas without interfering with the clarity of the medium.

Hayflick[3] suggested inclusion of 10% v/v of a 25% w/v extract of baker's yeast in the medium and Lemcke[4] used Oxoid Yeast Extract LP0021. The majority of mycoplasmas require media enriched with serum; horse serum (20% v/v) is commonly used. Swine or human sera may be substituted for horse serum but the possible presence of antibodies or antibiotics in human serum make media control of great importance (Fallon[6]). The addition of DNA to the medium to encourage the growth of bovine general genital strains and other mycoplasmas was suggested by Edward[7]. 20 mg of sodium deoxyribonucleate per ml of medium is quoted by Lemcke[4].

Antibacterial agents are necessary to prevent overgrowth of the slow-growing mycoplasmas by contaminating organisms. Penicillin and thallous acetate are the most common agents used but T-strain mycoplasma* are sensitive to thallous acetate. Hutchinson[5] and Fallon[6] state that ampicillin at 1 mg per ml of medium may be substituted for penicillin and thallous acetate.

Penicillin may be used at concentrations between 50 and 500 units per ml and thallous acetate between 1/2000 and 1/8000 (Lemcke)[4]. It is preferable to omit thallous acetate when searching for T-strain mycoplasma* (Shepherd & Lanceford[8]).

Two supplements, Mycoplasma Supplement-G SR0059 and Mycoplasma Supplement-P SR0060 have been developed for the improved growth of mycoplasmas. Mycoplasma Supplement-G SR0059 is a general supplement prepared to the formulation of Hayflick[3] which, when added to Oxoid Mycoplasma Broth or Agar Base produces a complete selective medium for the propagation of sterol-requiring *Mycoplasma* species of the classical type.

Mycoplasma Supplement-P SR0060 is a liquid supplement based on the formulation recommended by the Mycoplasma Reference Laboratory, CPHLS, Colindale, which is used in conjunction with Mycoplasma Agar Base CM0401 to form a bi-phasic medium for the isolation and preliminary identification of *Mycoplasma pneumoniae*.

Many species of mycoplasmas are aerobes or facultative anaerobes but some prefer micro-aerophilic conditions with the addition of carbon dioxide, or strict anaerobiosis.

Pathogenic strains grow best at 35°C while saprophytic strains often grow between 22°C and 30°C, T-strains* have an optimal temperature of 36°C.

Mycoplasma species grow best at pH 7.4-8.0 but T-strains* prefer pH 6.0-6.5.

T-strain mycoplasma = *Ureaplasma urealyticum

Technique

Agar plates

Material for cultivation is inoculated onto agar plates (usually 55 mm) prepared with Mycoplasma Agar Base CM0401 + Mycoplasma Supplement-G SR0059. Plates are incubated in moist chambers aerobically, anaerobically and in 10% CO2-90% N2 atmosphere. Examine the agar surface after 7 days incubation with a dissecting microscope at 60x magnification, using obliquely transmitted light. The colonies are characteristic with the centre of the colony embedded beneath the surface, giving a 'fried-egg' appearance.

Purification of the organism by further cloning sub-cultures is essential before identification. This may be carried out by removing a plug of agar containing a colony from the plate and using it to inoculate further plates of medium. Growth inhibition tests using specific antisera may then be carried out (Clyde[9]).

BI-phasic Medium

Bi-phasic media preparedwith 1 ml quantities of solid Mycoplasma Agar Base overlaid with 2 ml of reconstituted Mycoplasma Supplement-P SR0060. Bi-phasic medium bottles should be inoculated with a swab or a fleck of sputum and incubated at 35°C for up to three months. Any bottles showing gross turbidity due to growth of bacteria or fungi should be discarded.

Growth of *Mycoplasma pneumoniae* results in the reduction of methylene blue followed by production of acid due to the fermentation of glucose, resulting in a colour change of the phenol red indicator to yellow. Bottles showing such a colour change should be sub-cultured onto agar for further examination. Mycoplasma Agar Base supplemented with Mycoplasma Supplement-G SR0059 is suitable for this purpose.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured gel.

Quality Control

Positive control:	***Expected result at 35°C***
Mycoplasma pneumoniae ATCC® 15531	Microscopic examination – 'fried-egg' colonies
Negative control:	
Escherichia coli ATCC® 25922*	Inhibited

*This organism is available as a Culti-Loop®

Precautions
Thallous acetate is toxic, observe the precautions in the HAZARDS section.

Do not use thallous acetate media to isolate *Ureaplasma urealyticum*.

Mycoplasmas may be suspected if (1) typical morphology (2) no growth in media without serum (3) colonies are embedded below the agar surface.

References

1. Edward D. G. ff. (1971) *J. Gen. Microbiol.* 69. 205-210.
2. Lynn R. J. and Morton H. E. (1965) *Appl. Microbiol*. 4. 339-341.
3. Hayflick L. (1965) *Texas Rep. Biol. & Med. 23. suppl.* 1. 285-303.
4. Lemcke Ruth M. (1965) *'Media for the Mycoplasmataceae', Lab. Pract.* 14. 712.
5. Hutchinson D. (1969) *J. Med. Lab. Technol.* 26. 111-116.
6. Fallon R. J. (1969) *S. A. B. Technical series 3. Academic Press*. 41-50.
7. Edward D. G. ff. (1954) *J. Gen. Microbiol.* 10. 27-64.
8. Shepard M. C. and Lanceford C. D. (1970) *Appl. Microbiol.* 2. 539-543.
9. Clyde W. A. (1964) *J. Immun.* 92. 958-962.

MYP Agar (Mannitol Egg Yolk Polymyxin Agar)

Code: CM0929

A medium for the enumeration of Bacillus cereus in food samples.

Formula	***gm/litre***
Meat extract	1.0
Peptone	10.0
Mannitol	10.0
Sodium chloride	10.0
Phenol Red	0.025
Agar	12.0
pH 7.2 ± 0.2	

BACILLUS CEREUS SELECTIVE SUPPLEMENT

Code: SR0099

Vial contents (each vial 500 ml)	***per vial***	***per litre***
Polymyxin B	50,000 IU	100,000 IU

Directions
Suspend 21.5 g in 450 ml distilled water and bring gently to the boil to dissolve. Sterilise by autoclaving at 121°C for 15 minutes.

Cool to approximately 49°C and aseptically add 50 ml Egg Yolk Emulsion (SR0047) and 1 vial of Bacillus cereus Selective Supplement, reconstituted as directed. Mix well and pour into sterile Petri dishes.

Description

Bacillus cereus has been recognised as a causative agent of food poisonings since the 1950s. Many early isolation techniques used blood agar, relying on haemolysis and colony morphology for the detection of suspect *Bacillus cereus*, and then a range of tests for confirmation. The main problem with these media was that they were not selective and were generally only useful in detecting high numbers of *Bacillus cereus*.

Bacillus cereus is a Gram-positive, rod shaped, facultatively aerobic sporeforming organism. The cells are large (3.5 µm) and sometimes form short chains or long strings with central to terminal ellipsoidal spores that do not distend the cell. Unstained globules within the cells occur when they are grown on glucose-containing media.

Although the presence of *Bacillus cereus* has been determined in foods related to outbreaks of illness since the early years of this century[1], its role as a causative agent in food poisoning was not established until the 1950s.

Two distinct syndromes, emetic and diarrhoeal, may occur in *Bacillus cereus*-associated foodborne illnesses, related to two different metabolites. The diarrhoeal type of illness is caused by a heat labile, high molecular weight protein, while the vomiting (emetic) type is caused by a heat stable, low molecular weight peptide[2].

A wide range of foods have been associated with the diarrhoeal syndrome, including meat-based dishes, soups, vegetables, puddings and sauces[2]. The emetic syndrome seems to be associated to a more limited range of foods with approximately 95% of all cases related to fried or cooked rice[3]. Foods implicated in *Bacillus cereus*-induced illnesses usually contain at least 10^5 cfu/g, although about 10% of outbreaks have been associated with food containing less than this[2]. In almost all cases the implicated food has been held for too long at unsatisfactory storage temperatures.

MYP Agar is a selective and differential medium developed by Mossel *et al*[4]. The diagnostic features of the medium rely upon the failure of *Bacillus cereus* to utilise mannitol and the ability of most strains to produce phospholipase C. The medium is made selective by the addition of Polymyxin B which will inhibit Gram-negative bacteria. MYP Agar has proved to be very effective for detecting *Bacillus cereus* even for ratios as challenging as one cell of *Bacillus cereus* to 106 cells of other organisms[4].

Technique

1. Dry the surface of the agar medium. Prepare the food sample by making appropriate dilutions in Peptone Water.
2. Spread 0.1 ml of these dilutions over the surface of the agar plate using a sterile glass spreader.
3. Incubate at 30°C for 18-40 hours.
4. Count the number of typical colonies and calculate the viable count.
5. Typical colonies of *Bacillus cereus* are rough and dry with a violet pink background surrounded by an egg yolk precipitate.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label. Bacillus cereus Selective Supplement should be stored at 2-8°C. Store the prepared plates at 2-8°C.

Appearance

Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Red coloured gel.

Quality control

Positive control:	***Expected result***
Bacillus cereus ATCC® 10876	Good growth; bright pink colonies; zone of egg yolk precipitation
Negative control:	
Escherichia coli ATCC® 25922*	No growth

*This organism is available as a Culti-Loop®

References

1. Lübenau C. (1906) *Zbl. Bakt.*, I. 40: 433-437.
2. Kramer J. M. and Gilbert R. J. (1989) *Bacillus cereus* and other *Bacillus* species. In: *Foodborne Bacterial Pathogens*. pp. 21-70. Doyle M. P. (Ed.). Marcel Dekker, New York.
3. Jenson I. and Moir C. J. (1997) *Bacillus cereus* and other *Bacillus* species. In: *Foodborne Microorganisms of Public Health Significance*. 5th Edition. pp. 379-406. A. D. Hocking (Ed.). AIFST (NSW Branch) *Food Microbiology Group*, Australia.
4. Mossel D. A. A., Koopman M. J. and Jongerius E. (1967) *Appl. Microbiol.* 15. 650-653.

NEW YORK CITY MEDIUM VARIANTS

Media for the Isolation of Neisseria gonorrhoeae.

OXOID GC AGAR BASE

Code: CM0367

Formula	***gm/litre***
Special peptone	15.0
Corn starch	1.0
Sodium chloride	5.0
Dipotassium hydrogen phosphate	4.0
Potassium dihydrogen phosphate	1.0
Agar	10.0
pH 7.2 ± 0.2	

LCAT SELECTIVE SUPPLEMENT

Code: SR0095

Vial contents (each vial is sufficient for 500 ml of medium)	***per vial***	***per litre***
Lincomycin	0.5 mg	1.0 mg
Colistin sulphate	3.0 mg	6.0 mg
Amphotericin B	0.5 mg	1.0 mg
Trimethoprim	3.25 mg	6.5 mg

VCAT SELECTIVE SUPPLEMENT

Code: SR0104

Vial contents (each vial is sufficient for 500 ml of medium)	***per vial***	***per litre***
Vancomycin	1.0 mg	2.0 mg
Colistin sulphate	3.75 mg	7.5 mg
Amphotericin B	0.5 mg	1.0 mg
Trimethoprim	1.5 mg	3.0 mg

Directions

Suspend 18 g of Oxoid GC Agar Base in 425 ml of distilled water and bring gently to the boil to dissolve the agar. Sterilise by autoclaving at 121°C for 15 minutes. Dissolve the contents of a vial of sterile Yeast Autolysate Supplement SR0105 in 15 ml of sterile distilled water or the alternative product Liquid Autolysate SR0182 may be utilised.

Reconstitute as directed either LCAT SR0095 or VCAT SR0104 make final volume 10 ml.

Aseptically add 50 ml laked horse blood SR0048, Yeast Autolysate Supplement and the Antibiotic Supplement LCAT or VCAT to the sterile GC Agar Base cooled to 50°C. Mix gently to avoid trapping air bubbles in the agar and pour into sterile Petri dishes.

Description

Oxoid GC Agar Base has been formulated to include Special Peptone LP0072 which is a mixture of meat and plant enzymatic digests. The presence of starch ensures that toxic metabolites produced by *neisseria* are absorbed. Phosphate buffers are included to prevent changes in pH due to amine production that would affect the survival of the organism.

These are derivatives of NYC Medium[1,2,3] based on Young's publication[4] where the higher level of glucose recommended by the originators was reduced to allow sugar fermentation test to be carried out[5].

Storage conditions and Shelf life
Store the dehydrated medium below 30°C and use before the expiry date on the label.

Store the prepared plates at 2-8°C.

Appearance
Dehydrated medium: Light straw coloured, free-flowing powder.

Prepared medium (before additions): Straw coloured gel.

Quality Control

Positive control: with antibiotics	***Expected result***
Neisseria gonorrhoeae ATCC® 19424*	Good growth; grey-brown colonies
Neisseria meningitidis ATCC® 13090*	Good growth; grey-brown colonies
Negative control: with antibiotics	
Proteus hauseri ATCC® 13315 *	Inhibited
Staphylococcus aureus ATCC® 25923*	Inhibited
Negative control: without antibiotics	
Uninoculated medium	No change

References
1. Faur Y. C., Wiesburd M. H., Wilson M. E. and May P. S. (1973) *Health Lab. Sci.* **10.** 44-54.
2. Faur Y. C., Weisburd M. H. and Wilson M. E. (1973) *Health Lab. Sci.* **10.** 55-60.
3. Faur Y. C., Weisburd M. H. and Wilson M. E. (1977) *Health Lab. Sci.* **15.** 22-27.
4. Young H. (1978) *Brit. J. Ven. Diseases* **54.** 36-40.
5. Young H. (1978) *J. Clin. Microbiol.* **7.** 247-250.

NICKERSON MEDIUM – see BIGGY AGAR

NOVEL ENRICHMENT BROTH

Code: CM1066

A selective enrichment broth for Listeria species from food samples in 24 hours.

Formula	***gm/litre***
Peptone	28.0
Carbohydrate mix	6.0
Salt mix	10.0
Final pH 7.4 ± 0.2 at 25°C	

ONE BROTH SELECTIVE SUPPLEMENT

Code: SR0234

Directions
Suspend 22 g of ONE Broth Base in 500 ml of distilled water. Mix well and sterilize by autoclaving at 121°C for 15 minutes. Cool the medium to below 50°C and aseptically add the contents of one vial of ONE Broth Selective Supplement reconstituted as directed.

Description
A novel broth developed by Oxoid for the selective enrichment and isolation of Listeria species from food samples. Recovery of *Listeria spp*. after 24 hours using Oxoid Novel Enrichment Broth – Listeria (ONE Broth) has been shown to be comparable and in some cases better than using the ISO 11290-1[1] combined Fraser and Half Fraser broth enrichment procedure[2].

This novel enrichment broth incorporates a carefully balanced mixture of peptones, carbohydrates and salts to give optimal resuscitation, recovery and growth of even low numbers of *Listeria* spp. from food samples after 24 hours.

Convenient freeze-dried vials provide easy addition of selective agents to the broth base allowing recovery of Listeria spp. while inhibiting a wide range of competing flora.

Internal trials and an evaluation carried out on behalf of Oxoid at Leatherhead Food International show that at 24 hours ONE Broth is capable of the improved recovery of *Listeria* when compared to Half Fraser Broth[2] alone or Half Fraser followed by full Fraser Broth. In addition, the number of *Listeria* cells detected after broth enrichment for 24 hours was found to be higher with ONE Broth than with Half Fraser Broth.

ONE Broth offers the benefit of requiring a single enrichment medium at one temperature, with no need to sub-culture into a secondary enrichment broth. Listeria recovery levels at 24 hours are at least equivalent to the ISO enrichment method for and in some cases are improved[2,3].

Technique

1. Add 25 g of food sample to 225 ml of ONE Broth and stomach for a minimum of 30 seconds to mix the sample.
2. Incubate the broth without agitation at 30°C for 24 ± 2 hours.
3. Gently agitate the bag then, using a microbiological loop, remove 10 µl and inoculate onto a suitable agar medium e.g. Oxoid Chromogenic Listeria Agar (OCLA) and incubate appropriately (37°C for 24-48 hours).
4. Confirm presumptive colonies on the agar plates as *Listeria monocytogenes* or *Listeria* species by appropriate methods e.g. Gram-stain, catalase, oxidase, Oxoid Listeria Latex Test Kit DR1126, Oxoid O.B.I.S. mono ID0800, Microbact Listeria 12L MB1128.

Storage conditions and Shelf life

ONE Broth Base must be stored tightly capped in the original container at 10-30°C. ONE Broth Selective Supplement should be stored in the dark at 2-8°C.

Store the prepared medium for up to 4 weeks at 2-8°C.

Appearance

Dehydrated Medium: straw coloured, free-flowing powder.

Selective Supplement: orange/yellow, freeze-dried pellet.

Prepared Medium: dark straw coloured, clear liquid.

Quality Control

Positive control:	***Expected results***
Listeria Monocytogenes ATCC® 7644*	Turbid growth
Negative control:	
Staphylococcus aureus ATCC® 25923*	Inhibited

*This organism is available as a Culti-Loop®

Precautions

ONE Broth Base and ONE Broth Selective Supplement are for Laboratory Use Only.

ONE Broth Base is designated as ‘Irritant’ and ONE Broth Selective Supplement is designated as ‘Harmful’. For a full Material Safety Data Sheet (MSDS)

Do not use ONE Broth Base or ONE Broth Selective Supplement beyond the stated expiry dates, or if the products show any sign of deterioration.

References

1. ISO 11290-1 (Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of *Listeria monocytogenes* – part 1).
2. Oxoid Poster – Folio No. 1033, July 2004.
3. Data on file at Oxoid.

NUTRIENT AGAR

Code: CM0003 (Powder)

Code: CM0004 (Tablets)

A general purpose medium which may be enriched with 10% blood or other biological fluid.

Formula	***gm/litre***
'Lab-Lemco' powder	1.0
Yeast extract	2.0
Peptone	5.0
Sodium chloride	5.0
Agar	15.0
pH 7.4 ± 0.2	

Directions

Powder: Suspend 28 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

Tablets: Add one tablet to 5 ml of distilled water and soak for 5 minutes. Sterilise by autoclaving at 121°C for 15 minutes.

Description

Nutrient Agar is a basic culture medium used to sub-culture organisms for maintenance purposes or to check the purity of sub-cultures from isolation plates prior to biochemical or serological tests.

In semi-solid form, agar slopes or agar butts the medium is used to maintain control organisms[1].

Nutrient Agar is suitable for teaching and demonstration purposes. It contains a concentration of 1.5% agar to permit the addition of up to 10% blood or other biological fluid, as required. The medium, without additions, may be used for the cultivation of organisms which are not exacting in their nutritional requirements.

For a medium which is richer in nutrients, see Blood Agar Base No. 2 CM0271.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance

Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured gel.

Quality control

Positive controls:	***Expected result***
Staphylococcus aureus ATCC® 25923*	Good growth; straw colonies
Escherichia coli ATCC® 25922*	Good growth; straw/white colonies
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

Reference

1. Lapage S. P., Shelton J. E. and Mitchell T. G. (1970) in *Methods in Microbiology.* Eds. Norris J. R. and Ribbons D. W. Vol.3A. Academic Press. London. p.116.

NUTRIENT BROTH

Code: CM0001

A general purpose fluid medium for the cultivation of micro-organisms not exacting in their nutritional requirements. Blood, serum, sugars, etc., may be added as required for special purposes.

Formula	***gm/litre***
'Lab-Lemco' powder	1.0
Yeast extract	2.0
Peptone	5.0
Sodium chloride	5.0
pH 7.4 ± 0.2	

Directions
Add 13 g to 1 litre of distilled water. Mix well and distribute into final containers. Sterilise by autoclaving at 121°C for 15 minutes.

Description
Lab-Lemco beef extract is combined with peptone and sodium chloride to form the basic bouillon described by Loeffler and other early bacteriologists. Yeast extract is added to provide vitamins and minerals to help speed the growth of most organisms.

Nutrient Broth can be enriched with other ingredients such as carbohydrates, blood etc., for special purposes. See also Nutrient Broth No. 2 CM0067.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium below 25°C.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured solution.

Quality control

Positive controls:	***Expected results***
Staphylococcus aureus ATCC® 25923*	Turbid growth
Escherichia coli ATCC® 25922*	Turbid growth
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

NUTRIENT BROTH No. 2

Code: CM0067

A nutritious medium suitable for the cultivation of fastidious pathogens and other micro-organisms.

Formula	***gm/litre***
'Lab-Lemco' powder	10.0
Peptone	10.0
Sodium chloride	5.0
pH 7.5 ± 0.2	

Directions
Add 25 g to 1 litre of distilled water. Mix well, distribute into final containers and sterilise by autoclaving at 121°C for 15 minutes.

Directions (to prepare Preston Campylobacter Selective Enrichment Broth)

Dissolve 12.5 g of Nutrient Broth No. 2 in 475 ml of distilled water and sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C or below. Aseptically add 25 ml of Lysed Horse Blood SR0048, 1 vial of Preston

Campylobacter Selective Supplement SR0117 or SR0204 and 1 vial of Campylobacter Growth Supplement SR0084 or SR0232. Aseptically dispense 5 ml volumes in sterile small screw-capped bottles. The Selective Enrichment Broth may be stored for up to 7 days at 2-8°C.

It is essential that the head space above the liquid should be as small as possible to ensure microaerobic conditions.

Description

This medium is comparable to a meat infusion and is richer in nutrients than Nutrient Broth CM0001. It gives good growth from small inocula and is recommended for sterility testing for aerobic organisms. Nutrient Broth No. 2 complies with the recommendations in the *British Pharmacopoeia*[1] for the composition of a sterility testing medium for aerobes. The medium is ideally suited for sub-culture, particularly as a secondary growth medium for staphylococci which are to be tested for coagulase production. Nutrient Broth No. 2 made up at double strength corresponds to the medium recommended by the British Standards Institution[2] for use in the determination of the Rideal-Walker Coefficient of Disinfectants.

Nutrient Broth No. 2 is the basal medium for Preston Campylobacter Enrichment Broth[3,4].

Selective Enrichment Broth Technique

1. Prepare the Preston Selective Enrichment Broth as directed from CM0067, SR0117, SR0084 or SR0232 and Lysed Blood SR0048.
2. Emulsify the specimen under test in the selective enrichment broth.
3. Incubate the broth aerobically at 42°C for 24 hours.
4. Sub-culture on to Preston Campylobacter Selective Agar or Campylobacter Blood-Free Selective Agar.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance

Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured solution.

Quality control

Positive controls:	***Expected result***
Staphylococcus aureus ATCC® 25923*	Turbid growth
Pseudomonas aeruginosa ATCC® 27853*	Turbid growth
Negative control:	
Uninoculated medium	No change
Campylobacter Media	
Positive control:	***Expected results***
Campylobacter jejuni ATCC® 29428*	Turbid growth
Negative control:	
Escherichia coli ATCC® 25922*	Inhibited

*This organism is available as a Culti-Loop®

References

1. British Pharmacopoeia (1980) London, HMSO.
2. British Standard 541: (1934) *Determining the Rideal Walker Coefficient of Disinfectants* BSI London, p.9.
3. Bolton F. J. and Robertson L. (1982) *J. Clin. Pathol.* 35. 462-467.
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NUTRIENT GELATIN (CM135a)

Code: CM0635

For determination of gelatin liquefaction, and for the 20°C plate count.

Formula	***gm/litre***
'Lab-Lemco' powder	3.0
Peptone	5.0
Gelatin	120.0
pH 6.8 ± 0.2	

Directions
Suspend 128 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Mix well before pouring and cool below 20°C or leave to set in a refrigerator.

Description
Nutrient Gelatin, a solid medium at 22°C and below, is employed for the determination of gelatin liquefaction and for the 20°C plate count[1].

Gelatin is liquefied in a characteristic manner by certain proteolytic organisms; whereas gelatin media have been largely superseded by agar media for most purposes, nutrient gelatin is still employed for differentiation of micro-organisms by their proteolytic effects.

Technique
Test for gelatin liquefaction by incubating a Nutrient Gelatin stab or plate culture at 20-22°C. Alternatively, incubate at a higher temperature (usually optimum for the organism under investigation) and then transfer the tube to a refrigerator or into cold water before observation. The latter method not only allows determinations to be carried out on organisms which grow slowly or not at all at 20-22°C but also usually avoids false positive results produced by the release of enzymes after the death of the organisms[2].

If the medium is incubated at a higher temperature it is necessary to employ uninoculated controls to allow for the hydrolytic effect of heat and other factors. Rates of liquefaction vary considerably, so that some organisms produce liquefaction within a few days whereas others require several weeks. For practical purposes, a maximum incubation period of 14 days is suggested[3,4].

Considerably longer incubation may be necessary, some strains of *Enterobacter cloacae* liquefied gelatin only after 3 months at 20-22°C5. Especially where prolonged incubation is necessary, it is important to ensure adequate closure of the containers in order to prevent dehydration of the medium. Besides its presence or absence, the shape and nature of the liquefied portion of the stab culture are often useful identifying characteristics.

Particularly with plate cultures, gelatin liquefaction may be detected sooner by the 'Stone reaction' (Stone[6]): add a drop of saturated aqueous ammonium sulphate solution, or of fresh 20% aqueous sulpho-salicylic acid solution, to an individual colony growing on Nutrient Gelatin. A positive reaction (i.e. gelatin liquefaction) is indicated by the presence of a clear zone round the colony after 10 minutes contact with either reagent. This method is slightly less sensitive but several strains may be tested on one plate. The 'Stone reaction' is also employed with Staphylococcus Medium No. 110 CM0145 for the differentiation of staphylococci.

For the standard gelatin plate count on water (American Public Health Association[1]) dilute the original sample with sterile tap water and place 0.5 or 1 ml of the dilutions in each dish of at least two duplicate sets of sterile Petri dishes. Cool the sterile prepared Nutrient Gelatin to approximately 42°C and aseptically add 10 ml to each Petri dish. Mix the contents by tilting and rotation, allow to solidify as soon as possible after pouring and immediately place in an incubator at 19-21°C. Incubate for 48 ± 3 hours and count at least two plates made from the dilution giving between 30 and 300 colonies per plate.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance
Dehydrated Medium: Straw coloured, free-flowing powder/granules.

Prepared medium: Straw coloured gel.

Quality control

Positive controls:	***Expected results***
Serratia liquefaciens ATCC® 27592*	Gelatinase positive
Staphylococcus aureus ATCC® 25923*	Gelatinase positive
Negative control:	
Escherichia coli ATCC® 25922	Gelatinase negative

*This organism is available as a Culti-Loop®

Precautions

Do not shake the gelatin tubes whilst they are warm because growth and liquefaction of gelatin frequently occurs on the surface layer only[7].

In routine diagnostic work report gelatin liquefaction or not. The type or shape of liquefaction is of less importance[2].

References

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OCCA – see CHROMOGENIC CANDIDA AGAR

OCLA – see CHROMOGENIC LISTERIA AGAR

OGYE – see OXYTETRACYCLINE GLUCOSE YEAST EXTRACT AGAR

ONE BROTH – see NOVEL ENRICHMENT BROTH-LISTERIA

OPSP AGAR

PERFRINGENS AGAR

Code: CM0543

For the enumeration of Clostridium perfringens in foods.

Formula	***gm/litre***
Tryptone	15.0
Yeast extract	5.0
Soya peptone	5.0
Liver extract	7.0
Ferric ammonium citrate	1.0
Sodium metabisulphite	1.0
Tris buffer	1.5
Agar	10.0
pH 7.3 ± 0.2	

PERFRINGENS (OPSP) SELECTIVE SUPPLEMENT A

Code: SR0076

Vial contents (each vial is sufficient for 500 ml of medium)	***per vial***	***per litre***
Sodium sulphadiazine	50 mg	100 mg

PEFRINGENS (OPSP) SELECTIVE SUPPLEMENT B

Code: SR0077

Vial contents (each vial is sufficient for 500 ml of medium)	***per vial***	***per litre***
Oleandomycin phosphate	0.25 mg	0.5 mg
Polymyxin B	5,000 IU	10,000 IU

Directions

Suspend 22.8 g in 500 ml of distilled water and bring gently to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Allow to cool to 50°C and aseptically add the contents of one vial each of Perfringens Agar (OPSP) supplements A and B and which have been rehydrated as directed. Mix well and pour into sterile dishes.

Description

Oxoid Perfringens Agar (OPSP), is based on the formulation developed by Handford[1].

The medium utilises sulphadiazine (100 µg/ml), oleandomycin phosphate (0.5 µg/ml) and polymyxin B sulphate (10 IU/ml), presented as freeze-dried supplements SR0076 and SR0077 to give a high degree of selectivity and specificity for *Clostridium perfringens*.

Sodium metabisulphite and ammonium ferric citrate are used as an indicator of sulphite reduction by *Clostridium perfringens* which produces black colonies on this medium when using a pour plate technique.

Tests for confirmation of *Clostridium perfringens* are described in a study initiated by the International Commission on Microbiological Specifications for Foods (ICMSF)[2].

Sulphite-reducing bacteria other than *Clostridium perfringens* such as salmonellae, *Proteus* species and *Citrobacter freundii*, as well as staphylococci and *Bacillus* species, are inhibited on OPSP Agar.

Perfringens Agar (OPSP) has the advantage of inhibiting growth of *Clostridium bifermentans* and *Clostridium butyricum*. These sulphite-reducing organisms grow readily on Shahidi-Ferguson Perfringens Agar (SFP)[3] and Tryptone-Sulphite-Neomycin Agar (TSN)[4] as black colonies with a tendency to spread and obscure the whole surface of the medium.

Occasional strains of enterococci will grow on Perfringens Agar (OPSP) as white colonies, easily distinguished from the large black colonies of *Clostridium perfringens*.

Clostridium perfringens enumeration media which include egg yolk in order to detect lecithinase activity have not proved satisfactory partly because *Clostridium perfringens* colonies may frequently fail to produce haloes and thus appear falsely to be negative, and partly because counting is rendered impractical as the organism often grows in the form of large spreading colonies which completely blacken the medium[5].

Technique

Make up the medium according to the directions. Prepare pour plates, containing approximately 25 ml per plate, using 1 ml aliquots of a suitable series of dilutions of the homogenised test sample. Mix well before setting.

It is unlikely that colonies of *Clostridium perfringens* will blacken if plates are surface-inoculated unless the inoculum is covered with a layer of agar.

Incubate the plates at 35°C for 18-24 hours with an anaerobic Gas Generating Kit pack BR0038 in a conventional gas-jar. Alternatively use AnaeroGen AN0025 or AN0035. AnaeroGen does not require the addition of water or a catalyst.

Clostridium perfringens may be seen as large black colonies (2-4 mm diameter) within the depth of the agar.

Occasional strains of *Enterococcus faecalis* which may grow on Perfringens Agar (OPSP) as small colourless colonies are easily distinguished from *Clostridium perfringens*.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured gel.

Quality control

Positive control:	***Expected results***
Clostridium perfringens ATCC® 13124*	Good growth; black coloured colonies
Negative control:	
Clostridium sporogenes ATCC® 19404*	No growth

*This organism is available as a Culti-Loop®

Precautions
The production of black colonies on this medium is a presumptive identification of *Clostridium perfringens*. Further identification tests must be carried out.

References

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ORANGE SERUM AGAR

Code: CM0657

A medium for the isolation and enumeration of spoilage organisms of citrus products.

Formula	***gm/litre***
Tryptone	10.0
Yeast extract	3.0
Orange serum (equivalent solids)	3.5
Glucose	4.0
Dipotassium phosphate	2.5
Agar	14.0
pH 5.5 ± 0.2	

Directions
Suspend 37 g in 1 litre of distilled water and bring gently to the boil to dissolve completely. Dispense into final containers and sterilise by autoclaving at 121°C for 15 minutes.

Description
Orange Serum Agar was developed specifically for the isolation and enumeration of micro-organisms that are capable of surviving in citrus products[1]. The low pH of these products limits the growth of micro-organisms to those capable of tolerating the acidic environment.

Organisms known to grow in single strength and concentrated citrus juices are lactic acid and acetic acid bacteria, yeasts and moulds[2]. Of this group of organisms lactic acid bacteria have been mainly implicated as the cause of spoilage and these have been identified as *Lactobacillus plantarum*, *Lactobacillus brevis*, *Leuconostoc mesenteroides* and *Leuconostoc dextranicum*[3].

In comparative studies carried out by Murdock *et al*.[4] Orange Serum Agar was found to be a superior medium when compared to other media used for the total count and detection of spoilage organisms.

Technique

1. Prepare the medium as directed.
2. Add 1 ml of the test sample to a sterile Petri dish.
3. Add approximately 20 ml of cooled medium (50°C) to each plate and mix according to the plate count method[5].
4. Incubate at 30°C and examine daily for up to seven days.
5. Report as number of colony-forming units per ml of test material.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance

Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw-orange coloured gel.

Quality control

Positive controls:	***Expected results***
Lactobacillus fermentum ATCC® 9338*	Good growth; cream coloured colonies
Saccharomyces cerevisiae ATCC® 9763*	Good growth; cream coloured colonies
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

Precautions

Do not overheat the medium.

References

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OXACILLIN RESISTANCE SCREENING AGAR BASE

Code: CM1008

A medium for screening for methicillin-resistant Staphylococcus aureus (MRSA) directly from routine swab samples.

Formula	***gm/litre***
Peptone	11.8
Yeast extract	9.0
Mannitol	10.0
Sodium chloride	55.0
Lithium chloride	5.0
Aniline Blue	0.2
Agar	12.5
PH 7.2 ± 0.2	

ORSAB SELECTIVE SUPPLEMENT

Code: SR0195

Vial contents (each vial is sufficient to supplement 500 ml of medium)	***per vial***	***per litre***
Polymyxin B	25,000 IU	50,000 IU
Oxacillin	1 mg	2 mg

Directions
Suspend 51.75 g of Oxacillin Resistance Screening Agar Base in 500 ml of distilled water and bring gently to the boil to dissolve. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C and aseptically add the contents of one vial of ORSAB Selective Supplement (SR0195), reconstituted as directed below. Mix well and pour into sterile Petri dishes.

Description
ORSAB is intended as a medium for the screening for methicillin-resistant *Staphylococcus aureus* (MRSA) directly from routine swab samples. The screening of patients and staff for the early detection of MRSA colonisation is essential if epidemics are to be prevented. ORSAB is based on traditional Mannitol Salt Agar CM0085 with a reduction in salt concentration from 75 g/l (7.5%) to 55g/l (5.5%). This lower level of salt is still sufficient to inhibit most bacteria other than staphylococci whilst optimising growth of low numbers of MRSA.

Oxacillin Resistance Screening Agar Base is a nutritious and selective medium containing peptones for growth, a high salt concentration and lithium chloride to suppress non-staphylococcal growth with mannitol and aniline blue for the detection of mannitol fermentation.

The antibiotics contained in ORSAB Selective Supplement are oxacillin at 2 mg/litre to inhibit methicillin-sensitive *Staphylococcus aureus* (MSSA) and polymyxin B for the suppression of other bacteria able to grow at such a high salt concentration, e.g. *Proteus* spp.

Typical colonies of MRSA are intense blue in colour on a colourless background enabling the organism to be more easily identified in mixed culture than the pale yellow colonies seen on Mannitol Salt Agar.

Culture Medium	**Colony Colour**	
	Positive	***Negative***
ORSAB CM1008 & SR1095	Intense blue on colourless media	Straw/No Growth
Mannitol Salt Agar CM0085	Pale yellow on red media	Pink-Red/No Growth

Technique
Take a routine swab sample from the patient or person to be screened. Rub the swab onto an ORSAB plate in one set of streaks near the plate perimeter. The sample material should then be streaked across the plate using the diminishing sweep technique. Incubate at 37°C for 24 hours.

Examine after 24 hours for blue colonies. Confirm suspected MRSA with coagulase test Staphytect Plus DR0850 or Dryspot Staphytect Plus DR0100 and PBP2' DR0900. Re-incubate negative plates for a further 24 hours if necessary. Do not re-incubate positive plates.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label. Salmonella Selective Supplement should be stored at 2-8°C.

Store the prepared medium at 2-8°C.

Appearance
Dehydrated medium: Straw-grey coloured, free-flowing powder.

Prepared medium: Blue-grey coloured gel.

Quality control

Positive control:	***Expected results***
Staphylococcus aureus (MRSA strain) ATCC® 43300*	Good growth; blue coloured colonies
Negative controls:	
Staphylococcus aureus (MSSA strain) ATCC® 25923 *	No growth No growth
Escherichia coli ATCC® 25922*	No growth

*This organism is available as a Culti-Loop®

OXFORD LISTERIA AGAR – see LISTERIA SELECTIVE AGAR (OXFORD)

OXYTETRACYCLINE-GLUCOSE-YEAST EXTRACT AGAR (OGYE AGAR)

Code: CM0545

For the selective enumeration of moulds and yeasts.

Formula	***gm/litre***
Yeast extract	5.0
Glucose	20.0
Agar	12.0
pH 7.0 ± 0.2	

OXYTETRACYCLINE GYE SELECTIVE SUPPLEMENT

Code: SR0073

Vial contents (each vial is sufficient for 500 ml of medium)	***per vial***	***per litre***
Oxytetracycline (in buffered base)	50 mg	100 mg

NB: When re-constituted the resultant solution is photo-sensitive. It is recommended the solution is added immediately to the prepared agar base. Failure to do so may result in the solution becoming cloudy.

Directions

Suspend 18.5 g in 500 ml of distilled water and bring gently to the boil to dissolve completely. Sterilise by autoclaving at 115°C for 10 minutes. Allow the medium to cool to 50°C and aseptically add the contents of one vial of Oxytetracycline GYE Selective Supplement SR0073 reconstituted as directed. Mix thoroughly and pour into sterile Petri dishes.

Description

Oxytetracycline-Glucose-Yeast Extract Agar is recommended for the selection and enumeration of yeasts and moulds from foodstuffs[1,2]. The medium using oxytetracycline as the selective agent is based on the formulation developed by Mossel *et al*.[3], who stated that the use of this antibiotic in a medium with a neutral pH gave increased counts of yeasts and moulds from a variety of foodstuffs compared with media which relied on a low pH to suppress bacterial growth. Physically stressed yeast cells give higher counts on media which depend upon broad-spectrum antibiotics rather than a low pH for selectivity[4]. In earlier work Mossel[5] found that Glucose Yeast Extract Agar was as favourable a basal medium as 'Mycophil' Agar later recommended by Sharf[6]. Addition of the oxytetracycline was found to make the Glucose Yeast Extract Agar more selective than 'Mycophil' Agar by inhibiting the growth of lactobacilli, most of which grow at the acid pH of the latter medium.

The choice of a suitable medium for enumeration of yeasts and moulds is greatly dependent on the nature of the foodstuffs under investigation and the organisms that occur on them[7]. Oxytetracycline-Glucose-Yeast Extract Agar remains bacteriostatic when inoculated with not greater than 1 ml of a 10^{-1} dilution of foods and subsequently incubated for not greater than 5 days at 25°C as is the customary practice in food mycology[2].

For isolation of psychrotrophic yeasts from chilled proteinaceous foods a combination of oxytetracycline and gentamicin is effective[8].

Very proteinaceous foods and the higher incubation temperatures around 35°C required for some organisms will inactivate oxytetracycline allowing growth of Gram-positive and Gram-negative rods. For such applications Rose-Bengal Chloramphenicol Agar CM0549 may be substituted or Dichloran-Glycerol (DG18) Agar Base CM0729.

Technique
Transfer 1 ml aliquots of a series of suitable dilutions of the sample to empty 9 cm diameter Petri dishes. Two dishes are used for each of the dilutions. Add approximately 15 ml of medium prepared as described. Mix gently, turning the plates three times clockwise and three times counter-clockwise.

Incubate for 5 days at 22 ± 2°C with the Petri dishes upside down, checking for formation of aerial mycelia after 2 days.

Count the numbers of colonies in plates containing 50-100 colonies after 5 days, or in any countable plates when aerial mycelia threaten to obscure further readings after 2 days. The counts obtained for each dilution should be similar on both plates.

Calculate the number of yeasts or moulds per 1 g or 1 ml by multiplying the number of colonies by the dilution factor.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured gel.

Quality control

Positive controls:	***Expected results***
Aspergillus niger ATCC® 9642*	White mycelium, black spores
Saccharomyces cerevisiae ATCC® 9763*	Good growth; cream coloured colonies
Negative control:	
Escherichia coli ATCC® 25922*	Inhibited

*This organism is available as a Culti-Loop®

Precautions:
The lactic-acid bacteria are inhibited on this medium.

References

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2. Mossel D. A. A., Kleynen-Semmeling A. M. C., Vincentie H. M., Beerens H. and Catsaras M. (1970) *J. Appl. Bact.* 33. 454-457.
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PALCAM AGAR BASE

Code: CM0877

A selective and differential diagnostic medium for the detection of Listeria monocytogenes.

Formula	***gm/litre***
Columbia Blood Agar Base	39.0
Yeast extract	3.0
Glucose	0.5
Aesculin	0.8
Ferric ammonium citrate	0.5
Mannitol	10.0
Phenol red	0.08
Lithium chloride	15.0
pH 7.2 ± 0.2	

PALCAM SELECTIVE SUPPLEMENT

Code: SR0150

Vial contents (each vial is sufficient for 500 ml of medium)	***per vial***	***per litre***
Polymyxin B	5.0 mg	10.0 mg
Acriflavine hydrochloride	2.5 mg	5.0 mg
Ceftazidime	10.0 mg	20.0 mg

Directions

Suspend 34.5 g in 500 ml of distilled water. Bring gently to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C and aseptically add the contents of one vial of PALCAM Selective Supplement SR0150, reconstituted with 2 ml of sterile distilled water. Mix well and pour into sterile Petri dishes.

To prepare 2.5 litres of medium, suspend 172.5 g in 2.5 litres of distilled water. Sterilise and cool as above and add the contents of one vial of SR0150, reconstituted with 10ml of sterile distilled water.

The addition of 2.5% (v/v) Egg Yolk Emulsion (Oxoid code SR0047) to the medium may aid the recovery of damaged Listeria.

Description

PALCAM Medium is based on the formulation described by Van Netten *et al*.[1] and is recommended for the isolation of *Listeria monocytogenes* from foods.

The heightened awareness and concern surrounding the presence of Listeria monocytogenes in food has resulted in the development of many media for its isolation[2,3,4,5]. However, Cassiday and Brackett[6] conclude that no single method currently available is suitable for use with all types of food.

PALCAM Medium is highly selective due to the presence of lithium chloride, ceftazidime, polymyxin B and acriflavine hydrochloride. It allows the easy differential diagnosis of *Listeria monocytogenes* by utilising the double indicator system:

1. Aesculin and ferrous iron

2. Mannitol and phenol red

Listeria monocytogenes hydrolyses aesculin resulting in the formation of a black halo around colonies. *Listeria monocytogenes* does not ferment mannitol so easy differentiation from contaminants such as enterococci and staphylococci can be made as these will ferment mannitol. This formulation produces a change from red to yellow in the pH indicator phenol red.

Incubation under microaerobic conditions serves to inhibit strict aerobes such as *Bacillus* spp. and *Pseudomonas* spp. that might otherwise appear on the medium.

A modification to PALCAM medium in which incubated plates are overlaid with medium containing blood enables haemolytic Listeria species to be differentiated and enumerated[7].

The addition of egg yolk to PALCAM medium has been reported to aid repair of damaged cells[3].

Incubation under microaerophilic conditions serves to inhibit strict aerobes such as *Bacillus* spp. and *Pseudomonas* spp. that might otherwise appear on the medium.

Technique

Techniques for the isolation of *Listeria monocytogenes* will depend on the material under test. It is usual for the test sample to be first inoculated into an enrichment broth to allow multiplication before isolation and identification. Depending on the type of sample used, the appropriate method and selective enrichment broth should be used prior to inoculation onto PALCAM Medium plates. As a general rule use Listeria Selective Enrichment Medium (Oxoid codes CM0862 and SR0149) for dairy products and Listeria Selective Enrichment Media UVM and Fraser Broth (Oxoid codes CM0863, SR0142 and SR0143; CM0895 and SR0156) for meats and poultry.

1. Inoculate one loopful of the selective enrichment broth onto the PALCAM Medium plates.
2. Incubate at 37°C for 48 hours under micro-aerophilic conditions. The micro-aerophilic condition can be best achieved by using Oxoid Campylobacter Gas Generating Kit BR0056 in conjunction with the Oxoid Anaerobic Jar and an active catalyst BR0042. For jars of smaller capacity (2.5 litres) use the Oxoid Campylobacter Gas Generating Kit BR0060. Alternatively use CampyGen CN0025A or CN0035. CampyGen does not require the addition of water or a catalyst.
3. Examine for typical colonies of *Listeria* after 48 hours incubation.
4. Colonies identified as presumptive *Listeria* spp. must be confirmed by biochemical and serological testing[8].

After 48 hours incubation, typical *Listeria* spp. form colonies that are approximately 2 mm in diameter, grey-green in colour with a black sunken centre and a black halo against a cherry-red medium background.

Occasional enterococci or staphylococci develop on PALCAM Medium to forming grey colonies with a brown-green halo or yellow colonies with a yellow halo.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the selective supplement in the dark at 2-8°C and use before the expiry date on the label.

The prepared medium may be stored for up to 4 weeks at 2-8°C in the dark.

Appearance

Dehydrated Medium: Straw coloured, free-flowing powder.

Prepared medium: Red coloured gel.

Quality control

Positive controls:	***Expected results***
Listeria monocytogenes ATCC® 7644*	Good growth; dimpled brown/black coloured colonies with black halo
Negative control:	
Enterococcus faecalis ATCC® 29212*	Inhibited

*This organism is available as a Culti-Loop®

Precautions

Acriflavine hydrochloride is activated by light which may cause it to become inhibitory to *Listeria* growth.

References

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PEPTONE SALT BROTH – see MAXIMUM RECOVERY DILUENT

PEPTONE WATER (ANDRADE)

Code: CM0061

A nutrient base containing Andrade's indicator, to which carbohydrates and other organic compounds may be added for use in catabolic studies.

Formula	***gm/litre***
Peptone	10.0
Sodium chloride	5.0
Andrade's indicator (acid-Fuchsin)	0.1
pH 7.4 ± 0.2	

Directions
Suspend 15 g in 1 litre of distilled water. Mix well to dissolve, distribute into tubes or bottles, containing Durham tubes and sterilize by autoclaving at 121°C for 15 minutes.

Ensure that each individual bottle of peptone water sugar is correctly coded for the contained sugar. Some sugar solutions may affect the pH of the peptone water, check and correct if necessary.

Description
Andrade's indicator is a solution of acid-Fuchsin that when added to peptone water is colourless or slightly pink at pH 7.4. It becomes pink at acid pH levels and yellow at alkaline pH levels (working over a pH range of 5-8). Filter-sterilised carbohydrate solutions are added to the base medium post-sterilisation. These solutions are usually 10% w/v concentrations and it is important to allow for the dilution of the peptone water when making up the initial volume of medium. A final concentration of 1% w/v carbohydrate in peptone water is normally used.

The peptone used in this medium is free from fermentable carbohydrates and also free from nitrates which may interfere with gas production. The biochemical identification of organisms capable of growing in this medium is made by observing the catabolism of the various carbohydrates added to separate tubes of peptone water. To detect fermentation, which is the production of acid and gas, a small, inverted tube (Durham fermentation tube) is inserted into the peptone water to trap any gas produced by the organism. A single bubble in the tube is sufficient to label the organism positive. Some organisms will utilise the sugar to produce acid only, without gas formation.

Technique
The bottles should be inspected before inoculation to confirm that they are clear, that there is no colour or only a slight pinkness in the medium (pH 7.4) and that the glucose tube contains no air trapped in the Durham tube.

1. Aseptically inoculate each bottle with a single, well-separated colony or with a colony from a purity plate.
2. Incubate the bottles at 35-37°C for the required period of time. Incubation is normally carried out aerobically but if anaerobic incubation is required then fresh indicator may have to be added to the bottles after incubation at the time of examination.
3. Note which sugars give an acid reaction and look for bubbles of gas in the Durham tube of the glucose bottle.

Although most reactions are complete after 18-24 hours, it may be necessary to look for late reactions after prolonged incubation.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium in the dark at room temperature.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: reddish pink solution when hot. A colourless to a slight pink coloured solution at room temperature.

Quality control

Positive control:	***Expected result***
Salmonella typhimurium ATCC® 9642*	See table

*This organism is available as a Culti-Loop®

Table

Carbohydrate	***Growth***	***Acid***	***Gas***
Glucose	Good growth	+	+
Mannitol	Good growth	+	+
Lactose	Growth	No reaction	No gas
Sucrose	Growth	No reaction	No gas
Control (no carbohydrate)	Good growth	No reaction	No gas

One or two drops of 1N hydrochloric acid added to a control bottle will demonstrate the acid-reaction colour and prove that the indicator is active.

PEPTONE WATER

Code: CM0009

A basal medium to which carbohydrates and indicator may be added for fermentation studies.

Formula	***gm/litre***
Peptone	10.0
Sodium chloride	5.0
pH 7.2 ± 0.2	

Directions

Dissolve 15 g in 1 litre of distilled water. Mix well and distribute into final containers. Sterilise by autoclaving at 121°C for 15 minutes.

When sterile solutions are to be added after autoclaving, reduce the volume of water for reconstitution by an equal amount.

Description

Peptone Water may be used as a growth medium or as the basis of carbohydrate fermentation media, whilst a pure culture in Peptone Water is a convenient inoculum for a series of fermentation tubes or other diagnostic media.

Peptone Water, adjusted to pH 8.4, is suitable for the cultivation and enrichment of *Vibrio cholerae* from infected material[1].

The medium was formerly used for the performance of the indole test, but now better results can be obtained by the use of Tryptone Water CM0087.

Peptone Water may be modified for use in carbohydrate fermentation tests by the addition of Andrade's indicator. The indicator which is pink at pH 5.0 and yellow at pH 8.0 is prepared by adding sodium hydroxide to acid fuchsin until it becomes yellow. When added to Peptone Water it is colourless to slightly pink. Filter-sterilised 'sugar' solutions are added to the base medium after sterilisation. These solutions are usually at 10% w/v concentrations and it is important to allow for dilution of the Peptone Water when making up the initial volume of medium. A final concentration of 1% w/v sugar in Peptone Water is normally used but more expensive sugar can be used at 0.5%.

Andrade's indicator may be made by adding 1 N sodium hydroxide to a 0.5% solution of fuchsin until the colour just becomes yellow.

Appropriate safety precautions must be taken to avoid inhalation of, and skin contact with, acid fuchsin.

Both Peptone Water and Andrade Peptone Water are prepared and sterilised in the same manner except that an inverted fermentation tube (Durham tube) to detect gas production is included in Andrade Peptone Water containing glucose. Some organisms will utilise carbohydrate to produce acid only without gas formation. It is unnecessary to add Durham tubes to Peptone Water sugars other than glucose.

Precautions for Andrade Peptone Water sugars
Make sure that each individual bottle of Peptone Water sugar is correctly coded for the contained sugar.

Andrade Peptone Water is reddish-pink when hot; it should return to a colourless or a slightly pink colour when cooled to room temperature.

Some sugar solutions may affect the pH of the Peptone Water; check and correct if so.

Sub-cultures may be necessary to ensure purity of the inoculant. Mixed or contaminated cultures will give false reactions.

Andrade indicator will fade on prolonged storage; do not use beyond the expiry date.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at room temperature.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Light straw coloured solution.

Quality control
Maintain stock cultures of organisms which have known positive and negative reactions in each sugar. Using fresh sub-cultures, test each batch of sugar medium with the appropriate organisms.

Positive control:	***Expected results***
Escherichia coli ATCC® 25922*	Turbid growth
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

Reference
1. Cruickshank R. (1968) *Medical Microbiology* 11th ed., Livingstone Ltd., London, p.268.

PEMBA – see BACILLUS CEREUS SELECTIVE AGAR

PERFRINGENS OPSP – see OPSP AGAR

PERFRINGENS TSC – see TRYPTOSE SULPHITE CYCLOSERINE AGAR

PERFRINGENS SFP – see SHAHADI FERGUSON AGAR

PLATE COUNT AGAR
TRYPTONE GLUCOSE YEAST AGAR

Code: CM0325

A medium for the enumeration of viable organisms in milk and dairy products.

Formula	***gm/litre***
Tryptone	5.0
Yeast extract	2.5
Glucose	1.0
Agar	9.0
pH 7.0 ± 0.2	

Directions
Add 17.5 g to 1 litre of distilled water. Dissolve by bringing to the boil with frequent stirring, mix and distribute into final containers. Sterilise by autoclaving at 121°C for 15 minutes.

Description
Plate Count Agar is equivalent to the medium recommended by the APHA[1] and the PHLS[2] for the plate count of micro-organisms in food, milk and other dairy products.

Basically the APHA method consists of the preparation of pour-plates using diluted samples, and counting colonies after incubation. Incubation is for 48 hours at 32°C or at 35°C for the Standard Plate Count. For the enumeration of micro-organisms with other temperature requirements, plates may also be incubated for 7-10 days at 5-7°C: for 3-5 days at 20°C; for 2-3 days at 45°C; or for 48 hours at 55°C. See the APHA[1] publication for details.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared plates at 2-8°C.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured gel.

Quality control

Positive control:	***Expected results***
Escherichia coli ATCC® 11775*	Good growth; pale straw coloured colonies
Negative control:	
Uninoculated plate	No change

*This organism is available as a Culti-Loop®

Quality control
Compare with previous lot/batch using pasteurised and raw milk samples, incubated at 32-35°C for 48 hours.

Precautions
Make sure that the procedures and media used in milk product testing comply with the National Regulations required for each country.

References

1. American Public Health Association (1978) *Standard Methods for the Examination of Dairy Products.* 14th edn. APHA Inc. Washington DC.
2. PHLS Standard methods for the *Microbial Examination of Food and Dairy Samples* (1999) Aerobic plate count at 30°C – F10 & F11.

PLATE COUNT AGAR APHA – see STANDARD PLATE COUNT AGAR

PLATE COUNT AGAR WITH ANTIBIOTIC FREE SKIM MILK – see MILK PLATE COUNT AGAR

PLATE COUNT AGAR WATER (ISO) – see WATER PLATE COUNT AGAR

POTATO DEXTROSE AGAR

Code: CM0139

A medium recommended for the detection and enumeration of yeasts and moulds in butter and other dairy and food products.

Formula	***gm/litre***
Potato extract	4.0
Glucose	20.0
Agar	15.0
pH 5.6 ± 0.2	

Directions
Suspend 39 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Mix well before pouring.

In order to suppress bacterial growth it is sometimes desirable to acidify the medium to pH 3.5. This can be done by adding 1 ml of Lactic Acid 10% SR0021 to each 100 ml of sterilised medium at 50°C. The medium must not be heated after the addition of the acid, this would result in hydrolysis of the agar and destroy its gelling properties.

Description
A suitable medium for the isolation and count of yeasts and moulds in dairy products[1] or those occurring on the surface of fresh meats, cured meats and sausage products and other foods[2]. This medium is suitable for the detection and enumeration of heat-resistant moulds in thermally processed fruits and fruit products[2].

Work carried out in cooperation with CSIRO Melbourne had shown that the minerals present in agar could influence the pigment formation of certain fungi. Where pigment production is a critical part of the identification of the fungus it is clearly important to stabilise this characteristic. The agar used in Potato Dextrose Agar is carefully screened to ensure correct pigment production by fungi such as *Fusaria* species.

Technique
After sterilising the reconstituted medium adjust the reaction to pH 3.5 by adding 1 ml of Lactic Acid 10% SR0021 to each 100 ml of medium at 50°C. Do not reheat after acidification.

Prepare dilute emulsions or suspensions of the product to be tested, make pour-plates in the usual manner, and incubate for 5 days at 21°C. Count the number of yeast and mould colonies.

If a non-selective medium is required, it is suggested that Potato Dextrose Agar may be used without added acid or, alternatively, one may use a general purpose mycological medium such as Malt Extract Agar CM0059.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance
Dehydrated medium: Off white coloured, free-flowing powder.

Prepared medium: Light straw coloured gel.

Quality control
Compare with previous lot/batch using pasteurised and raw milk samples, incubated at 32-35°C for 48 hours.

Positive control:	***Expected result***
Aspergillus fumigatus ATCC® 9197*	White mycelium, blue-green spores
Negative controls:	
Uninoculated medium	No change
At pH 3.5 *Bacillus subtilis* ATCC® 6633*	No growth

*This organism is available as a Culti-Loop®

References

1. American Public Health Association (1992) *Standard Methods for the Examination of Dairy Products*. 16th edn. APHA Inc. Washington DC.
2. American Public Health Association (1992) *Compendium of Methods for the Microbiological Examination of Foods* 3rd edn. APHA Inc. Washington DC.

PRESTON SELECTIVE MEDIUM

A selective medium which when prepared from Campylobacter Agar Base CM0689, Preston Campylobacter Selective Supplement SR0117 and Lysed Horse Blood SR0048 can be used for the selective isolation of Campylobacter jejuni and C. coli from human, animal, avian and environmental specimens.

CAMPYLOBACTER AGAR BASE

Code: CM0689

Formula	***gm/litre***
'Lab-Lemco' powder	10.0
Peptone	10.0
Sodium chloride	5.0
Agar 12.0	12.0
pH 7.5 ± 0.2	

CAMPYLOBACTER SELECTIVE SUPPLEMENT (PRESTON)

Code: SR0117

Vial contents (each vial is sufficient for 500 ml of medium)	***per vial***	***per litre***
Polymyxin B	2,500 IU	5,000 IU
Rifampicin	5.0 mg	10.0 mg
Trimethoprim	5.0 mg	10.0 mg
Cycloheximide	50.0 mg	100.0 mg

MODIFIED PRESTON CAMPYLOBACTER SELECTIVE SUPPLEMENT

Code: SR0204

Vial contents (each vial is sufficient for 500 ml of medium)	***per vial***	***per litre***
Polymixin B	2,500 IU	5,000 IU
Rifampicin	5.0 mg	10.0 mg
Trimethoprim	5.0 mg	10.0 mg
Amphortericin B	5.0 mg	10.0 mg

Directions (to prepare Preston Campylobacter Selective Agar)
Suspend 18.5 g of Campylobacter Agar Base in 475 ml of distilled water and bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C. Aseptically add 25 ml of Lysed Horse Blood SR0048, and 1 vial of Preston Campylobacter Selective Supplement SR0117 or SR0204 reconstituted as directed and one vial of Campylobacter Growth Supplement SR0084 or SR0232. Mix well and pour into sterile Petri dishes.

Directions (to prepare Preston Campylobacter Selective Enrichment Broth)
Dissolve 12.5 g of Nutrient Broth No. 2 CM0067 in 475 ml of distilled water and sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C or below. Aseptically add 25 ml of Lysed Horse Blood SR0048, 1 vial of Preston Campylobacter Selective Supplement SR0117 or SR0204 and 1 vial of Campylobacter Growth Supplement SR0084 or SR0232. Aseptically dispense 5 ml volumes in sterile small screw-capped bottles. The Selective Enrichment Broth may be stored for up to 7 days at 2-8°C.

It is essential that the head space above the liquid should be as small as possible to ensure microaerobic conditions.

Description

The Preston Campylobacter Selective Agar is based on the formulation described by Bolton and Robertson[1]. This medium was specifically formulated to be suitable for isolation of *Campylobacter* species from all types of specimens (human, animal, avian and environmental).

In comparative studies[2] of the selective media of Skirrow, Butzler, Blaser, Campy-BAP and Preston, the Preston medium was found to give the maximum isolation rate of Campylobacter species from all types of specimens tested and also to be the most selective.

Oxoid Campylobacter Agar Base has been prepared from materials described by Bolton and Robertson[1]. It is suitable as a basal medium for the selective supplements of Blaser-Wang, Skirrow and Butzler.

Preston Campylobacter Selective Supplement SR0117 can also be used to prepare Preston Campylobacter Selective Enrichment Broth[2].

The selective enrichment technique is recommended for specimens and food samples that are expected to be heavily contaminated and/or carry small numbers of viable colony forming units. The Preston Campylobacter Selective Enrichment Broth which is supplemented with the growth supplement SR0084, made to the formulation of George *et al*.[3] effectively quenches toxic compounds which may form on exposure of the medium to light and air[4].

Technique

Direct Selective Plating Method

1. Prepare the Preston Campylobacter Selective Agar as directed from CM0689, SR0117 or SR0204 and Lysed Blood (SR0048) and SR0084 or SR0232 as required.
2. Emulsify the specimen under test in 2 ml of 0.1% peptone water.
3. Inoculate onto the selective medium with cotton tipped swabs so that single isolated colonies are formed.
4. Incubate the plates in an atmosphere consisting of approximately 5-6% oxygen, 10% carbon dioxide and 84-85% nitrogen for 24-48 hours† at 42°C. This can best be achieved by using the Oxoid Gas Generating Kit for Campylobacters BR0056 in conjunction with the Oxoid Anaerobic Jar and an active catalyst. For jars of smaller capacity (2.5 litres) use the Oxoid Gas Generating Kit for Campylobacters BR0060. Alternatively use CampyGen CN0025 or CN0035. CampyGen does not require the addition of water or a catalyst.
5. Examine the plates and confirm the typical colonies as *Campylobacter jejuni* or *Campylobacter coli* by the standard methods.

† When few Campylobacter colony forming units are present 48 hours incubation is necessary.

Selective Enrichment Broth Technique

1. Prepare the Preston Selective Enrichment Broth as directed from CM0067, SR0117, SR0084 or SR0232 and Lysed Blood (SR0048).
2. Emulsify the specimen under test in the selective enrichment broth.
3. Incubate the broth aerobically at 42°C for 24 hours.
4. Subculture on to Preston Campylobacter Selective Agar or Campylobacter Blood-Free Selective Agar.

CAMPYLOBACTER TRANSPORT MEDIUM

Campylobacter Selective Supplement (Preston) is incorporated in an improved medium for storage and transportation of thermophilic Campylobacters[5].

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared plates at 2-8°C.

Appearance

Dehydrated Medium: Straw coloured, free-flowing powder.

Prepared medium: Agar: Straw coloured gel. Broth: Straw coloured solution.

Quality control

Positive controls:	***Expected results***
Campylobacter jejuni ATCC® 29428*	Good growth; grey-brown coloured colonies
Negative control:	
Escherichia coli ATCC® 25922*	Inhibited

*This organism is available as a Culti-Loop®

References

1. Bolton F. J. and Robertson L. (1982) *J. Clin. Pathol.* 35. 462-467.
2. Bolton F. J., Coates D., Hinchliffe P.M. and Robertson L. (1983) *J. Clin. Pathol.* 36. 78-83.
3. George H. A., Hoffman P. S., Kreig N. R. and Smibert R. M. (1979) *Can. J. Microbiol.* 25. 8-16.
4. Bolton F. J., Coates D. and Hutchinson D. N. (1984) *J. Appl. Bact.* 56. 151-157.
5. Rogol M., Schnaidman B., Katzenelso E. and Sechter I. (1990) *Eur. J. Clin. Microbiol. Inf. Dis.* 9. 760-762.

PSEUDOMONAS AGAR BASE

Code: CM0559

For the selective isolation of Pseudomonas species when supplemented with SR0102 or SR0103.

Formula	***gm/litre***
Gelatin peptone	16.0
Casein hydrolysate	10.0
Potassium sulphate	10.0
Magnesium chloride	1.4
Agar	11.0
pH 7.1 ± 0.2	

CN SELECTIVE SUPPLEMENT

Code: SR0102

Vial contents (each vial is sufficient for 500 ml of medium)	***per vial***	***per litre***
Cetrimide	100 mg	200 mg
Sodium nalidixate	7.5 mg	15 mg

CFC SELECTIVE AGAR SUPPLEMENT

Code: SR0103

Vial contents (each vial is sufficient for 500 ml of medium)	***per vial***	***per litre***
Cetrimide	5.0 mg	10 mg
Fucidin	5.0 mg	10 mg
Cephalosporin	25.0 mg	50 mg

Directions

To Prepare the Agar Base
Suspend 24.2 g of the agar base, CM0559, in 500 ml of distilled water. Add 5 ml of glycerol. Bring to the boil to dissolve completely, sterilise by autoclaving at 121°C for 15 minutes. Allow the medium to cool to 50°C.

To Prepare Pseudomonas C-N Agar
To 500 ml of agar base cooled to 50°C add the contents of 1 vial of Pseudomonas C-N Supplement rehydrated as directed. Mix well and pour into sterile Petri dishes.

To Prepare Pseudomonas C-F-C Agar
To 500 ml of agar base cooled to 50°C add the contents of 1 vial of Pseudomonas C-F-C Supplement rehydrated as directed. Mix well and pour into sterile Petri dishes.

Description
Pseudomonas Agar Base is designed so that by the addition of the appropriate supplement, the medium becomes selective for *Pseudomonas aeruginosa* or *Pseudomonas* spp. generally. The base medium is a modification of King's A Medium[1] in which magnesium chloride and potassium sulphate are present to enhance pigment production.

Pseudomonas C-N Supplement is recommended for the selective isolation of *Pseudomonas aeruginosa*. The formula of the supplement was described by Goto and Enomoto[2] following the demonstration of cetrimide as a selective agent by Lowbury and Collins[3]. Goto and Enomoto showed that addition of nalidixic acid at 15 µg/ml, while at the same time reducing the cetrimide content to 200 mg, improved performance. The medium gave better recovery of *Pseudomonas aeruginosa* with enhanced pigment formation whilst strongly suppressing *Klebsiella*, *Proteus* and *Providencia* spp., the latter organisms being the troublesome contaminants of conventional cetrimide media.

Pseudomonas C-F-C Supplement is recommended for the selective isolation of *Pseudomonas* spp. generally. Mead and Adams[4] showed that reducing the cetrimide content to 10 mg/ml allowed the growth of all pigmented and non-pigmented psychrophilic pseudomonads. To improve its selective action they added cephaloridine (50 µg/ml) and fucidin (10 µg/ml). This combination of agents gave a new and more specific medium for isolating pseudomonads from chilled foods and processing plants. Incubation should be carried out at 25-30°C for 48 hours.

Considerable importance is given to detection of *Burkholderia cepacia* (formerly *Pseudomonas cepacia*) in water systems, particularly where the water is to be used for the preparation of pharmaceuticals and cosmetics[5]. The organism is resistant to many commonly-used disinfectants. *Burkholderia cepacia* has emerged as an important opportunistic pathogen in urinary, abdominal, respiratory and other infections.

Growth characteristics of *Pseudomonas* species on Oxoid Pseudomonas Agar Base with Supplements

Pseudomonas species	*Amount of growth on Supplement C-N (SR0102)*	*Amount of growth on Supplement C-F-C (SR0103)*
B. cepacia ATCC® 17759	±	+++
B. cepacia ATCC® 25416	+	+++
Ps. aeruginosa	+++	+++
Ps. putida	++	+++
Ps. fluorescens	+++	+++

Incubation carried out at 30°C and plates read after 18 hours incubation.

Technique

Clinical Specimens for *Ps. aeruginosa* Investigations
1. Prepare Pseudomonas C-N Medium as directed.
2. Pour plates and dry the surface.
3. Swab a large inoculum over half the area of the plate.
4. Using a sterile loop, streak out the inoculum over the remainder of the plate to obtain isolated colonies.
5. Incubate at 35°C and examine after 24 and 48 hours, using both white and ultraviolet light.

Food, Water and Environmental Samples for Pseudomonads
1. Prepare Pseudomonas C-F-C Medium as directed.
2. Pour plates and dry the surface.
3. Prepare food samples by diluting 1 in 5 or 1 in 10 with 1% (w/v) sterile Peptone Water, CM0009, and homogenise in a 'Stomacher' or a laboratory blender.
4. Pipette 0.5 or 1 ml of the homogenate onto the plate and spread over the surface with a sterile glass spreader. Inoculate water and swab samples directly on the surface of the medium.
5. Incubate at 25°C and examine after 24 and 48 hours, using both white and ultraviolet light.

Colonial Appearance
Growth on C-N or C-F-C Medium is usually limited to *Pseudomonas* spp. but some members of the family Enterobacteriaceae may also be present. The presence of blue-green or brown pigmentation, or fluorescence may be taken as presumptive evidence of *Pseudomonas* spp. but further tests must be carried out to confirm the identity of the organism.

Stanbridge and Board[6] modified C-F-C Medium to differentiate pseudomonads from Enterobacteriaceae developing on beef steaks packaged in modified atmospheres. Arginine 1% w/v and phenol red 0.002% w/v were added to the medium.

Pseudomonads produce ammonia from the arginine and colonies may be distinguished by a pink coloration.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured gel.

Quality control

C-N formulation	
Positive control:	***Expected results***
Pseudomonas aeruginosa ATCC® 27853*	Good growth; straw coloured colonies with green pigmentation
Negative control:	
Proteus vulgaris ATCC® 13315*	Inhibited
C-F-C formulation	
Positive control:	
Burkholderia cepacia ATCC® 25416*	Good growth; straw coloured colonies with brown pigmentation
Negative control:	
Staphylococcus aureus ATCC® 25923*	Inhibited

*This organism is available as a Culti-Loop®

Precautions
Fresh media should be prepared as required. Molten agar should not be kept longer than 4 hours. Medium should not be stored and remelted. If swarming colonies of *Proteus* species are a problem in food samples then the incubation temperature can be lowered to 20°C for a period of 3-5 days. Chilled foods may carry a wide range of pseudomonads and the colonies on C-F-C Medium, incubated at lower temperatures, may be *Pseudomonas fluorescens* or *Pseudomonas putida* as well as *Pseudomonas aeruginosa*. *Aeromonas* species will also appear as pink/brown colonies, particularly from fish products.

References
1. King E. O., Ward M. K. and Raney D. E. (1954) *J. Lab. & Clin. Med.* 44. 301-307.
2. Goto S. and Enomoto S. (1970) *Jap. J. Microbiol.* 14. 65-72.
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4. Mead G. C. and Adams B. W. (1977) *Br. Poult. Sci.* 18. 661-667.
5. Geftic S. G., Heymann H. and Adair F. W. (1970) *App. & Environmental Microbiol.* 37. 505-510.
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PSEUDOMONAS CETRIMIDE AGAR (USP, EP)

Code: CM0579

Pseudomonas Cetrimide Agar is used for the selective isolation and identification of Pseudomonas aeruginosa.

Formula	***gm/litre***
Peptone	20.0
Magnesium chloride	1.4
Potassium sulphate	10.0
Cetrimide	0.3
Agar	13.6
Final pH 7.2 ± 0.2	

Directions
Suspend 45.3 g of Pseudomonas Cetrimide Agar in 1 litre of distilled water. Add 10 ml of glycerol and boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Cool the medium to approximately 50°C and pour into sterile Petri dishes.

Description
A modification of the medium described by Brown and Lowbury[3] for the selective isolation and differentiation of *Pseudomonas aeruginosa* from a range of samples.

Cetrimide is a quarternary ammonium compound with bactericidal activity against a broad range of Gram-positive organisms and some Gram-negative organisms.

Pseudomonas aeruginosa produces a number of water-soluble iron chelators, including the yellow-green or yellow-brown fluorescent pyoverdin. When pyoverdin combines with the blue water-soluble pyocyanin, the bright green colour characteristic of *Pseudomonas aeruginosa* is created. The addition of magnesium chloride and potassium sulphate enhances the production of these chelators.

Cetrimide Agar is recommended in the United States Pharmacopoeia XXVI[1] and European Pharmacopoeia IV[2] for use in Microbial Limit Tests. The formulation is also in the AOAC guidelines[4] for isolation of *Pseudomonas aeruginosa* from cosmetics and in the AOAC method[5] for testing disinfectants on hard surfaces.

Microbial Limit Tests
The US and EU Pharmacopoeia state that pharmaceutical articles ranging from raw materials to finished products must be monitored for the total number of viable aerobic organisms present (Total Aerobic Microbial Count) and also that they must be completely free from the following organisms; *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella* species and *Escherichia coli*.

For the examination of materials for the absence of specific organisms the sample is first dissolved or suspended in a recovery medium such as Tryptone Soya Broth (CM0129, CM0876 or CM1065). The sample should be incubated in this medium at 35-37°C for long enough to allow sub-lethally injured organisms to be revived, but not to multiply (usually 2-5 hours). If antimicrobials are present in the material to be examined, they must be adequately neutralised. This may be achieved by the addition of 0.1-1% of a neutralising agent such as Polysorbate and/or lecithin to the recovery broth. The level of neutralising agent required for each type of sample will need to have been predetermined using recommended control strains.

After the appropriate incubation time a portion of any positive broth is inoculated onto a range of recommended selective media which are incubated appropriately and examined for bacterial growth. Further tests are carried out on any colonies present on the selective media to confirm identification.

Technique
Follow the methods and procedures stated in the appropriate standard method. Plates are usually inoculated by streak or spread method from non-selective medium or directly from the specimen. Incubate the plates at 35-37°C for up to 48 hours.

Pseudomonas aeruginosa colonies are yellow-green or yellow-brown in colour and fluoresce under uv light. Presumptive identification by colonial morphology should be confirmed using further tests such as oxidase and inoculation onto media for the detection of pyoverdin and pyocyanin.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared plates at 2-8°C.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Translucent, light straw coloured gel.

Quality control

Positive control:	***Expected results***
Pseudomonas aeruginosa ATCC® 27853*	Good growth; yellow/green colonies
Negative control:	
Escherichia coli ATCC® 25922*	Inhibited

*This organism is available as a Culti-Loop®

Precautions
Cetrimide Agar should be used for *in vitro* diagnostic purposes only.

Do not use Cetrimide Agar beyond the stated expiry date, or if the product shows any signs of deterioration.

References

1. United States Pharmacopoeia 2002. Microbiological Limit Tests, United States Pharmacopoeia, 26th edn. United States Pharmacopial Convention, Rockville, MD.
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PSUDOMONAS CEPACIA MEDIUM – see BURKHOLDERIA CEPACIA MEDIUM

R2A AGAR

Code: CM0906

A medium for the bacterial examination of drinking water.

Formula	***gm/litre***
Yeast extract	0.5
Proteose peptone	0.5
Casein hydrolysate	0.5
Glucose	0.5
Starch	0.5
Di-potassium phosphate	0.3
Magnesium sulphate	0.024
Sodium pyruvate	0.3
Agar	15.0
pH 7.2 ± 0.2	

Directions
Suspend 18.1 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Mix well and pour into sterile Petri dishes.

Description
Standard methods for enumeration of heterotrophic bacteria in water have traditionally used nutritionally rich media, such as Plate Count Agar, with incubation at 35°C[1]. Organisms isolated under these conditions may represent only a small percentage of the bacteria present in the sample[2].

R2A Agar developed by Reasoner and Geldreich[3] is a nutritionally reduced medium. It was demonstrated

that using this medium and incubating for longer at lower temperatures enhanced the recovery of stressed and chlorine damaged bacteria from treated waters resulting in higher, more realistic bacterial counts.

Technique
R2A Agar may be used in poured plate, spread plate and membrane filtration procedures. Refer to standard methods for sample collection and testing[1,2,4].

Recommended incubation is for 5-7 days at 20°C or 28°C, or for 3 days at 35°C.

Storage conditions and Shelf life
R2A Agar should be stored tightly capped in the original container at 10-30°C. When stored as directed the medium will remain stable until the expiry date printed on the label.

The prepared medium may be stored for up to 2 weeks at 2-8°C.

Appearance
Dehydrated medium: Pale straw coloured, free-flowing powder.

Prepared medium: Light straw coloured gel.

Quality control

Positive control:	***Expected results***
Staphylococcus aureus ATCC® 6538*	Good growth; white coloured colonies
Negative control:	
Uninoculated medium	No change

* This organism is available as a Culti-Loop®

References

1. Greenbreg, Trussell and Clesceri (ed) (1998) *Standard Methods for the Examination of Drinking Water and Waste Water.* 20th Ed. APHA, Washington DC.
2. Reasoner and Geldreich (1985) *Appl. Environ. Microbiol.* 49. 1.
3. Environment Agency – *The Microbiology of Drinking Water* 2002.
4. *European Pharmacopoeia 2002*, supplement 4.6.

RAKA-RAY AGAR

Code: CM0777

The addition of phenylethanol and cycloheximide forms a selective medium for the isolation of lactic acid bacteria in beer and brewing processes.

Formula	***gm/litre***
Yeast extract	5.0
Tryptone	20.0
Liver concentrate	1.0
Maltose	10.0
Fructose	5.0
Glucose	5.0
Betaine HCL	2.0
Diammonium hydrogen-citrate	2.0
Potassium aspartate	2.5
Potassium glutamate	2.5
Magnesium sulphate. $7H_2O$	2.0
Manganese sulphate. $4H_2O$	0.66
Potassium phosphate	2.0
N-acetyl glucosamine	0.5
Agar	17.0
pH 5.4 ± 0.2	

Supplement	***per litre***
0.1% cycloheximide solution†	7 ml
Sorbitan mono-oleate	10 ml
2-Phenylethanol	3 g

†0.1% Cycloheximide Solution SR0222

Suspend 77.1 g in 1 litre of distilled water. Add 10 ml of sorbitan mono-oleate and 7 ml of 0.1% Cycloheximide Solution SR0222. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50-55°C and aseptically add 3 g of phenylethanol. Pour into sterile Petri dishes or distribute into 4 ml volumes held at 55°C if the overlay technique is to be used.

Description

Raka-Ray Agar, is based on the formula of Saha, Sondag and Middlekauff for the detection of lactic acid bacteria in beer and brewing processes[1]. Its use is recommended by the American Society of Brewing Chemists (ASBC)[2], and the European Brewing Convention (EBC)[3].

Members of the family Lactobacillaceae occurring in the brewing process are important spoilage organisms because products arising from their growth and metabolism are often seriously detrimental to flavour. Detection is complicated by the diverse nutritional and environmental requirements of the family and a considerable number of formulations have been described arising from attempts to optimise conditions.

Raka-Ray 3 Medium[1] was developed to enable brewers to monitor in-process beer quickly and accurately for a wide range of organisms including pediococci.

Investigations in which various combinations of growth stimulating agents were added to Universal Beer Agar led to the recognition of a number of agents including sorbitan mono-oleate, liver extract, yeast extract and N-acetyl glucosamine which gave superior results in respect of colony size, colony numbers and incubation time when compared with unmodified Universal Beer Agar.

These investigations provided the basis for the formula of Raka-Ray 3 Medium in which sorbitan mono-oleate is included as a stimulant for lactic acid bacteria in general[4]. Fructose is present as the essential carbohydrate source for *Lactobacillus fructivorans*[5] while maltose is present to detect lactobacilli which cannot utilise glucose[6].

Detailed changes to the published Raka-Ray 3 formula are common, arising from attempts to further improve the performance for particular organisms and strains. Pediococci appear to have a universal ability to utilise glucose[7]. The value of partial substitution by glucose of the fructose content has been noted.

Selectivity is achieved by the addition of 3 gm/litre of 2-phenylethanol to inhibit Gram-negative organisms and 7 mg of cycloheximide to inhibit yeasts[8].

In a review of the performance of various media, Van Keer *et al*.[5] found that Raka-Ray 3 yielded the highest colony count and allowed the enumeration of the greatest number of strains within 48 hours from a total of 30 strains of Lactobacillus taken from different origins and incubated under semi-anaerobic conditions.

Hsu and Taparowsky[9], when comparing Raka-Ray 3 and MRS Agar found the Raka-Ray formulation to be superior for *Pediococcus cerevisiae* although it was not as efficient for *Lactobacillus gayonii*. In another study Hug, Schlienger and Pfenniger[10] compared Raka-Ray 3 with a number of other Lactobacillus media including MRS and sucrose agars and concluded that Raka-Ray 3 and MRS were the best.

Technique

Surface Inoculation

0.1 ml of the sample is spread on agar plates. Incubate at 25-30°C under anaerobic conditions using the Oxoid Gas Generating Kit BR0038 with the Oxoid Anaerobic Jar, alternatively use AnaeroGen AN0025 or AN0035. AnaeroGen does not require the addition of water or the use of an active catalyst. Alternatively, the specimen can be filtered and the membrane placed on the agar surface for incubation.

Overlay Technique

Aseptically dispense 4 ml volumes of Raka-Ray Agar into small test tubes and keep molten in a water bath at 55°C.

Mix 1 ml of the test sample with 4 ml of molten agar and immediately pour the contents into a Petri dish containing 15-20 ml of solid Raka-Ray Agar to give well distributed colonies. Incubate under anaerobic conditions at 25-30°C.

Because the agar layer is very thin, individual colonies can be picked easily for further examination.

Incubation Conditions
An incubation period of 4 days is generally sufficient but slower growing organisms may require up to 7 days.

Because of the diversity of environmental conditions required for growth of lactic acid bacteria a semi-anaerobic atmosphere may be needed. This is achieved using Oxoid Gas Generating Kit BR0056 in the Oxoid Anaerobic Jar. Alternatively use CampyGen CN0025 or CN0035. CampyGen does not require the addition of water or the use of an active catalyst.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured gel.

Quality control

Positive control:	***Expected results***
Lactobacillus fermentum ATCC® 9338	Good growth; white cream coloured colonies
Negative control:	
Escherichia coli ATCC® 25922*	Inhibited

*This organism is available as a Culti-Loop®

Precautions
Although the concentration of cycloheximide in the medium is below toxic levels, precautions should be observed as detailed under HAZARDS section.

References

1. Saha R. B., Sondag R. J. and Middlekauff J. E. (1974) *Proceedings of the American Society of Brewing Chemists*, 9th Congress, 1974.
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9. Hsu W. P. and Taparowsky J. A. (1977) *Brewers Digest* 52. 48.
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RAPPAPORT-VASSILIADIS (RV) ENRICHMENT BROTH

Code: CM0669

A selective enrichment broth for the isolation of salmonellae.

Formula (Classical)	***gm/litre***
Soya peptone	5.0
Sodium chloride	8.0
Potassium dihydrogen phosphate	1.6
Magnesium chloride $6H_2O$	40.0
Malachite green	0.04
pH 5.2 ± 0.2	

THIS MEDIUM IS VERY HYGROSCOPIC AND MUST BE PROTECTED FROM MOISTURE.

The quantities given for the formula as classically described made 1110 ml of medium. They have been published this way in the Oxoid literature to coincide with the scientific literature.

The directions for reconstituting Oxoid Rappaport-Vassiliadis (RV) Enrichment Broth follow usual Oxoid practice and specify the weight needed for 1 litre of medium.

Directions
Add 30 g (the equivalent weight of dehydrated medium per litre) to 1 litre of distilled water. Heat gently until dissolved completely. Dispense 10 ml volumes into screw-capped bottles or tubes and sterilise by autoclaving at 115°C for 15 minutes.

Description
Rappaport-Vassiliadis (RV) Enrichment Broth is based on the formulation described by van Schothorst and Renaud[1] and is recommended as the selective enrichment medium when isolating *Salmonella* species from food and environmental specimens. It can also be used to isolate *Salmonella* from human faeces without pre-enrichment but the inoculum must be small. The original formulation described by Rappaport *et al*.[2] was specifically developed to exploit the four characteristics of *Salmonella* species when compared with other Enterobacteriaceae.

1. The ability to survive at relatively high osmotic pressures.
2. To multiply at relatively low pH values.
3. To be relatively more resistant to malachite green.
4. To have relatively less demanding nutritional requirements.

Oxoid's Rappaport-Vassiliadis (RV) Enrichment Broth is similar to that described by Vassiliadis *et al*.[3] except that the peptone used is soya peptone, which has been reported to enhance the growth of salmonellae[1,11].

Rappaport Broth was found[2] to be superior to Selenite Enrichment Broth and Tetrathionate Broth for enrichment of salmonellae with the exception of *Salmonella typhi*. Vassiliadis *et al*.[3] modified Rappaport Broth by lowering the concentration of malachite green and raising the incubation temperature to 43°C. This modified Rappaport Enrichment Broth is RV or Rappaport-Vassiliadis Medium and has been found to be superior to other *Salmonella* selective enrichment media, especially when small inocula of pre-enrichment broth are used[4,5,6,7,8].

In an evaluation of different enrichment media for isolation of *Salmonella* from seawater, Rappaport-Vassiliadis (RV) Broth and the same broth supplemented with novobiocin were the best for detection and enumeration of salmonellae in samples with low and moderate pollution levels[9].

In another study[10], Rappaport-Vassiliadis (RV) Broth was found to be superior to tetrathionate-brilliant green broth for the detection of salmonellae in artificially contaminated fluid whole milk.

It is important that the inoculum size used for enrichment culture in RV Broth is sufficiently small not to interfere with its selectivity. Inoculum/broth ratios 1:100-1:2000 have been suggested[12].

Technique

Food and Environmental Specimens
1. Prepare Buffered Peptone Water (Oxoid CM0509) as directed in containers containing 225 ml of the medium.
2. Prepare Rappaport-Vassiliadis (RV) Enrichment Broth as directed.
3. Add 25 g of the test specimen to 225 ml of Buffered Peptone Water and incubate at 35°C for 16-20 hours.
4. Inoculate 0.1 ml of the pre-enrichment peptone water culture to 10 ml of Rappaport-Vassiliadis (RV) Enrichment Broth and incubate at 42 ± 1°C for 24-48 hours.‡
5. Sub-culture the broth by streaking onto plates of Brilliant Green Agar (Modified) CM0329. Incubate at 35°C for 18-24 hours.
6. Colonies showing typical *Salmonella* colonial morphology should be confirmed by biochemical or serological methods.

‡The recommended incubation temperature is 43°C but this is a critical upper limit. To allow for incubator temperature fluctuation 42 ± 1°C is a preferred recommendation with 42 ± 0.1°C for water baths.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance
Dehydrated medium: Pale green coloured, free-flowing powder.

Prepared medium: Blue coloured solution.

Quality control

Positive control:	***Expected results***
Salmonella typhimurium ATCC® 14028*	Good growth
Negative control:	
Escherichia coli ATCC® 25922*	Inhibited

*This organism is available as a Culti-Loop®

Precautions

RV Broth should not be used if *Salmonella typhi* is suspected.

Note the difference in weight between the classical formula on the label and the reduced weight per litre, using anhydrous magnesium chloride.

References

1. van Schothorst M. and Renaud A. M. (1983) *J. Appl. Bact.* 54. 209-215.
2. Rappaport F., Konforti N. and Navon B. (1956) *J. Clin. Path.* 9. 261-266.
3. Vassiliadis P., Pateraki E., Papaiconomou N., Papadakis J. A. and Trichopoulos D. (1976a) *Annales de Microbiologie* (Institut Pasteur) 127B. 195-200.
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RAPPAPORT-VASSILIADIS SOYA (RVS) PEPTONE BROTH

Code: CM0866

A selective enrichment broth for the isolation of salmonellae.

Formula	***gm/litre***
Soya peptone	4.5
Sodium chloride	7.2
Potassium dihydrogen phosphate	1.26
Di-potassium hydrogen phosphate	0.18
Magnesium chloride (anhydrous)	13.58
Malachite green	0.036
pH 5.2 ± 0.2	

Directions

Suspend 26.75 g in 1 litre of distilled water and heat gently to dissolve. Dispense 10 ml volumes into screw-capped bottles or tubes and sterilise by autoclaving at 115°C for 15 minutes.

Description

Rappaport-Vassiliadis Soya (RVS) Peptone Broth is recommended as a selective enrichment medium for the isolation of salmonellae from food and environmental specimens.

RVS Broth shares with the original formulation[1], the ability to exploit the full characteristics of *Salmonella* species when compared with other Enterobacteriaceae. These are:

1. The ability to survive at relatively high osmotic pressure.
2. To multiply at relatively low pH values.
3. To be relatively more resistant to malachite green.
4. To have relatively less demanding nutritional requirements.

RVS Broth is based on the revised formulation described by van Schothorst *et al.*[2], and is recommended as the selective enrichment medium for the isolation of salmonellae from food and environmental specimens. It can also be used to isolate salmonellae from human faeces without the need for pre-enrichment.

RVS Broth is a modification of the Rappaport Vassiliadis (RV) Enrichment Broth described earlier by van Schothorst and Renauld[3]. The modifications to their earlier formula are:

1. The addition of di-potassium hydrogen phosphate to buffer the medium so that the pH is maintained during storage of the prepared broth.
2. Clarifying the optimum concentration of magnesium chloride $6H_2O$.

The two modifications are said to enhance the reliability of the enrichment broth[1]. Peterz *et al*.[4] have also highlighted the importance of the concentration of magnesium chloride in the final medium.

Technique

Food and environmental specimens.

1. Prepare Buffered Peptone Water (ISO CM1049) as instructed on the label in volumes of 225 ml.
2. Prepare RVS Broth as instructed.
3. Add 25 g or 25 ml of the test sample to 225 ml of Buffered Peptone Water and incubate at 37°C for 16-20 hours. Transfer 0.1 ml of the pre-enrichment peptone water culture to 10 ml of RVS Broth and incubate at 42 ± 1°C for 24 hours.
4. Sub-culture the enrichment broth by streaking onto plates eg XLD Medium (CM0469) MLCB Agar CM0783 or Brilliant Green Agar (Modified) CM0329. Incubate at 35°C for 18-24 hours. Colonies suspected as salmonellae should be confirmed by biochemical or serological methods.

Faecal specimens – no pre-enrichment needed. Add one or two 3 mm loopfuls of liquid faeces (or an emulsion of faeces in saline) to 10 ml of RVS Broth CM0866 pre-warmed to 42°C. Incubate at 42°C ± 1°C for 24 hours, and then streak onto selective agars of choice.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance

Dehydrated medium: Pale green coloured, free-flowing powder.

Prepared medium: Blue coloured solution.

Quality control

Positive control:	***Expected results***
Salmonella typhimurium ATCC® 14028*	Good growth
Negative control:	
Escherichia coli ATCC® 25922*	Inhibited

*This organism is available as a Culti-Loop®

Precautions

RVS Broth should not be used if *Salmonella typhi* is suspected.

In order to achieve optimum recovery it is recommended that the enrichment broth is incubated at 42 ± 1°C.

References

1. Rappaport F., Konforti N. and Navon B. (1956) *J. Clin. Path.* 9. 261-266.
2. van Schothorst M., Renauld A. and van Beek C. (1987) *Food Microbiology* 4. 11-18.
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REINFORCED CLOSTRIDIAL AGAR (RCM AGAR)

Code: CM0151

A solid medium for the cultivation and enumeration of anaerobes, especially Clostridium species.

Formula	***gm/litre***
Yeast extract	3.0
'Lab-Lemco' powder	10.0
Peptone	10.0
Glucose	5.0
Soluble starch	1.0
Sodium chloride	5.0
Sodium acetate	3.0
Cysteine hydrochloride	0.5
Agar	15.0
pH 6.8 ± 0.2	

Directions
Suspend 52.5 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

Description
Reinforced Clostridial Agar is a solid version of Oxoid Reinforced Clostridial Medium CM0149, suitable for the cultivation and enumeration of clostridia and other anaerobes, lactobacilli, and many other species of bacteria. Reinforced Clostridial Agar does not differ significantly in performance from the pork-starch-pea agar of Anderson[1] for the count of anaerobes. It is employed for the estimation of clostridia in food – see below (also Barnes *et al*.[2] and Angelotti[3]). Attenborough and Scarr[4] employed RCM Agar, in conjunction with Membrane Filters, for the count of *Clostridium thermosaccharolyticum* in sugar.

Reinforced Clostridial Agar is also frequently employed for the investigation of intestinal flora: Perry *et al*.[5] for investigation of bovine rumen streptococci; Williams Smith & Crabb[6] used the Oxoid medium with added sodium chloride or blood for counts on human or animal faeces; Barnes & Goldberg[7] employed the Oxoid medium with added chlortetracycline hydrochloride or sodium azide and ethyl violet, for the examination of poultry faeces; the Oxoid medium was also used by Goldberg *et al*.[8] for the examination of poultry faecal samples. Williams Smith[9] employed Oxoid Reinforced Clostridial Agar with added blood for the 'total' and *Lactobacillus* count of human and animal faeces; with added blood and neomycin for determination of *Bacteroides*, anaerobic Gram-negative cocci, 'total' streptococci, sporeformers, yeasts, and 'actino' types.

Sneath[10] used Oxoid RCM Agar and other media for the anaerobic count of micro-organisms from soil samples up to approximately 300 years old. Gregory *et al*.[11] also employed the Oxoid medium for the estimation of anaerobes in moulding hay.

Technique
Barnes and Ingram[12,13] described the use of RCM Agar for the total viable count of clostridia employing their black glass rod technique. In this method the diluted sample is added to plugged test tubes containing about 9 ml of RCM Agar held at 48°C. The tubes are quickly rotated to mix the contents, and a sterile black glass rod inserted into each tube before the agar sets. They are then sealed with about 1.5 cm of RCM Agar containing 1/20,000 methylene blue. Growth is very rapid in this medium and it is necessary to count the colonies before gas production disrupts the agar. The authors suggest that the tubes should be incubated overnight at 25°C and then at 35°C for several hours. The colonies are clearly visible against the black background.

Oxoid RCM Agar may also be used for enumerating anaerobes using the Miller-Prickett technique (Miller *et al*.[14]). The Miller-Prickett tube is a flattened test tube 15 x 2.5 x 1.3 cm.

Mossel *et al*.[15], although working with other media, suggested the following procedure which may be used with RCM Agar:

1. Transfer, in triplicate, 1 ml of serial decimal dilutions of the food under investigation into sterilised, plugged Miller-Prickett tubes. Cool the freshly prepared medium to approximately 50°C and, without shaking, add about 15 ml to each tube.
2. Seal immediately with melted sterile paraffin, and allow to set in a water bath at about 15°C.
3. Incubate for 1 to 10 days at a temperature between 30°C and 55°C, depending on the type of clostridia expected.

4. Run at least one blank to detect contamination occurring during the procedure.

5. Count colonies.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Light straw to straw coloured gel.

Quality control

Positive control:	***Expected results***
Clostridium perfringens ATCC® 13124	Good growth; gas production
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

Precautions
Further identification tests must be carried out on organisms isolated from this medium.

References

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REINFORCED CLOSTRIDIAL MEDIUM (RCM)

Code: CM0149

A semi-solid medium for the enumeration and cultivation of clostridia and other anaerobes occurring in food and pathological specimens. It is the basal medium for Differential Reinforced Clostridial Medium.

Formula	***gm/litre***
Yeast extract	3.0
'Lab-Lemco' powder	10.0
Peptone	10.0
Glucose	5.0
Soluble starch	1.0
Sodium chloride	5.0
Sodium acetate	3.0
Cysteine hydrochloride	0.5
Agar	0.5
pH 6.8 ± 0.2	

Directions
Suspend 38 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

Description
A semi-solid medium for the enumeration and cultivation of anaerobes. Recommended for the isolation and cultivation of anaerobic organisms occurring in a variety of habitats, including food and pathological specimens.

Reinforced Clostridial Medium (RCM) was designed by Hirsch & Grinstead[1] for the cultivation and enumeration of clostridia. They showed that the medium was more fertile and enabled growth to be initiated from small inocula more readily than five other media tested. In a further comparison, the highest viable count obtainable was the criterion used, and again, RCM proved superior. Compared with the spleen infusion medium of Mundt & Jones[2], RCM gave somewhat higher counts (Gibbs & Hirsch[3]).

Reinforced Clostridial Medium can be made differential for sulphite-reducing clostridia by the addition of sodium sulphite and ferric citrate[4]. Differential Reinforced Clostridial Medium is recommended for detection of sulphite-reducing clostridia and *Clostridium perfringens* in drinking water[5].

Preparation of Differential Reinforced Clostridial Medium
Make separate solutions of 4% sodium sulphite (anhydrous) and 7% ferric citrate in distilled water. Heat the ferric citrate solution to dissolve. Sterilise both solutions by filtration. The solutions may be stored at 4°C for up to 14 days.

On the day of use mix equal volumes of the two solutions. Add 0.5 ml of the mixture to each 25 ml volume of single-strength freshly steamed and cooled Reinforced Clostridial Medium. To each 10 ml and 50 ml volume of double-strength medium add 0.4 ml and 2 ml respectively of the mixed solutions.

All cultures showing blackening must be sub-cultured for confirmatory tests.

Weenk, Fitzmaurice and Mossel[6] modified Differential Reinforced Clostridial Medium by increasing the iron content to 1 gram/litre of ferric ammonium citrate and accurately adjusting the sulphite concentration to 0.05% disodium sulphite heptahydrate. The time required for sulphite-reducing clostridium colonies to blacken was significantly shorter than that when using iron sulphite agar. The modified medium to a great extent suppressed the formation of black colonies by hydrogen sulphide-positive *Bacillus* spp. Resistance to metronidazole and growth on aerobically incubated tryptone soya agar are reliable criteria for recognising *Bacillus* spp. colonies that develop.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured solution.

Quality control

Positive control:	***Expected results***
Clostridium perfringens ATCC® 13124*	Turbid growth
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

Precautions
Further identification tests must be carried out on organisms isolated from this medium.

References

1. Hirsch A. and Grinstead E. (1954) *J. Dairy Res.* 21. 101-110.
2. Mundt J. O. and Jones V. W. (1952) *Bact. Proc.* p. 106.
3. Gibbs B. M. and Hirsch A. (1956) *J. Appl. Bact.* 19. 129-141.
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5. The Microbiology of Water 1994 Part 1 – Drinking Water. Report on Public Health and Medical Subjects Number 71: *Methods for the Examination of Waters and Associated Materials*. HMSO London.
6. Weenk G., Fitzmaurice E. and Mossel D. A. A. (1991) *J. Appl. Bact.* 70. 135-143.

ROGOSA AGAR

Code: CM0627

A medium for the selective isolation and enumeration of lactobacilli.

Formula	***gm/litre***
Tryptone	10.0
Yeast extract	5.0
Glucose	20.0
Sorbitan mono-oleate 'Tween 80'	1.0ml
Potassium dihydrogen phosphate	6.0
Ammonium citrate	2.0
Sodium acetate, anhydrous	17.0
Magnesium sulphate	0.575
Manganese sulphate	0.12
Ferrous sulphate	0.034
Agar	20.0
pH 5.4 ± 0.2	

Directions
Suspend 82 g in 1 litre of distilled water and bring to the boil to dissolve completely. Add 1.32 ml glacial acetic acid and mix thoroughly. Heat to 90-100°C for 2-3 minutes with frequent agitation. Distribute into sterile tubes, Petri dishes or bottles.

DO NOT AUTOCLAVE.

Description
Rogosa Agar, a modification of the medium described by Rogosa *et al*.[1], is a selective medium for the isolation and enumeration of *Lactobacilli.* The medium has given excellent results when used in quantitative and qualitative studies of *Lactobacilli* in faeces, saliva and mouth rinses, and in dairy products. It is an effective, selective medium for *Lactobacilli* but the high acetate concentration and low pH suppresses many strains of other lactic acid bacteria.

If the pH of the medium is adjusted to 6.2 without adding acetic acid then the selectivity of the medium is altered to include the whole group of lactic acid bacteria[2,3].

Technique
For the isolation of *Lactobacilli*, Sharpe[4] recommends that Rogosa Agar plates should be incubated for 3 days at 35°C or for 5 days at 30°C. *Lactobacilli* prefer a microaerophilic atmosphere, however, if a suitable container is not available, overlay the inoculated plate with a second layer of Rogosa Agar, before incubation.

Thermophilic lactic acid bacteria are incubated at 42°C for 48 hours and suspected psychrotrophic organisms can be incubated at 30°C for 2 days and at 22°C for a further day. *Leuconostocs* from meat are incubated at 25°C for 3 days.

After incubation all well grown colonies may be considered as lactic acid bacteria although enterococci and pediococci show a reduced growth rate. Some *Leuconostocs* from meat show slime production at 25°C.

Colony characteristics
Small greyish-white, flat or raised, smooth, rough or intermediate.

Size
Lactobacilli and other lactic acid bacteria 0.5-2.5 mm diameter; *Enterococci* 0.5-1.0 mm diameter; non-lactic acid bacteria >2.5 mm after prolonged incubation at room temperature.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured gel.

Quality control

Positive control:	***Expected results***
Lactobacillus gasseri ATCC® 19992*	Good growth; grey/white coloured colonies
Negative control:	
Escherichia coli ATCC® 25923*	No growth

*This organism is available as a Culti-Loop®

Precautions
Lactobacillus carnis does not grow on this medium.

All colonies must be tested by Gram stain and catalase test before carrying out further identification tests.

References
1. Rogosa M., Mitchell J. A. and Wiseman R. F. (1951) *J. Bact.* 62. 132-133.
2. Reuter G. (1985) *Int. J. Food Microbiol.* 2. 55-68.
3. ISO/TC34/SC6/WG15. (1984) *Enumeration of Lactobacteriaceae in meat and meat products.*
4. Sharpe M. Elizabeth (1960) *Lab. Practice* 9. 223-227.

ROTHE BROTH – see AZIDE DEXTROSE BROTH

ROSE-BENGAL CHLORAMPHENICOL AGAR

Code: CM0549

For the selective enumeration of moulds and yeasts from foods.

Formula	***gm/litre***
Mycological peptone	5.0
Glucose	10.0
Dipotassium phosphate	1.0
Magnesium sulphate	0.5
Rose-Bengal	0.05
Agar	15.5
pH 7.2 ± 0.2	

CHLORAMPHENICOL SELECTIVE SUPPLEMENT

Code: SR0078

Vial contents (each vial is sufficient or 500 ml of medium)	***per vial***	***per litre***
Chloramphenicol	50 mg	100 mg

Directions
Suspend 16.0 g in 500 ml of distilled water and bring to the boil to dissolve completely. Add the contents of one vial of Chloramphenicol Selective Supplement reconstituted as directed and mix gently. Sterilise by autoclaving at 121°C for 5 minutes. Cool to 50°C, mix gently and pour into sterile Petri dishes.

Description
Rose-Bengal Chloramphenicol Agar is a selective medium for the enumeration of yeasts and moulds from a wide variety of foodstuffs. The medium has a neutral pH and chloramphenicol is used as a selective agent to suppress the growth of bacteria. Several investigators have noted advantages in the use of media at neutral pH containing antibiotics[1,2].

Rose-Bengal is taken up by mould and yeast colonies thereby assisting enumeration of small colonies[3]. Rose-Bengal also controls the size and height of mould colonies, such as *Neurospora* and *Rhizopus* spp.

Over-growth of slow growing strains by more luxuriant species is thus prevented and plate counting is assisted.

The choice of a suitable medium for enumeration of yeasts and moulds is greatly dependent on the nature of the foodstuffs under investigation and the organisms that occur on them[4]. Rose-Bengal Chloramphenicol Agar is recommended for fresh proteinaceous foods whose associated flora consists mainly of Gram-negative rod-shaped bacteria although it should be noted that chloramphenicol alone may not be sufficient to inhibit the bacterial background. Because of the stability of chloramphenicol, Rose-Bengal Chloramphenicol Agar is also suitable when higher and prolonged incubation temperatures around 35°C are required.

Technique

Add 1 ml aliquots of a suitable series of decimal dilutions to empty 9 cm Petri dishes. Two dishes are used for each dilution. Then add to each dish approximately 15 ml of medium cooled to 50°C. Mix gently, turning the plates three times clockwise and three times counter clockwise.

Allow the medium to gel then turn the Petri dishes upside down and incubate them for 5 days at 22 ± 2°C.

Inspect the dishes and count the colonies on those that contain an estimated 50-100 colonies.

Calculate the number of yeasts or moulds per 1 g or 1 ml by multiplying the number of colonies by the dilution factor.

Consult the appropiate references for further information[5,6,7].

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C away from light.

Note: ROSE-BENGAL PHOTO-OXIDISES TO FORM TOXIC COMPOUNDS. STORE PLATES OF THE MEDIUM IN THE DARK AND AVOID EXPOSURE TO LIGHT[8].

Appearance

Dehydrated medium: Straw-pink coloured, free-flowing powder.

Prepared medium: Pink coloured gel.

Quality control

Positive controls:	***Expected results***
Saccharomyces cerevisiae ATCC® 9763*	Good growth; pink coloured colonies
Aspergillus niger ATCC® 9642*	White mycelium; black spores
Negative control:	
Escherichia coli ATCC® 25922*	No growth
Enterococcus faecalis ATCC® 29212*	No growth

*This organism is available as a Culti-Loop®

Precautions

It is essential to store plates of media containing Rose-Bengal in the dark to prevent toxic photo-oxidation of the dye. See above.

Identify moulds and yeasts by morphological appearance and microscopic examination. Colonies of bacteria and yeasts can be confused.

References

1. Mossel D. A. A., Visser M. and Mengerink W. H. J. (1962) *Lab. Pract.* 11. 109-112.
2. Koburger J. A. (1968) *Bact. Proc.* 13. A73.
3. Jarvis B. (1973) *J. Appl. Bact.* 36. 723-727.
4. Mossel D. A. A., Vega C. L. and Put H. M. C. (1975) *J. Appl. Bact.* 39. 15-22.
5. American Public Health Association (1976) *Compendium of Methods for the Microbiological Examination of Foods.* APHA Inc. Washington DC.
6. American Public Health Association (1978) *Standard Methods for the Examination of Dairy Products.* 14th edn. APHA Inc. Washington DC.
7. American Public Health Association (1981) *Standard Methods for the Examination of Water and Wastewater.* 15th edn. APHA Inc. Washington DC.
8. Kramer C. L. and Pady S. M. (1961) Kansas Academy of Science Vol. 64 No. 2 1961. *Inhibition of growth of Fungi on Rose-Bengal media by light.*

RPF – see BAIRD PARKER RPF

RV – see RAPPAPORT-VASSILLIADIS ENRICHMENT BROTH

RVS – see RAPPAPORT VASSILLIADIS SOYA PEPTONE BROTH

RYANS AGAR – see AEROMONAS MEDIUM

SABOURAUD DEXTROSE AGAR

Code: CM0041

An acidic pH medium for the isolation of dermatophytes, other fungi and yeasts.

Formula	***gm/litre***
Mycological peptone	10.0
Glucose	40.0
Agar	15.0
pH 5.6 ± 0.2	

Directions

Add 65 g to 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Mix well and pour into sterile Petri dishes.

Description

This modification of Sabouraud agar (Carlier[1]) is suitable for the cultivation and differentiation of fungi.

Carlier showed that the medium gives reliable results with *Microsporum audouini*, *Microsporum canis*, *Trichophyton mentagrophytes*, *Trichophyton flavum*, *Trichophyton rubrum* and *Candida albicans*. Sabouraud Dextrose Agar may be used in place of the Standard American medium of Hodges[2]. The fungi maintain their typical cultural appearance and thus may be readily identified according to the standard macroscopic characters described by Sabouraud[3].

The medium is often used with antibiotics for the isolation of pathogenic fungi from material containing large numbers of other fungi or bacteria.

Georg *et al*.[4] aseptically added 0.5 gram cycloheximide, 20,000 units penicillin and 40,000 units streptomycin to each litre of autoclaved, cooled medium. *Cryptococcus neoformans*, *Aspergillus fumigatus* and *Allescheria boydii* are sensitive to cycloheximide; *Actinomyces bovis* and *Nocardia asteroides* are sensitive to penicillin and streptomycin.

Alternatively, one may add 0.4 gram chloramphenicol and 0.05 gram cycloheximide to each litre of reconstituted medium before autoclaving (Ajello[5]). The same micro-organisms are sensitive to this new combination – see Dermasel Selective Supplement SR0075.

Williams Smith & Jones[6] employed Oxoid Sabouraud Dextrose Agar, containing 20,000 units penicillin and 0.04 gram neomycin per litre, for the count of yeasts in the alimentary tract of the pig.

Hantschke[7] used colistin, novobiocin and cycloheximide to isolate *Candida albicans*. Dolan[8] used gentamicin, chloramphenicol and cycloheximide for the selective isolation of pathogenic fungi.

Oxoid Sabouraud Dextrose Agar may also be used as the basis of a Pagano-Levin medium[9] for the isolation of *Candida albicans*. 0.1 gram of triphenyltetrazolium chloride (as a filter sterilised solution) is added to each litre of autoclaved molten medium cooled to 55°C. The medium is usually made inhibitory to most non-pathogenic fungi and bacteria by the addition of antibiotics as above. After incubation for 3 days at 25°C, *Candida albicans* colonies are unpigmented or pale pink whilst other *Candida* species and other fungi form deeper pink or red colonies. The test is adequate for screening purposes but other diagnostic criteria should also be utilised for the identification of *Candida albicans*[10,11,12,13].

Technique

1. Inoculate each specimen in duplicate.
2. Incubate one set of media aerobically at 22-25°C and the other set at 35°C for 5-30 days. Loosen the caps of tubes and ensure adequate moisture for the plates to compensate for loss of water vapour. DO NOT SEAL THE PLATES.
3. Examine every 2-4 days.
4. Describe each specific type of colony morphology and sub-culture to appropriate media for further identification tests.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance

Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Light straw to straw coloured gel.

Quality control

Positive controls:	***Expected results***
Candida albicans ATCC® 10231*	Good growth; cream colonies
Aspergillus niger ATCC® 16404*	White mycelium, black spores
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

Precautions

Some of the pathogenic fungi may produce infective spores which are are easily dispersed into the laboratory. Such organisms should be examined only within a protective cabinet.

The combination of cycloheximide and chloramphenicol inhibits many pathogenic fungi[4]. However, the mycelial phase of *Histoplasma capsulatum*, *Paracoccidioides brasiliensis*, *Sporothrix schoenckii* and *Blastomyces dermatitidis* is not inhibited by these antibiotics when incubated at 25-30°C[14].

Note the precautions in handling cycloheximide described in HAZARDS section.

References

1. Carlier Gwendoline I. M. (1948) *Brit. J. Derm. Syph.* 60. 61-63.
2. Hodges R. S. (1928) *Arch. Derm. Syph*., New York, 18. 852.
3. Sabouraud R. (1910) *Les Teignes*, Masson, Paris.
4. Georg Lucille K., Ajello L. and Papageorge Calomira (1954) *J. Lab. Clin. Med.* 44. 422-428.
5. Ajello Libero (1957) *J. Chron. Dis.* 5. 545-551.
6. Williams Smith H. and Jones J. E. T. (1963) *J. Path. Bact.* 86. 387-412.
7. Hantschke D. (1968) *Mykosen*. 11. 113-115.
8. Dolan C. T. (1971) *Appl. Microbiol.* 21. 195-197.
9. Pagano J., Levin J. G. and Trejo W. (1957-58) *Antibiotics Annual* 1957-58, 137-143.
10. Kutscher A. H., Seguin L., Zegarelli E. V., Rankow R. M., Mercadante J. and Piro J. D. (1959a) *J. Invest. Derm.* 33. 41-47.
11. Kutscher A. H., Seguin L., Zegarelli E. V., Rankow R. M., Campbell J. B. and Mercadante J. (1959b) *Antibiotics and Chemotherapy* 9. 649-659.
12. Sinski J. T. (1960) *J. Invest. Dermat.* 35. 131-133.
13. Ridley M. F. (1960) *Australian J. Dermat.* 5. 209-213.
14. McDonough E. S., Georg L. K., Ajello L. and Brinkman S. (1960) *Mycopath. Mycol. Appl.* 13. 113-116.

SABOURAUD LIQUID MEDIUM

Code: CM0147

A liquid medium recommended for sterility testing and for the determination of the fungistatic activity of pharmaceutical products.

Formula	***gm/litre***
Pancreatic digest of casein	5.0
Peptic digest of fresh meat	5.0
Glucose	20.0
pH 5.7 ± 0.2	

Directions
Dissolve 30 g in 1 litre of distilled water. Mix well, distribute into final containers and sterilise by autoclaving at 121°C for 15 minutes.

Description
Sabouraud Liquid Medium is a mycological sterility test medium conforming to the medium described in the USP[1] for the determination of the fungistatic activity of pharmaceutical and cosmetic products in order to avoid false sterility tests. The medium may also be used for the cultivation of moulds, yeasts, and acidophilic bacteria.

In clinical microbiology, the use of Sabouraud Liquid Medium has been shown to increase the isolation rate of *Candida albicans* in blood culture[2].

Technique
The USP recommends that the fungistatic activity of pharmaceutical products be determined as follows:

1. Test Culture
   A 1 in 1,000 dilution of a 24-28 hour culture of *Candida albicans* in Sabouraud Liquid Medium and inoculate with the Test Culture.
2. Tests
   Add specified amounts of the product to be tested to volumes of Sabouraud Liquid Medium and inoculate with the Test Culture.
3. Controls
   Inoculate tubes of Sabouraud Liquid Medium only, with the Test Culture.
4. Incubate at 22-25°C for at least 10 days.
5. If growth in the test series is comparable to that in the control tubes, then the product is not fungistatic – therefore use the amount of product and medium specified for all routine sterility tests on the product.

If the product is fungistatic when tested as above, add a suitable sterile inactivating reagent, or, use a larger ratio of medium to product in order to determine the ratio of product to medium in which growth of the test organism is not affected.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium below 25°C.

Appearance
Dehydrated medium: Light straw coloured, free-flowing powder.

Prepared medium: Straw coloured solution.

Quality control

Positive controls:	***Expected results***
Candida albicans ATCC® 10231*	Turbid growth
Aspergillus niger ATCC® 9642*	Surface growth; white mycelium black spores
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

References
1. Pharmacopoeia of the United States: *1995 Sterility Testing*.
2. Reeder J. C., Ganguli L. A., Drucker D. B., Keaney M. G. L. and Gibbs A. C. C. (1989) *Microbios.* 60. 71-77.

SABOURAUD MALTOSE AGAR

Code: CM0541 (CM41a)

An acid medium for the isolation of dermatophytes, other fungi and yeasts.

Formula	***gm/litre***
Mycological peptone	10.0
Maltose	40.0
Agar	15.0
pH 5.6 ± 0.2	

Directions
Suspend 65 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Mix well and pour in to sterile Petri dishes.

Description
This medium differs from Sabouraud Dextrose Agar, only in the carbohydrate incorporated. Sabouraud Maltose Agar may be used, with or without antibiotics, where a maltose medium is preferred.

Sabouraud Maltose Agar may be modified to form a selective indicator medium for the isolation of *Candida albicans* by the addition of Tergitol-7, bromocresol purple, potassium tellurite and triphenyltetrazolium chloride (Chapman[1]).

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance
Dehydrated Medium: Straw coloured, free-flowing powder.

Prepared medium: Light straw to straw coloured gel.

Quality Control

Positive control:	***Expected results***
Candida albicans ATCC® 10231*	Good growth; cream colonies
Aspergillus niger ATCC® 16404*	White mycelium, black spores
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

Precautions
Some of the pathogenic fungi may produce infective spores which are are easily dispersed into the laboratory. Such organisms should be examined only within a protective cabinet.

The combination of cycloheximide and chloramphenicol inhibits many pathogenic fungi. However, the mycelial phase of Histoplasma capsulatum, *Paracoccidioides brasiliensis*, *Sporothrix schoenckii* and *Blastomyces dermatitidis* is not inhibited by these antibiotics when incubated at 25-30°C .

Note the precautions in handling cycloheximide described in HAZARDS section.

Reference
1. Chapman G. H. (1952) *Trans. New York Acad. Sci., Series II* 14(6). 254.

SALMONELLA/SHIGELLA AGAR – see SS AGAR

SALMONELLA/SHIGELLA MODIFIED – see SS AGAR MODIFIED

SALT MEAT BROTH

Code: CM0094 (Tablets)

An enrichment broth for halophilic organisms, especially staphylococci.

Formula	***gm/litre***
Peptone	10.0
'Lab-Lemco' powder	10.0
Neutral heart muscle	30.0
Sodium chloride	100.0
pH 7.6 ± 0.2	

Directions

Add 2 tablets to 10 ml of distilled water in an appropriately sized test tube and soak for 5 minutes. Sterilise by autoclaving at 121°C for 15 minutes.

Description

Salt Meat Broth is an enrichment medium for the isolation of staphylococci from grossly contaminated specimens such as faeces, particularly during the investigation of staphylococcal food poisoning. Salt meat medium will detect small numbers of staphylococci when mixed with large numbers of other bacteria[1,2].

The medium is also an excellent substrate for the cultivation of some of the halophilic micrococci associated with hides and raw salt supplies. It should be noted that staphylococci growing in this medium cannot be directly tested for coagulase production – they should first be sub-cultured on a medium which contains less salt. Blood Agar Base CM0055 is recommended for this purpose.

Technique

For the isolation of *Staphylococcus aureus* from samples of food, emulsify the specimen in Peptone Water CM0009, and inoculate a tube of Salt Meat Broth. After 24 to 48 hours incubation at 35°C, discrete colonies may be obtained by plating out a small portion of the culture onto Mannitol Salt Agar CM0085 or Staphylococcus Medium No. 110 CM0145.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at room temperature.

Appearance

Tablets: Straw coloured, with brown granules.

Prepared medium: Straw coloured solution, with brown granules.

Quality control

Positive controls:	***Expected results***
Staphylococcus aureus ATCC® 25923*	Good growth
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

References

1. Maitland H. B. and Martyn G. (1948) *J. Path. Bact.* 60. 553-557.
2. Fairbrother R. W. and Southall J. E. (1950) *Mon. Bull. Min. Hlth Pub. Hlth Serv.* 9. 170-172.

SCHAEDLER ANAEROBE AGAR

Code: CM0437

A medium free from thioglycollate for the growth of aerobic and anaerobic organisms.

Formula	***gm/litre***
Tryptone Soya Broth (Oxoid CM0129)	10.0
Special peptone	5.0
Yeast extract	5.0
Glucose	5.0
Cysteine HCl	0.4
Haemin	0.01
Tris Buffer	0.75
Agar	13.5
pH 7.6 ± 0.2	

Directions

Suspend 40 g in 1 litre of distilled water and boil to dissolve the medium completely. Sterilise by autoclaving at 121°C for 15 minutes. Mix well before pouring into sterile Petri dishes.

Description

Schaedler, Dubos and Costello[1] formulated this medium for the isolation of aerobic and anaerobic micro-organisms from the gastro-intestinal tract of mice. Mata, Carrillo and Villatoro[2] modified the formula in their studies on anaerobic human faecal microflora. The modified formula has been used in Oxoid Schaedler Anaerobe Agar/Broth and the medium can be used to create selective conditions under which the required, delicate and more nutritionally exacting micro-organisms of the intestinal tract would develop, despite the presence of antagonistic organisms. Normally such fastidious micro-organisms would be swamped by the growth of enterococci, coliform bacilli and other Gram-negative bacilli.

In both studies[1,2] the use of the base medium with selective agents (shown overleaf) for the isolation and enumeration of *Lactobacillus*, *Streptococcus*, *Clostridium*, *Bacteroides* and *Flavobacterium* species from faecal samples and various organs of the digestive tract.

Although thioglycollate is widely used in anaerobic media, to lower the redox potential in order to favour growth of anaerobic organisms, some workers have reported it to be inhibitory to some anaerobes[3,4].

Schaedler Anaerobe Agar contains cysteine hydrochloride and glucose, as reducing substances, with the advantage that cysteine inhibits the growth of *Escherichia coli*. Kari, Nagy, Kovacs & Hernadi[5] have reported the inhibitory effect of cysteine on several enzymatic reactions of *Escherichia coli in vitro*.

Schaedler Anaerobe Agar has been shown to be a suitable alternative to blood agar for the enumeration of *Clostridia*[6] and has been used for the examination of food, waste products and ditch water[7]. These authors showed the necessity for strict anaerobic conditions for the successful recovery of obligate anaerobes when using this medium without the addition of blood.

Investigations at Oxoid have shown that Schaedler Anaerobe Agar gave similar results in recovery and colonial appearance to Oxoid Blood Agar Base No. 2 when tested with the same organisms.

Technique

The sample suspension is diluted as necessary in order to obtain separated and countable colonies. A calibrated loopful is then spread on the surface of a previously dried Schaedler Anaerobe Agar plate. Conditions of incubation will vary according to the type of culture under test. Pure cultures may grow on the base medium and this is also used for general aerobic and anaerobic counts.

In the enumeration of *Enterococcus faecalis* (facultatively anaerobic) as an indicator organism in dehydrated or frozen foods and water, and for the detection of *Clostridium*, the medium can be used as follows:

Food sample (e.g. pre-cooked frozen food) suspensions are plated out by the surface spread technique and an aerobic viable count may be carried out at 25°C and 35°C. For pre-cooked meat products, an anaerobic viable count and a selective plate examination for *Clostridium perfringens* should also be performed.

Addition of Selective Agents

To 1,000 ml of base agar, the following selective agents may be added:

1. Medium for anaerobic *Lactobacilli* and anaerobic *Streptococci*: NaCl 10.0 g; Neomycin 0.002 g. Incubate anaerobically at 35°C.

2. Medium for *Bacteroides* and *Clostridia*: Placenta powder 2.0 g; Neomycin 0.002 g. Incubate anaerobically at 35°C.
3. Medium for *Flavobacteria*: 7 ml of 0.5% tyrothricin in ethanol. Incubate aerobically at 35°C.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Prepared plates may be stored at 2-8°C if suitably protected.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Light straw to straw coloured gel.

Quality control

Positive controls:	***Expected results***
Staphylococcus aureus ATCC® 25923*	Good growth; pale straw coloured colonies
Clostridium sporogenes ATCC® 19404*	Good growth; pale straw coloured colonies
Bacteroides fragilis ATCC® 23745*	Good growth; pale straw coloured colonies
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

Precautions
Note the comment on strict anaerobic conditions for obligate anaerobe isolation without blood.

References

1. Schaedler R. W., Dubos R. and Castello R. (1965) *J. Exp. Med.* 122. 59-66.
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4. Mossel D. A. A., Beerens H., Tahon-Castel Baron G. and Potspeel B. (1965) *Ann. Inst. Pasteur de Lille* 16. 147-156.
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SCHAEDLER ANAEROBE BROTH

Code: CM0497

A broth version of Schaedler Anaerobe Agar CM0437 for the general growth of anaerobes and for use in blood cultures and antibiotic MIC studies of these organisms.

Formula	***gm/litre***
Tryptone Soya Broth (Oxoid CM129)	10.0
Special peptone	5.0
Yeast extract	5.0
Glucose	5.0
Cysteine HCl	0.4
Haemin	0.01
Tris Buffer	0.75
pH 7.6 ± 0.2	

Directions
Add 26.5 g to 1 litre of distilled water and mix to dissolve completely. Dispense into final containers and sterilise by autoclaving at 121°C for 15 minutes.

Description
Schaedler Anaerobe Broth is a clear medium which can support the growth of those anaerobic bacteria commonly associated with human and veterinary disease. It is identical to the formula of Schaedler Anaerobe Agar CM0437, except that the agar has been omitted.

Used as a fluid medium, under the appropriate atmosphere, Schaedler Anaerobe Broth showed enhanced growth with a number of demanding anaerobic organisms when compared with seven other commonly used broth media[1].

Schaedler Anaerobe Broth can also be used to determine antibiotic MIC levels of anaerobic organisms. The extreme variations in growth rates usually prevent the existing linear regression plots of MIC versus zone diameter being used. The use of tube methods overcomes this problem[1].

Fass, Prior and Rotilie[2] described a simple tube method that does not require special atmospheres or special equipment to carry out the test. By adding a 6 mm solid glass bead to the tube of broth prior to autoclaving, growth of most organisms could be detected after incubation for one day, by slowly rotating the tube and observing the swirl of organisms. The addition of 0.0001 of w/v resazurin to the medium was used to determine whether oxidation had occurred in stored media. For anaerobic cocci, heat-inactivated horse serum was added to a final concentration of 1% v/v before use[3].

The addition of menadione (0.1 g/litre), sodium polyanethol-sulphonate (SPS, 0.3 g/litre) and carbon dioxide (3% v/v) to Schaedler Broth enables it to be used as a blood culture medium and for the cultivation of especially fastidious *Bacteroides* species.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store prepared broth in the dark at <15°C.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured solution.

Quality Control

Positive control:	***Expected Results***
Clostridium perfringens ATCC® 13124*	Turbid growth
Bacteroides fragilis ATCC® 25285*	Turbid growth
Prevotella loescheii ATCC® 15930 (with menadione addition)	Turbid growth
Negative control:	
Uninoculated.	No change

*This organism is available as a Culti-loop®

Precautions
As with all anaerobic broth media, it is important to avoid chemo-oxidation (overheating) and photo-oxidation (storage in the light) because such oxidative effects cause the inhibition of growth.

References
1. Stalons D. R., Thornsberry C. and Dowel V. R. (1974) *Appl. Microbiol.* 27. 1098-1104.
2. Fass R. J., Prior R. B. and Rotilie C. A. (1975) *Antimicrob. Agents Chemother.* 8. 444-452.
3. Rotilie C. A., Fass R. J., Prior R. B. and Perkins R. L. (1975) *Antimicrob. Agents Chemother.* 7. 311-315.

SCHIEMMANN'S CIN AGAR – see YERSINIA SELECTIVE CIN AGAR

SELENITE BROTH BASE (LACTOSE)

Code: CM0395

An enriched medium for the isolation of Salmonella from faeces and food products.

Formula	***gm/litre***
Peptone	5.0
Lactose	4.0
Sodium phosphate	10.0
pH 7.1 ± 0.2	

Directions

Dissolve 4 g of sodium biselenite LP0121 in 1 litre of distilled water and then add 19 g of Selenite Broth Base. Warm to dissolve, mix well and fill out into containers. Sterilise in a boiling water bath, or in free flowing steam, for 10 minutes. **DO NOT AUTOCLAVE.**

To minimise any possible risk of teratogenicity to laboratory workers, the sodium biselenite must be added as a solution to this medium.

Robertson[7] reported miscarriages and possible teratogenic effects on pregnant laboratory assistants which may have been caused by ingested sodium biselenite. Oxoid therefore removed this substance from the powdered medium.

Although no further reports have been received sodium biselenite is now considered to be very toxic and should be handled with great care.

SODIUM BISELENITE (SODIUM HYDROGEN SELENITE)

Code: LP0121

Directions

Dissolve 4 g in 1 litre of distilled water and use this solution to reconstitute the base medium.

Toxic by inhalation and if swallowed. Danger of cumulative effects.

Description

Klett[1] first demonstrated the selective inhibitory effects of selenite and Guth[2] used it to isolate *Salmonella typhi*. It was twenty years later before Leifson[3] fully investigated selenite and promoted wide use of the medium.

Selenium toxicity to certain micro-organisms is not fully understood but it is suggested that it reacts with sulphur and sulphydral groups in critical cell components[4,5]. Liefson[3] suggested that it is best to tube the medium to a depth of 2 inches (50 mm) or more.

Proteus and *Pseudomonas* species appear to be resistant to its effects[4]. Lactose is added as a fermentable carbohydrate to prevent a rise in pH value during incubation because any increase in pH will reduce the selective activity of selenite. The fact that *Proteus* and *Pseudomonas* species do not ferment lactose may explain why they escape inhibition.

There have been many modifications and alterations to the original medium described by Leifson, including mannitol to replace lactose (Mannitol Selenite Broth CM0399), addition of cystine (Selenite Cystine Broth CM0699), brilliant green, sodium taurocholate, sulphapyridine and streptomycin. The performance of these modifications has been investigated but with no overall agreement[6].

Technique

For routine purposes Selenite Broth cultures should be incubated at 35°C for 18 to 24 hours and then sub-cultured on any combination of greater and lesser inhibitory selective agars for Enterobacteriaceae. The development of *Escherichia coli* and *Proteus* species is not indefinitely retarded in selenite media. Where the initial proportion of these organisms is high, it is often advantageous to sub-culture onto the solid media after 6 hours as well as after 18 hours.

If a high proportion of debris is present, in the sample of material being examined, the selective powers of the selenite may be nullified. This is well established in the examination of faeces and egg powder. It is common practice to emulsify the specimen in sterile saline, allow the gross particles to settle, and inoculate the medium with the supernatant. An alternative method is as follows: Add 2-3 g of solid specimen to 15 ml of saline in a wide-necked 1oz. bottle, emulsify, separate the debris by slowly pressing a plug of cotton-wool

down through the suspension. Withdraw approximately 1 ml of the supernatant and inoculate 10 ml of Selenite Broth.

Harvey & Scott Thomson[8] showed that incubation of the selenite broth at 43°C facilitated the isolation of *Salmonella paratyphi B* from faeces. They recommended the use of this principle for the examination of sewage and river water containing large numbers of other bacteria that preferred a lower temperature for growth. The authors also suggested that the procedure was of value for all salmonellae except *Salmonella typhi.* For urines, the broth should be made double strength and inoculated with its own volume of the specimen.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C away from light.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Light straw coloured solution.

Quality control

Positive control:	***Expected results***
Salmonella typhimurium ATCC® 14028*	Good growth
Negative control:	
Escherichia coli ATCC® 25922* Sub-culture to MacConkey Agar	Inhibited or no growth

*This organism is available as a Culti-Loop®

Precautions
Discard the prepared medium if large amounts of reduced selenite can be seen as a red precipitate in the bottom of the bottles.

Do not incubate longer than 24 hours because the inhibitory effect of selenite is reduced after 6-12 hours incubation[10].

Take sub-cultures of broth from the upper third of the broth column, which should be at least 5 cm in depth.

References

1. Klett A. (1900) *Zeitsch. für Hyg. und Infekt.* 33. 137-160.
2. Guth F. (1916) *Zbl. Bakt. I. Orig.* 77. 487-496.
3. Leifson E. (1936) *Amer. J. Hyg.* 24. 423-432.
4. Weiss K. F., Ayres J. C. and Kraft A. A. (1965) *J. Bact.* 90. 857-862.
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7. Robertson D. S. F. (1970) *Lancet* i. 518-519.
8. Harvey R. W. S. and Scott T. (1953) *Mon. Bull. Min. Hlth & PHLS*. 12. 149-150.
9. Harvey R. W. S. and Price T. H. (1979) *J. Appl. Bact.* 46. 27-56.
10. Chattopadhyay W. and Pilford J. N. (1976) *Med. Lab. Sci.* 33. 191-194.

SELENITE CYSTINE BROTH BASE

Code: CM0699

An enrichment medium for the isolation of salmonellae from faeces and food products.

Formula	***gm/litre***
Tryptone	5.0
Lactose	4.0
Disodium phosphate	10.0
L-Cystine	0.01
pH 7.0 ± 0.2	

Directions
Dissolve 4 g of sodium biselenite LP0121 in 1 litre of distilled water and then add 19 g of Selenite Cystine Broth Base. Warm to dissolve and dispense into containers to a depth of at least 60 mm. Sterilise by placing in free flowing steam for 15 minutes. DO NOT AUTOCLAVE.

To minimise any possible risk of teratogenicity to laboratory workers the sodium biselenite is not included in the dry powder but should be prepared separately as a solution to which the Selenite Cystine Broth Base is added.

Description
Selenite Cystine Broth Base is modified from the formula of Leifson[1] with added cystine[2]. This addition has given favourable results in many studies[3]. Liefson[1] suggested that it is best to tube the medium to a depth of 2 inches (50 mm) or more.

The effect of the cystine may be due to its reducing abilities which will lower the toxicity of selenite to micro-organisms and/or the extra organic sulphur provided may have a sparing effect on the critical sulphur components of the bacteria, again reducing the selective effect of the selenite.

Selenite Cystine Broth is used for enrichment culture of salmonellae from faeces, foodstuffs and other materials. The formulation corresponds to that recommended by the AOAC[4] for detection of *Salmonella* in foodstuffs, in particular egg products. It is included among the standard methods media of the American Public Health Association[5,6]. It also complies with the requirements of the United States Pharmacopoeia[7].

Technique
The proportion of sample in the enrichment broth should not exceed 10-20% (1 or 2 g in 10-15 ml). Solid material is added to the normal strength broth. Liquid samples are mixed with double strength medium in the ratio of 1 to 1. Incubate for 12-24 hours at 35-37°C. Some workers have recommended that 43°C be used[8,9].

Sub-culture to any combination of greater and lesser inhibitory, selective agars for the Enterobacteriaceae.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C away from light.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Light straw coloured solution.

Quality control

Positive control:	***Expected results***
Salmonella typhimurium ATCC® 14028*	Good growth
Negative control:	
Escherichia coli ATCC® 25922*	Inhibited or no growth
Sub-culture to MacConkey Agar	

*This organism is available as a Culti-Loop®

Precautions
Observe the precautionary comments made about sodium biselenite in Selenite Broth Base CM0395.

Discard the prepared medium if large amounts of reduced selenite can be seen as a red precipitate in the bottom of the bottle.

Do not incubate longer than 24 hours because the inhibitory effect of selenite is reduced after 6-12 hours incubation[10].

Take sub-cultures of broth from the upper third of the broth column which should be at least 5 cm in depth.

References

1. Leifson E. (1936) *Am. J. Hyg.* 24(2). 423-432.
2. North W. R and Bartram M. T. (1953) *Appl. Microbiol.* I. 130-134.
3. Fricker C. R. (1987) *J. Appl. Bact.* 63. 99-116.
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6. American Public Health Association (1978) *Standard Methods for the Examination of Dairy Products*. 14th edn. APHA Inc. Washington DC.
7. United States Pharmacopoeia XXI (1980) *Microbial Test Limits.*
8. Harvey R. W. S. and Scott T. (1953) *Mon. Bull. Min. Hlth & PHLS.* 12. 149-150.
9. Harvey R. W. S. and Price T. H. (1979) *J. Appl. Bact.* 46. 27-56.

SELENITE BROTH WITH MANNITOL – see MANNITOL SELENITE BROTH

SENSITEST AGAR

Code: CM0409

A medium specially designed to give large, clear zones with all antibiotics, without the addition of lysed or whole blood.

Formula	***gm/litre***
Hydrolysed casein	11.0
Peptones	3.0
Sodium chloride	3.0
Glucose	2.0
Starch	1.0
Buffer salts	3.3
Nucleoside bases	0.02
Thiamine	0.00002
Agar	8.0
pH 7.4 ± 0.2	

Directions
Suspend 32 g in 1 litre of distilled water and bring to the boil to dissolve the agar. Sterilise by autoclaving at 121°C for 15 minutes.

Description
Sensitest Agar was developed in the Oxoid laboratories as an antimicrobial susceptibility testing medium that did not require the addition of lysed horse blood to overcome sulphonamide and trimethoprim antagonists.

Using hydrolysed casein as the major source of amino-nitrogen in the medium, it was possible to lower the peptone content to the minimum necessary to supply essential peptides and other growth factors. Careful control of the hydrolysis of the peptones ensures that antagonists to critical antibiotics do not arise.

Bell[1] in a monograph on antimicrobial susceptibility testing chose Oxoid Sensitest Agar as the preferred medium, from those tested, on the following criteria:

1. Ability to support the growth of common Gram positive and Gram negative organisms under the conditions of test.
2. Ability to yield reproducible results.
3. Did not require the addition of lysed horse blood when a heavy inoculum method was employed.
4. Ability to demonstrate standard zones of inhibition with reference organisms and antibiotics.

In the final development of the CDS method Bell selected Sensitest Agar because of its superiority in sulphonamide testing, easier reconstitution of the dehydrated powder and stability of the powdered medium on storage.

Whilst the addition of 5% horse blood to the medium is required for demanding strains, e.g. *Streptococcus pyogenes* and *Streptococcus pneumoniae*, there is no significant difference in zone sizes from the addition of blood.

The agar used in the medium has been specially processed to yield a gel that does not impede the diffusion of antimicrobials.

For further details of antimicrobial susceptibility testing see relevant section.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared plates of agar at 2-8°C.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured gel.

Quality control

Positive controls:	***Expected results***
Escherichia coli ATCC® 25922*	Good growth; straw coloured colonies
Pseudomonas aeruginosa ATCC® 27853*	Good growth; straw coloured colonies with green pigmentation
Staphylococcus aureus ATCC® 25923*	Good growth; white coloured colonies
Enterococcus faecalis ATCC® 29212*	Good growth; white/grey coloured colonies
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

Precautions
As with other susceptibility testing media, Sensitest Agar should be used for rapidly growing aerobic organisms only. It should not be modified by the addition of carbohydrates or incubated in a CO_2 enriched atmosphere.

If the medium is used for Bell's CDS method then the specified discs and technique must be used. Refer to the monograph cited in the References.

Reference
1. Bell S. M. (1975) Supplement to Pathology (*Journal of the Royal College of Pathology of Australia*) Vol. 7 No. 4. pp.1-48.

SHAHIDI-FERGUSON MEDIUM

PERFRINGENS AGAR BASE (TSC AND SFP)

Code: CM0587

A basal medium for use with selective agents to make either TSC agar or SFP agar for the presumptive identification and enumeration of Clostridium perfringens.

Formula	***gm/litre***
Tryptose	15.0
Soya peptone	5.0
Yeast extract	5.0
Sodium metabisulphite	1.0
Ferric ammonium citrate	1.0
Agar	19.0
Final pH 7.6 ± 0.2	

PERFRINGENS (SFP) SELECTIVE SUPPLEMENT

Code: SR0093

Vial contents (each vial is sufficient for 500 ml of medium)	***per vial***	***per litre***
Kanamycin sulphate	6.0 mg	12.0 mg
Polymyxin B	15,000 IU	30,000 IU

PERFRINGENS (TSC) SELECTIVE SUPPLEMENT B

Code: SR0088

Vial contents (each vial is sufficient for 500 ml of medium)	***per vial***	***per litre***
D-cycloserine	200.0 mg	400.0 mg

Directions

To Prepare the Agar Base
Suspend 23 g in 500 ml of distilled water and heat gently until the agar is completely dissolved. Sterilise by autoclaving at 121°C for 10 minutes. Allow the medium to cool to 50°C.

To Prepare Tryptose Sulphite Cycloserine Agar (TSC Agar)
To 500 ml of Agar base cooled to 50°C add the rehydrated contents of 1 vial of TSC supplement, SR0088 and 25 ml of egg yolk emulsion, SR0047. Mix well and pour into sterile Petri dishes.

To Prepare Egg Yolk Free TSC Agar
To 500 ml of Agar base cooled to 50°C add the rehydrated contents of 1 vial of TSC supplement SR0088. Mix well and pour into sterile Petri dishes.

To Prepare Shahidi-Ferguson Perfringens Agar (SFP Agar)
To 500 ml of Agar base cooled to 50°C add the rehydrated contents of 1 vial of SFP supplement SR0093 and 25 ml of egg yolk emulsion SR0047, mix well and pour into sterile Petri dishes.

To Prepare Agar for an Overlay
For TSC or SFP Agar used as an overlay, the egg yolk emulsion, SR0047, is omitted. Its inclusion does not improve the lecithinase reaction and diminishes the visibility of the colonies.

Description
Perfringens Agar Base (TSC and SFP) is a nutrient medium to which is added egg yolk emulsion SR0047 and the appropriate antibiotic supplement to prepare either Shahidi-Ferguson Perfringens (SFP)[1] Agar using SR0093 or Tryptose Sulphite Cycloserine (TSC)[2] Agar using SR0088.

An egg yolk free TSC Agar had been described[4,5] which has the advantage that smaller colonies are formed. This can simplify the counting of plates with high numbers of colonies. Higher counts have been demonstrated by using it with a pour plate technique. The differences were thought to be due to exposure of the *Clostridium perfringens* cells to high oxygen tension in the surface plating procedure[4].

Shahidi-Ferguson Perfringens Agar is based on the formulation developed by Shahidi and Ferguson1. The medium utilises kanamycin sulphate (12 mg/litre) and polymyxin B sulphate (30,000 IU/litre) as the selective agents to give a high degree of selectivity and specificity for *Clostridium perfringens*.

Tryptose Sulphite Cycloserine Agar was developed using the same basal medium as SFP Agar[2] but with 400 mg/litre of D-cycloserine as the selective agent.

Sodium metabisulphite and ferric ammonium citrate are used as an indicator of sulphite reduction by *Clostridium perfringens* which produces black colonies in both media.

Trials[3] have indicated that polymyxin B and kanamycin sulphate used in SFP Agar allow a greater recovery of both vegetative cells and spores of *Clostridium perfringens* than either polymyxin B and sulphadiazine used in Sulphite Polymyxin Sulphadiazine Agar, or neomycin, used in Tryptone Sulphite Neomycin Agar. However, a greater number of non-specific colonies were found on SFP Agar.

In another study[2], *Serratia marcescens* and *Streptococcus lactis* were the only faculative anaerobes to grow

on TSC Agar, whereas SFP Agar also allowed the growth of *Enterococcus*, *Proteus* and *Enterobacter* strains, but allowed a slightly higher rate of recovery of *Clostridium perfringens* than TSC Agar.

Both SFP Agar and TSC Agar permitted growth of other sulphite-reducing Clostridium species tested.

Some strains of *Clostridium perfringens* may produce an opaque zone around the colony due to lecithinase activity, but this is not considered to be universal for all *Clostridium perfringens* strains after overnight incubation[4] and both black lecithinase-positive and black lecithinase-negative colonies should be considered as presumptive *Clostridium perfringens* on TSC or SFP Agars and confirmatory tests carried out. Egg yolk positive facultative anaerobes may grow on SFP Agar to produce completely opaque plates thus masking the egg yolk reaction of *Clostridium perfringens*.

Technique

1. Make up the medium according to the directions and prepare plates containing approximately 20 ml of a basal layer of TSC or SFP Agar containing egg yolk.
2. Prepare 0.1 ml aliquots of a suitable series of dilutions of the homogenised test sample and spread over the surface of the basal layer using a sterile swab.
3. Overlay with an additional 10 ml of egg yolk free TSC or SFP Agar. Cultures which are not overlaid with agar are unlikely to grow as black colonies
4. Incubate the plates at 35°C for 18-24 hours with an anaerobic Gas Generating Kit, BR0038, in a gas-jar. Alternatively use AnaeroGen AN0025A or AN0035A. AnaeroGen does not require the addition of water or a catalyst.

Alternatively, pour-plates using approximately 25 ml per plate of TSC or SFP Agar containing egg yolk may be prepared using 1 ml aliquots of a suitable series of dilutions of the homogenised test sample. Mix the plates well before the agar gels. With this technique, lecithinase activity of *Clostridium perfringens* colonies is more difficult to see. *Clostridium perfringens* colonies may be seen as large, black (2-4 mm diameter) colonies within the depth of the agar.

Egg yolk free TSC Agar is used with the techniques described above. *Clostridium perfringens* colonies are black but in the absence of egg yolk no lecithinase activity can be detected.

Tests for confirmation are described in a study initiated by the International Commission on Microbiological Specifications for Foods[6] involving nitrate reduction, lactose fermentation, gelatin liquefaction and the absence of motility. All black colonies growing on TSC or SFP Agars should be tested.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance

Dehydrated Medium: Straw coloured, free-flowing powder.

Prepared medium: Straw green coloured gel.

Quality control

Positive controls:	***Expected results***
Clostridium perfringens ATCC® 13124*	Good growth; black coloured colonies with opaque halo
Negative control:	
Escherichia coli ATCC® 25922 *	Inhibited

*This organism is available as a Culti-Loop®

Precautions

Black colonies appearing on these two media may be organisms other than *Clostridium perfringens.*

References

1. Shahidi S. A. and Ferguson A. R. (1971) *Appl. Microbiol.* 21. 500-506.
2. Harmon S. M., Kauttar D. A. and Peeler J. T. (1971) *Appl. Micobiol*. 22. 688-692.
3. Harmon S. M., Kautter D. A. and Peeler J. T. (1971) *Appl. Microbiol.* 21. 922-927.
4. Hauschild A. H. W. and Hilsheimar R. (1974) *Appl. Microbiol.* 27. 78-82.
5. Hauschild A. H. W. and Hilsheimar R. (1974) *Appl. Microbiol.* 27. 521-526.
6. Hauschild A. H. W., Gilbert R. J., Harmon S. M., O'Keefe M. F. and Vahlfeld R. (1977) *Can. J. Microbiol.* 23. 884-892.

SHEEP BLOOD AGAR BASE

Code: CM0854

A Blood Agar Base that has been specifically formulated to give improved haemolytic reactions with sheep blood.

Formula	***gm/litre***
Tryptone	14.0
Peptone Neutralised	4.5
Yeast extract	4.5
Sodium chloride	5.0
Agar	12.0
Final pH 7.3 ± 0.2	

Directions
Suspend 40 g in 1 litre of distilled water and bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C and aseptically add 7% sterile sheep blood.

Description
Sheep Blood Agar Base was developed to meet the demand for an especially nutritious blood agar base which would permit the maximum recovery of organisms without interfering with their haemolytic reactions when used with sheep blood. Sheep Blood Agar Base is based on the formulation of Blood Agar Base No. 2 CM0271.

Blood Agar Base No. 2 when supplemented with sheep blood was occasionally found to result in a mixed haemolytic reaction (alpha and beta haemolysis) for some Group A Streptococci (*Streptococcus pyogenes*). These mixed haemolytic reactions were due to trace amounts of fermentable carbohydrates in yeast extract and the physiological differences of sheep blood when compared to horse blood[1].

Having identified the causes of the mixed haemolytic reactions, the Sheep Blood Agar Base was formulated to be compatible with sheep blood. Comparisons with other blood agar bases supplemented with sheep blood have shown that with Sheep Blood Agar Base the growth of many bacteria – especially the fastidious streptococci – is considerably improved, and the expected beta haemolytic reaction is achieved with *Streptococcus pyogenes*.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured gel.

Quality control

Positive controls:	***Expected results***
Streptococcus pyogenes ATCC® 19615*	Good growth; colourless/white colonies
Streptococcus pneumoniae ATCC® 6303*	Good growth; colourless colonies
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

Reference
1. Spector W. S. (1961) Handbook of Biological Data, P51 and 53. In W. S. Spector (Ed.) *Handbook of Biological Data*. W B Saunder Company, Philadelphia and London.

SIM MEDIUM

Code: CM0435

A medium for the differentiation of enteric bacteria on the basis of sulphide production, indole production and motility.

Formula	***gm/litre***
Tryptone	20.0
Peptone	6.1
Ferrous ammonium sulphate	0.2
Sodium thiosulphate	0.2
Agar	3.5
pH 7.3 ± 0.2	

Directions
Suspend 30 g in 1 litre of distilled water and boil to dissolve the medium completely. Dispense into final containers and sterilise by autoclaving for 15 minutes at 121°C.

Description
A motility-indole medium has been found to be helpful in the identification of the Enterobacteriaceae; e.g. in the differentiation of *Klebsiella* from *Enterobacter* and *Serratia* species[1]. For convenience, these two important tests have been combined with sulphide-production in one tube. The production of hydrogen sulphide is a useful diagnostic test in the identification of enteric bacteria and is helpful in the differentiation between *Salmonella* and *Shigella*. The sulphate-reducing bacteria will produce hydrogen sulphide and further chemical substitution results in ferrous sulphide being formed along the line of inoculation.

The presence of fermentable sugars may suppress the enzyme mechanism which forms hydrogen sulphide, as a result of the acid products formed (Bulmash and Fulton[2]) and therefore sugars are not included in the medium. Oxoid SIM Medium can be used in conjunction with Triple Sugar Iron Agar CM0277 to assess the ability of the culture to ferment lactose, sucrose and glucose.

The production of indole from tryptophan is one of the diagnostic tests used in identifying enteric bacteria. For example, unless it is an unusual form, a *Salmonella* culture never produces indole from tryptophan in amounts detectable in usual tests. Tryptone is incorporated into the medium since it is a tryptophan-rich peptone, and after incubation, indole can be identified by a red dye complex reaction with one of several reagents, e.g. Kovac's Reagent which consists of amyl alcohol, para-dimethylaminobenzaldehyde and concentrated hydrochloric acid[3].

The presence of glucose in the medium is avoided as recommended[4]. False negative reactions have been recorded when fermentation has occurred[5].

The use of only 0.35% agar in the medium results in the production of a semi-solid medium, ideal for the examination of motility. Non-motile organisms will grow along the line of inoculation only, whereas motile species will grow away from it.

SIM Medium is therefore designed to determine three characteristics: hydrogen sulphide production, indole production and motility.

Technique
The medium should be dispensed in tubes or bottles and when cool, inoculated once with a pure culture, by inserting a straight wire to about one-third of the depth of the medium. If papers are used for the detection of indole, then these are wedged between the cotton wool plug or cap, and side of the container.

The inoculated medium is incubated at 35°C for 18 hours or longer, if necessary, and examined for motility, hydrogen sulphide production and finally indole production from tryptophan.

To Test for Indole Production:

1. Add 0.2 ml of Kovac's Reagent to the tube and allow to stand for 10 minutes. A dark red colour in the reagent constitutes a positive indole test. No change in the original colour of the reagent constitutes a negative test.

   Or

2. Suspend a strip of filter paper, soaked in a solution of saturated oxalic acid and dried, over the medium[4]. Indole formed by positive organisms is volatile and causes the test paper to turn pink.

Colonial Appearances
Non-motile organisms grow only along the line of inoculation, whereas motile species show either a diffuse even growth spreading from the inoculum, turbidity of the whole medium, or more rarely, localised outgrowths which are usually fan-shaped or occasionally nodular.

Hydrogen sulphide production is shown by blackening of the line of inoculation.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw green coloured semi-solid gel.

Quality control

Organism	*Motility*	*H_2S*	*Indole*
Escherichia coli ATCC® 25922*	V	–	+
Proteus vulgaris ATCC® 13315*	+	+	+
Shigella sonnei ATCC® 25931*	–	–	–

*This organism is available as a Culti-Loop®

Precautions
To avoid delay in initiating growth always sub-culture from solid media. The reactions given by SIM Medium are not sufficient to speciate organisms. Additional biochemical and serological tests are required for confirmation.

References
1. Blazevic D. J. (1968) *Appl. Microbiol.* 16. 668.
2. Bulmash J. M. and Fulton M. (1964) *J. Bact.* 88. 1813.
3. Harrigan W. F. and McCance M. E. (1966) *Laboratory Methods in Microbiology* Academic Press. 53.
4. Wilson G. S. and Miles A. A. (1964) Topley and Wilson's *Principles of Bacteriology and Immunity* 5th edn., Arnold, 1. 490.
5. Giles R. R. (1956) *J. Clin. Path.* 9. 368-371.

SIMMONS CITRATE AGAR

Code: CM0155

An agar medium for the differentiation of Enterobacteriaceae based on the utilisation of citrate as the sole source of carbon.

Formula	*gm/litre*
Magnesium sulphate	0.2
Ammonium dihydrogen phosphate	0.2
Sodium ammonium phosphate	0.8
Sodium citrate, tribasic	2.0
Sodium chloride	5.0
Bromothymol blue	0.08
Agar	15.0
pH 7.0 ± 0.2	

Directions
Suspend 23 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

Description

Simmons Citrate Agar is recommended (Ewing and Edwards[1]) for the differentiation of the family Enterobacteriaceae based on whether or not citrate is utilised as the sole source of carbon.

The medium is virtually a solidified form of Koser citrate medium which, in its original form, suffered from the disadvantage that false appearance of growth occurred when large inocula were employed. The addition of bromothymol blue indicator to the medium was a distinct improvement.

Simmons Citrate Agar complies with the recommendations of the APHA[2].

Technique

The medium may be used either as slopes in test tubes or as a plate medium in Petri dishes. In both cases the surface of the medium is lightly inoculated by streaking and, where slopes are used, the butt of medium is inoculated by stabbing. Incubation for 48 hours at 35°C is recommended.

Positive growth (i.e. citrate utilisation) produces an alkaline reaction and changes the colour of the medium from green to bright blue, whilst in a negative test (i.e. no citrate utilisation) the colour of the medium remains unchanged.

Escherichia coli including serotypes from epidemic infantile enteritis, as well as *Shigella*, *Yersinia* and *Edwardsiella* species do not grow on the medium. *Serratia* and the majority of the *Enterobacter*, *Citrobacter*, *Klebsiella*, *Proteus* and *Providencia* species, except *Morganella morganii* and *Klebsiella rhinoscleromatis* utilise citrate and produce the characteristic blue coloration[3].

Simmons Citrate Agar may be used to differentiate citrate-positive *Salmonella enteritidis* and members of *Salmonella* subgenus II, III and IV from the citrate-negative *Salmonella typhi*, *Salmonella paratyphi A*, *Salmonella pullorum* and *Salmonella gallinarum*.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance

Dehydrated medium: Yellow coloured, free-flowing powder.

Prepared medium: Dark blue-green coloured gel.

Quality control

Positive control:	***Expected result***
Klebsiella pneumoniae ATCC® 13883*	Growth; colour change of medium to blue
Negative control:	
Escherichia coli ATCC® 25922*	Inhibited or no growth; no colour change of medium

*This organism is available as a Culti-Loop®

Precautions

It is important not to carry over any nutrients into the citrate medium because this will result in false positive tests. Dilute the inoculum in saline before inoculating the citrate medium to avoid a carry-over of other carbon sources[4].

References

1. Ewing W. H. and Edwards P. R. (1960) *Bull. Bact. Nomen. and Taxon*. 10. 1-12.
2. American Public Health Association (1998) *Standard Methods for the Examination of Water and Wastewater.* 20th edn. APHA Inc. Washington DC.
3. Kauffman F. (1954) *Enterobacteriaceae* 2nd edn., Munksgaard, Copenhagen.
4. Matsen J. M. and Sherris J. C. (1969) *Appl. Microbiol.* 18. 452-454.

SKIRROW SELECTIVE MEDIUM

A selective medium for the isolation of campylobacter species at 42°C.

COLUMBIA BLOOD AGAR BASE

Code: CM0331

Formula	***gm/litre***
Special peptone	23.0
Starch	1.0
Sodium chloride	5.0
Agar	10.0
pH 7.3 ± 0.2	

Directions
Add 39 g to 1 litre of distilled water. Boil to dissolve and sterilise by autoclaving at 121°C for 15 minutes.

CAMPYLOBACTER SELECTIVE SUPPLEMENT (SKIRROW)

Code: SR0069

Vial contents (each vial is sufficient for 500 ml of medium)	
Vancomycin	5.0 mg
Trimethoprim	2.5 mg
Polymyxin B	1250 IU

Directions
Reconstitute one vial as directed aseptically add the contents to 500 ml of a nutrient medium cooled to approximately 50°C prepared from Columbia Agar or Blood Agar Base No. 2 , with 10% defibrinated horse or sheep blood SR0050,SR0051 or 5-7% laked horse blood SR0048. Mix gently and pour into sterile Petri dishes.

Description
Campylobacter Selective Supplement is based on the formulation of Skirrow[1]. The antibiotic supplement is designed to be used at 42°C for optimum selective effect.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance
Dehydrated Medium: Straw coloured, free-flowing powder.

Prepared medium: Opaque red coloured gel.

Reference
1. Skirrow M. B. (1977) *BMJ* 2. 9-11.

SLANETZ AND BARTLEY MEDIUM

Code: CM0377

A medium for the detection of enterococci.

Formula	***gm/litre***
Tryptose	20.0
Yeast extract	5.0
Glucose	2.0
Di-potassium hydrogen phosphate	4.0
Sodium azide	0.4
Tetrazolium chloride	0.1
Agar	10.0
pH 7.2 ± 0.2	

Directions

Suspend 42 g in 1 litre of distilled water and bring to the boil to dissolve the agar completely.

EXCESSIVE HEATING MUST BE AVOIDED. Dispense into Petri dishes and allow to solidify. It should not be remelted. The medium may be used with membrane filters or by spreading dilutions of the sample over the surface of the agar with a glass rod.

Description

Slanetz and Bartley[1] originally devised this medium to detect and enumerate enterococci by the technique of membrane filtration, but it has also proved useful as a direct plating medium[2,3].

The medium is very selective for enterococci and when it is incubated at elevated temperatures (44-45°C), all red or maroon colonies may be accepted as presumptive enterococci[4,5].

Burkwall and Hartman showed that the addition of 0.5 ml of 'Tween 80' and 20 ml of a 10% solution of sodium carbonate or bicarbonate to each litre of medium of a modified formulation of Slanetz and Bartley Medium was of value when examining frozen foods for enterococci; the original article should be consulted for procedural details[2].

Technique

The Environment Agency 'Microbiology of Drinking Water 2002'[6] recommend the use of Slanetz and Bartley medium for the enumeration of enterococci in water supplies, as do ISO in the standard for water quality[7]. The water is filtered through a membrane filter which is then placed on the surface of a well dried plate of the medium. Plates are incubated at 35°C for 4 hours and then at 44-45°C for 44 hours. Membranes are examined, with a hand lens in a good light, and all red or maroon colonies counted as enterococci.

Food samples can be examined for enterococci by the method suggested by the Nordic Committee of Food Analysis[3]. Samples are homogenised and so diluted with physiological saline that only 15-150 colonies grow on each Petri dish. Homogenates or dilutions are spread evenly over the agar surface with a glass rod and allowed to soak in. Dishes are inverted and incubated at 35°C for 48 hours, after which typical colonies (pink or dark red, with a narrow whitish border) are counted.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C away from light.

Appearance

Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured gel.

Quality control

Positive control:	***Expected results***
Enterococcus faecalis ATCC® 29212*	Good growth; deep red coloured colonies
Negative control:	
Escherichia coli ATCC® 25922*	No growth

*This organism is available as a Culti-Loop®

Precautions
Count all red, maroon or pink colonies as presumptive enterococci. Not all species reduce TTC therefore pale colonies should not be ignored.

Although incubation at 35°C yields a higher count, it allows the growth of organisms which do not conform to the definition of enterococci. Incubation at 44-45°C has a selective effect and produces fewer false-positives. However, the preliminary incubation at 35°C encourages the recovery of stressed organisms.

Although the selective properties of this medium are very good it is advisable to regard the colony count as a presumptive or unconfirmed count. Further identification may be required depending on the scope of the examination.

References

1. Slanetz L. W. and Bartley C. H. (1957) *J. Bact.* 74. 591-595.
2. Burkwall M. K. and Hartman P. A. (1964) *Appl. Microbiol.* 12. 18-23.
3. Nordic Committee on Food Analysis (1968) *Leaflet* 68.
4. Taylor E. W. and Burman N. P. (1964) *J. Appl. Bact.* 27. 294-303.
5. Mead G. C. (1966) *Proc. Soc. Wat. Treat. Exam.* 15. 207-221.
6. Environment Agency 'Microbiology of Drinking Water 2002'. *Methods for Examination of Waters and Associated Materials*.
7. ISO Standard for Water Quality – Detection and enumeration of intestinal enterococci – Part 2: Membrane filtration method.

SORBITOL MacCONKEY AGAR

Code: CM0813

A selective and differential medium for the detection of Escherichia coli O157.

Formula	***gm/litre***
Peptone	20.0
Sorbitol	10.0
Bile salts No. 3	1.5
Sodium chloride	5.0
Neutral red	0.03
Crystal violet	0.001
Agar	15.0
pH 7.1 ± 0.2	

Directions
Suspend 51.5 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

Description
Sorbitol MacConkey Agar is based on the formulation described by Rappaport and Henig[1], and is recommended for the isolation of pathogenic *Escherichia coli* O157. The formulation is identical to MacConkey Agar No. 3 except that lactose has been replaced with sorbitol. *Escherichia coli* O157 does not ferment sorbitol and therefore produces colourless colonies. In contrast, most *Escherichia coli* strains ferment sorbitol and form pink colonies. The efficiency of Sorbitol MacConkey Agar has been confirmed by March and Ratnam[2]. These workers reported that the detection of *Escherichia coli* O157 on this medium had a sensitivity of 100% and a specificity of 85%. They recommended the medium as a simple, inexpensive, rapid and reliable means of screening *Escherichia coli* O157.

Escherichia coli O157 causes haemorrhagic colitis, an illness characterised by bloody diarrhoea and severe abdominal pain and haemolytic uraemic syndrome (HUS)[3,4,5,6,7].

Technique
1. Make up the agar according to the directions and pour into plates. If necessary dry the surface of the agar.
2. Inoculate the plates with a suspension of the food, faeces, etc. to produce separated colonies.
3. Incubate at 35°C for 24 hours. Doyle and Schoeni[8] have reported that 35-37°C is the optimal temperature for growth of *Escherichia coli* O157. At 44-45.5°C this *Escherichia coli* serotype does not grow well even after 48 hours incubation.

Delay in reading plates beyond 24 hours should be avoided because the colour intensity of sorbitol-fermenting colonies fades, reducing the contrast with non-fermenting colonies.

Other Gram negative organisms including *Pseudomonas*, *Proteus* and *Klebsiella* species are able to grow on Sorbitol MacConkey Agar but may generally be differentiated by the appearance of their colonies.

A diagnostic reagent Escherichia coli O157 latex test DR0620 is available so that instant confirmatory tests can be made from suspicious colonies.

Colonial Morphology
Escherichia coli O157 will form colourless but otherwise typical *Escherichia coli* colonies.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared plates at 2-8°C.

Appearance
Dehydrated medium: Straw/pink coloured, free-flowing powder.

Prepared medium: Dark red coloured gel.

Quality control

Positive control:	***Expected result***
Escherichia coli O157:H7 NCTC 12900*	Good growth; straw colonies
Negative control:	
Escherichia coli ATCC® 25922*	Good growth; pink/red colonies

*This organism is available as a Culti-Loop®

Precautions
Although the great majority of *Escherichia coli* O157 strains have a typical appearance on Sorbitol MacConkey Agar, some strains are atypical[9].

Sorbitol MacConkey Agar cannot be used solely to detect VTEC strains of *Escherichia coli* as some non-toxic strains will not ferment sorbitol[10].

References

1. Rappaport F. and Henig E. (1952) *J. Clin. Path.* 5. 361.
2. March S. B. and Ratnam S. (1986) *J. Clin. Microbiol.* 23. 869-872.
3. Centers for Disease Control 1985 – *United States, 1984, Morbid Mortal Weekly Rep*., 34. 20-21.
4. Karmali M. A., Petric, M., Lim C., Fleming P. C., Arbus G. S. and Lior H. (1985) *J. Infest. Dis.* 151. 775-782.
5. Karmali M. A., Steele B. T., Petric M. and Lim C. (1983) *Lancet i*: 619-620.
6. Pai C. H., Gordon R., Sims H. V. and Bryant L. E. (1984) *Ann. Intern. Med.* 101. 738-742.
7. Waters J. R. (1985) *Can. Dis. Weekly Rep*. 11. 123-124.
8. Doyle M. P. and Schoeni S. L. (1984) *Appl. and Envir. Microbiol.* 48. 855-856.
9. Karmali M. A. (1988) *Culture* 9. 2.
10. Lior H. and Borcryk A. (1987) *Lancet* i. 333.

CEFIXIME-TELLURITE SUPPLEMENT

Code: SR0172

A freeze-dried supplement for use with Sorbitol MacConkey Agar, CM0813, for the selective isolation of Escherichia coli O157.

Vial contents (each vial is sufficient for 500 ml of medium	***per vial***	***per litre***
Potassium tellurite	1.25 mg	2.5 mg
Cefixime	0.025 mg	0.05 mg

Directions
Reconstitute 1 vial of Cefixime-Tellurite Supplement as directed. Mix gently to dissolve the contents completely.

Add the vial contents to 500 ml of Sorbitol MacConkey Agar prepared as directed and cooled to 50°C. Mix well and pour the medium into plates.

Description
Chapman and co-workers[1], added cefixime and potassium tellurite to Sorbitol MacConkey Agar to improve the selectivity of the medium. The level of potassium tellurite selects serogroup O157 from other *Escherichia coli* serogroups and inhibits *Providencia* and *Aeromonas* species. Cefixime is inhibitory to *Proteus* spp.

The use of cefixime and tellurite in Sorbitol MacConkey Agar for isolation of *Escherichia coli* O157:H7 is described in the FDA Bacteriological Analytical Manual[2].

Storage conditions and Shelf life
Cefixime-Tellurite Supplement should be stored in the dark at temperatures below 0°C.

Prepared medium may be stored for up to 2 weeks in plastic bags.

References
1. Zadik P. M., Chapman P. A. and Siddons C. A. (1993) *J. Med. Microbiol.* 39. 155-158.
2. Food and Drug Administration (1995) *Bacteriological Analytical Manual.* 8th Edition. AOAC International. Gaitherburg, MD. Chapter 4, 20-23.

SORBITAL MacCONKEY AGAR (SMAC) with BCIG

Code: CM0981

A selective and differential medium for the detection of Escherichia coli O157 incorporating the chromogen 5-bromo-4-chloro-3-indolyl-β-D-glucuronide (BCIG)

Formula	***gm/litre***
Peptone	20.0
Sorbitol	10.0
Bile Salts No. 3	1.5
Sodium chloride	5.0
Neutral red	0.03
Crystal violet	0.001
Agar	15.0
5-bromo-4-chloro-3-indolyl-β-D-glucuronide (BCIG)	0.1
pH 7.1 ± 0.2	

Directions
Suspend 51.6 g of SMAC with BCIG in 1 litre of distilled water. Mix well and sterilise by autoclaving at 121°C for 15 minutes. Pour into sterile Petri dishes.

Description
SMAC with BCIG combines two different screening mechanisms for the detection of *Escherichia coli* O157, the failure to ferment sorbitol and the absence of β-glucuronidase activity. In a study with artificially contaminated meat samples, SMAC with BCIG reduced the number of false suspect *Escherichia coli* O157 colonies by 36%[1].

The formulation includes 5-bromo-4-chloro-3-indolyl-β-D-glucuronide (BCIG) as a substrate for β-glucuronidase, an enzyme which is not usually present in *Escherichia coli* O157 strains. The non-sorbitol-fermenting and β-glucuronidase-negative *Escherichia coli* O157 will appear as straw coloured colonies. Organisms with β-glucuronidase activity will cleave the substrate and release indoxyl (or halogenated indoxyl) which is rapidly oxidised to the insoluble indigo or its analogue, leading to a distinct blue-green colouration of the colonies[2].

Enterohaemorrhagic strains of *Escherichia coli* and in particular the serotype O157:H7 have presented an increasing concern among food and clinical microbiologists since their role as a causative agent in two major food related outbreaks of haemorrhagic colitis was established in 1982. Although a variety of serotypes are known to produce verotoxins a three-year study in England and Wales showed that 99% of isolates from patients suffering diarrhoea, bloody diarrhoea or haemolytic uraemic syndrome (HUS) belonged to serogroup O157[1].

The intestinal tract of ruminants is the prime reservoir of *Escherichia coli* O157 and other enterohaemorrhagic

Escherichia coli (EHEC) strains, therefore meats derived from cattle, sheep, goat and deer can be expected to be contaminated. Foods implicated in human illness related to *Escherichia coli* O157 include meats, dairy products, vegetables, salads, apple juice and water[3].

Technique
Inoculate the plates with a suspension of the food, faeces, etc. to produce separated colonies. Incubate for 24 hours at 35-37°C. Examine the plates for straw coloured colonies which are sorbitol-negative and glucuronidase-negative organisms. Confirm suspected *Escherichia coli* O157 with the *Escherichia coli* O157 Latex Test DR0620 or Dryspot *Escherichia coli* O157 DR0120.

Escherichia coli O157 do not usually ferment sorbitol and are glucuronidase-negative, so the colonies will appear colourless. Most other *Escherichia coli* serogroups are glucuronidase-positive and sorbitol-fermenting and will therefore appear as blue to purple coloured colonies.

When high numbers of Gram-negative organisms are expected to be present, the medium may be made more selective by the addition of Cefixime-Tellurite (CT) Selective Supplement.

SMAC with BCIG is a USDA/FSIS recommended medium for the presumptive identification of *Escherichia coli* O157:H7/NM.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared plates at 2-8°C.

Appearance
Dehydrated medium: Straw/pink coloured, free-flowing powder.

Prepared medium: Dark red coloured gel.

Quality Control

Positive control:	
Escherichia coli O157:H7 (glucuronidase-negative) NCTC 12900	Good growth: colourless colonies
Negative control:	
Escherichia coli (glucuronidase-positive) ATCC® 25922	Good growth: blue/purple coloured colonies
Enterococcus faecalis ATCC® 19433*	Inhibited

References

1. Thomas, A., Cheasty, T., Frost, J. A., Chart, H., Smith, H. R. and Rowe, B. (1996) *Epidemiol Infect*. 117, 1-10.
2. Desmarchelier, P. M. and Grau, F. H. (1997) *Escherichia coli*. In: Foodborne Microorganisms of Public Health Significance. 5th Edition. pp.231-264. A. D. Hocking (Ed.). AIFST (NSW Branch) Food Microbiology Group, Australia.
3. Okrend, A. J. G., Rose, B. E. and Lattuada, C. P. (1990) *J. Food Prot.* 53, 941-943.

SOYA CASEIN DIGEST MEDIUM – see TRYPTONE SOYA BROTH

SALMONELLA SHIGELLA AGAR (SS AGAR)

Code: CM0099

A differential selective medium for the isolation of Salmonella and some Shigella species from clinical specimens, foods etc.

Formula	***gm/litre***
'Lab-Lemco' powder	5.0
Peptone	5.0
Lactose	10.0
Bile salts	8.5
Sodium citrate	10.0
Sodium thiosulphate	8.5
Ferric citrate	1.0
Brilliant green	0.00033
Neutral red	0.025
Agar	15.0
pH 7.0 ± 0.2	

Directions
Suspend 63 g in 1 litre of distilled water. Bring to the boil with frequent agitation and allow to simmer gently to dissolve the agar. **DO NOT AUTOCLAVE**. Cool to about 50°C, mix and pour into sterile Petri dishes.

Description
SS Agar is a differential, selective medium for the isolation of *Shigella* and *Salmonella* species from pathological specimens, suspected foodstuffs, etc. Gram-positive and coliform organisms are inhibited by the action of the selective inhibitory components brilliant green, bile salts, thiosulphate and citrate.

Thiosulphate in combination with iron also acts as an indicator for sulphide production, which is indicated by blackening in the centres of the colonies.

Technique
Inoculate the medium heavily with the specimen, spreading a portion of the original inoculum in order to obtain well separated colonies on some part of the plate. Incubate for 18 to 24 hours at 35°C; non-lactose fermenters form colourless colonies, whilst occasional resistant coliforms or other lactose fermenters produce pink or red colonies.

In parallel with the SS Agar plate, inoculate a tube of Selenite Broth CM0395 enrichment medium, incubate for 12 hours at 35°C, and sub-culture onto another SS Agar plate.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance
Dehydrated medium: Straw pink coloured, free-flowing powder.

Prepared medium: Pink-red coloured gel.

Quality control

Positive controls:	***Expected results***
Salmonella enteritidis ATCC® 13076*	Good growth; straw coloured colonies with black centres
Shigella sonnei ATCC® 25931*	Good growth; straw coloured colonies
Negative control:	
Enterococcus faecalis ATCC® 29212*	No growth

*This organism is available as a Culti-Loop®

Precautions
This medium is highly selective and R-strains of shigellae will not grow on it. It is not recommended for the primary isolation of shigellae[1,2].

References
1. Leifson E. (1935) *J. Path. Bact.* 40. 581-
2. Taylor W. I. and Harris B. (1965) *Am. J. Clin. Path.* 44. 476-477.

SALMONELLA SHIGELLA AGAR (SS AGAR MODIFIED)

Code: CM0533

An improved formulation which gives better growth of shigellae and better colony characteristics for salmonellae.

Formula	***gm/litre***
'Lab-Lemco' powder	5.0
Peptone	5.0
Lactose	10.0
Bile salts	5.5
Sodium citrate	10.0
Sodium thiosulphate	8.5
Ferric citrate	1.0
Brilliant green	0.00033
Neutral red	0.025
Agar	12.0
pH 7.3 ± 0.2	

Directions
Suspend 57 g in 1 litre of distilled water. Bring to the boil with frequent agitation, and allow to simmer gently to dissolve the agar. **DO NOT AUTOCLAVE.** Cool to about 50°C and pour into Petri dishes.

Description
Although widely used, SS Agar has been criticised because of excessive inhibition of *Shigella* species.

Investigation has shown that modification to the formulation by alterations to the bile salt mixture, peptone and pH value considerably improve its performance in the growth of shigellae without too much increased growth of commensal organisms.

Salmonella colonies are also larger with improved blackening at the centre. The change in formulation has reduced the number of gm/litre from 63 g to 57 g.

Technique
Inoculate the medium heavily with the specimen, spreading a portion of the original inoculum in order to obtain well separated colonies on some part of the plate. Incubate for 18-24 hours at 35°C; non-lactose-fermenters form colourless colonies. Occasional resistant coliforms and other lactose-fermenters produce pink or red colonies.

In addition to the SS Agar (Modified) plate, inoculate a tube of Selenite Broth Enrichment Medium, CM0395. Incubate it for 12 hours at 35°C, and sub-culture onto another SS Agar (Modified) plate.

Colonial Characteristics

Non-Lactose-Fermenting Organisms

Salmonella species	Transparent colonies usually with black centres
Shigella species	Transparent colonies
Proteus species, *Citrobacter* species	Transparent colonies grey-black centres

Late-lactose-fermenting organisms will develop colonies with pink centres after 48 hours incubation.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance
Dehydrated medium: Pink-red coloured, free-flowing powder.

Prepared medium: Pink-red coloured gel.

Quality control

Positive controls:	***Expected results***
Salmonella enteritidis ATCC® 13076*	Good growth; straw coloured colonies with black centres
Shigella sonnei ATCC® 25931*	Good growth; straw coloured colonies
Negative control:	
Enterococcus faecalis ATCC® 29212*	No growth

*This organism is available as a Culti-Loop®

References
See SS Agar CM0099 as well.

STAA AGAR BASE

Code: CM0881
A medium for the isolation of Brochothrix thermosphacta from food samples.

Formula	***gm/litre***
Peptone	20.0
Yeast extract	2.0
Dipotassium hydrogen phosphate	1.0
Magnesium sulphate	1.0
Agar	13.0
pH 7.0 ± 0.2	

STAA Selective Supplement

Code: SR0151

Vial contents: (each vial is sufficient for 500 ml of medium)	***per vial***	***per litre***
Streptomycin sulphate	250.0 mg	500.0 mg
Thallous acetate	25.0 mg	50.0 mg
Cycloheximide	25.0 mg	50.0 mg

STA Selective Supplement

Code: SR0162

Vial contents: (each vial is sufficient for 500 ml of medium)	***per vial***	***per litre***
Streptomycin sulphate	250.0 mg	500.0 mg
Thallous acetate	25.0 mg	50.0 mg

Directions
Suspend 18.5 g of STAA Agar Base in 500 ml distilled water and bring gently to the boil to dissolve completely. Add 7.5 g of glycerol and sterilise by autoclaving at 121° C for 15 minutes. Cool to 50°C and aseptically add:

(a) for STAA Medium the contents of 1 vial of STAA Selective Supplement reconstituted as directed.

(b) for STA Medium the contents of 1 vial of STA Selective Supplement reconstituted as directed.

Mix well and distribute into sterile Petri dishes.

Description
STAA Medium CM0881+ SR0151 is based on the formulation described by Gardner[2] and is recommended

for the microbiological examination of meat and meat products in the ISO Standard 13722:1996. When made up as STA Medium CM0881+SR0162 it complies with the method described by the Nordic Committee on Food Analysis (NMKL)[3].

Brochothrix thermosphacta is a Gram-positive, non-motile, facultatively anaerobic rod-shaped micro-organism which occurs singly, in short chains or in long filamentous-like chains. It constitutes a significant proportion of the spoilage flora of meat and meat products stored aerobically or vacuum packed at chill temperatures, and is occasionally the dominant organism. It is, therefore, responsible for some of the off-odours which signal the onset of spoilage in vacuum packed meat products.

Although *Brochothrix thermosphacta* is not considered to be pathogenic, it is an economically important meat-spoilage organism because it grows in a wide variety of meats and meat products and produces malodorous metabolic end products which make affected meat unpalatable[1].

STAA Medium is made selective by the inclusion of streptomycin sulphate, thallous acetate and actidione (cycloheximide). STA Medium is modified from the original formulation by removal of the toxic selective agent cycloheximide as recommended by the NMKL. According to this method the medium is applicable for meat, meat products and some fish products.

Streptomycin sulphate inhibits some Gram-positive organisms and most Gram-negatives at higher concentrations, whilst *Brochothrix thermosphacta* remains resistant. Thallous acetate inhibits most yeasts as well as many aerobic and facultatively anaerobic bacteria. The incorporation of cycloheximide serves to further inhibit yeasts and filamentous fungi.

Technique
Homogenise the test sample in sterile 0.1% peptone water or Maximum Recovery Diluent CM0733 and prepare appropriate dilutions. Transfer 0.1 ml volumes to the agar plate and spread across the surface. Incubate at 22°C for 48 hours aerobically. Typical colonies of *Brochothrix thermosphacta* will grow as straw coloured colonies, 0.5-1.0 mm in diameter. Pseudomonads able to grow on STAA and STA Media may be differentiated from *Brochothrix thermosphacta* by performing an oxidase test using Oxoid Oxidase Touch Sticks BR0064. Pseudomonads are oxidase positive.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the selective supplement in the dark at 2-8°C and use before the expiry date on the label.

The prepared medium may be stored for up to 2 weeks at 2-8°C.

Appearance
Dehydrated Medium: Straw coloured free flowing powder

Prepared medium: Staw coloured gel.

Quality control

Positive controls:	***Expected results***
Brochothrix thermosphacta ATCC® 11509*	Good growth straw colonies
Negative control:	
Staphylococcus aureus ATCC® 25923*	No growth

*This organism is available as a Culti-Loop®.

Precautions
STAA Selective Supplement contains cycloheximide and thallous acatate and is toxic if swallowed, inhaled or if in contact with skin.

STA Selective Supplement contains thallous acatate and is toxic if swallowed, inhaled or if in contact with skin.

References

1. Sneath, P. H. A and D. Jones (1986) *Genus Brochothrix*. In: *Bergey's Manual of Systematic Bacteriology*, Vol.2, pp.1249-1253. Sneath, P. H. A., Mair, N. S. *et al*. (eds.). Williams & Wilkins, Baltimore.
2. Gardner,G. A.(1966) *J. Appl. Bacteriol.* 29 (3), 455-460.
3. Nordic Committee on Food Analysis: *Brochothrix thermosphacta*. Determination in Meat and Meat Products, No.141, 1991.

STANDARD PLATE COUNT AGAR (APHA)

Code: CM0463

A standard medium corresponding to the APHA formulation for milk, water, food and dairy products.

Formula	***gm/litre***
Yeast extract	2.5
Pancreatic digest of casein	5.0
Glucose	1.0
Agar	15.0
pH 7.0 ± 0.2	

Directions
Suspend 23.5 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Dispense into bottles and sterilise by autoclaving at 121°C for 15 minutes.

Description
Standard Plate Count Agar was developed by Buchbinder *et al*.[1] who wished to use an agar without milk solids in the formulation and investigated the control tests necessary to give standard results in dairy products with statistically valid counts.

Standard Plate Count Agar meets the prescribed standards of the APHA[2], AOAC[3] and PHLS[4].

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared plates at 2-8°C.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured gel.

Quality control

Positive control:	***Expected result***
Escherichia coli ATCC® 11775*	Good growth; pale straw coloured colonies
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

Precautions
When carrying out prescribed Standard Methods it is essential to follow the protocols given in precise detail.

References

1. Buchbinder *et al.* (1951) *Public Health Reports.* 66. 327-329.
2. American Public Health Association (1976) *Compendium of Methods for the Microbiological Examination of Foods.* APHA Inc. Washington DC.
3. Association of Official Analytical Chemists (1995) *Bacteriological Analytical Manual.* 8th edn. AOAC. Washington DC.
4. PHLS Standard Methods for the Microbiological Examination of Food and Dairy Samples (1999) Aerobic plate count at 30°C F10 & F11.

STAPHYLOCOCCUS MEDIUM No. 110

Code: CM0145

A selective medium for the isolation and differentiation of pathogenic staphylococci based on salt tolerance, pigmentation, mannitol fermentation and gelatin liquefaction.

Formula	***gm/litre***
Yeast extract	2.5
Tryptone	10.0
Lactose	2.0
Mannitol	10.0
Sodium chloride	75.0
Dipotassium hydrogen phosphate	5.0
Gelatin	30.0
Agar	15.0
pH 7.1 ± 0.2	

Directions
Suspend 150 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Disperse the precipitate by gentle agitation before pouring.

Description
Staphylococcus Medium No. 110 is a selective medium for isolation and differentiation of pathogenic *Staphylococci* (Chapman[1,2]) on a basis of salt tolerance, pigmentation, mannitol fermentation, and gelatin liquefaction. Pathogenic *Staphylococci* (coagulase-positive) are able to grow on the high-salt mannitol medium to form orange colonies which give positive reactions for acid production and gelatin liquefaction.

Stone[3] suggested that gelatinase activity was indicative of food poisoning strains but Chapman *et al*.[4] reported that typical food poisoning *Staphylococci* should also produce an orange pigment, be haemolytic, be coagulase-positive, and ferment mannitol. Chapman[5] showed that incubation at 30°C produced deeper pigmentation and no interference with the Stone reaction or with acid production from mannitol – both of the latter being about as intense as at 35°C.

Smuckler & Appleman[6] made Staphylococcus Medium No. 110 selective, for the determination of coagulase-positive staphylococci in meat pies containing large numbers of *Bacillus* species, by the addition of sodium azide 0.75 mM (4.875 g per litre).

Staphylococcus Medium No. 110 is formulated according to the APHA[7] and AOAC[8] specifications. Carter[9] modified the medium by adding egg yolk (5% v/v SR0047) so that the characteristic egg yolk reactions of staphylococci can be seen.

Technique
Streak or smear the Staphylococcus Medium No. 110 plate with the specimen and incubate for 43 hours at 35°C or for 48 hours at 30°C. Pigmented colonies are a deep orange colour, whilst non-pigmented colonies are white.

Acid production from mannitol is best demonstrated by adding a drop of 0.04% bromothymol blue indicator to the sites of the individual colonies; yellow indicates acid production.

Gelatin hydrolysis may be demonstrated by adding a drop of a saturated aqueous solution of ammonium sulphate or, preferably, of a 20% aqueous solution of sulphosalicylic acid to an individual colony ('Stone reaction'). A positive 'Stone reaction' is denoted by the presence of a clear zone round gelatinase-producing colonies after 10 minutes' contact with the reagent.

The above reactions may be conveniently performed using short sleeves, 5 mm long and 10 mm diameter, cut from polythene tubing. The sleeves act as receptacles for the reagents when placed over discrete colonies, and may be stored in 70% alcohol prior to use.

Coagulase tests should not be carried out without first sub-culturing in Nutrient Broth No. 2 CM0067 or on Blood Agar Base CM0055.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance
Dehydrated medium: Light straw coloured, free-flowing powder.

Prepared medium: Straw coloured gel.

Quality control

Positive control:	***Expected results***
Staphylococcus aureus ATCC® 25923*	Good growth; cream coloured colonies
	Gelatinase positive; mannitol negative
Negative control:	
Escherichia coli ATCC® 25922*	No growth

*This organism is available as a Culti-Loop®

Precautions
Enterococcus faecalis may grow on this medium as tiny colonies with slight mannitol fermentation.

The high salt content in Staphylococcus Medium No. 110 may interfere with the coagulase reaction. Always sub-culture to a non-inhibitory medium before testing.

References

1. Chapman G. H. (1946) *J. Bact.* 51. 409-410.
2. Chapman G. H. (1952) *J. Bact.* 63. 147-150.
3. Stone R. V. (1935) *Proc. Soc. Exper. Biol. & Med.* 33. 185-187.
4. Chapman G. H., Lieb C. W. and Cumco L. G. (1937) *Food Research* 2. 349-367.
5. Chapman G. H. (1947) *J. Bact.* 53. 365-366.
6. Smuckler S. A. and Appleman M. D. (1964) *Appl. Microbiol.* 12. 335-339.
7. American Public Health Association (1992) *Compendium of Methods for the Microbiological Examination of Foods.* APHA Inc. Washington DC.
8. Association of Official Analytical Chemists (1998) *Bacteriological Analytical Manual.* 8th edn. AOAC. Washington DC.
9. Carter C. H. (1960) *J. Bact.* 79. 753-756.

STAPH/STREP SELECTIVE MEDIUM – see COLUMBIA CNA AGAR

STREPTOCOCCUS SELECTIVE AGAR – see COBA SELECTIVE MEDIUM

STUART TRANSPORT MEDIUM

Code: CM0111

A transport medium for fastidious pathogenic organisms.

Formula	***gm/litre***
Sodium glycerophosphate	10.0
Sodium thioglycollate	0.5
Cysteine hydrochloride	0.5
Calcium chloride	0.1
Methylene blue	0.001
Agar	5.0
pH 7.4 ± 0.2	

Directions
Suspend 16 g in 1 litre of distilled water. Bring to the boil to dissolve completely and dispense into screw-capped 7 ml bottles. Fill each bottle to the brim, tighten the cap and sterilise by autoclaving at 121°C for 15 minutes. When sufficiently cool to handle, mix by inversion.

Description

This improved medium originally described by Moffett *et al*.[1] and Stuart *et al*.[2], is a non-nutritional semi-solid substrate for the preservation of *Neisseria* species and other fastidious organisms during their transport from clinic to laboratory. Originally formulated for the conservation of *Neisseria gonorrhoeae* and *Trichomonas vaginalis*, it may also be used for the transport of other bacteriological specimens. Stuart *et al*.[2] noted that the transport medium may also be used for *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Streptococcus pyogenes* and *Corynebacterium diphtheriae*. Cooper[3] investigated the extension of Stuart's method to the transport of swabs of clinical material containing upper respiratory tract and enteric pathogens. Stuart[4] published an account of his experiences of the medium in a public health bacteriology, whilst Crookes and Stuart[5] used the transport medium in combination with polymyxin for the cultivation of *Neisseria gonorrhoeae*.

Preparation of Charcoal Swabs for use with Transport Medium

1. Prepare swabs by rolling absorbent cotton-wool on wooden sticks.
2. Boil the swabs in a phosphate buffer solution of the following composition: Disodium hydrogen phosphate 0.81 g, Potassium dihydrogen phosphate 0.18 g, Distilled water 100 ml, pH 7.4.
3. Immediately dip the swabs into a 1% suspension of charcoal (pharmaceutical grade).
4. Place in cotton-wool plugged test tubes and sterilise in the autoclave at 121°C for 15 minutes. Dry at 100°C to remove any excess moisture.

Transport of Swabs

After collection of the specimen, place the swab in the middle of the bottle of Stuart Transport Medium. Break off the stick, replace the screw cap tightly and transport to the laboratory as soon as possible.

The transport method will allow the isolation of gonococci from approximately 90% of cases of female gonorrhoea, provided the transport period is under 24 hours; for longer periods the method is still useful up to 3 days[2].

In all cases, specimens should be cultivated as soon as possible or stored in the refrigerator if delay is unavoidable. Wilkinson[6] reported successful isolation after as long as six days storage in a refrigerator.

Trichomonas vaginalis remains viable, in the medium, up to 24 hours whilst Cooper[3] has reported the recovery of upper respiratory tract and enteric pathogens after 8-12 weeks storage. Stuart *et al.*[2] successfully used the transport method for the recovery of *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Streptococcus pyogenes* and *Corynebacterium diphtheriae* from specimens which had been in transit for 3 to 5 days.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 15-25°C.

Appearance

Dehydrated medium: Blue green coloured, free-flowing powder.

Prepared medium: Off white coloured gel, semi-solid gel.

Quality control

Positive control:	***Expected results***
Streptococcus pyogenes ATCC® 19615*	Good growth; white
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

Precautions

A small amount of blue colour at the top of the bottle indicates oxidation. If this colour extends down into the medium it should be discarded.

Avoid prolonged heating in open flasks, during the preparation of the medium, because thioglycollate is volatile.

Sodium glycerophosphate may be metabolised by some organisms and thus promote their growth.

References
1. Moffett M., Young J. L. and Stuart R. D. (1945) *BMJ.* 2. 421-424.
2. Stuart R. D., Toshach S. R. and Patsula T. M. (1954) *Canad. J. Publ. Hlth* 45. 13-83.
3. Cooper G. N. (1967) *J. Clin. Path.* 10. 226-230.
4. Stuart R. D. (1959) *Pub. Hlth Rep. Wash.* 74. 431-438.
5. Crookes E. M. L. and Stuart R. D. (1959) *J. Path. Bact.* 78. 283-288.
6. Wilkinson A. E. (1955) *J. Med. Lab. Technol.* 15. 184-195.

TBX MEDIUM – TRYPTONE BILE X-GLUCURONIDE

Code: CM0945

A selective, chromogenic medium for the detection and enumeration of Escherichia coli in food.

Formula	***gm/litre***
Tryptone	20.0
Bile Salts No.3	1.5
Agar	15.0
X-glucuronide	0.075
pH 7.2 ± 0.2	

Directions
Suspend 36.6 g of TBX Medium in 1 litre of distilled water. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C and pour the medium into sterile Petri dishes.

Description
TBX Medium is based on Tryptone Bile Agar CM0595. Tryptone Bile Agar was originally formulated to improve on earlier methods used to detect *Escherichia coli* in foods[1,2] in terms of speed, reliability, better recovery from frozen samples and the detection of poor lactose-fermenters.

TBX Medium builds on these advantages through the addition of a chromogenic agent – X-glucuronide – which detects glucuronidase activity. This is the same enzyme detected by MUG reagent[3], and has been shown to be highly specific for *Escherichia coli*[4]. However, approximately 3-4% of *Escherichia coli* are glucuronidase negative, notably *Escherichia coli* O157 strains[5].

Unlike MUG, where the flurophore leaches out of the cell into the surrounding agar, the released chromophore in TBX Medium is insoluble and accumulates within the cell. This ensures that coloured target colonies are easy to identify.

Most *Escherichia coli* strains can be differentiated from other coliforms by the presence of the enzyme glucuronidase. The chromogen in TBX Medium is 5-bromo-4-chloro-3-indolyl-beta-D-glucuronide (X-glucuronide), and is targeted by this enzyme. *Escherichia coli* cells are able to absorb this complex intact and intracellular glucuronidase splits the bond between the chromophore and the glucuronide. The released chromophore is coloured and builds up within the cells, causing *Escherichia coli* colonies to be coloured blue/green.

Technique
Dry the surface of the medium in the prepared plates. Dilute the food sample according to the method being followed e.g. 1 in 10 with Maximum Recovery Diluent (CM0733). Homogenise in a stomacher or a laboratory blender.

The following incubation techniques may be used (consult the relevant standard for the complete method):

1. Pipette 0.1 ml of the homogenate onto the plate and spread over the surface with a sterile glass spreader. Incubate the plates for 24 hours at 37°C[6].
2. Pipette 0.5 ml of the homogenate onto the plate and spread over the surface with a sterile glass spreader. Incubate the plates for 4 hours at 30°C, then 18-24 hours at 44°C[7].
3. Place a cellulose membrane onto the surface of a Minerals Modified Glutamate Medium (CM0607) prepared plate. Pipette 1ml of the homogenate onto the membrane. Incubate for 4 hours at 37°C. Transfer the membrane to a TBX prepared plate and incubate for 18-24 hours at 44°C[8].
4. Pipette 1 ml of the homogenate into a sterile Petri dish. Add TBX Medium, cooled to 45°C. Mix well and allow to set. Incubate for 18-24 hours at 44°C. If the presence of stressed cells is suspected pre-incubate the plates for 4 hours at 37°C[9].

Multiply the numbers of blue/green colonies by the dilution factor and express the result as the number of *Escherichia coli* per gram of food.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared plates of medium at 2-8°C.

Appearance

Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured gel.

Quality control

Positive control:	***Expected results***
Escherichia coli ATCC® 25922*	Good growth; blue/green coloured colonies
Negative control:	
Klebsiella pneumoniae ATCC® 29665	Good growth; straw coloured colonies

*This organism is available as a Culti-Loop®

References

1. Gross R. J. and Rowe B. (1985) *J. Hyg. Camb.* 95. 513-550.
2. Anderson J. M. and Baird-Parker A. C. (1975) *J. Appl. Bact.* 39. 111-117.
3. Feng P. C. S. and Hartmann P. A. (1982) *Appl. Environ. Microbiol.* 43. 1320-1329.
4. Hansen W. and Yourassowsky E. (1984) *J. Clin. Microbiol.* 20. 1177-1179.
5. Ratnam S., March S. B., Almed R., Bezanson G. S. and Kasatiya S. (1988) *J. Clin. Microbiol.* 26. 2006-2012.
6. Donovan T. J. *et al.* (1998) *Communicable Disease and Public Health* 1. 188-196.
7. PHLS Standard Methods for Microbiological Examination of Food, Dairy and Water Samples. F20: Direct Enumeration of *Escherichia coli*.
8. ISO 16649-1: 2001. Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of β-glucuronidase-positive *Escherichia coli*. Part 1: Colony-count technique at 44°C using membranes and 5-bromo-4-chloro-3-indoyl-beta-D-glucuronide.
9. ISO 16649-2: 2001. Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of β-glucuronidase-positive *Escherichia coli*. Part 2: Colony-count technique a 44°C using 5-bromo-4-chloro-3-indoyl-beta-D-glucuronide.

TCBS MEDIUM – see CHOLERA MEDIUM TCBS

TERGITOL-7 AGAR

Code: CM0793

A selective medium for the detection and enumeration of coliforms.

Formula	***gm/litre***
Peptone	10.0
Yeast extract	6.0
Meat extract	5.0
Lactose	20.0
Bromothymol blue	0.05
Tergitol-7	0.1
Agar	13.0
pH 7.2 ± 0.2	

TTC Solution (SR0148) is supplied as 2 ml of filtered 0.125% aqueous solution of tri-phenyltetrazolium chloride (TTC).

Directions
Suspend 54.15 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Dispense in 100 ml volumes and sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C and add the contents of 1 vial of SR0148. Mix well and pour into sterile Petri dishes.

Description
Tergitol-7 Agar is a selective and differential medium for the detection and enumeration of coliforms in food and water samples.

Tergitol-7 Agar is based on the formulation described by Chapman[1] and is recommended for the selective isolation and differentiation of the coliform group. The use of Tergitol-7 as a selective agent had been described earlier[2].

The addition of tri-phenyltetrazolium chloride (TTC)[3] allows earlier recognition and identification of *Escherichia coli* and *Enterobacter aerogenes*. This medium has been recommended for examining foodstuffs for faecal contamination[4] and has been successfully used in routine water analysis[5].

Tergitol-7 inhibits Gram-positive organisms and minimises the swarming of *Proteus* allowing superior recovery of coliforms. Fermentation of lactose is seen by a change in colour of the pH indicator bromothymol blue. TTC is rapidly reduced to insoluble red formazan by most coliform organisms except *Escherichia coli* and *Enterobacter aerogenes*, thus allowing easy differentiation. Tergitol-7 Agar is quoted in the EU Directive for the detection and enumeration of coliforms in drinking water[6].

Technique
Inoculate by spreading the sample on the surface of the agar. Incubate at 35°C for up to 24 hours.

Escherichia coli	Yellow colonies with yellow zone. Sometimes with rust coloured centre
Enterobacter/Klebsiella species	Greenish/yellow colonies
Salmonella species	Red colony with bluish zone
Shigella species	Red colony with bluish zone
Proteus species	Red colony with bluish zone
Pseudomonas species	Red colony with bluish zone
Gram-positive bacteria	No growth to slight growth

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance
Dehydrated medium: Pale green coloured, free-flowing powder.

Prepared medium: Dark green coloured gel.

Quality control

Positive control:	***Expected results***
Escherichia coli ATCC® 25922*	Good growth; yellow orange coloured colonies with yellow zone
Negative control:	
Staphylococcus aureus ATCC® 25923*	Inhibited or no growth

*This organism is available as a Culti-Loop®

Precautions
Tergitol-7 Agar is designed for early detection of *Escherichia coli*, i.e. 6-10 hours incubation[3].

Incubation at 44°C has been recommended[4,5].

References

1. Chapman G. H. (1947) *J. Bact.* 53. 504.
2. Pollard A. L. (1946) *Science* 103. 758-759.
3. Chapman G. H. (1951) *Am. J. Pub. Hlth* 41. 1381.
4. Mossel D. A. A. (1962) *J. Appl. Bact.* 25. 20-29.
5. Kulp W., Mascoli C. and Tavshanjian O. (1953) *Am. J. Pub. Hlth* 43. 1111-1113.
6. E.U. (1998) 98/83/EC of Council of 3rd of November 1998 on the quality of water intended for human consumption. *Off. J. Eur. Commun*., L3330, 32-54.

TETRATHIONATE BROTH BASE

Code: CM0029

Formula	***gm/litre***
'Lab-Lemco' powder	0.9
Peptone	4.5
Yeast extract	1.8
Sodium chloride	4.5
Calcium carbonate	25.0
Sodium thiosulphate	40.7
pH 8.0 ± 0.2	

Directions
Add 77 g to 1 litre of distilled water and bring to the boil. Cool below 45°C and add 20 ml of iodine solution. Mix well and tube in 10 ml quantities. The prepared base will keep for several weeks at 4°C but should be used soon after the addition of the iodine solution.

Iodine Solution

Iodine	6 g
Potassium iodide	5 g
Distilled water	20 ml

Description
Tetrathionate Broth is recommended for the selective enrichment method of isolating *Salmonella typhi* and other salmonellae from faeces, sewage, etc.

Organisms which reduce tetrathionate, such as salmonellae, flourish in the medium whilst many faecal organisms are inhibited[1]. Members of the *Proteus* group reduce tetrathionate and may consequently impair the value of this medium for the isolation of salmonellae; this disadvantage of the medium is largely overcome by the addition of 40 µg of novobiocin to each millilitre of the incomplete medium before the addition of iodine[2,3].

Technique
Inoculate the broth with about 2 g of the specimen and mix thoroughly to disperse particulate matter. A loose cotton-wool plug may be passed down through the inoculated medium in order to carry gross particles to the bottom of the tube. Incubate for 12 to 24 hours at 35°C and then sub-culture onto Bismuth Sulphite Agar CM0201, SS Agar CM0099 or Desoxycholate Citrate Agar (Hynes) CM0227, etc.

The complete medium (with added iodine) should be used the same day as it is prepared, but the sterilised basal medium will keep for many weeks at 4°C. Jeffries[2] showed that novobiocin, at a concentration of 40 µg/ml in the medium, remained stable for at least 48 hours at 35°C, and for one month at room temperature.

This medium is frequently used in parallel with Selenite Broth Base CM0395.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium (without iodine solution) at 2-8°C.

Appearance
Dehydrated medium: White, free-flowing powder.

Prepared medium: Off white opaque suspension.

Quality control

Positive control:	***Expected results***
Salmonella typhimurium ATCC® 14028*	Good growth
Negative control:	
Escherichia coli ATCC® 25922*	Inhibited or no growth

* This organism is available as a Culti-Loop®

References
1. Knox R., Gell P. G. H. and Pollock M. R. (1942) *J. Path. Bact.* 54. 469-483.
2. Jeffries L. (1959) *J. Clin. Path.* 12. 568-571.
3. Buttiaux R., Catsaras M. and Verdant M. (1961) *Ann. Inst. Pasteur de Lille* 12. 13-18.

TETRATHIONATE BROTH (USA)

Code: CM0671

An American formulation which complies with the description given in the US Pharmacopoeia for the enrichment of specimens undergoing examination for salmonellae.

Formula	***gm/litre***
Casein peptone	2.5
Meat peptone	2.5
Bile salts	1.0
Calcium carbonate	10.0
Sodium thiosulphate	30.0

Directions
Suspend 46 g in 1 litre of distilled water and bring to the boil. Cool to below 45°C and add 20 ml of iodine-iodide solution immediately before use. Mix continuously whilst dispensing 10 ml volumes into sterile tubes. Use the complete medium (with added iodine) on the day of preparation.

Iodine-Iodide Solution

Iodine	6 g
Potassium iodide	5 g
Distilled water	20 ml

The base may be prepared beforehand and kept for several weeks at 4°C. Iodine-iodide solution can then be added at the time of use to the quantity of medium needed.

Description
Tetrathionate Broth USA complies with the description given in the United States Pharmacopoeia[1].

Tetrathionate Broth is specified by the 15th edition of *Standard Methods for the Examination of Water and Wastewater*[2] and *Compendium of Methods for the Microbiological Examination of Foods*[3] for the enrichment of specimens undergoing examination for salmonellae.

The selectivity of the medium depends on the ability of thiosulphate and tetrathionate in combination to suppress commensal coliform organisms[4]. Organisms which possess the enzyme tetrathionate reductase grow in the medium. *Salmonella* and *Proteus* species possess the enzyme; *Escherichia coli* and shigellae do not.

Proteus can be suppressed by adding 40 mg per ml of novobiocin[6] to the incomplete medium before the addition of iodine.

Bile salts are present to inhibit those organisms which do not live in the intestine.

Brilliant Green 0.001% w/v can be added to the broth[1] but it should be remembered that *Salmonella typhi* and some other salmonellae are inhibited by this compound.

The role of calcium carbonate is to neutralise the acidic tetrathionate decomposition products.

Technique
Inoculate the broth with 1-2 g of the specimen and mix thoroughly to disperse the sample.

Incubate at 35°C and sub-culture after 18-24 hours to XLD Agar CM0469, SS Agars CM0099 or CM0533, Bismuth Sulphite Agar CM0201, or similar selective/indicator media for *Salmonella* isolation.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.
Store the base broth at 2-8°C. Use the medium immediately after adding the iodine solution.

Appearance
Dehydrated medium: White coloured, free-flowing powder.

Prepared medium: Off white coloured solution.

Quality control

Positive control:	***Expected results***
Salmonella typhimurium ATCC® 14028	Good growth
Negative control:	
Escherichia coli ATCC® 25922*	No growth

*This organism is available as a Culti-Loop®

References

1. United States Pharmacopoeia XXI (1985) *Microbial Limit Tests*. Rockville, Md.
2. American Public Health Association (1998) *Standard Methods for the Examination of Water and Wastewater.* 20th edn. APHA Inc. Washington DC.
3. American Public Health Association (1992) *Compendium of Methods for the Microbiological Examination of Foods.* 3rd edn. APHA Inc. Washington DC.
4. Pollock M. R. and Knox R. (1943) *Biochem. J.* 37. 476-481.
5. Papavassiliou J., Samaraki-Lyberopoulou V. and Piperakis G. (1969) *Can. J. Microbiol.* 15. 238-240.
6. Jeffries L. (1959) *J. Clin. Path.* 12. 568-571.

THAYER MARTIN MEDIUM AND VARIANTS

Media for the Isolation of Neisseria gonorrhoeae and Neisseria meningitides.

OXOID GC AGAR BASE

Code: CM0367

Formula	***gm/litre***
Special peptone	15.0
Corn starch	1.0
Sodium chloride	5.0
Dipotassium hydrogen phosphate	4.0
Potassium dihydrogen phosphate	1.0
Agar	10.0
pH 7.2 ± 0.2	

VCN SELECTIVE SUPPLEMENT

Code: SR0101

Vial contents (each vial is sufficient for 500 ml of medium)	***per vial***	***per litre***
Vancomycin	1.5 mg	3.0 mg
Colistin sulphate	3.75 mg	7.5 mg
Nystatin	6,250 IU	12,500 IU

VCNT SELECTIVE SUPPLEMENT

Code: SR0091

Vial contents (each vial is sufficient for 500 ml of medium)	***per vial***	***per litre***
Vancomycin	1.5 mg	3.0 mg
Colistin sulphate	3.75 mg	7.5 mg
Nystatin	6,250 IU	12,500 IU
Trimethoprim	2.5 mg	5.0 mg

Directions

1. Suspend 18 g of GC Agar Base in 240 ml of distilled water and bring gently to the boil to dissolve the agar. Sterilise by autoclaving at 121° C for 15 minutes. Prepare a 2% solution of Soluble Haemoglobin Powder LP0053 by adding 250 ml of warm distilled water to 5 g of haemoglobin powder. Continually stir the solution during the addition of water. Sterilise by autoclaving at 121°C for 15 minutes.

Reconstitute one vial of Vitox SR0090 as directed on the vial label.

Dissolve the contents of a vial of either VCN Antibiotic Supplement or VCNT Antibiotic Supplement as directed on the vial label.

Aseptically add the Vitox solution to 240 ml of sterile GC Agar Base cooled to 50°C.

Aseptically add the reconstituted antibiotic supplement VCN or VCNT to the GC Agar Base-Vitox solution.

Aseptically add the 250 ml of sterile haemoglobin solution, cooled to 50°C to the GC Agar Base-Vitox-Antibiotic Supplement solution. Mix gently to avoid trapping air bubbles in the agar and pour into sterile Petri dishes.

Description

Oxoid GC Agar Base has been formulated to include Special Peptone LP0072 which is a mixture of meat and plant enzymatic digests. The presence of starch ensures that toxic metabolites produced by neisseria are absorbed. Phosphate buffers are included to prevent changes in pH due to amine production that would affect the survival of the organism.

Thayer and Martin[1] described a medium to which the antibiotics in VCN selective supplement are added. This medium has been widely accepted for the primary isolation of *Neisseria gonorrhoeae* and *Neisseria meningitides* from conspicuously contaminated sites.

Seth[2] described a modification of Thayer Martin Medium in which Trimethoprim at 5 µg/ml was added to the VCN supplement to prevent the swarming of Proteus species. Several other workers[3,4,5] confirmed the non-inhibitory effect on isolation of *Neisseria gonorrhoeae* and its effectivness in the prevention of swarming.

Storage conditions and Shelf life

Store the dehydrated medium below 30°C and use before the expiry date on the label.

Store the prepared plates at 2-8°C.

Appearance

Dehydrated medium: Light straw coloured, free-flowing powder.

Prepared medium (before additions): Straw coloured gel.

Quality Control

Positive control: with antibiotics	***Expected result***
Neisseria gonorrhoeae ATCC® 19424*	Good growth; grey-brown colonies
Neisseria meningitidis ATCC® 13090*	Good growth; grey-brown colonies
Negative control: with antibiotics	
Proteus hauseri ATCC® 13315*	Inhibited
Staphylococcus aureus ATCC® 25923*	Inhibited
Negative control: without antibiotics	
Uninoculated medium	No change

References
1. Thayer J. D. and Martin J. E. (1966) *Public Health Rep*., 81. 6. 559-562.
2. Seth A. (1970) *Brit. J. Vener. Dis.* 46. 201-202.
3. Riddel R. H. and Buck A. C. (1970) *J. Clin. Path.* 23. 481-483.
4. Odegaard K. (1971) *Acta Path. Microbiol. Scand*., Sect. B. 79. 545-548.
5. Martin J. E. and Lester A. (1971) *HSMHA Health Reports*. 86. No.1 30-33.

THIOGLYCOLLATE BROTH USP – ALTERNATIVE

Code: CM0391

Used in place of Thioglycollate Medium USP CM0173 for testing turbid or viscous products.

Formula	*gm/litre*
L-cystine	0.5
Sodium chloride	2.5
Glucose	5.5
Yeast extract	5.0
Pancreatic digest of casein	15.0
Sodium thioglycollate	0.5
pH 7.1 ± 0.2	

Directions
Suspend 29 g in 1 litre of distilled water. Bring to the boil and dissolve the medium completely. Distribute into tubes or bottles and sterilise by autoclaving at 121°C for 15 minutes.

PREPARE FRESHLY OR BOIL AND COOL THE MEDIUM JUST BEFORE USE.

Description
Thioglycollate Medium USP – Alternative, is intended for sterility testing with certain biological products that are turbid or otherwise do not lend themselves readily to culturing in Thioglycollate Medium USP (CM0173) because of its viscosity.

The formulation, which omits the agar and resazurin present in Thioglycollate Medium USP, is described in the N.I.H. Memorandum[1] and the U.S. Pharmacopoeia XXI. These omissions make it essential that the medium should be freshly prepared or boiled and cooled within four hours of use.

The containers of choice for the medium are tubes of 20 x 150 mm size. The use of 15 ml of medium in this tube provides adequate medium for inocula up to 3 ml and sufficient thioglycollate to inactivate a mercurial preservative, when present in the inoculum, in a concentration not greater than 0.03% w/v. Other preservatives will need to be inactivated by adequate dilution unless an effective inactivator is used.

If larger volumes of medium are required, cylindrical or square bottles or containers which provide approximately the same ratio of surface exposed to depth of medium as in the test-tube above, should be used.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Whilst storage of the prepared medium is not recommended, autoclaved volumes of Thioglycollate Medium USP – Alternative, should be held at 20-30°C in the dark. Storage at lower temperatures increases oxygen absorption.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured solution.

Quality control

Positive controls:	***Expected results***
Candida albicans ATCC® 10231*	Flocculent growth
Clostridium sporogenes ATCC® 19404*	Turbid growth and or colonies
Peptostreptococcus anaerobius ATCC® 27337*	Turbid growth and or colonies
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

Precautions
This medium lacks agar and reducing indicator, therefore it is essential that the medium is freshly prepared and used within four hours of preparation.

Thioglycollate media should not be reheated more than once because toxic oxygen radicles are formed on reheating.

References
1. N.I.H. (1955) Memorandum: *Culture media for sterility tests* 4th Revision.
2. US Pharmocopoeia XXI (1985) *Sterility tests*.

THIOGLYCOLLATE MEDIUM USP

Code: CM0173

A medium for the cultivation of both aerobic and anaerobic organisms in the performance of sterility tests.

Formula	***gm/litre***
Yeast extract	5.0
Tryptone	15.0
Glucose	5.5
Sodium thioglycollate	0.5
Sodium chloride	2.5
L-cystine	0.5
Resazurin	0.001
Agar	0.75
pH 7.1 ± 0.2	

Directions
Suspend 29.5 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Distribute into final containers, sterilise by autoclaving at 121°C for 15 minutes. Mix well and cool to room temperature.

Description
This medium is prepared according to the formula specified in the US Pharmacopoeia[1] for the performance of sterility tests, and conforms to formulations detailed in the British and European Pharmacopoeia[2,3]. It is suitable for the cultivation of both aerobic and anaerobic organisms. No paraffin or special seal is necessary, nor is an anaerobic jar required for the cultivation of anaerobes. It is well buffered so that acid or alkaline inocula produce negligible alteration in the reaction of the medium. The sodium thioglycollate content of the medium will neutralise the bacteriostatic effect of mercurial compounds used as preservatives in solutions for injection, etc. If the solution undergoing test contains a bacteriostatic substance it is necessary, in order to avoid a false negative result, to establish the bacteriostatic activity of the product by the method described in the US Pharmacopoeia[1]. Thioglycollate Medium USP is also recommended for the cultivation of *Clostridium* species. Sealey[2] found that, of the media tested, Thioglycollate Medium USP gave the best results for the cultivation and maintenance of *Desulfotomaculum nigrificans*.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium away from light at room temperature.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw yellow coloured solution.

Quality control[1]

Positive controls:	***Expected results***
Staphylococcus aureus ATCC® 25923*	Turbid growth
Bacteroides vulgatus ATCC® 8482*	Turbid growth
Candida albicans ATCC® 10231*	Turbid growth
Bacillus subtilis ATCC® 6633*	Turbid growth
Negative control:	
Uninoculated medium	No change

* This organism is available as a Culti-Loop®

Precautions
If the upper portion of the medium is pink because of oxidation, anaerobic conditions can be restored by reheating for 10 minutes in boiling water or steam. Do not reheat more than once.

If more than one-third of the medium is oxidised then it should be discarded.

Some glucose-fermenting organisms which are able to reduce the pH of the medium to a critical level may not survive in this medium. Early sub-culture is necessary to isolate these organisms.

References
1. US Pharmacopoeia. XXIV (2000) *Sterility Testing*.
2. Sealey J. Q. (1951) *Thesis of the University of Texas.*
3. British Pharmocopoeia. Volume II (2000).
4. European Pharmocopoeia. 3rd Edition (2001).

THIOGLYCOLLATE MEDIUM (BREWER)

Code: CM0023

An anaerobic medium especially useful for the sterility control of solutions containing mercury preservatives.

Formula	***gm/litre***
'Lab-Lemco' powder	1.0
Yeast extract	2.0
Peptone	5.0
Glucose	5.0
Sodium chloride	5.0
Sodium thioglycollate	1.1
Methylene blue	0.002
Agar	1.0
pH 7.2 ± 0.2	

Directions
Suspend 20 g in 1 litre of distilled water. Bring to the boil, mix well and allow to stand until completely dissolved. Distribute into final containers and sterilise by autoclaving at 121°C for 15 minutes.

Media containing small quantities of agar are liable to separate if cooled rapidly. Tubes of reconstituted and autoclaved medium should be allowed to cool slowly on a wooden surface in a draught-free atmosphere.

Description
This anaerobic medium, developed by Brewer[1,2,3] is used principally for testing the sterility of biological products.

The medium contains a small concentration of methylene blue as an oxidation-reduction indicator. If more than 20% of the uppermost portion of the stored medium has changed to a green colour, anaerobic conditions may be restored by heating in a boiling water bath or steamer for 5 to 10 minutes. This treatment must not be repeated.

Thioglycollate Medium (Brewer) is especially useful for the control of biological solutions containing mercurial preservatives, the toxicity of the latter being neutralised by the thioglycollate.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

The prepared medium should be stored away from light at room temperature.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw/yellow coloured solution with green layer.

Quality control

Positive controls:	***Expected results***
Bacterioides vulgatus ATCC® 8482	Turbid growth; and/or single colonies
Clostridium sporogenes ATCC® 19404*	Turbid growth; and/or single colonies
Negative control:	
Uninoculated medium	No change

* This organism is available as a Culti-Loop®

Precautions
Check the upper portion of the prepared medium before inoculation. If more than one-third of the fluid is oxidised i.e. green coloured, discard the bottle.

If one-third or less fluid is oxidised, then heat in boiling water with the cap loosened to drive off oxygen. Cool to room temperature before inoculation. This reheating process can only be carried out once because of the formation of toxic radicles in the medium.

Organisms which ferment glucose and lower the pH to critical levels may not survive in this medium after growth has taken place.

References
1. Brewer J. H. (1940) *JAMA.* 115. 598-600.
2. Brewer J. H. (1940) *J. Bact.* 39. 10-13.
3. Brewer J. H. (1943) *J. Bact.* 46. 395-398.

TINSDALE AGAR BASE

Code: CM0487

A medium for the isolation and identification of C. diphtheriae.

Formula	***gm/litre***
Proteose peptone	20.0
Yeast extract	5.0
Sodium chloride	5.0
L-cystine	0.24
Agar	15.0
pH 7.9 ± 0.2	

Directions
Suspend 9 g of agar base in 200 ml of distilled water. Bring to the boil and dissolve completely. **DO NOT AUTOCLAVE**. Allow to cool to approximately 50°C and add the contents of one vial of Oxoid Tinsdale Supplement reconstiuted as directed. Mix throughly and pour into sterile Petri dishes.

TINSDALE SUPPLEMENT

Code: SR0065

Vial contents (each vial is sufficient for 200 ml of medium)	***per vial***	***per litre***
Serum equiv.	20.0 ml	100.0 ml
Potassium tellurite	0.069 g	0.3445 g
Sodium thiosulphate	0.085 g	0.425 g

Description

Tinsdale's original agar medium[1] containing serum, tellurite, cystine and formolised blood was formulated to differentiate between *Corynebacterium diphtheriae* and the diphtheroids found in the upper respiratory tract. This differentiation was based on the ability of *Corynebacterium diphtheriae* to produce black colonies, surrounded by a brown/black halo, after incubation at 35°C for 48 hours. Diphtheroids do not have this ability. The dark halo is due to the production of H_2S from cystine, interacting with the tellurite salt.

Oxoid Tinsdale Base and Supplement are based on Billings'[2] modification of Tinsdale's Medium, which improved the differential qualities as well as the reproducibility of the medium. Moore and Parsons[3] using Billings' modification confirmed the stability of halo formation on the clear medium and its specificity for *Corynebacterium diphtheriae* and *Corynebacterium ulcerans*.

They considered that as the incidence of diphtheria gets smaller, it becomes more essential to have a medium which gives a distinctive, characteristic colony.

Technique

Inoculate the medium to obtain well separated colonies. Stab deep into the agar at intervals in order to initiate browning at an early stage (10-12 hours incubation).

Plates are incubated at 35°C and examined after 24 hours and 48 hours incubation. Growth of *Corynebacterium diphtheriae* may be inhibited if Tinsdale Agar is incubated in carbon dioxide-enriched air e.g. in a CO_2 incubator.

Browning may be regarded as presumptive evidence of the presence of *Corynebacterium diphtheriae* although 48 hours incubation may be necessary for the recognition of characteristic colonies.

Colonial Characteristics

C. diphtheriae biotype gravis	Small, shiny black
C. diphtheriae biotype mitis *C. ulcerans*	Convex colonies with dark brown halos after 24 hours incubation
C. diphtheriae biotype intermedius	Pale brown colonies which form halos after 36 hours incubation
Diphtheroids (*C. pseudodiphtheriticum*) *Haemophilus, Klebsiella, Neisseria*, *Staphylococcus* and *Streptococcus* species	Dark, brown colonies without halos. Minute colonies showing no discoloration of the medium
Proteus species	Brown-black colonies showing characteristic odour and morphology

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C for not more than 4 days.

Appearance

Dehydrated Medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured gel.

Quality control

Positive controls:	***Expected results***
Corynebacterium diphtheriae gravis ATCC® 19409	Good growth, black colonies, black halos
Negative control:	
Staphylococcus aureus ATCC® 25923*	Inhibited, black colonies, no halos

*This organism is available as a Culti-Loop®

Precautions
Further tests must be carried out on colonies suspected as *Corynebacterium diphtheriae*, including cell morphology after sub-culture to Loeffler's Medium and examination for toxin production.

Do not incubate Tinsdale's Agar plates in enhanced CO_2 atmosphere (5-10% v/v).

References

1. Tinsdale G. F. (1947) *J. Path. Bact.* 59. 461-464.
2. Billings E. (1956) '*An Investigation of Tinsdale's Tellurite Medium, its Usefulness and Mechanism of Halo Formation*'. Thesis Univ. Michigan.
3. Moore Mary S. and Parsons Eliz. I. (1958) *J. Infect. Dis.* 102. 88-91.

TODD-HEWITT BROTH

Code: CM0189

A medium for the production of antigenic streptococcal haemolysin and the cultivation of streptococci prior to serological grouping.

Formula	***gm/litre***
Infusion from 450g fat-free minced meat	10.0
Tryptone	20.0
Glucose	2.0
Sodium bicarbonate	2.0
Sodium chloride	2.0
Disodium phosphate	0.4
pH 7.8 ± 0.2	

Directions
Dissolve 36.4 g in 1 litre of distilled water. Mix well, distribute into containers and sterilise by autoclaving at 115°C for 10 minutes.

Description
An easily reconstituted, dehydrated modification of the medium originally described by Todd and Hewitt[1] for the production of antigenic streptococcal haemolysin. Fermentation of glucose, which is included as a growth stimulant, would lead to the destruction of haemolysin by the acid produced; consequently, the medium is buffered with sodium bicarbonate and sodium phosphate.

It has been found that inorganic phosphates have a stimulating effect on the growth of pneumococci quite apart from their buffering power.

Todd-Hewitt Broth may be employed as an alternative to serum broth or horse-flesh digest broth, for the cultivation of streptococci prior to serological grouping[2].

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured solution.

Quality control

Positive controls:	***Expected results***
Streptococcus pyogenes ATCC® 19615*	Turbid growth
Streptococcus pneumoniae ATCC® 6303*	Turbid growth
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

Precautions
Streptococcus species grown in Todd-Hewitt Broth and harvested as antigens to raise antibodies, will sometimes carry antigenic material from the broth. This problem must be looked for in the antisera. If detected it would be preferable to use another medium to grow the test *Streptococci*.

References
1. Todd E. W. and Hewitt L. F. (1932) *J. Path. Bact.* 35(1). 973-974.
2. Finegold S. M. and Martin W. J. (1982) *Diagnostic Microbiology*. C. V. Mosby Co. St. Louis. USA. p.645.

TOMATO JUICE AGAR

Code: CM0113

A medium for the cultivation and enumeration of Lactobacillus species.

Formula	***gm/litre***
Tomato juice (solids from 400 ml)	20.0
Peptone	10.0
Peptonised milk	10.0
Agar	12.0
pH 6.1 ± 0.2	

Directions
Suspend 52 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Mix well and pour into sterile Petri dishes.

Description
Tomato Juice Agar is recommended for the cultivation and enumeration of *Lactobacillus* species. This is a modification of Kulp's medium for the culture of lactobacilli, its preparation differing only slightly from the method described by Kulp & White[1].

When a more acid medium is required, for the direct plate count of lactobacilli and other organisms from saliva, the pH of the medium may be adjusted to approximately 5.1 by the addition of 1ml of 10% Lactic Acid SR0021 to each 100 ml of sterilised medium.

Davis[2] compared the morphology of *Lactobacillus* colonies on Oxoid Tomato Juice Agar and other media.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the plates of prepared medium at 2-8°C.

Appearance
Dehydrated Medium: Straw coloured, free-flowing powder.

Prepared medium: Dark straw coloured gel.

Quality Control

Positive control:	***Expected results***
Lactobacillus fermentum ATCC® 9338	Good growth, cream colonies
Negative control:	
Uninoculated medium	No change

References
1. Kulp J. W. L. and White V. (1932) *Science* 76. 17-18.
2. Davis G. H. G. (1959) *Lab. Prac.* 8(5). 161-167.

TRICHOMONAS MEDIUM

Code: CM0161

A medium for the cultivation of Trichomonas vaginalis.

Formula	***gm/litre***
Liver digest	25.0
Glucose	5.0
Sodium chloride	6.5
Agar	1.0
pH 6.4 ± 0.2	

Directions

Suspend 37.5 g in 1 litre of distilled water and bring to the boil to dissolve. Sterilise by autoclaving at 121°C for 15 minutes. Cool to approximately 50°C. Inactivate 80 ml of horse serum†, adjust to pH 6.0 and add it to the medium. For diagnostic work, bacterial growth may be suppressed by the addition of 1000 units of penicillin and 500 mg of streptomycin per ml of medium or 100 µg of chloramphenicol per ml of medium (1 vial of SR0078 per 500 ml medium).

†Prior to incorporation in the medium, Horse Serum SR0035 is inactivated by maintaining at a temperature of 56°C for 30 minutes, and then acidified with 1N hydrochloric acid to pH 6.0.

Description

A medium based on that of Feinberg & Whittington[1] for the detection of *Trichomonas vaginalis* and *Candida* species. The authors, after a series of investigations on 1,704 genito-urinary specimens, found that *Trichomonas vaginalis* would not have been detected in 23.5% of the samples had they not been cultured. Furthermore, out of 747 vaginal specimens, 63% which were positive would have been dismissed as negative without use of the medium.

Stenton[2] noted that the success of an earlier version of the Trichomonas Medium was largely dependent on the choice and percentage of liver incorporated and he selected Oxoid Liver Infusion. Trichomonas Medium has been slightly modified by the incorporation of 0.1% w/v of agar which leads to reduced oxygen tension and consequently more prolific growth of trichomonads. Good growth of both *Trichomonas* and *Candida* may be obtained in mixed culture – the growth of *Candida* species seldom interferes with trichomonads.

Technique

Inoculate Trichomonas Medium and incubate at 35°C for three to five days. At intervals, microscopically examine medium taken from the bottom of the tube. In addition, microscopically examine fresh wet smears of the specimen at the time of initial culture.

The medium is equally suitable for the examination of urethral and vaginal swabs, and urine specimens.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance

Dehydrated Medium: Light straw coloured, free-flowing powder.

Prepared medium: Dark straw coloured solution.

Quality Control

Positive controls:	***Expected results***
Trichomonas vaginalis ATCC® 30001	Viable organisms
Candida albicans ATCC® 10231*	Turbid growth
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

References

1. Feinberg J. G. and Whittington Joan M. (1957) *J. Clin. Path*. 10. 327-329.
2. Stenton P. (1957) *J. Med. Lab. Technol.* 14. 228-230.

TRIPLE SUGAR IRON AGAR

Code: CM0277

A composite medium for the differentiation of Enterobacteriaceae by three sugar fermentations and hydrogen sulphide production.

Formula	***gm/litre***
'Lab-Lemco' powder	3.0
Yeast extract	3.0
Peptone	20.0
Sodium chloride	5.0
Lactose	10.0
Sucrose	10.0
Glucose	1.0
Ferric citrate	0.3
Sodium thiosulphate	0.3
Phenol red	0.024
Agar	12.0
pH 7.4 ± 0.2	

Directions
Suspend 65 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Mix well and distribute. Sterilise by autoclaving at 121°C for 15 minutes. Allow the medium to set in sloped form with a butt about 1 in. deep.

Description
A composite medium for the differentiation of Enterobacteriaceae according to their ability to ferment lactose, sucrose and glucose, and to produce hydrogen sulphide.

Not only does this medium perform most of the functions of Kligler Iron Agar but, in addition, its sucrose content permits the recognition and exclusion of sucrose-fermenting species. These organisms may ferment lactose slowly or not at all during the incubation period, but they attack sucrose readily. Some *Proteus* and other species may give similar reactions to *Salmonellae* and *Shigellae* and it is necessary to distinguish them by their ability to hydrolyse urea. For this reason Triple Sugar Iron Agar should be used in parallel with Urea Broth or Urea Agar.

This medium was formerly considered to be interchangeable with Kligler medium for the detection of hydrogen sulphide-producing Enterobacteriaceae. It is now thought that Triple Sugar Iron Agar is not suitable for the detection of hydrogen sulphide production by sucrose-fermenting organisms, such as some *Citrobacter* and *Proteus* species, in which the sucrose fermentation masks the hydrogen sulphide indicator in the medium[†1].

Triple Sugar Iron Agar is recommended for the presumptive identification of colonies or sub-cultures from plating media such as Salmonella Shigella Agar (Modified) CM0533, Bismuth Sulphite Agar CM0201, Brilliant Green Agar CM0263, MacConkey Agar No. 3 CM0115, or Desoxycholate Citrate Agar (Hynes) CM0227.

Technique
The USA techniques are described elsewhere[2,3] but the following is suggested as a simple method:

1. Pick a single colony from the surface of a selective plating medium and smear a MacConkey Agar CM0007 plate. Incubate for 18 hours at 37°C and inoculate two separate tubes of media from one single isolated colony:
   (i) Triple Sugar Iron Agar – smear the slope and stab the butt.
   (ii) Urea Broth Base CM0071 (with added Urea Solution SR0020).
2. Incubate at 35°C.
3. Examine the Urea Broth tube after 5 hours and again after 18 hours incubation. Discard tubes showing a red or pink coloration, which is due to urea hydrolysis by *Proteus* or other organisms.
4. Where there is no urea hydrolysis, examine the Triple Sugar Iron Agar tubes after 18 hours and 48 hours. The following are typical reactions:

Organism	*Butt*	*Slope*	*H_2S*
Enterobacter aerogenes	AG	A	–
Enterobacter cloacae	AG	A	–
Escherichia coli	AG	A	–
Proteus vulgaris	AG	A	+
Morganella morganii	A or AG	NC or ALK	–
Shigella dysenteriae	A	NC or ALK	–
Shigella sonnei	A	NC or ALK	–
Salmonella typhi	A	NC or ALK	+
Salmonella paratyphi	AG	NC or ALK	–
Salmonella enteritidis	AG	NC or ALK	+
Salmonella typhimurium	AG	NC or ALK	+

AG = acid (yellow) and gas formation; A = acid (yellow); NC = no change; ALK = alkaline (red); + = hydrogen sulphide (black)†
– = no hydrogen sulphide (no black)†
†See note in text.

The presumptive evidence so obtained may be confirmed serologically after sub-culturing the organism from the Triple Sugar Iron Agar slope in Nutrient Broth No. 2 CM0067.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Quality control

Typical reactions of organisms in Triple Sugar Iron Agar.

Organism	*Slant*	*Butt*	*Gas*	*H_2S*
Escherichia coli ATCC® 8739*	A	A	+	-
Proteus vulgaris ATCC® 13315*	A	A	+	+
Pseudomonas aeruginosa ATCC® 9027*	ALK	ALK	-	-
Salmonella enteritidis ATCC® 13076*	ALK	ALK	+	+

*This organism is available as a Culti-Loop®

References

1. Bulmash J. M. and Fulton M. (1966) *J. Bact.* 88. 1813.
2. American Public Health Association (1976) *Compendium of Methods for the Microbiological Examination of Foods.* APHA Inc. Washington DC.
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TRYPTONE BILE AGAR

Code: CM0595

A rapid and direct plate method for the enumeration of Escherichia coli in food.

Formula	***gm/litre***
Tryptone	20.0
Bile salts No.3	1.5
Agar	15.0
pH 7.2 ± 0.2	

Directions
Suspend 36.5 g in 1 litre of distilled water and bring gently to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C and pour 12-15 ml of the medium into sterile dishes.

Description
Tryptone Bile Agar has been developed according to the formulation of Anderson and Baird-Parker[1] for the detection and enumeration of *Escherichia coli* in foods.

It has several advantages over older methods:

1. It is faster.

2. It is less variable.

3. It gives better recovery from frozen samples.

4. It detects anaerogenic and poor lactose-fermenting strains.

The Direct Plating Method (DPM) described by Anderson and Baird-Parker is a modification of that described by Delaney *et al*[2]. This method, developed for the detection and enumeration of *Escherichia coli* in water and food samples, utilises the ability of *Escherichia coli* to produce indole from tryptophan at 44°C when grown on a cellulose acetate membrane on plates of Tryptone Bile Agar.

The authors concluded that the formation of indole was a more reliable characteristic for both enterotoxigenic and non-enterotoxigenic strains of *Escherichia coli* than lactose fermentation. Ewing[3] found that only 90% of *Escherichia* strains produce acid from lactose within two days, whereas 99% of strains produce indole.

The International Commission on Microbiological Specifications for Foods (CMSF)[4] compared the Most Probable Number (MPN) and the Anderson-Baird-Parker Direct Plating Method (DPM) and concluded that the DPM was preferable to the MPN method of enumeration of *Escherichia coli* in raw meats, because of less variability, better recovery from frozen samples, greater rapidity and the smaller quantity of medium needed.

The Direct Plating Method will enumerate both anaerogenic and late lactose-fermenting strains of *Escherichia coli* which would be missed by the MPN method. According to Ewing[3] these organisms comprise as many as 10% of *Escherichia* strains.

Holbrook *et al*.[5] have further modified the Direct Plating Method for detection and enumeration of sub-lethally damaged cells of *Escherichia coli* in frozen, dried, heat processed or acid foods. In this modification the inoculum is applied to a cellulose acetate membrane on Minerals Modified Glutamate Agar and incubated for 4 hours at 37°C. The resuscitation step permits the repair of stressed cells before the transfer of the membrane to a Tryptone Bile Agar plate.

It has been shown that the presence of high levels of fermentable carbohydrates will inhibit the synthesis of tryptophanase[6] and thereby stop indole formation. Holbrook *et al*. have demonstrated that the resuscitation step reduces the high concentration of sugar present in the inoculum to a level which does not interfere with the production of indole by *Escherichia coli* when grown on Tryptone Bile Agar. The resuscitation step should always be carried out when testing dairy or other products containing high concentrations of sugars.

The indole reagent described by Vracko and Sherris[7] was found to be the most suitable, giving the most distinct reaction and reproducibility. The reagent, 5% p-dimethylaminobenzaldehyde in 1N hydrochloric acid is easy to prepare and will not deteriorate when kept for three months in the dark at room temperature.

All indole positive strains give well defined pink colonies when ‘stained’ using the indole reagent; colonies that do not produce indole are straw coloured.

The growth of indole positive organisms other than *Escherichia coli* is inhibited by the selective action of the bile salts and the elevated incubation temperature.

The 'stained' membranes may be 'fixed' by drying in direct sunlight or under a low pressure fluorescent ultra violet lamp with a 'Woods' type filter. When dried the intensity of the staining reaction is improved, and such membranes may be stored for reference.

Technique

Direct Plating Method

1. Prepare plates of Tryptone Bile Agar and dry the surface.
2. Place a cellulose acetate filter membrane (85 mm diameter, 0.45 µm pore size), which need not be sterilised, on the surface of the medium. Gently flatten with a sterile spreader to remove trapped air.
3. Prepare the food sample by diluting 1 in 5 or 1 in 10 with 0.1% (w/v) sterile Peptone Water CM0009 and homogenise in a 'Stomacher' or a laboratory blender[8].
4. Pipette 0.5 or 1.0 ml of the homogenate onto the membrane and spread over the surface with a sterile glass spreader.
5. Allow the homogenate to soak in and incubate plates, stacked not more than three high, with lids uppermost, for 18-24 hours in a water-jacketed incubator at 44 ± 1°C.
6. Remove the plates from the incubator and pipette 1-2 ml of the indole reagent into each labelled lid.
7. Lift the membrane with a pair of forceps from the plate and lower onto the reagent.
8. Place the stained membranes in direct sunlight or under a low pressure uv lamp for 5-10 minutes. Indole positive colonies are stained pink.
9. Multiply the number of pink colonies by the dilution factor and express the result as the number of *Escherichia coli* per gram of food.
10. The 'stained' membrane may be 'fixed' by prolonged drying in direct sunlight or under a uv lamp, and kept for reference.

Resuscitation Procedure

1. Preparation of Minerals Modified Glutamate Agar plates.

   Make up 1 litre of Minerals Modified Glutamate Medium CM0607 and add 12 g of Agar No.1 LP0011. Bring gently to the boil until dissolved completely and sterilise by autoclaving at 116°C for 10 minutes. Cool to 50°C and pour 12-15 ml of the medium into sterile dishes.
2. Place a cellulose acetate filter membrane onto the well dried surface of a plate of Minerals Modified Glutamate Agar. Gently flatten with a sterile spreader to remove trapped air.
3. Prepare the food sample by diluting 1 in 5 or 1 in 10 with 0.1% (w/v) Peptone Water CM0009 and homogenise in a 'Stomacher' or a laboratory blender.
4. Pipette 0.5 or 1.0 ml of the homogenate onto the membrane and spread completely over the surface with a sterile glass spreader.
5. Allow the homogenate to soak in, and incubate the plates with the lids uppermost in piles of not more than three for 4 hours at 35°C.
6. Transfer the membrane filter from the plate using sterile forceps and gently lower onto the dried surface of a Tryptone Bile Agar plate.
7. Incubate the plates as described for the Direct Plating Method, strain, and count the number of pink indole positive colonies.

If required the unstained plates may be placed in the refrigerator overnight and the indole test carried out the following morning.

Indole Reagent

5% p-dimethylaminobenzaldehyde in 1N hydrochloric acid.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared plates at 2-8°C.

Appearance

Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Light straw coloured gel.

Quality control

Positive control:	***Expected results***
Escherichia coli ATCC® 25922*	Good growth; straw coloured colonies; indole positive
Negative control:	
Enterobacter aerogenes ATCC® 13048*	Good growth; straw coloured colonies; indole negative

*This organism is available as a Culti-Loop®

References

1. Anderson J. M. and Baird-Parker A. C. (1975) *J. Appl. Bact.* 39. 111-117.
2. Delaney J. E., McCarthy J. A. and Grasso R. J. (1962) *Wat. Sewage Works* 109. 289.
3. Ewing W. H. (1972) COC Atlanta, US Dept. of Health, Education & Welfare.
4. International Commission on Microbiological Specifications for Foods (1979) *Can. J. Microbiol.* 25. 1321-1327.
5. Holbrook R., Anderson J. M. and Baird-Parker A. C. (1980) *Food Technol. in Aust.* 32. 78-83.
6. Clarke P. H. and Cowen S. T. (1952) *J. Gen. Microbiol.* 6. 187-197.
7. Vracko R. and Sherris J. C. (1963) *Amer. J. Clin. Path.* 39. 429-432.
8. Sharpe A. N. and Jackson A. K. (1972) *Appl. Microbiol.* 24. 175-178.

TRYPTONE BILE X-GLUCORONIDE MEDIUM – see TBX

TRYPTONE GLUCOSE EXTRACT AGAR

Code: CM0127

For the plate count of water, dairy products and for the detection of thermophilic organisms.

Formula	***gm/litre***
'Lab-Lemco' powder	3.0
Tryptone	5.0
Glucose	1.0
Agar	15.0
pH 7.0 ± 0.2	

Directions

Suspend 24 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Mix well before pouring. When the dilution of the original specimen is greater than 1 in 10, add 10 ml of sterile 10% solution of Skim Milk Powder LP0031 per litre.

Description

This medium, of American origin, is recommended for the plate count of water and dairy products[1,2]. It can also be used for the detection of thermophilic bacteria in dairy products. The Standard Methods Committee of the American Public Health Association recommended that sterile milk should be added to this medium only when the dilution of the original specimen was greater than 1 in 10. The addition of 10 ml of a sterile 10% solution of Skim Milk Powder LP0031 per litre is suitable for this purpose. It is essential that this medium is not overheated during sterilisation.

See also Plate Count Agar CM0325.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance

Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured gel.

Quality Control

Positive control:	***Expected result***
Enterococcus faecalis ATCC® 19433*	Good growth; white/cream colonies
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

Quality Control
Compare with previous lot/batch of medium using samples of pasteurised and unpasteurised milk.

References

1. American Public Health Association (1980) Standard Methods for the Examination of Water and Wastewater. 15th Edn. APHA Inc. Washington DC.
2. American Public Hea.lth Association (1972) Standard Methods for the Examination of Dairy Products. 13th Edn. APHA Inc. Washington DC.

TRYPTONE SOYA AGAR

Code: CM0131

A general purpose medium for the growth of a wide variety of organisms.

Formula	***gm/litre***
Tryptone	15.0
Soya peptone	5.0
Sodium chloride	5.0
Agar	15.0
pH 7.3 ± 0.2	

Directions
Add 40 g to 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

Description
A general purpose agar medium, containing two peptones, which will support the growth of a wide variety of organisms. It is suitable for the cultivation both of aerobes and anaerobes, the latter being grown either in deep cultures or by incubation under anaerobic conditions. The medium may also be used as a blood agar base – for this purpose 7% of sterile blood should be added to the sterile molten medium which has been cooled to approximately 45°C. Tryptone Soya Agar can also be used for the preparation of 'chocolate' agar.

Since Tryptone Soya Agar contains no added carbohydrate it may be used, with added blood, in the determination of haemolysis.

Horse blood agar plates prepared with Oxoid Tryptone Soya Agar are used for the colicine typing of *Shigella sonnei*[1,2,3,4].

The Oxoid medium has also been used as a replacement for yeastrel-milk agar plates in the Lisboa test[5] and for bacterial counts on eviscerated poultry[6].

When supplemented with 0.7 g lecithin and 5 g Polysorbate (Tween 80) per litre of Tryptone Soya Agar, the medium can be used as Microbial Content Test Agar for testing quaternary ammonium compounds[7].

Tryptone Soya Agar is recommended as a reference medium when testing selective media, to measure the degree of inhibition[8].

A medium for isolation of *Bacteroides gracilis* is prepared from Tryptone Soya Agar by adding formate, fumarate and nitrate. The medium is made selective using nalidixic acid and teicoplanin[9].

Enchanced haemolysis agar (EHA) used to improve detection of *Listeria monocytogenes* when present amongst other listeriae has been modified to optimise its performance by substituting Tryptone Soya Agar for Columbia Agar in the original formulation[10].

Oxoid Tryptone Soya Agar conforms to formulations detailed in various international pharmacopoeia[11,12,13,14].

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared plates of medium at 2-8°C.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Light straw to straw coloured gel.

Quality control

Positive controls:	***Expected results***
Staphylococcus aureus ATCC® 25923*	Good growth; straw coloured colonies
Streptococcus pyogenes ATCC® 19615*	Good growth; pale straw coloured colonies
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

Precautions
It should be noted that haemolytic reactions of streptococci on Tryptone Soya Agar can vary according to the origin of the blood e.g. horse or sheep. Tryptone Soya Agar designed for sheep blood show significant differences when used with horse blood and vice versa.

References

1. Abbott J. D. and Graham J. M. (1961) *Mon. Bull. Min. Hlth Pub. Hlth Lab. Serv.* 20. 51-58.
2. Barrow G. I. and Ellis C. (1962) *Mon. Bull. Min. Hlth Pub. Hlth Lab. Serv.* 21. 141-147.
3. Cooke G. T. and Daines C. F. (1964) *Mon. Bull. Min. Hlth Publ. Hlth Lab. Serv*. 23. 81-85.
4. Gillies R. R. (1964) *J. Hyg. Camb.* 62. 1-9.
5. Mitchell T. G. (1964) *J. Appl. Bact.* 27. 45-52.
6. Barnes Ella M. and Shrimpton D. H. (1958) *J. Appl. Bact.* 2. 313-329.
7. American Public Health Association (1978) *Standard Methods for the Examination of Dairy Products*. 14th edn. APHA Inc. Washington DC.
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11. *British Pharmacopoeia* Volume II (2000).
12. *US Pharmacopoeia* XXIV, (2000).
13. *European Pharmacopoeia*. 3rd Edition (2001).
14. *Japanese Pharmacopoeia*. 12th Edition. (2000).

TRYPTONE SOYA BROTH – SOYBEAN CASEIN DIGEST MEDIUM USP

Code: CM0129

A highly nutritious general purpose medium for the growth of bacteria and fungi.

Formula	***gm/litre***
Pancreatic digest of casein	17.0
Papaic digest of soybean meal	3.0
Sodium chloride	5.0
Dibasic potassium phosphate	2.5
Glucose	2.5
pH 7.3 ± 0.2	

Directions
Add 30 g to 1 litre of distilled water, mix well and distribute into final containers. Sterilise by autoclaving at 121°C for 15 minutes.

Description
A highly nutritious versatile medium which is recommended for general laboratory use. Due to the inclusion

of both Tryptone and Soya Peptone, the medium will support a luxuriant growth of many fastidious organisms without the addition of serum, etc.

Oxoid Tryptone Soya Broth conforms to formulations detailed in various international pharmacopoeia[1,4,5,6].

Technique

Aerobic Cultivation
Tryptone Soya Broth may be used for the cultivation of aerobes and facultative anaerobes, including some fungi. Cultures should be examined at frequent intervals, as maximum growth is reached earlier than with less nutritious media and the phase of decline consequently begins sooner.

Anaerobic Cultivation
The addition of a small amount of agar (e.g. up to one agar tablet CM0049 to every 100 ml of reconstituted Tryptone Soya Broth, prior to sterilisation) renders the broth suitable for the cultivation of obligatory anaerobes, such as *Clostridium* species. For this purpose, the broth (with added agar) should be used soon after sterilisation, or, heated and cooled just before inoculation.

Blood Culture
The superior growth-promoting properties of Tryptone Soya Broth make it especially useful for the isolation of organisms from blood or other body fluids. Anticoagulants such as 'liquoid'† (sodium polyanethyl sulphonate) or sodium citrate may be added to the broth prior to sterilisation. Five to 10 ml of blood may be added to 50 ml of medium.

Tryptone Soya Broth is especially suitable for the tube dilution method of antibiotic susceptibility testing.

Oxoid laboratory tests have shown that Tryptone Soya Broth has a greater ability to resuscitate heated spores of *Bacillus stearothermophilus* than Dextrose Tryptone Broth. Tryptone Soya Broth is recommended in the USP XXIV (Soybean Casein Digest Medium) for the recovery of organisms after sterilisation processes[1]. A positive result is revealed after 24-48 hours incubation at 55°C, by a heavy growth of organisms causing turbidity in the broth.

Selective Culture Media
Tryptone Soya Broth is used in food bacteriology as the basal medium to which a variety selective agents are added for selective enrichment of *Staphylococcus aureus*[2] and *Escherichia coli* O157[3].

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at room temperature.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured solution.

Quality control

Positive controls:	***Expected results***
Streptococcus pneumoniae ATCC® 6305*	Turbid growth
Staphylococcus aureus ATCC® 25923*	Turbid growth
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

References

1. US Pharmacopoeia. XXIV. (2000) Bethesda MD.
2. *Compendium of Methods for the Microbiological Examination of Foods*. 3rd edition. Vanderzant C. and Splittstoesser D. F. (eds). APHA. Washington DC.
3. *Practical Food Microbiology*. Roberts D., Hooper W. and Greenwood M. (eds). Public Health Laboratory Service, London 1995.
4. *British Pharmacopoeia* Volume II (2000).
5. *European Pharmacopoeia*. 3rd Edition (2001).
6. *Japanese Pharmacopoeia*. 12th Edition. (2000).

†Roche Products Ltd. Welwyn Garden City, Herts.

TRYPTONSE SOYA BROTH (COLD FILTERABLE) – see COLD FILTERABLE TRYPTONE SOYA BROTH

TRYPTONE SOYA BROTH MODIFIED (mTSB)

Code: CM0989

Two products designed specifically for the selective enrichment of Escherichia coli O157 from food or faecal samples

Formula	***gm/litre***
Pancreatic digest of casein	17.0
Papaic digest of soybean meal	3.0
Sodium chloride	5.0
Dipotassium hydrogen phosphate	4.0
Glucose	2.5
Bile Salts	1.5
pH 7.4 ± 0.2	

VCC SELECTIVE SUPPLEMENT

Code: SR0190

Vial contents: (each vial is sufficient for 225 ml of medium)	***per vial***	***per litre***
Vancomycin	2.0 mg	8.0 mg
Cefixime	0.0125 mg	0.05 mg
Cefsulodin	2.5 mg	10.0 mg

Directions

Suspend 33 g of Modified Tryptone Soya Broth in 1 litre of distilled water. Mix well, dispense into final containers and sterilise by autoclaving at 121°C for 15 minutes. Allow the medium to cool to 50°C before adding a selective supplement as appropriate.

Description

Many selective broths have been formulated for the enrichment of *Escherichia coli* O157. Modified Tryptone Soya Broth was devised by Doyle and Shoeni[1]. The broth was incubated for at 37°C for 24 hours in shaken flasks. Szabo and co-workers[2] also used mTSB and found incubation at 43°C with agitation gave better recovery rates from inoculated food samples.

Buffered Peptone Water (BPW) CM0509, supplemented with VCC Selective Supplement SR0190 was used for the isolation of *Escherichia coli* O157 from beef carcasses, bovine faeces and from milk and minced beef[3].

In EHEC enrichment broth (EEB), VCC Selective Supplement SR0190 is added to mTSB, which is used instead of BPW CM0509 to overcome some of the inhibitory effects of the antibiotics on *Escherichia coli* O1574.

Both BPW-VCC CM0509 + SR0190 and EEB CM0989 + SR0190 are incubated for 6 hours at 37°C3.

Tryptone Soya Broth CM0129 has been modified by increasing the concentration of the buffer, dipotassium hydrogen phosphate, to 4 g/l and the addition of 1.5 g/l Bile Salts. The bile salts inhibit the growth of spore formers and enterococci, but allow the growth of *Escherichia coli* O157.

VCC Selective Supplement SR0190 contains three antibiotics vancomycin for inhibition of Gram-positive organisms. Cefixime for the inhibition of *Proteus* spp. cefsulodin suppresses *Pseudomonas* spp.

Technique

Modified Tryptone Soya Broth is a nutritious, selective base, which can be used with either Novobiocin Selective Supplement SR0181 or VCC Selective Supplement SR0190 depending on which protocol is followed.

VCC Selective Supplement SR0190 is a highly selective supplement, which can be used either with Modified Tryptone Soya Broth, to give EHEC Enrichment Broth (EEB), or with Buffered Peptone Water CM0509 depending on which protocol is followed.

1. ISO[5]/PHLS[6]/FDA/BAM[7]/NMKL[9]

mTSB+n
500 ml of Modified Tryptone Soya Broth and 1 vial of Novobiocin Selective Supplement SR0181. Incubate at 41.5°C for 6 and 22 hours.

2. FDA/BAM[7]

EEB
225 ml of Modified Tryptone Soya Broth and 1 vial of VCC Selective Supplement SR0190. Incubate at 37°C with agitation for 6 and 24 hours.

3. CCFRA[8]

BPW-VCC
225 ml of Buffered Peptone Water CM0509 and 1 vial of VCC Selective Supplement SR0190. Incubate at 37°C for 6 and 24 hours.

Storage conditions and Shelf Life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Prepared medium should only be used fresh.

Appearance
Dehydrated Medium: Straw coloured free flowing powder

Prepared medium: Staw coloured solution.

Quality control

Positive controls:	***Expected results***
Escherichia coli 0157:H7 NCTC 12900	Turbid growth
Negative control:	
Staphylococcus aureus ATCC® 25923*	Inhibited or no growth

This organism is available as a Culti-Loop®.

References

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2. Szabo R. E. *et al* (1990) *Applied Environmental Microbiology* 56: 3546-3549
3. Desmarchelier, P. M. and Grau, F. H. (1997) *Escherichia coli.* In: *Foodborne Microorganisms of Public Health Significance*. 5th Edition. pp.231-264. A. D. Hocking (Ed.). AIFST (NSW Branch) Food Microbiology Group, Australia
4. Weagant S. D. *et al* (1995) *Journal of Food Protection* 58: 7-12
5. ISO:16654:2001 Microbiology of food and animal feed stuffs. Horizontal method for the detection of E. coli O157 (Pub March 22, 1999, expired June 30 1999)
6. PHLS Standard Methods F17 & W16: Detection of *E. coli* O157
7. FDA/BAM 8th Edition (Revision A) (1998) Chapter 4: 4.20-4.26
8. Campden and Chorleywood Food Research Association Method 19.1: 1997. Detection of *E. coli* O157
9. Nordic Committee on Food Analysis No 164 1999 *Escherichia coli* O157. Detection in food and feeding stuffs

TSC AGAR – see TRYPTONE SULPHITE CYCLOSERINE AGAR

TRYPTONE WATER

Code: CM0087

A liquid medium for the production of indole by micro-organisms.

Formula	***gm/litre***
Tryptone	10.0
Sodium chloride	5.0
pH 7.5 ± 0.2	

Directions
Dissolve 15 g in 1 litre of distilled water and distribute into final containers. Sterilise by autoclaving at 121°C for 15 minutes.

Description
Tryptone Water is a good substrate for the production of indole because of its high content of tryptophan and it is more reliable than Peptone Water for this purpose. The ability of certain organisms to break down the amino-acid tryptophan with formation of indole is an important property which is used for the classification and identification of bacteria[1,2].

Technique

Kovac's reagent:	
paradimethylaminobenzaldehyde	5 g
amyl alcohol	75 ml
concentrated hydrochloric acid	25 ml

1. Inoculate tubes of Tryptone Water, and incubate for 24-48 hours at 35°C.

2. Add 0.2 ml of Kovac's reagent and shake. Allow to stand for 10 minutes and observe the result.

A dark red colour in the amyl alcohol surface layer constitutes a positive indole test; no change in the original colour of the reagent constitutes a negative test.

Ehrlich reagent:	
paradimethylaminobenzaldehyde	4 g
absolute alcohol	380 ml
concentrated hydrochloric acid	80 ml

1. Inoculate tubes of Tryptone Water, and incubate for 24-48 hours at 35°C.

2. Withdraw a small portion of the culture and add an equal volume of the reagent. Shake the mixture and allow to stand for a few minutes; a rose colour indicates the presence of indole. If indole is present, this colour change may be accelerated by the addition of saturated potassium persulphate solution.

As an alternative, extract the indole by shaking the cultures with 1 ml of ether, allow the mixture to stand for a few minutes and then add 0.5 ml of Ehrlich reagent.

Incubation at 44°C for 24 hours has the specific advantage of detecting *Escherichia coli*, as this is the only organism present in water capable of producing indole at this temperature[3].

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at room temperature.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Light straw coloured solution.

Quality control

Positive control:	***Expected result***
Escherichia coli ATCC® 25922*	Turbid growth; indole positive
Negative control:	
Enterobacter aerogenes ATCC® 13048*	Turbid growth; indole negative

* This organism is available as a Culti-Loop®

References
1. American Public Health Association (1998) S*tandard Methods for the Examination of Water and Wastewater.* 20th edn. APHA Inc. Washington DC.
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TRYPTOSE BLOOD AGAR BASE

Code: CM0233

A highly nutritious medium specially developed for the preparation of a blood agar which will support the growth of fastidious organisms.

Formula	***gm/litre***
Tryptose	10.0
'Lab-Lemco' powder	3.0
Sodium chloride	5.0
Agar	12.0
pH 7.2 ± 0.2	

Directions

Suspend 30 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

For blood agar, cool the basal medium to 45-50°C and add 7% of sterile blood. Mix thoroughly, taking care to avoid incorporation of air bubbles, and dispense into Petri dishes or other containers.

Description

A highly nutritious medium specially developed by Casman[1,2] for the preparation of a blood agar which will support the growth of many fastidious organisms.

The original formulation included 0.3 g dextrose per litre which interfered with the haemolytic reactions. It is now used without dextrose as a standard medium[3].

Tryptose Blood Agar Base with added blood gives good haemolytic reactions and without blood it will sustain good to excellent growth of many demanding organisms. However, to improve the growth of certain organisms e.g. *Neisseria meningitidis* and *Streptococcus pneumoniae*, the addition of 1 g of yeast extract (LP0021) to one litre of Tryptose Blood Agar Base can be made.

Technique

Lightly inoculate the surface of the medium and incubate aerobically at 35°C for 18-24 hours in 5-10% carbon dioxide (if necessary) or incubate anaerobically at 35°C for 48 hours to enhance haemolysis and to restrict unwanted commensal growth.

Carry out microscopy and other identification tests on the isolated colonies.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared plates of medium at 2-8°C.

Appearance

Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured gel.

Quality control

Positive controls:	***Expected result***
Staphylococcus aureus ATCC® 25923*	Good growth; white colonies
Streptococcus pyogenes ATCC® 19615*	Good growth; pale straw coloured colonies; zone of β-haemolysis
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

References

1. Casman E. P. (1942) *J. Bact.* 43. 33-37.
2. Casman E. P. (1947) *Amer. J. Clin. Path.* 17. 281-282.
3. American Public Health Association (1970) *Diagnostic Procedures and Reagents*. 5th edn. APHA Inc. New York.

TRYPTOSE PHOSPHATE BROTH

Code: CM0283

A buffered glucose broth for the cultivation of fastidious bacteria.

Formula	***gm/litre***
Tryptose	20.0
Glucose	2.0
Sodium chloride	5.0
Disodium hydrogen phosphate	2.5
pH 7.3 ± 0.2	

Directions

Dissolve 29.5 g in 1 litre of warm distilled water and distribute into final containers. Sterilise by autoclaving at 121°C for 15 minutes.

For the cultivation of anaerobes, if the reconstituted medium has been stored prior to use, remove dissolved oxygen by placing the tubes in a boiling water bath for 15 minutes and cool without agitation before inoculation.

Description

A buffered dextrose broth for use as an adjuvant to tissue culture media and for the cultivation of fastidious bacteria. Ginsberg *et al*.[1] maintained tissue cultures of HeLa cells for at least 10 days in a mixture of Tryptose Phosphate Broth 15-25%, Scheren maintenance solution 67.5-77.5% and chicken serum 7.5%. The cells increased 3-5 fold in number during this period, smaller quantities of ARD, AD and type 1 poliomyelitis virus could be detected and more ARD virus could be propagated in HeLa cells in the Tryptose Phosphate.

Broth supplemented medium

Tryptose Phosphate Broth is recommended for the cultivation of *Streptococci*, *Pneumococci*, *Meningococci* and other fastidious organisms. Tryptose Phosphate Broth with added agar and sodium azide is recommended for the isolation of pathogenic streptococci from cheese and other dairy products[2,3].

Tryptose Phosphate Broth with added agar is also recommended by the American Public Health Association for the examination of throat swabs and blood for *Streptococcus pneumoniae* and as a growth medium for *Pneumococci* prior to the bile solubility test.

Tryptose Phosphate Agar Broth may also be employed for the emulsification of cheese prior to the plate isolation method for *Brucella* species[2].

The small proportion of agar (0.1-0.2%) necessary for the above methods may be most conveniently added to the Oxoid broth before sterilisation, in the form of Oxoid Agar Tablets CM0049 (one tablet per 100ml).

Technique

Examination of throat swabs for *Streptococcus pneumoniae*[4]

1. Prepare Tryptose Phosphate Broth in the usual manner but add a small amount of agar by dissolving 10 Agar Tablets CM0049 per litre of broth before autoclaving.
2. Place a pharyngeal or sputum swab in 3 ml of Tryptose Phosphate (Agar) Broth and incubate for 2 hours at 35°C. If sufficient *Pneumococci* are present, type directly or preferably employ the culture for mouse inoculation.

Examination of cheese for Lancefield Group A *Streptococci*[2]

1. Heat 25 ml of Tryptose Phosphate (Agar) Broth (prepared as above) to 45°C.
2. Immediately emulsify 5 g of cheese in the broth, using a heavy sterile glass rod. Plate suitable dilutions of the emulsion on Tryptose Agar plates containing 1.5% agar and 0.04% of sodium azide.

Alternatively, add sterile aqueous sodium azide to the emulsion to give a final concentration of 2.5%. Incubate for 12-14 hours at 35°C, then shake and plate on Tryptose Agar plates containing 1.5% agar and 0.04% of sodium azide.

The sodium azide suppresses the growth of most bacteria except *Streptococci* and some *Lactobacilli*.

Blood Culture

1. Add up to 10 ml of blood to 150 ml of Tryptose Phosphate Broth in a 300 ml flask or bottle.
2. Incubate and sub-culture onto other media in the usual manner, according to the exact purpose of the investigation.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Light straw coloured solution.

Quality control

Positive control:	***Expected results***
Streptococcus pneumoniae ATCC® 6303*	Turbid growth
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

References

1. Ginsberg H. S. *et al*. (1955) *Proc. Soc. Exper. Biol. Med.* 89. 66-71.
2. American Public Health Association (1953) *Standard Methods for the Examination of Dairy Products* 10th edn., APHA Inc., New York, pp.179, 180, 181.
3. Newman R. W. (1950) *J. Milk Food Tech.* 13. 226-233.
4. American Public Health Association (1953) *Diagnostic Procedures and Reagents* 4th edn., APHA Inc., New York, p.141.

TRYPTOSE SULPHITE CYCLOSERINE AGAR

Code: CM0587

A basal medium for use with selective agents to make either TSC agar or SFP agar for the presumptive identification and enumeration of Clostridium perfringens.

Formula	***gm/litre***
Tryptose	15.0
Soya peptone	5.0
Yeast extract	5.0
Sodium metabisulphite	1.0
Ferric ammonium citrate	1.0
Agar	19.0
Final pH 7.6 ± 0.2	

PERFRINGENS (SFP) SELECTIVE SUPPLEMENT

Code: SR0093

Vial contents (each vial is sufficient for 500 ml of medium)	***per vial***	***per litre***
Kanamycin sulphate	6.0 mg	12 mg
Polymyxin B	15,000 IU	30,000 IU

PERFRINGENS (TSC) SELECTIVE SUPPLEMENT B

Code: SR0088

Vial contents (each vial is sufficient for 500 ml of medium)	***per vial***	***per litre***
D-cycloserine	200 mg	400 mg

Directions

To Prepare the Agar Base
Suspend 23 g in 500 ml of distilled water and heat gently until the agar is completely dissolved. Sterilise by autoclaving at 121°C for 10 minutes. Allow the medium to cool to 50°C.

To Prepare Tryptose Sulphite Cycloserine Agar (TSC Agar)
To 500 ml of Agar base cooled to 50°C add the rehydrated contents of 1 vial of TSC supplement, SR0088 and 25 ml of egg yolk emulsion, SR0047. Mix well and pour into sterile Petri dishes.

To Prepare Egg Yolk Free TSC Agar
To 500 ml of Agar base cooled to 50°C add the rehydrated contents of 1 vial of TSC supplement SR0088. Mix well and pour into sterile Petri dishes.

To Prepare Shahidi-Ferguson Perfringens Agar (SFP Agar)
To 500 ml of Agar base cooled to 50°C add the rehydrated contents of 1 vial of SFP supplement SR0093 and 25 ml of egg yolk emulsion SR0047, mix well and pour into sterile Petri dishes.

To Prepare Agar for an Overlay
For TSC or SFP Agar used as an overlay, the egg yolk emulsion, SR0047, is omitted. Its inclusion does not improve the lecithinase reaction and diminishes the visibility of the colonies.

Description
Perfringens Agar Base (TSC and SFP) CM0587 is a nutrient medium to which is added egg yolk emulsion SR0047 and the appropriate antibiotic supplement to prepare either Shahidi-Ferguson Perfringens (SFP) Agar using SR0093 or Tryptose Sulphite Cycloserine (TSC) Agar using SR0088.

An egg yolk-free TSC Agar had been described[4,5] which has the advantage that smaller colonies are formed. This can simplify the counting of plates with high numbers of colonies. Higher counts have been demonstrated by using it with a pour plate technique. The differences were thought to be due to exposure of the *Clostridium perfringens* cells to high oxygen tension in the surface plating procedure[4].

Shahidi-Ferguson Perfringens Agar is based on the formulation developed by Shahidi and Ferguson[1]. The medium utilises kanamycin sulphate (12 mg/litre) and polymyxin B sulphate (30,000 IU/litre) as the selective agents to give a high degree of selectivity and specificity for *Clostridium perfringens*.

Tryptose Sulphite Cycloserine Agar was developed using the same basal medium as SFP Agar[2] but with 400 mg/litre of D-cycloserine as the selective agent.

Sodium metabisulphite and ferric ammonium citrate are used as an indicator of sulphite reduction by *Clostridium perfringens* which produces black colonies in both media.

Trials[3] have indicated that polymyxin B and kanamycin sulphate used in SFP Agar allow a greater recovery of both vegetative cells and spores of *Clostridium perfringens* than either polymyxin B and sulphadiazine used in Sulphite Polymyxin Sulphadiazine Agar, or neomycin, used in Tryptone Sulphite Neomycin Agar. However, a greater number of non-specific colonies were found on SFP Agar.

In another study[2], *Serratia marcescens* and *Streptococcus lactis* were the only faculative anaerobes to grow on TSC Agar, whereas SFP Agar also allowed the growth of *Enterococcus*, *Proteus* and *Enterobacter* strains, but allowed a slightly higher rate of recovery of *Clostridium perfringens* than TSC Agar.

Both SFP Agar and TSC Agar permitted growth of other sulphite-reducing *Clostridium* species tested.

Some strains of *Clostridium perfringens* may produce an opaque zone around the colony due to lecithinase activity, but this is not considered to be universal for all *Clostridium perfringens* strains after overnight incubation[4] and both black lecithinase-positive and black lecithinase-negative colonies should be considered as presumptive *Clostridium perfringens* on TSC or SFP Agars and confirmatory tests carried out. Egg yolk positive facultative anaerobes may grow on SFP Agar to produce completely opaque plates thus masking the egg yolk reaction of *Clostridium perfringens*.

Technique

1. Make up the medium according to the directions and prepare plates containing approximately 20ml of a basal layer of TSC or SFP Agar containing egg yolk.
2. Prepare 0.1 ml aliquots of a suitable series of dilutions of the homogenised test sample and spread over the surface of the basal layer using a sterile swab.
3. Overlay with an additional 10 ml of egg yolk free TSC or SFP Agar. Cultures which are not overlaid with agar are unlikely to grow as black colonies.
4. Incubate the plates at 35°C for 18-24 hours with an anaerobic Gas Generating Kit, BR0038, in a gas-jar. Alternatively use AnaeroGen AN0025A or AN0035A. AnaeroGen does not require the addition of water or a catalyst.

Alternatively, pour-plates using approximately 25ml per plate of TSC or SFP Agar containing egg yolk may be prepared using 1 ml aliquots of a suitable series of dilutions of the homogenised test sample. Mix the plates well before the agar gels. With this technique, lecithinase activity of *Clostridium perfringens* colonies is more difficult to see. *Clostridium perfringens* colonies may be seen as large, black (2-4 mm diameter) colonies within the depth of the agar.

Egg yolk-free TSC Agar is used with the techniques described above. *Clostridium perfringens* colonies are black but in the absence of egg yolk no lecithinase activity can be detected.

Tests for confirmation are described in a study initiated by the International Commission on Microbiological Specifications for Foods[6] involving nitrate reduction, lactose fermentation, gelatin liquefaction and the absence of motility. All black colonies growing on TSC or SFP Agars should be tested.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance

Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw/green coloured gel.

Quality control

Positive control:	***Expected results***
Clostridium perfringens ATCC® 13124*	Good growth; black coloured colonies with opaque halo
Negative control:	
Escherichia coli ATCC® 25922*	Inhibited

*This organism is available as a Culti-Loop®

Precautions

Black colonies appearing on these two media may be organisms other than *Clostridium perfringens*.

References

1. Shahidi S. A. and Ferguson A. R. (1971) *Appl. Microbiol.* 21. 500-506.
2. Harmon S. M., Kauttar D. A. and Peeler J. T. (1971) *Appl. Micobiol.* 22. 688-692.
3. Harmon S. M., Kautter D. A. and Peeler J. T. (1971) *Appl. Microbiol.* 21. 922-927.
4. Hauschild A. H. W. and Hilsheimar R. (1973) *Appl. Microbiol.* 27. 78-82.
5. Hauschild A. H. W. and Hilsheimar R. (1973) *Appl. Microbiol.* 27. 521-526.
6. Hauschild A. H. W., Gilbert R. J., Harmon S. M., O'Keefe M. F. and Vahlfeld R. (1977) *Can. J. Microbiol*. 23. 884-892.

UNIVERSAL BEER AGAR

Code: CM0651

For the isolation of beer spoilage organisms.

Formula	***gm/litre***
Peptonised milk	15.0
Yeast extract	6.1
Glucose	16.1
Tomato supplement	12.2
Dipotassium hydrogen phosphate	0.31
Potassium dihydrogen phosphate	0.31
Sodium chloride	0.006
Ferrous sulphate	0.006
Manganese sulphate	0.006
Magnesium sulphate	0.12
Agar	12.0
pH 6.1 ± 0.2	

Directions

Suspend 62 g in 750 ml of distilled water and bring to the boil to dissolve completely. Add 250 ml beer, without degassing, to the hot medium and mix gently. Distribute into final containers and sterilise by autoclaving at 121°C for 10 minutes.

Description

Universal Beer Agar is presented as a basal medium to which beer alone or beer and cycloheximide may be added for the detection and culture of microbial contaminants in beer. The medium is based on the formula developed by Kozulis and Page[1], who recommended that beer must be incorporated in the medium in order to increase selectivity by stimulating the growth of beer spoilage organisms. The presence of hop constituents and alcohol eliminates many airborne contaminants not originating in pitching yeasts, wort or beer; thus minimising false positive results.

Oxoid Universal Beer Agar supports the growth of *Lactobacilli*, *Pediococci*, *Acetobacter*, *Zymomonas* species and wild yeast strains which may be found infecting the pitching yeast, the cooled wort, or during fermentation or storage of the finished beer.

Technique

The presence of microbial spoilage organisms in pitching yeast, the cooled wort or beer in storage may be detected and enumerated using Universal Beer Agar. Either direct surface plating or pour plate techniques with serial dilutions of the sample can be employed. Plates are incubated both aerobically to detect *Acetobacter* species and anaerobically to detect the micro-aerophilic *Lactobacilli* and *Pediococci* species as well as the anaerobic *Zymomonas* spp. Plates are incubated at 28-30°C for three days and examined daily.

To increase the differentiation of the colonies, Bromocresol Green (20 mg/litre) and powdered chalk (3 g/litre) may be added to the medium before sterilisation[2]. Zones of decolorisation are seen around *Pediococcus* and some *Lactobacillus* colonies.

The addition of cycloheximide (SR0222) at 0.001 g/litre to suppress yeast growth gives a medium that is selective for the detection of bacterial contaminants in yeast cultures.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance

Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured gel.

Quality control

Positive controls:	***Expected results***
Lactobacillus fermentum ATCC® 9338	Good growth; straw coloured colonies
Negative control:	
Saccharomyces cerevisciae ATCC® 9763* (when cycloheximide is added to the medium)	Inhibited

*This organism is available as a Culti-Loop®

Precautions
When handling cycloheximide observe the precautions to be taken under HAZARDS.

References
1. Kozulis J. A. and Page H. E. (1968) *Proc. Am. Soc. Brew. Chem.* 52-58.
2. Boatwright J. and Kirsop B. H. (1976) *J. Inst. Brew.* 82. 343-346.

UREA AGAR BASE

Code: CM0053

An agar base for the preparation of Christensen's medium to detect rapid urease activity of the Proteae and non-rapid urease activity of some Enterobacteriaceae.

Formula	***gm/litre***
Peptone	1.0
Glucose	1.0
Sodium chloride	5.0
Disodium phosphate	1.2
Potassium dihydrogen phosphate	0.8
Phenol red	0.012
Agar	15.0
pH 6.8 ± 0.2	

Directions
Suspend 2.4 g in 95 ml of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 115°C for 20 minutes. Cool to 50°C and aseptically introduce 5 ml of sterile 40% Urea Solution SR0020. Mix well, distribute 10 ml amounts into sterile containers and allow to set in the slope position.

Description
Urea Agar Base is recommended for the preparation of Christensen medium[1] for the detection of rapid urease activity of the urease-positive *Proteae*. The urea medium may be used for the detection of urea hydrolysis by some other Enterobacteriaceae but the incubation period is much longer, 24-48 hours.

Technique
Heavily inoculate the surface of a Urea Agar slope with a pure culture of the organism to be tested. When inoculated with urease-positive Proteae the reaction is usually complete after 3-5 hours at 35°C: urease-producing organisms hydrolyse the urea to form ammonia, and the medium changes from orange to pink. 40% Urea Solution SR0020 is supplied, as a sterile solution in ampoules, for the convenient preparation of this medium.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Orange coloured gel.

Quality control

Positive control:	***Expected results***
Proteus mirabilis ATCC® 29906*	Urease positive; pink slope
Negative control:	
Escherichia coli ATCC® 25922*	Urease negative; no colour change

*This organism is available as a Culti-Loop®

Precautions
The alkaline reaction produced in this medium after prolonged incubation may not be caused by urease activity. Check using medium without urea.

Do not heat or reheat the medium because urea decomposes very easily.

For the detection of urease-positive *Proteae* the reaction must be read within the first 2-5 hours of incubation.

Reference
1. Christensen W. B. (1946) *J. Bact.* 52. 461-466.

UREA BROTH BASE

Code: CM0071

A liquid version of Christensen's medium for the differentiation of urease-producing Enterobacteriaceae.

Formula	***gm/litre***
Peptone	1.0
Glucose	1.0
Sodium chloride	5.0
Disodium phosphate	1.2
Potassium dihydrogen phosphate	0.8
Phenol red	0.004
pH 6.8 ± 0.2	

Directions
Add 0.9 g to 95 ml of distilled water. Sterilise by autoclaving at 115°C for 20 minutes. Cool to 55°C and aseptically introduce 5 ml of sterile 40% Urea Solution SR0020. Mix well and distribute 10 ml amounts into sterile containers.

Description
This is a liquid modification of Christensen medium[1]. The modification is suitable for the differentiation of urease-producing organisms from members of the *Salmonella* and *Shigella* groups, during the routine examination of rectal swabs and faeces. Maslen noted that in the routine examination of faeces for *Salmonella* and *Shigella* organisms many non-lactose-fermenting colonies isolated were later found to belong to the urease-positive Proteae. He evolved this medium as a means whereby the latter organisms could be rapidly detected and eliminated – thus saving a considerable amount of time and media. Maslen claimed that the advantages of the fluid medium were:

1. A Pasteur pipette could be used to inoculate other diagnostic media.

2. Rapid growth ensued and it was possible to discern a clear-cut positive reaction within two to five hours at 35°C.

3. It was easier to detect any contamination during storage.

Technique
For the examination of faeces, specimens are cultured in enrichment and selective media in the usual manner. Discrete colonies are then picked off the surface of the solid selective media.

Inoculate tubes of Urea Broth with single colonies of non-lactose-fermenting organisms and incubate for 2 to 6 hours at 35°C. (Maslen states that the cultures should be incubated in a water bath in order to obtain the highest proportion of positive reactions within 5 hours.) Regard all organisms producing a pink coloration in the medium (i.e. due to the alkalinity caused by urea hydrolysis) as not belonging to the *Salmonella* or *Shigella* groups, and discard.

Inoculate all cultures showing no colour change (i.e. no urea hydrolysis) into 'sugar' peptone waters (Andrade Peptone Water CM0061) plus the appropriate carbohydrate and onto a Blood Agar Base CM0055 slope.

Incubate the new cultures, together with the Urea Broth, until the next morning. No further examination is necessary if the urea tube now shows an alkaline reaction (pink colour), otherwise continue the diagnostic tests – including slide agglutinations from the Blood Agar Base culture, if necessary.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Orange coloured solution.

Quality control

Positive control:	***Expected results***
Proteus mirabilis ATCC® 29906	Pink broth; urease positive
Negative control:	
Escherichia coli ATCC® 25922*	No colour change; urease negative

*This organism is available as a Culti-Loop®

Precautions
It is preferable that the medium be used on the day of preparation. If not, examine the tubes carefully to ensure sterility.

After overnight incubation other members of the Enterobacteriaceae may show alkaline reactions.

Reference
1. Maslen L. G. C. (1952) *Brit. Med. J.* 2. 545-546.

UVM MEDIA – see LISTERIA SELECTIVE ENRICHMENT (UVM)

VCC BROTH – see BUFFERED PEPTONE WATER (VCC)

VIOLET RED BILE LACTOSE AGAR

Code: CM0107

A lactose-containing selective medium for the detection and enumeration of coliform organisms in water, food and dairy products.

Formula	***gm/litre***
Yeast extract	3.0
Peptone	7.0
Sodium chloride	5.0
Bile Salts No. 3	1.5
Lactose	10.0
Neutral red	0.03
Crystal violet	0.002
Agar	12.0
pH 7.4 ± 0.2	

Directions
Suspend 38.5 g in 1 litre of distilled water. Bring to the boil. Continue to boil for 2 minutes or for the minimum time necessary to dissolve completely and ensure that there are no remaining flecks of unmelted agar. No further sterilisation is necessary or desirable. Mix well before pouring.

Description

Violet Red Bile Lactose Agar is a selective medium for the detection and enumeration of coliform organisms. The medium has been used for the determination of the coli-aerogenes content of water, milk and other dairy products, dairy equipment, and food products etc[1,2].

Organisms which rapidly attack lactose produce purple colonies surrounded by purple haloes. Non-lactose or late-lactose fermenters produce pale colonies with greenish zones. Other related Gram-negative bacteria may grow but can be suppressed by incubation at >42°C or by anaerobic incubation.

Druce *et al*.[3] found that Violet Red Bile Lactose Agar was as good an indicator of coli-aerogenes bacteria in milk as MacConkey Broth, and that the Oxoid medium was suitable for determining the coli-aerogenes content of milk.

Technique

Druce *et al*. recommended the following procedures:

For the routine determination of the coli-aerogenes content of raw milk, prepare pour-plates containing 1.0, 0.1 and 0.01 ml of the sample in Violet Red Bile Lactose Agar, and incubate for 20-24 hours at 35°C.

For coli-aerogenes counts of pasteurised milk, employ 4 pour-plates of Violet Red Bile Lactose Agar. Divide 10 ml of the sample among three of the plates, and add 1 ml of the sample to the remaining plate. Incubate for 20-24 hours at 30°C. Similarly the examination of rinses and swabs from dairy equipment and apparatus, should include the spreading of 10 ml of solution on each of 3 plates and of 1 ml on a single plate. Coliform organisms form dark red colonies which are 1-2 mm in diameter, usually surrounded by a reddish zone. Occasionally colonies may be considerably smaller (less than 0.5 mm in diameter).

When preparing pour-plates the medium should be freshly made up, cooled to 47°C and used within 3 hours.

An overlay method is helpful to improve the specificity of the medium. In this case a thin layer of cooled molten medium is poured over the inoculated base layer and allowed to set before incubation. Incubation may be carried out at >42°C for 18 hours, 32°C for 24-48 hours or 4°C for 10 days, depending on the temperature characteristics of the organisms to be recovered. For *Escherichia coli* a temperature of 44 ± 1°C is specifically recommended[4].

Characteristic appearance of colonies

Round, purple-red may be surrounded by purple-red haloes (lactose-positive organisms).

Pale, may have greenish haloes (lactose-negative organisms).

Confirmation of the identity of red colonies must be made by further tests.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C and use as freshly as possible.

Appearance

Dehydrated medium: Straw-pink coloured, free-flowing powder.

Prepared medium: Dark purple coloured gel.

Quality control

Positive control:	***Expected results***
Escherichia coli ATCC® 25922*	Good growth; purple-pink colonies with haloes
Negative control:	
Staphylococcus aureus ATCC® 25923*	No growth

*This organism is available as a Culti-Loop®

Precautions

This medium is not completely specific for Enterobacteriaceae, other organisms e.g. *Aeromonas* and *Yersinia* species may give similar reactions.

The selectivity of the medium diminishes after 24 hours incubation and organisms previously suppressed may exhibit growth.

References
1. American Public Health Association (1978) *Standard Methods for the Examination of Dairy Products*. 14th edn. APHA Inc. Washington DC.
2. American Public Health Association (1992) *Compendium of Methods for the Microbiological Examination of Foods* 3rd edn. APHA Inc. Washington DC.
3. Druce R. G., Bebbington N. N., Elson K., Harcombe J. M. and Thomas S. B. (1957) *J. Appl. Bact.* 20. 1-10.
4. Mossel D. A. A. and Vega C. L. (1973) *Hlth Lab. Sci.* 11. 303-307.

VIOLET RED BILE AGAR (VRBA) WITH MUG

Code: CM0978

A lactose-containing selective medium for the detection and enumeration of coliform organisms in water, food and dairy products.

Formula	***gm/litre***
Yeast extract	3.0
Peptone	7.0
Sodium chloride	5.0
Bile Salts No.3	1.5
Lactose	10.0
Neutral red	0.03
Crystal violet	0.002
Agar	12.0
4-methylumbelliferyl-β-D-glucuronide (MUG)	0.1
pH 7.4 ± 0.2	

Directions
Suspend 38.6 g of VRBA with MUG in 1 litre of distilled water. Bring to the boil to dissolve completely. No further sterilisation is necessary or desirable. Mix well before pouring into sterile Petri dishes.

Description
VRBA with MUG is a Standard Methods medium recommended by FDA/BAM[1].

The medium is based on Violet Red Bile Lactose Agar (VRBLA) (CM0107) which is a selective medium for the detection and enumeration of coliform organisms. The medium has been used for the determination of the coli-aerogenes content of water, milk and other dairy products, dairy equipment, and food products etc.

Organisms which rapidly attack lactose produce purple colonies surrounded by purple haloes. Non-lactose or late-lactose-fermenters produce pale colonies with greenish zones. Other related Gram-negative bacteria may grow but can be suppressed by incubation at >42°C or by anaerobic incubation.

Druce *et al*. found that Violet Red Bile Lactose Agar was as good an indicator of coli-aerogenes bacteria in milk as MacConkey Broth, and that the Oxoid medium was suitable for determining the coli-aerogenes content of milk.

VRBA with MUG contains 4-methylumbelliferyl-β-D-glucuronide (MUG) as a substrate which is cleaved by the enzyme β-glucuronidase produced by *Escherichia coli* resulting in a flourescent end-product.

Storage conditions and Shelf Life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C and use as freshly as possible.

Appearance
Dehydrated medium: Straw pink coloured, free-flowing powder.

Prepared medium: Dark purple coloured gel.

Quality control

Positive control:	***Expected results***
Escherichia coli ATCC® 25922*	Good growth; purple-pink colonies with flourescence
Negative control:	
Staphylococcus aureus ATCC® 25923*	No growth

*This organism is available as a Culti-Loop®

References

1. 'Association of Official Analytical Chemists' *F.D.A. Bacteriological Analytical Manual* 8th Edition (Revision A/1998) AOAC, Arlington Va.

VIOLET RED BILE GLUCOSE AGAR

Code: CM0485

A glucose-containing selective medium for the detection and enumeration of Enterobacteriaceae in food products.

Formula	***gm/litre***
Yeast extract	3.0
Peptone	7.0
Sodium chloride	5.0
Bile Salts No. 3	1.5
Glucose	10.0
Neutral red	0.03
Crystal violet	0.002
Agar	12.0
pH 7.4 ± 0.2	

Directions

Suspend 38.5 g in 1 litre of distilled water. Bring to the boil. Continue to boil for 2 minutes or for the minimum time necessary to dissolve completely and ensure that there are no remaining flecks of unmelted agar. No further sterilisation is necessary or desirable. Mix well and dispense into tubes or dishes.

Description

Results from tests that may be applied to water to detect coli-aerogenes organisms as possible indicators of faecal contamination possess far less significance when applied to raw foods. In the examination of foodstuffs, detection of a more defined group of organisms, the Enterobacteriaceae, that ferment glucose to produce acid and/or gas has been recommended[1,2]. In addition to coliforms this group includes salmonellae and shigellae, which do not ferment lactose, and enterotoxigenic *Escherichia coli.* It also contains organisms, such as *Klebsiella* and *Citrobacter*, which are more resistant to heat than coliforms and are therefore better indicators of failure of processes that use minimal heat.

The difficulties of measuring the total Enterobacteriaceae content of foodstuffs have been studied by Mossel *et al*.[3], who showed that the addition of glucose to an existing medium for the detection of coliforms improves the performance. They added 10 g per litre of glucose to crystal violet neutral red bile lactose agar (Violet Red Bile Agar CM0107), and named the modified formulation MacConkey Glucose Agar.

Further work by Mossel *et al*.[4,5] showed that the lactose could be omitted resulting in the formulation of Violet Red Bile Glucose Agar. The continued inclusion of lactose would not provide test results leading to more accurate identification. Exclusion of lactose renders the medium more economical to make as less weight is required per litre.

Media that contain bile salts have an intrinsic toxicity for Enterobacteriaceae, even for cells that have not been under stress[6,7,8,9,10,11].

Considerable differences have been observed among six commercial preparations of Violet Red Bile Agar[4,5] with regard to productivity for Enterobacteriaceae[12], and the intensity of their metabolism. In conjunction with Oxoid the components of the medium were examined and Mossel drafted a specification as follows:

1. Approved media have to be clear and yield colonies of satisfactory size. They have to give reproducible counts of typical colonies of Enterobacteriaceae.
2. When challenged for intrinsic toxicity by an anaerobic metabolic test[13] using a strain of *Yersinia enterocolitica* (Serotype 03) as a sensitive indicator, media must promote adequate growth, acid formation and, where required, adequate gas formation.
3. Media have to satisfy the confirmation rate of typical colonies, i.e. the number of colonies confirmed as Enterobacteriaceae divided by the number of colonies tested.

Violet Red Bile Glucose Agar has been developed to satisfy all of these criteria and complies with the recommendations of ISO[14].

Technique

Prepare a series of dilutions of the samples so that at least one will be included that will yield 100-200 colonies from a 1 ml aliquot. Transfer 1 ml aliquots of each dilution to 9 cm Petri dishes using 2 plates for each dilution. Add 15 ml of medium, cool to 47°C. Gently swirl the plates 3 times clockwise and 3 times anti-clockwise. After the medium has solidified overlay with 10 ml of the same medium and leave to solidify. Invert the dishes and incubate at >42°C for 18 hours, 32°C for 24-48 hours or 4°C for 10 days depending on the groups of Enterobacteriaceae to be recovered[15].

The agar overlay ensures anaerobic conditions which suppress the growth of non-fermentative Gram-negative bacteria. It also encourages the fermentation of glucose which favours the formation of clearly visible purple colonies, surrounded by a purple halo.

Characteristic appearance of colonies

Round, purple 1-2 mm diameter surrounded by purple haloes.

Although colony size is generally 1-2 mm, size can be affected by a number of influences and all red colonies should be counted. Confirmation of the identity of these colonies must be made by further tests.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C and use as freshly as possible.

Appearance

Dehydrated medium: Straw-pink coloured, free-flowing powder.

Prepared medium: Purple coloured gel.

Quality Control

Positive control:	***Expected results***
Escherichia coli ATCC® 25922*	Good growth; purple/pink colonies with halo
Negative control:	
Staphylococcus aureus ATCC® 6538*	No growth

*This organism is available as a Culti-Loop®

Precautions

This medium is not completely specific for Enterobacteriaceae, other organisms may grow e.g. *Aeromonas* and *Yersinia* species.

The selective activity of this medium diminishes after 24 hours incubation and organisms previously suppressed may exhibit growth.

Medium for the poured plate procedure should be freshly prepared, cooled to 47°C and used within 3 hours.

References

1. WHO Technical Report Series N.598 (1976) Geneva, p.51.
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5. Mossel D. A. A., Eelderink I., Koopmans M. and van Rossem F. (1979) *J. Food Protect.* 42. 470-475.
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8. Bridson E. Y. (1978-79) in *Van Monster tot Resultaat* Nederland Society for Microbiology. Wageningen, pp.58-67.

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10. Mossel D. A. A. and Harrewijn G. A. (1972) *Alimenta* 11. 29-30.
11. Mossel D. A. A., Harrewijn G. A. and Nesselrooy-van Zadelhoff C. F. M. (1974) *Health Labor. Sci.* 11. 260-267.
12. Mossel D. A. A. (1971) Miscell. Papers Agricult. University Wageningen, The Netherlands 9. 29-39.
13. Mossel D. A. A., Eelderink I. and Sutherland J. P. (1977) *Zbl. Bakt. I. Orig.* A238. 66-79.
14. International Organization for Standardization: *Meat and meat products – detection and enumeration of Enterobacteriaceae.* ISO/DIS 5552. 1997.
15. Mossel D. A. A., van der Zee H., Hardon A. P. and van Netten P. (1986) *J. Appl. Bact.* 60. 289-295.

VOGEL-JOHNSON AGAR

Code: CM0641

A selective medium for the isolation of Staphylococcus aureus from clinical specimens and food.

Formula	***gm/litre***
Tryptone	10.0
Yeast extract	5.0
Mannitol	10.0
Dipotassium phosphate	5.0
Lithium chloride	5.0
Glycine	10.0
Phenol red	0.025
Agar	16.0
pH 7.2 ± 0.2	

Directions
Suspend 61 g in 1 litre of distilled water and bring gently to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C and add 5.7 ml of sterile 3.5% potassium tellurite solution SR0030 (equivalent to 20ml of 1% potassium tellurite).

Description
Vogel-Johnson Agar, by selecting and identifying coagulase positive and mannitol-fermenting strains, permits the early detection of *Staphylococcus aureus* from heavily contaminated foods and clinical specimens. It corresponds to the specification of the United States Pharmacopoeia[1] in terms of its formula.

Vogel and Johnson[2] modified the Tellurite Glycine Agar formula of Zebovitz *et al*.[3] by doubling the mannitol concentration to 1% (w/v) and adding Phenol Red as a pH indicator. The enhanced fermentation reaction which occurs as a result of the increase in mannitol content is clearly indicated by the development of yellow zones surrounding the colonies.

Staphylococcus aureus is able to reduce tellurite to metallic tellurium resulting in growth as black colonies.

During the first 24 hours of incubation contaminating organisms are almost completely inhibited by tellurite, lithium chloride and the high glycine concentration. Virtually all the organisms that grow in this time are coagulase positive.

Organisms that grow as black colonies surrounded by a yellow zone after incubation at 35-37°C for 24 hours may be presumed to be *Staphylococcus aureus*.

Prolonged incubation may result in the growth of black coagulase-negative colonies and if these organisms also ferment mannitol they may be falsely identified from their appearance as *Staphylococcus aureus*. In these circumstances further tests of identity should be carried out before concluding that the organism is *Staphylococcus aureus*.

Techniques
Food samples

1. Dry the surface of the plates.
2. With a sterile glass spatula spread from 0.1-1.0 ml of diluted food (macerated in 0.1% Peptone Water) over the surface of each well dried plate.
3. Incubate at 35-37°C and examine after 24 and 48 hours.

Clinical specimens

1. Dry the surface of the prepared plates.
2. Inoculate directly with the specimen.
3. Incubate at 35-37°C and examine after 24 and 48 hours.

Colonial appearance

Staphylococcus aureus appear as black, convex shiny colonies surrounded by a yellow zone.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance

Dehydrated medium: Straw-pink coloured, free-flowing powder.

Prepared medium: Orange coloured gel.

Quality control

Positive control:	***Expected results***
Staphylococcus aureus ATCC® 6538*	Good growth; black colonies with yellow zones
Negative control:	
Escherichia coli ATCC® 8739*	No growth

*This organism is available as a Culti-Loop®

Precautions

All presumptive *Staphylococcus aureus* colonies should be confirmed with further tests. Do not heat the medium after the addition of potassium tellurite.

References

1. United States Pharmacopoeia XXI (1985) *Microbial. Limit Tests.* Rockville. Md.
2. Vogel R. A. and Johnson M. J. (1961) *Pub. Hlth Lab.* 18. 131.
3. Zeboritz E., Evans J. B. and Niven C. F. (1955) *J. Bact.* 70. 687.

VRBA – see VIOLET RED BILE (LACTOSE) AGAR

VRBGA – see VIOLET RED BILE GLUCOSE AGAR

VRE BROTH BASE

Code: CM0984

Selective media for the isolation of Vancomycin Resistant Enterococci (VRE) and High Level Aminoglycoside Resistant Enterococci (HLARE) from clinical samples.

Formula	***gm/litre***
Calf brain infusion solids	12.5
Beef heart infusion solids	5.0
Proteose peptone	10.0
Glucose	2.0
Sodium chloride	5.0
Disodium phosphate	2.5
pH 7.4 ± 0.2	

VRE AGAR BASE

Code: CM0985

Formula	*gm/litre*
Tryptone	20.0
Yeast extract	5.0
Sodium chloride	5.0
Sodium citrate	1.0
Aesculin	1.0
Ferric ammonium citrate	0.5
Sodium azide	0.15
Agar	10.0
pH 7.0 ± 0.2	

MEROPENEM SUPPLEMENT

Code: SR0184

Vial Contents (each vial is sufficient for 500 ml of medium)	*per vial*	*per litre*
Meropenem	1.0 mg	2.0 mg

GENTAMICIN SUPPLEMENT

Code: SR0185

Vial Contents (each vial is sufficient for 500 mls of medium)	*per vial*	*per litre*
Gentamicin	256.0 mg	512.0 mg

VANCOMYCIN SUPPLEMENT

Code: SR0186

Vial Contents (each vial is sufficient for 500 mls of medium)	*per vial*	*per litre*
Vancomycin	3.0 mg	6.0 mg

Directions

VRE BROTH
Suspend 37.0 g of VRE Broth Base in 1 litre of distilled water. Warm to dissolve completely, sterilise by autoclaving at 121°C for 15 minutes and cool to 50°C. Supplement the medium as shown in the table below. Then mix well and distribute into final sterile containers.

VRE AGAR
Suspend 42.6 g of VRE Agar Base in 1 litre of distilled water. Warm to dissolve completely, sterilise by autoclaving at 121°C for 15 minutes and cool to 50°C. Supplement the medium as shown in the table below. Then mix agar well and distribute into sterile Petri dishes.

Supplement (mg per vial)	Reconstitution Volume (Sterile distilled water)	VRE Agar Base (per litre)		VRE Broth (per litre)
		VRE's	HLARE's	
Meropenem (SR0184) 1 mg	2 ml	1 vial	–	2 vials
Gentamicin (SR0185) 256 mg	3 ml	–	2 vials	–
Vancomycin (SR0186) 3 mg	2 ml	2 vials	–	–

Description

Selective media for the isolation of Vancomycin Resistant Enterococci (VRE) and High Level Aminoglycoside Resistant Enterococci (HLARE) from clinical samples. NB Enterococci containing the Van C genes will not be isolated on this medium.

The proliferation of enterococci, resistant to many commonly used antimicrobials is on the increase[1]. The recent emergence of VRE is of great concern as enterococci can cause bacteraemia, endocarditis and urinary tract infections. The use of VRE Broth Base and VRE Agar Base complies with recommendations from the Centre for Disease Control and Prevention (CDC) to detect VRE infection in its early stages[2].

Resistant enterococci can be isolated either directly by inoculation of the clinical sample onto supplemented VRE Agar, or indirectly isolated with a selective enrichment through VRE Broth followed by inoculation onto supplemented VRE Agar. VRE Agar Base contains an indicator system to detect the growth of aesculin-hydrolysing organisms. Enterococci produce black zones around the colonies from the formation of black iron phenolic compounds derived from aesculin-hydrolyis products and ferrous iron.

Oxoid has developed three antibiotic supplements to selectively isolate antibiotic-resistant populations amongst pathogenic Enterococci:

Meropenem Supplement is used at 2 mg/l in VRE Broth Bas), and 1 mg/l in VRE Agar Base for the suppression of contaminating flora, particularly Gram-negatives and *Enterococcus gallinarum*. It has been reported that some *Enterococcus faecalis* can be sensitive to meropenem. To isolate these strains the level of meropenem may need to be reduced, or the supplement omitted from the formulation.

Gentamicin Supplement is used at 512 mg/l[4] in VRE Agar Base for the selective isolation of HLARE.

Vancomycin Supplement is used at 6 mg/l in VRE Agar Base for the selective isolation of VRE.

Technique

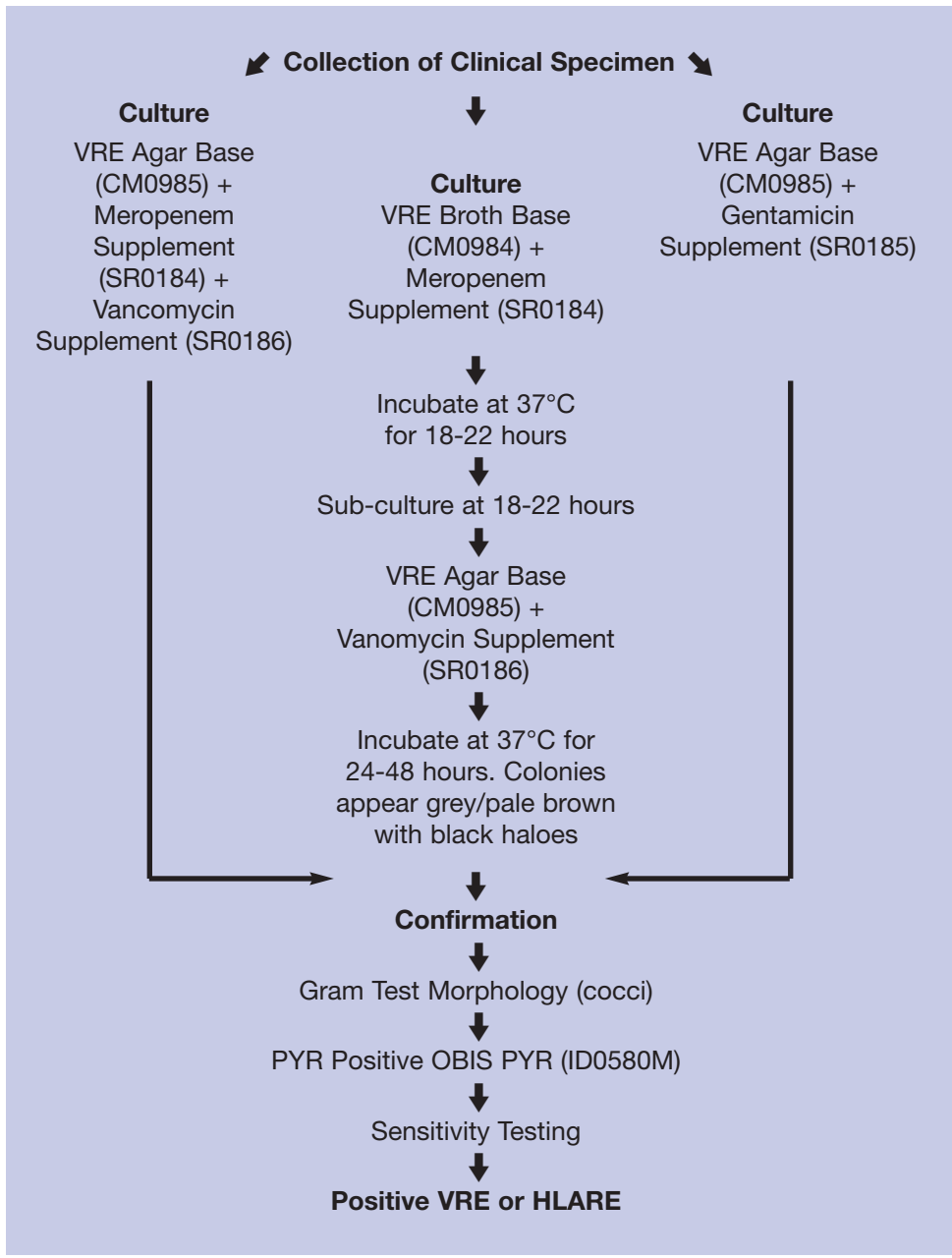
Broth

Add 'pea-sized' amounts of faecal samples directly to the supplemented VRE Broth and vortex to ensure emulsification. Incubate at 37°C for a minimum of 18 hours and sub-culture onto VRE Agar.

Agar

Inoculate faecal samples or VRE Broth culture onto supplemented VRE Agar plates with a sterile swab and spread with a loop using the diminishing sweep technique. Incubate at 37°C for 48 hours and examine at 24 hours. Re-incubate negative plates for a further 24 hours.

Enterococci appear as round grey/pale brown colonies about 1 mm in diameter surrounded by black zones.



Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Meropenem Supplement, Gentamicin Supplement and Vancomycin Supplement should be stored in the dark at 2-8°C. Prepared medium may be stored for up to two weeks at 2-8°C in the dark.

Appearence
Dehydrated medium: Broth: Cream coloured, free-flowing powder. Agar: Cream coloured, free-flowing powder.

Prepared medium: Broth: Straw coloured solution. Agar: Straw green coloured gel with blue hue.

Quality control

VRE Broth

Positive control:	
Enterococcus faecalis NCTC 12201	Growth
Negative control:	
Escherichia coli ATCC® 25922*	Inhibited

*This organism is available as a Culti-loop®

VRE Agar

Positive control:	
Enterococcus faecalis NCTC 12201	Growth
Negative controls:	
Enterococcus faecalis ATCC® 33186	Inhibited
Escherichia coli ATCC® 25922*	Inhibited

*This organism is available as a Culti-loop®

HLARE Agar

Positive control:	
Enterococcus faecalis ATCC® 51299*	Growth
Negative control:	
Enterococcus faecalis ATCC® 29212*	Inhibited

*This organism is available as a Culti-loop®

Warning: Sodium azide is harmful if swallowed, please take all necessary precautions.

References

1. King W. K. (1996) *Bug Bytes* Vol. 2 No. 19.
2. CDC *Preventing the spread of vancomycin resistance*: a report from the Hospital Infection Control Practices Advisory Committee (1994). *Fed. Regist.* May 17.
4. Weinbren M. J., Johnson A. P. and Woodford N. (2000) *J. Antimicrobial Chemotherapy* 45. 404-405.

WADOWSKY YEE MEDIUM (MWY)

A selective medium for the isolation of Legionella pnumophila from potable water samples.

LEGIONELLA CYE AGAR BASE

Code: CM0655

Formula	***gm/litre***
Activated charcoal	2.0
Yeast extract	10
Agar	13.0

LEGIONELLA MWY SELECTIVE SUPPLEMENT

Code: SR0118

Vial contents	***per 100 ml vial***	***per 500 ml vial***	***per litre***
Glycine	0.3 g	1.5 g	3.0 g
Polymyxin	5,000 IU	25,000 IU	50,000 IU
Anisomycin	8 mg	40 mg	80 mg
Vancomycin	100 µg	500 µg	100 mg
Bromothymol blue	1.0 mg	5.0 mg	10 mg
Bromocresol purple	1.0 mg	5.0 mg	10 mg

Direction

Suspend 2.5 g of Legionella CYE Agar Base in 90 ml of distilled water and bring gently to the boil to dissolve completely. Sterilise by autoclaving at 121° C for 15 minutes. Allow to cool to 50°C and aseptically add the contents of one vial of Legionella BYCE Growth Supplement SR0110 and one vial of Legionella MWY

Selective Supplement reconstituted as directed. Mix gently and pour into sterile Petri dishes. The final pH of both media should be 6.9 ± 0.2.

Description
Wadowsky and Yee Medium[1] modified by Edelstein[2] (MWY Medium) containing polymyxin 50 IU/ml, anisomycin 80 µg/ml, vancomycin 1 µg/ml and glycine 0.3% w/v. Bromocresol purple 10 µg/ml and Bromothymol blue 10 µg/ml colour the colonies and aid in the identification of the organisms. Edelstein considered the medium to be the best for isolating *Legionella pneumophila* from potable water samples. This medium can be prepared by supplementing BCYE Agar with Legionella MWY Selective Supplement.

Environmental samples should be pre-treated with an acid buffer (pH 2.2) or by heat treatment. They should be plated both before and after treatment to maximise recovery (see Technique).

MWY medium has been used successfully for examination of clinical specimens.

Technique
For each sample, three plates should be inoculated: one after pretreatment with heat, one after pretreatment with acid and one that has received neither pretreatment.

Heat pre-treatment
1. Take 10 ml of concentrated sample and place in a water bath at 50°C for 30 minutes.

Acid pre-treatment
1. Take 10 ml of concentrated sample and centrifuge in sealed buckets at 2,500 rpm for 20 minutes.
2. Decant the supernatant to leave approximately 1 ml of fluid.
3. Add 9 ml of HCl-KCl buffer (see below) and resuspend by gentle shaking. Leave to stand for 5 minutes and inoculate without further delay.

HCl-KCl Buffer
3.9 ml of 1.2M HCl
25 ml of 0.2M KCl
Adjust to pH 2.2 using 1M KOH

Directions

Environmental Samples
1. Take 10 ml of the concentrated sample and centrifuge at 2,500 rpm for 20 minutes (using sealed buckets).
2. Remove the supernatant to leave approximately 1 ml of fluid. Resuspend the deposit. This constitutes the inoculum.
3. Spread 0.1 ml on to plates of BCYE Medium with and without selective agents using a sterile spreader.
4. Add 9 ml of HCl-KCl buffer* (pH 2.2); shake gently and leave for 5 minutes.

*HCl-KCl buffer: 3.9 ml of 0.2 M HCl; 25 ml of 0.2 M KCl; Adjust the pH to 2.2 using 1M KOH.

Alternatively
Heat 10 ml of the sample concentrate in a 50°C water bath for 30 minutes.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the selective supplement in the dark at 2-8°C and use before the expiry date on the label.

Appearance
Dehydrated Medium: Black free flowing powder

Prepared medium: Black coloured gel.

References
1. Wadowsky R. M. and Yee R. B. (1981) *Clin. Microbiol. Newsletter* 4. 768-772.
2. Edelstein P. H. (1982) *J. Clin. Micro.* 16. 697-699.A

WATER PLATE COUNT AGAR (ISO)

Code: CM1012

A medium for the enumeration of culturable micro-organisms from water using a colony count technique.

Formula	***gm/litre***
Tryptone	6.0
Yeast extract	3.0
Agar	15.0
pH 7.2 ± 0.2	

Directions
Suspend 24 g of Water Plate Count Agar (ISO) in 1 litre of distilled water and bring gently to the boil to dissolve. If desired, distribute volumes of 15 ml to 20 ml in tubes, bottles or other containers. Sterilise by autoclaving at 121°C for 15 minutes. After sterilisation, allow to cool and maintain at 45 ± 1°C using a water bath.

Description
A nutritious non-selective medium for all culturable organisms in water which conforms to ISO 6222:1999 (E) Water quality – Enumeration of culturable micro-organisms – Colony count by inoculation in a nutrient agar culture medium.

Waters of all kinds invariably contain a variety of micro-organisms derived from various sources, such as soil and vegetation. Estimation of the overall numbers provides useful information for the assessment and surveillance of water quality. Separate counts are usually made of the micro-organisms which are able to grow and form colonies on nutrient agar media at 36°C and 22°C.

Colony counts are useful for assessing the integrity of ground water sources and the efficiency of water treatment processes such as coagulation, filtration and disinfection and provide an indication of the cleanliness and integrity of the distribution system. They can also be used to assess the suitability of a water supply for the preparation of food and drink to avoid contaminating the product with spoilage organisms.

The main value of colony counts lies in the detection of changes from expected counts, based on frequent, long-term monitoring. Any sudden increase in the count can be an early warning of serious pollution and a call for immediate investigation[1].

Technique
Prepare the sample, in accordance with current procedures, and make dilutions if necessary. Place a volume of the test sample (or its dilution), not exceeding 2 ml, in a Petri dish. Add 15 ml to 20 ml of molten Water Plate Count Agar (ISO) and mix carefully by gentle rotation. Allow the medium to set.

The time between addition of the test sample (or its dilution) and the addition of the molten medium shall not exceed 15 minutes. Inoculate at least one plate for incubation at each temperature.

Invert the plates and incubate one set at 36°C ± 2°C for 44 ± 4 hours. Incubate the other set at 22°C ± 2°C for 68 ± 4 hours. Examine the plates immediately after incubation. If this is not possible refrigerate the plates and examine within 48 hours. Reject any plate with confluent growth.

For each temperature of incubation, count the colonies present in each plate and calculate the estimated number of colony-forming units (cfu) present in 1 ml of sample.

Express the results as the number of colony-forming units per millilitre (cfu/ml) of the sample for each temperature of incubation.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared plates of medium at 2-8°C.

Appearence
Dehydrated medium: Straw coloured, free flowing powder.

Prepared medium: Straw coloured gel.

Quality control

Positive control:	***Expected results***
Escherichia coli ATCC® 25922*	Good growth: pale cream colonies
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

Reference

1. ISO 6222:1999 Water quality – Enumeration of culturable micro-organisms – Colony count by inoculation in a nutrient agar culture medium.

WILKINS-CHALGREN ANAEROBE AGAR

Code: CM0619

A medium for the general growth of anaerobes, recommended for antimicrobial susceptibility testing. See Antimicrobial Susceptibility Testing Section for details of the use of this medium in AST methodology.

Formula	***gm/litre***
Tryptone	10.0
Gelatin peptone	10.0
Yeast extract	5.0
Glucose	1.0
Sodium chloride	5.0
L-Arginine	1.0
Sodium pyruvate	1.0
Menadione	0.0005
Haemin	0.005
Agar	10.0
pH 7.1 ± 0.2	

Directions

Suspend 43 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

Description

Recognising the need for a standard medium for antimicrobial susceptibility testing of anaerobic bacteria, Wilkins and Chalgren[1] developed a new medium which would not require the addition of blood. Their formulation included yeast extract to supply vitamins and other growth factors such as purines and pyrimidines, that are necessary for good growth of *Peptostreptococcus anaerobius* and *Prevotella melaninogenica.* Arginine was added to ensure sufficient amino acid was available for the growth of *Eubacterium lentum*. Pyruvate was added as an energy source, for asaccharolytic cocci such as *Veillonella*[2]. It also acts similarly to catalase and degrades traces of hydrogen peroxide, which may be produced by the action of molecular oxygen on medium constituents and interfere with the metabolism of anaerobes[3]. Haemin was found to be essential for the growth of *Bacteroides* species[4] and menadione for *Prevotella melaninogenica*[5].

Peptones derived from the single protein sources casein and gelatin, were used to improve standardisation of the medium. Wilkins and Chalgren[1] considered that this medium consistently grew anaerobes as well or better than media such as Brucella Agar or Schaedler Anaerobe Agar. A collaborative study in ten laboratories showed that it could be used in an agar dilution method for susceptibility testing of anaerobic bacteria and recommended a procedure as a reference method[6].

The value of such a procedure was further confirmed by Brown and Waatti[7], who found that the incidence of resistance of anaerobic bacteria to frequently used antibiotics had increased. They considered it essential that diagnostic laboratories should have the capability of carrying out susceptibility tests on anaerobic bacteria.

Wilkins-Chalgren Agar has been recommended for the susceptibility testing of anaerobic bacteria using the Receiver Operating Characteristic (ROC) procedure.

Wilkins-Chalgren Anaerobe Agar is also recommended for the isolation of anaerobic organisms from clinical specimens. It has been shown to function well both in Petri dishes and roll tubes. (B. S. Drasar, personal communication.)

References

1. Wilkins T. D. and Chalgren S. (1976) *Antimicrob. Agents Chemother.* 10. 926-928.
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6. Sutter V. L., Barry A. L., Wilkins T. D. and Zabransky R. J. (1979) *Anti-Microb. Agents Chemother.* 16. 495-502.
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8. Castel O., Grollier G., Agius G. *et al.* (1990) *Eur. J. Clin. Microbiol. Inf. Dis.* 9. 667-671.
9. Drasar B. S., Personal communication.

N-S ANAEROBE SELECTIVE SUPPLEMENT

Code: SR0107

For the selective isolation of non-sporing anaerobes.

Vial contents (each vial is sufficient for 500 ml of medium)	***per vial***	***per litre***
Haemin	2.5 mg	5.0 mg
Menadione	0.25 mg	0.5 mg
Sodium pyruvate	500 mg	1000 mg
Nalidixic acid	5.0 mg	10.0 mg

G-N ANAEROBE SELECTIVE SUPPLEMENT

Code: SR0108

For the selective isolation of Gram-negative anaerobes.

Vial contents (each vial is sufficient for 500ml of medium)	***per vial***	***per litre***
Haemin	2.5 mg	5.0 mg
Menadione	0.25 mg	0.5 mg
Sodium succinate	1.25 g	2.5 mg
Nalidixic acid	5.0 mg	10 mg
Vancomycin	1.25 mg	2.5 mg

Directions

To prepare Non-Selective Medium for all anaerobic organisms
Suspend 21.5 g of Wilkins-Chalgren Anaerobe Agar in 475 ml of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C and aseptically add 25 ml defibrinated blood SR0050/SR0051. Mix gently, and pour into sterile Petri dishes (see plate 1).

To prepare Selective Medium for Non-Sporing Anaerobes
Suspend 21.5 g of Wilkins-Chalgren Anaerobe Agar in 475 ml of distilled water containing 0.5 ml ‘Tween 80’. Bring to the boil to dissolve completely and sterilise by autoclaving at 121°C for 15 minutes. Cool to 50-55°C and aseptically add the contents of 1 vial of N-S Anaerobe Supplement rehydrated as directed, together with 25 ml of defibrinated blood SR0050/SR0051. Mix gently and pour into sterile Petri dishes (see plate 2).

To prepare Selective Medium for Gram-Negative Anaerobes
Suspend 21.5 g of Wilkins-Chalgren Anaerobe Agar in 475 ml of distilled water. Bring to the boil to dissolve completely and sterilise by autoclaving at 121°C for 15 minutes. Cool to 50-55°C and aseptically add the contents of 1 vial of G-N Anaerobe Supplement rehydrated as directed, together with 25 ml defibrinated blood SR0050/SR0051. Mix gently and pour into sterile Petri dishes (see plate 3).

Wilkins-Chalgren Anaerobe Agar is recommended but other media may be used satisfactorily, e.g. Columbia Agar Base CM0331 and Blood Agar Base No. 2 CM0271. Sufficient haemin and menadione are contained in N-S and G-N Supplements to provide adequate levels in these media when used as directed.

Note
Use N-S Supplement with Wilkins-Chalgren Medium for NAT medium. Use G-N Supplement with Wilkins-Chalgren Medium for NAV Medium[1].

Discussion
Selective Medium for Non-Sporing Anaerobes

This medium is referred to in the published literature[1] as NAT Medium and is recommended for the isolation of non-sporing anaerobes from clinical specimens.

The recovery of non-sporing anaerobes from clinical material may sometimes prove difficult in specimens containing mixtures of aerobic and anaerobic bacteria. A medium which contains nalidixic acid as the selective agent was described by Wren[1] for isolating these organisms. It was shown to be virtually non-inhibitory to most non-sporing anaerobes whilst retaining good selectivity for these organisms when present in mixed cultures. The medium is particularly useful for the recovery of non-sporing Gram-positive anaerobes since the presence of 'Tween 80' stimulates their growth[2].

Another advantage of this medium is the earlier colonial pigmentation of the *Prevotella melaninogenica* group due to the slow lysis of the blood by 'Tween 80' during incubation. It is also a less inhibitory medium than aminoglycoside-containing media for non-sporing anaerobes in general.

The NS Anaerobe Supplement for non-sporing anaerobes contains nalidixic acid as the selective agent, together with haemin, menadione and sodium pyruvate as an additional energy source[1,4].

Haemin was found to be essential for the growth of *Bacteroides* species[5] and menadione for *Bacteroides melaninogenicus*[6]. Pyruvate, in addition to being an energy source, acts similarly to catalase and degrades traces of hydrogen peroxide which may be produced by the action of molecular oxygen on media constituents. Hydrogen peroxide is known to affect the metabolism of anaerobes[7].

Downes *et al*.[8] showed that NAT Medium was superior to kanamycin agar (KA) and neomycin agar (NA) in the recovery of all non-clostridial anaerobes. The major superiority was in the recovery of anaerobic, Gram-positive cocci.

Selective Medium for Gram-Negative Anaerobes

This medium is described[9] as NAV Medium and is recommended for the isolation of Gram-negative anaerobes from clinical specimens.

NAV Medium is a modification of NAT Medium[1] in which 'Tween 80' and sodium pyruvate have been replaced by sodium succinate. Vancomycin has been added, thus making the medium totally selective for Gram-negative anaerobes.

G-N Anaerobe Supplement contains nalidixic acid and vancomycin as selective agents; haemin, menadione and sodium succinate as growth factors. Haemin was found to be essential for the growth of *Bacteroides* species[5] and menadione for *Prevotella melaninogenica*[6]. Some Gram-negative anaerobes require succinate as a source of energy[10].

The recovery of Gram-negative anaerobes on NAV Medium has been shown[8] to be superior to that on media containing neomycin and kanamycin as selective agents.

In order to isolate the maximum non-sporing anaerobic bacteria from clinical specimens the following scheme must be followed.

Specimen
Inoculate onto each of the following media and incubate anaerobically for 48 hours.

Plate 1	***Plate 2***	***Plate 3***
Wilkins-Chalgren Anaerobe Agar CM0619 + 5% (v/v) defibrinated blood	CM0619 + 'Tween 80' + 5%(v/v) defibrinated blood + SR0107	CM0619 + 5% (v/v) defibrinated blood + SR0108
All bacteria capable of growing under anaerobic conditions	(NAT Medium) Non-Sporing Gram +ve and Gram -ve anaerobic bacteria	(NAV Medium) Gram-negative anaerobic bacteria

A non-selective plate is included for attempted isolation of any strain, in particular *Bacteroides corrodens* which is sensitive to the selective agents.

Technique

1. Prepare supplies of Plate 1 (CM0619 + blood), Plate 2 (CM0619 + blood + 'Tween 80' + SR0107) and Plate 3 (CM0619 + blood + SR0108) as described in the section marked Directions.
2. Inoculate the specimens onto plates of each medium. Best results are obtained if freshly prepared plates are used but plates may be stored at 4°C for up to 3 days.
3. Incubate the plates anaerobically at 35°C for 48 hours. The Oxoid Anaerobic System with a Gas Generating Kit BR0038 is recommended. Alternatively use AnaeroGen AN0025 or AN0035. AnaeroGen does not require the addition of water or a catalyst.
4. Examine the plates. If no growth has occurred then incubation should be continued up to 5 days before plates are discarded, as up to 20% of non-sporing anaerobes require prolonged incubation under unbroken anaerobic conditions.
5. Carry out confirmatory tests on the isolates and record the results as follows:
   (i) all facultative anaerobes and obligate anaerobes isolated on the Wilkins-Chalgren Anaerobe Agar Medium plate.
   (ii) all non-sporing anaerobes isolated on the medium for non-sporing anaerobes.
   (iii) all Gram-negative anaerobes isolated on the medium for Gram-negative anaerobes.

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared plates of medium at 2-8°C away from light.

Appearance

Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured gel.

Quality control

Positive controls:	***Expected results***
Clostridium perfringens ATCC® 13124*	Good growth; straw coloured colonies
Bacteroides fragilis ATCC® 25285*	Good growth; straw coloured colonies
Negative control:	
Uninoculated medium	No change
NAT Medium modification ***Positive controls:***	
Prevotella loescheii ATCC® 15930†	Good growth; grey/white colonies
Peptostreptcoccus anaerobius ATCC®	Good growth; grey/white colonies
14956*	
Negative control:	
Escherichia coli ATCC® 25922*	Inhibited
NAV Medium modification ***Positive controls:***	
Bacteroides fragilis ATCC® 25285*	Good growth; grey/white colonies
Fusobacterium necrophorum ATCC®	Good growth; grey/white colonies
25286*	
Negative control:	
Escherichia coli ATCC® 25922*	Inhibited

*This organism is available as a Culti-Loop®; †Formally known as *Bacteroides melanogenicus.*

References

1. Wren M. W. D. (1977) *J. Med. Microbiol.* 10. 195-201.
2. Holdeman L. V and Moore W. E. C. (1977) *Anaerobe Lab. Manual* (4th edition).
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WILKINS-CHALGREN ANAEROBE BROTH

Code: CM0643

A medium for the general growth and antimicrobial susceptibility testing of anaerobic organisms. See Antimicrobial Susceptibility Testing Section 6 for details of the use of this medium in AST methodology.

Formula	***gm/litre***
Tryptone	10.0
Gelatin peptone	10.0
Yeast extract	5.0
Glucose	1.0
Sodium chloride	5.0
L-Arginine	1.0
Sodium pyruvate	1.0
Menadione	0.0005
Haemin	0.005
pH 7.1 ± 0.2	

Directions
Suspend 33 g in 1 litre of warm distilled water. Mix well, distribute into final containers and sterilise by autoclaving at 121°C for 15 minutes.

Description
Wilkins-Chalgren Anaerobe Broth is derived from Wilkins-Chalgren Anaerobe Agar[1] CM0619.

Where studies on antimicrobial susceptibilities are being made both in broth and agar, standardisation is improved by using media of identical nutrient formulation.

The growth of anaerobic organisms in this broth is particularly good. The formulation includes yeast extract to supply vitamins and other growth factors such as purines and pyrimidines, that are necessary for good growth of *Peptostreptococcus anaerobius* and *Bacteroides melaninogenicus*. Arginine is added to ensure sufficient amino-acid is available for the growth of *Eubacterium lentum*. Pyruvate is present as an energy source for asaccharolytic cocci such as *Veillonella*. It also acts similarly to catalase and degrades traces of hydrogen peroxide, which may be produced by the action of molecular oxygen on medium constituents and interfere with the metabolism of anaerobes. Haemin is found to be essential for the growth of *Bacteroides* species and menadione for *Bacteroides melaninogenicus*.

Peptones derived from the single protein sources casein and gelatin are used to improve standardisation of the medium. The early heavy growth that is usual may reflect the absence of Eh-reducing substances that can be inhibitory to some organisms.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C away from light.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured solution.

Quality control

Positive controls:	***Expected results***
Peptostreptococcus anaerobius ATCC® 27337*	Turbid growth
Clostridium sporogenes ATCC® 19404*	Turbid growth
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

Reference

1. Wilkins T. D. and Chalgren S. (1976) *Antimicrob. Agents Chemother.* 10. 926-928.

WILSON AND BLAIR – see BISMUTH SULPHATE AGAR

WL NUTRIENT AGAR (MEDIUM)

Code: CM0309

A medium for the determination of the microbiological flora in brewing and fermentation which can be made selective for bacteria with cycloheximide.

Formula	***gm/litre***
Yeast extract	4.0
Tryptone	5.0
Glucose	50.0
Potassium dihydrogen phosphate	0.55
Potassium chloride	0.425
Calcium chloride	0.125
Magnesium sulphate	0.125
Ferric chloride	0.0025
Manganese sulphate	0.0025
Bromocresol green	0.022
Agar	15.0
pH 5.5 ± 0.2	

Directions

Suspend 75 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. If required the pH may be adjusted to 6.5 by the addition of 1% sodium bicarbonate solution.

Description

WL Nutrient Agar (Medium), based on that of Green and Gray[1] is recommended for the determination of the microbiological flora in brewing and fermentation processes. The medium is suitable for the differentiation of 'wild' yeasts from brewing yeasts[2].

Reliable counts for brewers' yeast are obtained with the medium at pH 5.5. Adjustment to pH 6.5 facilitates the counting of bakers' and distillers' yeasts.

Cycloheximide (SR0222) may be added to the medium at a final concentration of 4 mg/litre, to suppress yeast growth, the medium becomes selective for the bacterial contaminants of yeast cultures.

When making microbial counts with this medium the time and temperature of incubation will vary according to the materials tested and the organisms sought. Temperatures of 25°C are used for brewing materials and 30°C for bakers' yeasts.

Incubation times can vary from 2-14 days. Aerobic or anaerobic incubation conditions will depend on the characteristics of the organisms.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance
Dehydrated medium: White or pale straw coloured, free-flowing powder.

Prepared medium: Blue coloured gel.

Quality control

w/o cycloheximide ***Positive control:***	***Expected results***
Saccharomyces cerevisiae ATCC® 9763*	Good growth; green colonies with dark green centres
Negative control:	
Uninoculated medium	No change
with cycloheximide	
Positive control:	
Escherichia coli ATCC® 25922*	Good growth; green coloured colonies
Negative control:	
Saccharomyces cerevisiae ATCC® 9763*	No growth

*This organism is available as a Culti-Loop®

Precautions
When handling cycloheximide observe the precautions to be taken under HAZARDS.

References
1. Green S. R. and Gray P. P. (1950) *Wallerstein Lab. Comm.* 13. 357.
2. Hall Jean F. (1971) *J. Inst. Brewing* 77. 513-516.

WL NUTRIENT BROTH

Code: CM0501

A liquid medium for use in the control of brewing and other fermentation processes.

Formula	***gm/litre***
Yeast extract	4.0
Tryptone 5.0	5.0
Glucose	50.0
Potassium dihydrogen phosphate	0.55
Potassium chloride	0.425
Calcium chloride	0.125
Magnesium sulphate	0.125
Ferric chloride	0.0025
Manganese sulphate	0.0025
Bromocresol green	0.022
pH 5.5 ± 0.2	

Directions
Dissolve 60 g in 1 litre of distilled water. Mix well and distribute into containers. Sterilise by autoclaving at 121°C for 15 minutes.

The pH may be raised to 6.5 by the addition of 1% w/v sodium bicarbonate solution. Adding 0.004 g/litre of cycloheximide per litre (4ml SR0222) will form WL Differential Broth. Note the precautions to be taken under HAZARDS.

Description
WL Nutrient Broth is based on the formulation of Green and Gray[1] and is used where there are advantages for broth media e.g. using larger samples of liquid products or for enrichment cultures with cycloheximide.

Addition of 0.004 g/litre of cycloheximide suppresses yeast growth and renders the medium selective for bacterial contaminants. Adjustment of the medium to pH 6.5 facilitates growth of bakers' and distillers' yeasts; the medium at pH 5.5 is used for growth of bakers' yeasts.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance
Dehydrated Medium: Pale blue coloured, free-flowing powder.

Prepared medium: Blue/green coloured solution.

Quality control

Positive controls:	***Expected results***
w/o cycloheximide	
Saccharomyces cerevisiae ATCC® 9763*	Turbid growth
Negative control:	
Uninoculated medium	No change
Positive controls:	
with cycloheximide	
Escherichia coli ATCC® 25922*	Turbid growth
Negative control:	
Saccharomyces cerevisiae ATCC® 9763*	No growth

*This organism is available as a Culti-Loop®

Precautions
When handling cycloheximide observe the precautions to be taken under HAZARDS.

References
1. Green S. R. and Gray P. P. (1950) *Wallerstein Lab. Comm*. 13. 357.
2. Hall Jean F. (1971) *J. Inst. Brewing* 77. 513-516.

WORT AGAR

Code: CM0247

A medium for the cultivation and enumeration of yeasts.

Formula	***gm/litre***
Malt extract	15.0
Peptone	0.78
Maltose	12.75
Dextrin	2.75
Glycerol	2.35
Dipotassium phosphate	1.0
Ammonium chloride	1.0
Agar	15.0
pH 4.8 ± 0.2	

Directions
Suspend 50 g in 1 litre of distilled water and bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

PROLONGED OR EXCESSIVE HEATING WILL DIMINISH THE GEL STRENGTH OF THE AGAR.

Description
Wort Agar is a general purpose mycological medium, equivalent to the medium described by Parfitt[1] and especially suitable for the cultivation and enumeration of yeasts. The medium which duplicates the composition of natural wort, is of an acidity which is optimal for many yeasts but inhibitory to most bacteria. Parfitt investigated the relative merits of wort agar and other media for the count of yeasts and moulds in butter, and recommended the use of dehydrated whey, malt or wort agar for the purpose. Scarr[2] employed a modified wort agar ('osmophilic agar') for the examination of sugar products for osmophilic yeasts. Scarr's technique is also used for the determination of osmophilic yeasts occurring in materials used in the manufacture of soft drinks.

Technique
For the microbiological examination of butter, make suitable dilutions in quarter-strength Ringer solution (prepared with Ringer Solution Tablets BR0052). Transfer 1 ml of each dilution to a separate Petri dish; add 15 ml of melted Wort Agar, cooled to 45-48°C; mix by rotary movements in a horizontal plane; allow the poured-plates to set (protected from the light) at room temperature for 30-50 minutes. Incubate in an inverted position, e.g. for 5 days at 25°C, and count the number of yeasts and mould colonies which develop.

For the examination of sugar products for osmophilic yeasts, dissolve dehydrated Wort Agar in a syrup containing 35 parts w/w of sucrose and 10 parts w/w of glucose, and autoclave for 20 minutes at 110°C. Inoculate and mix as above. Scarr recommends incubation at 27°C for 3-4 days for *Zygosaccharomyces* species and for 5 days for less common osmophilic yeasts. Colonies on the medium are well defined and normally opaque.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance
Dehydrated medium: Light straw coloured, free-flowing powder.

Prepared medium: Straw coloured gel.

Quality control

Positive control:	***Expected results***
Saccharomyces cerevisiae ATCC® 9763*	Good growth; cream coloured colonies
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

Precautions
Do not remelt the solid agar, it will destroy the gel. The surface of the agar is soft but suitable for poured inocula.

References
1. Parfitt E. H. (1933) *J. Dairy Sci.* 16. 141-147.
2. Scarr M. Pamela (1959) *J. Sci. Food Agric.* 10(12). 678-681.

XLD MEDIUM

Code: CM0469

A selective medium for the isolation of salmonellae and shigellae from clinical specimens and foods.

Formula	***gm/litre***
Yeast extract	3.0
L-Lysine HCl	5.0
Xylose	3.75
Lactose	7.5
Sucrose	7.5
Sodium desoxycholate	1.0
Sodium chloride	5.0
Sodium thiosulphate	6.8
Ferric ammonium citrate	0.8
Phenol red	0.08
Agar	12.5
pH 7.4 ± 0.2	

Directions

Suspend 53 g in 1 litre of distilled water. Heat with frequent agitation until the medium boils. **DO NOT OVERHEAT**. Transfer immediately to a water bath at 50°C. Pour into plates as soon as the medium has cooled.

It is important to avoid preparing large volumes which will cause prolonged heating.

Description

Xylose-Lysine-Desoxycholate Agar was originally formulated by Taylor[1] for the isolation and identification of *Shigellae* from stool specimens. It has since been found to be a satisfactory medium for the isolation and presumptive identification of both *Salmonellae* and *Shigellae*[2]. It relies on xylose fermentation, lysine decarboxylation and production of hydrogen sulphide for the primary differentiation of *Shigellae* and *Salmonellae* from non-pathogenic bacteria.

Rapid xylose fermentation is almost universal amongst enteric bacteria, except for members of the *Shigella*, *Providencia* and *Edwardsiella* genera[1]. Xylose is thus included in the medium so that *Shigella* spp. may be identified by a negative reaction.

Salmonella spp. are differentiated from non-pathogenic xylose fermenters by the incorporation of lysine in the medium. *Salmonellae* exhaust the xylose and decarboxylate the lysine, thus altering the pH to alkaline and mimicking the *Shigella* reaction. However, the presence of *Salmonella* and *Edwardsiella* spp. is differentiated from that of *Shigellae* by a hydrogen sulphide indicator.

The high acid level produced by fermentation of lactose and sucrose, prevents lysine-positive coliforms from reverting the pH to an alkaline value, and non-pathogenic hydrogen sulphide producers do not decarboxylate lysine. The acid level also prevents blackening by these micro-organisms until after the 18-24 hour examination for pathogens.

Sodium desoxycholate is incorporated as an inhibitor in the medium. The concentration used allows for the inhibition of coliforms without decreasing the ability to support *Shigellae* and *Salmonellae*.

The recovery of *Shigella* spp. has previously been neglected despite the high incidence of shigellosis. This has been due to inadequate isolation media[3]. The sensitivity and selectivity of XLD Agar exceeds that of the traditional plating media, e.g. Eosin Methylene Blue, Salmonella-Shigella, and Bismuth Sulphite Agars, which tend to suppress the growth of shigellae. Many favourable comparisons between XLD Agar and these other media have been recorded in the literature[4,2,5,6,7,8,9,10].

The recovery of *Salmonellae* and *Shigellae* is not obscured by profuse growth of other species[3] therefore XLD Agar is ideal for the screening of samples containing mixed flora and suspected of harbouring enteric pathogens, e.g. medical specimens or food products. Chadwick, Delisle and Byer[11] recommended the use of this medium as a diagnostic aid in the identification of Enterobacteriaceae.

XLD Agar, in conjunction with MLCB Agar CM0783, is specified for use following enrichment culture in Modified Semi-Solid Rappaport Medium (MSRV) CM0910 when examining faeces for *Salmonella* spp.[12].

It is also used for the isolation of *Salmonella* from food and animal feedstuffs (ISO: 6579:2002)[13].

Technique
Faeces or rectal swabs may be plated directly[14] or selective enrichment broths may be used prior to streaking out. Selenite Broth CM0395 or Tetrathionate Broth CM0029 may be used for *Salmonella* enrichment.

1. Inoculate the poured, dried plates with a loopful of inoculum either from a suitable enrichment broth, from stool samples or rectal swabs.
2. Incubate the plates at 35-37°C for 18-24 hours.

Colonial Appearances

Organisms	***Appearance***
Salmonella, Edwardsiella	Red colonies with black centres
Shigella, Providencia, H_2S-negative *Salmonella* (e.g. *S. paratyphi A*)	Red colonies
Escherichia, Enterobacter, Klebsiella, Citrobacter, Proteus, Serratia	Yellow, opaque colonies

Note
Red colonies may occur with some *Proteus* and *Pseudomonas* species.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance
Dehydrated medium: Straw-pink coloured, free-flowing powder.

Prepared medium: Red coloured gel.

Quality control

Positive control:	***Expected Results (48 hours)***
Salmonella typhimurium ATCC® 14028*	Good growth; red colonies with black centre
Negative control:	
Escherichia coli ATCC® 25922*	No growth

*This organism is available as a Culti-Loop®

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1. Taylor W. I. (1965) *Am. J. Clin. Path.* 44. 471-475.
2. McCarthy M. D. (1966) *N.Z. J. Med. Lab. Technol.* 20. 127-131.
3. Isenberg H. D., Kominos S. and Sigeal M. (1969) *Appl. Microbiol.* 18. 656-659.
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11. Chadwick P., Delisle G. H. and Byer M. (1974) *Can. J. Microbiol.* 20. 1653-1664.
12. Aspinall S. T., Hindle M. A. and Hutchinson D. N. (1992) *Eur. J. Clin. Microbiol. Inf. Dis.* 11. 936-939.
13. Microbiology of Food and Animal Feeding Stuffs Horizontal Method for detection of *Salmonella* species BS: ISO 6579:2002.
14. Weissman J. B., Gangarosa E. J., Schmerler A., Marier R. L. and Lewis J. N. (1975) *Lancet* I. 1898. 88-90.

YEAST AND MOULD AGAR

Code: CM0920

A medium for the isolation of yeasts and moulds.

Formula	*gm/litre*
Yeast extract	3.0
Malt extract	3.0
Peptone	5.0
Dextrose	10.0
Agar	20.0
Final pH 6.2 ± 0.2	

Directions
Suspend 41.0 g in 1 litre of distilled water and bring to the boil to dissolve. Sterilise by autoclaving at 121°C for 15 minutes. The medium may be rendered selective after sterilisation by acidifying to pH 4.0 with 12-15 ml of Lactic Acid SR0021 after cooling to 50°C. Do not reheat after making this addition. Mix well and pour into sterile Petri dishes.

Description
Yeast and Mould Agar is based on the formulation described by Wickerham[1,2]. The medium is recommended for the isolation and maintenance of yeasts and moulds. Yeast and Mould Agar may be rendered selective by the addition of acid to reduce the pH of the medium to pH 4.0. A suitable acid for use is Lactic Acid SR0021.

Yeast and Mould Agar has now been formulated specifically for suitability within the Brewing Industry, for detection of both saccharomyces and non-saccharomyces wild yeasts in the presence of culture yeast.

The use of MYGP + Copper Medium was described by Taylor and Marsh for selective isolation of wild yeasts in the presence of culture ale or larger yeasts[3]. The medium utilises the the different sensitivities of wild yeast vs. culture ale or larger yeast to the inhibitory action of copper. This enables wild yeasts to grow on the medium whilst suppressing growth of culture yeast.

The authors recognised that variation in raw materials, including peptones, malt and yeast extract and agar, in the formulation could markedly affect the performance of the medium by neutralising the inhibitory action of the copper, resulting in overgrowth of the wild yeasts by culture yeast.

Careful selection of the raw materials by Oxoid has eliminated this variability, and the medium has been optimised for addition of copper, usually within the range 50-300 mg/litre depending on the sensitivity of the culture yeast to copper, as recommended by the Institute of Brewing Methods of Analysis[4].

The complete medium is recommended for examination of the microbiological quality of beers in process, pitching yeasts and packaged beers.

Technique
1. Prepare Yeast and Mould Agar plates as directed in the directions for use.

2. Inoculate the medium by surface or poured plate procedures.

3. Incubate the plates for 48-72 hours at 25-30°C.

Detection and enumeration of yeasts in the presence of moulds may be made easier by using a combined anaerobic/aerobic incubation procedure[5].

Cultures are initially incubated for 3 days under anaerobic conditions and then for a further 2 days aerobically. Development of mould colonies is impeded during the anaerobic phase of incubation. Dimorphic moulds e.g. *Mucor* spp., may form yeast-like colonies during anaerobic incubation.

Technique
Cultures on plates or membrane filters are incubated for 3 days at 25°C under strictly anaerobic conditions. Continue incubation of the cultures under aerobic conditions for a further 2 days. Yeast colonies may be very small immediately following anaerobic incubation but will increase in size in air. Mould growth may become completely unrestricted after 3 days in air.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

The prepared medium may be stored for up to 2 weeks at 2-8°C.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Straw coloured gel.

Quality control

Positive controls	***Expected result***	
	pH 6.2	***pH 4.0***
Aspergillus niger ATCC® 16404*	Good growth; white mycelium, black spores	Good growth; white mycelium, black spores
Candida albicans ATCC® 10231*	Good growth; cream-coloured colonies	Good growth; cream-coloured colonies
Eschericia coli ATCC® 25922*	Good growth; straw-coloured colonies	Partial to complete inhibition

*This organism is available as a Culti-Loop®

References

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YEAST EXTRACT AGAR

Code: CM0019

A nutrient agar for the plate count of organisms in water.

Formula	***gm/litre***
Yeast extract	3.0
Peptone	5.0
Agar	15.0
pH 7.2 ± 0.2	

Directions
Suspend 23 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

Description
This medium is made to the formula described by Windle Taylor for the plate count of micro-organisms in water[1].

In the UK the usual method of counting heterotrophic bacteria in water is by the poured plate method with Yeast Extract Agar. Separate counts are made of those aerobic mesophilic organisms which form visible colonies in this medium after 24 hours incubation at 35°C and those which form colonies after 3 days at 20-22°C. The two methods give different results[2].

The organisms growing under these conditions comprise bacteria, yeasts and moulds.

Technique
1. Prepare appropriate decimal dilutions of the water sample (with Ringer solution) and pipette 1 ml portions of the water and each dilution into duplicate sterile Petri dishes.
2. Add 15 ml of Yeast Extract Agar (previously melted and cooled to 45-50°C) to each plate. Mix the contents by a combination of rapid to-and-fro shaking and circular movements lasting over a period of 5-10 seconds.
3. Allow to solidify, and incubate duplicate sets of plates for 24 hours at 37°C and 3 days at 20-22°C respectively.
4. Select plates containing 30-300 colonies for counting. No count should be made on a plate containing less than 30 colonies unless the plates from the undiluted water contain less than this number.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C.

Appearance
Dehydrated medium: Light straw coloured, free-flowing powder.

Prepared medium: Straw coloured gel.

Quality control

Positive control:	***Expected results***
Escherichia coli ATCC® 25922*	Good growth; pale straw coloured colonies
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®

References

1. Windle Taylor E. (1958) *The Examination of Waters and Water Supplies*, 7th ed., Churchill Ltd., London, pp.394-398 and 778.
2. Environment Agency *Microbiology of Drinking Water 2002*. Methods for Examination of Waters and Associated Materials.

YERSINIA SELECTIVE AGAR BASE

Code: CM0653

A selective medium for Yersinia enterocolitica when used with Yersinia Selective Supplement (Schiemann CIN Medium).

Formula	***gm/litre***
Special peptone	20.0
Yeast extract	2.0
Mannitol	20.0
Sodium pyruvate	2.0
Sodium chloride	1.0
Magnesium sulphate	0.01
Sodium desoxycholate	0.5
Neutral red	0.03
Crystal violet	0.001
Agar	12.5
pH 7.4 ± 0.2	

YERSINIA SELECTIVE SUPPLEMENT

Code: SR0109

Vial contents (each vial is sufficient for 500 ml of medium)	***per vial***	***per litre***
Cefsulodin	7.5 mg	15 mg
Irgasan	2.0 mg	4 mg
Novobiocin	1.25 mg	2.5 mg

Directions
Suspend 29 g in 500 ml of distilled water and bring gently to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Allow to cool to 50°C and aseptically add the contents of one vial of Yersinia Selective Supplement reconstituted as directed. Mix gently and pour into sterile Petri dishes.

Description
Yersinia Selective Medium (CIN Medium) is based on the formulation of Schiemann[1,2] and is recommended for the isolation and enumeration of *Yersinia enterocolitica* from clinical specimens and food.

Yersinia enterocolitica is becoming increasingly recognised as a cause of diarrhoeal disease of man. Infection by the organisms results in diarrhoea, malaise, nausea and fever, plus constant abdominal pain over a period of 1-2 days. The organism has also been shown as a cause of polyarthritis, mesenteric adenitis and septicaemia. It is likely that human infections are directly or indirectly derived from animal sources and may be contracted through the ingestion of contaminated food. Initially serotypes 0:3 and 0:9 were implicated in human infections but since then other serotypes, mainly 0:5 and 0:8 have also been involved[3]. It is important to note that incidence of disease caused by the various serotypes of *Yersinia enteroccolitica* is currently reported to vary considerably with geographical location. It is expected that with provision of a selective medium, a higher isolation rate will result, and *Yersinia enterocolitica* will be recognised as more common and widespread than previously suspected.

Yersinia Selective Agar Base and the selective supplement have been developed specifically for the optimum growth and recovery of *Yersinia enterocolitica* after 18-24 hours incubation at 32°C. Schiemann[2] modified his earlier formulation for CIN Medium by replacing bile salts with sodium desoxycholate (0.5 g/l) and by reducing the concentration of novobiocin from 15 to 2.5 mg/l in order to eliminate the inhibition of some strains of serotype 0:8.

The typical colonies of *Yersinia enterocolitica* will develop as a dark red 'bullseye' surrounded by a transparent border and will vary considerably among serotypes in colony size, smoothness and the ratio of the border to centre diameter. Most other organisms that are capable of growing will produce larger colonies (>2 mm in diameter) with diffuse pinkish centres and opaque outer zones. *Serratia liquefaciens*, *Citrobacter freundii* and *Enterobacter agglomerans* may give a colonial morphology resembling *Yersinia enterocolitica*. These organisms can be differentiated from *Yersinia enterocolitica* by biochemical tests.

Test for growth on Nutrient and MacConkey Agars, test for indole and urease production and for acid reactions from sucrose, cellobiose, amygdalin, melibiose, rhamnose and reffinose. Carry out tests at 30°C rather than 37°C[4,5].

Technique for Culture

Direct Plate Method
1. Pour plates of Yersinia Selective Agar and dry the surface.
2. Inoculate the plates with a suspension of the food, faeces, etc., to produce single colonies.
3. Incubate at 32°C for 24 hours.

Cold Enrichment in Phosphate Buffered Saline[6]
1. Inoculate food, faeces, etc., into M/15 phosphate buffered saline.*
2. Hold at 4°C for up to 21 days.
3. Periodically sub-culture samples on to plates of Yersinia Selective Agar.
4. Incubate at 32°C for 24 hours.

*To prepare an M/15 buffer dissolve one tablet of Oxoid Dulbecco 'A' BR0014 in 223 ml of distilled water. Distribute into final containers and sterilise by autoclaving at 115°C for 10 minutes.

CIN Agar had been used for isolation of *Leptospira* spp[7]. With enhancement of its nutritional properties and addition of 5-fluorouracil to increase selectivity it has also been used to demonstrate the presence of *Arcobacter* spp. in ground pork[8].

Colonial Morphology
The typical colonies of *Yersinia enterocolitica* will develop a dark red 'bullseye' surrounded by a transparent border. The colony size, smoothness and the ratio of the border to centre diameter will vary considerably among serotypes.

Identification of Isolates

Table 1 ***Yersinia enterocolitica***
Growth at 4°C and on Nutrient/MacConkey Agars
Motile at 22°C
Indole production variable
Urease positive
Ornithine decarboxylase positive
Acid production from sucrose, cellobiose, amygdalin, rhamnose and raffinose
No acid production from melibiose

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Store the prepared medium at 2-8°C for not more than 24 hours.

Appearance

Dehydrated medium: Straw coloured, free-flowing powder.

Prepared medium: Red coloured gel.

Quality control

Positive control:	***Expected results***
Yersinia enterocolitica ATCC® 27729*	Good growth; pink/red coloured colonies
Negative control:	
Escherichia coli ATCC® 25922*	Inhibited

*This organism is available as a Culti-Loop®

Precautions

Some strains of *Yersinia enterocolitica* may grow poorly or not at all. Other species of *Yersinia* may grow along with some enteric organisms. It is therefore essential that full identification tests are carried out on suspect colonies.

References

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3
SUPPLEMENTARY REAGENTS

2006

SUPPLEMENTARY REAGENTS

EGG YOLK EMULSION

Code: SR0047

Description
A stabilised emulsion of egg yolk for use in culture media. It may be added directly to nutrient media for the identification of *Clostridium*, *Bacillus* and *Staphylococcus* species by their lipase activity.

Technique
Examination of Bacteria for Lecithinase

For demonstration of lecithinase activity (especially in the investigation of 'bitty cream' conditions) add 0.5 or 1.0 ml of the emulsion to 10 ml of Blood Agar Base CM0055 or Nutrient Broth No.2 CM0067 - in both cases to clear the medium, raise the final salt concentration by the addition of 1% of sodium chloride. After incubation for up to 5 days at 35°C, lecithinase-producers render the broth opalescent, whilst, on the solid medium, their colonies are surrounded by zones of opacity.

Lactose Egg-Yolk Milk Agar[1,2] - a medium for the identification of anaerobes which, in addition to serving as a half antitoxin-Nagler plate, also demonstrates lactose fermentation and proteolysis. Egg Yolk Emulsion SR0047 is recommended for use in the preparation of the medium.

References
1. Willis A. T. and Hobbs G. (1959) *J. Path. Bact.* 77. 511-521.
2. Willis A. T. (1977) *Anaerobic Bacteriology* 3rd Edn. Butterworths, London.

EGG YOLK TELLURITE EMULSION

Code: SR0054

Description
An emulsion of egg yolk containing potassium tellurite for use in Baird-Parker Medium CM0275. Baird-Parker Medium is widely used in the food industry for the detection of pathnogenic staphylococci. Baird-Parker plates incorporating Egg Yolk Tellurite Emulsion should be protected from moisture loss by enclosure in plastic or other vapour proof packaging.

Directions
Add 50 ml to 1 litre of Baird-Parker Medium CM0275. (50 ml Egg Yolk Tellurite Emulsion contains the equivalent of 3 ml of 3.5% potassium tellurite. This is the amount recommended for 1 litre of Baird-Paker Medium, i.e. concentration in SR0054 is 0.21% w/v. Final concentration in Baird-Parker Medium is 0.01% w/v).

FILDES PEPTIC DIGEST OF BLOOD

Code: SR0046

Description
Fildes Extract is prepared by the action of the enzyme pepsin on defibrinated horse blood at optimum temperature and pH value, as described by Fildes[1]. It is a rich source of growth factors, including haemin and coenzyme, derived from the blood cells from which it is prepared. As some of the growth factors are thermolabile it should not be heated above 55°C.

The extract is supplied in screw-capped bottles. Add to the appropriate medium only after the medium has been sterilised.

Technique
Fildes Extract is recommended for the preparation of many culture media among which are the following examples:

Nagler Medium
Melt 100 ml sterile nutrient agar, Blood Agar Base CM0055 is recommended, and cool to approximately 50°C. Add 5 ml of Fildes Extract and 20 ml serum or plasma. Pour plates and dry. Concentrated Egg Yolk Emulsion SR0047 may be used in 5% concentration.

Fildes Broth (Fildes Peptic Blood Broth)
Add 5 ml of Fildes Extract to 100 ml of Nutrient Broth No. 2 CM0067 or other liquid nutrient media.

Fildes Agar (Fildes Peptic Blood Agar)
Melt 100 ml of nutrient agar or Blood Agar Base CM0055, cool to 50°C and add 5 ml of Fildes Extract.

Fildes Broth and Agar, which are transparent and have the colour of nutrient broth or agar, give copious growths of Haemophilus influenzae and are admirably suited for the primary isolation of this organism.

References
1. Willis A. T. and Hobbs G. (1959) *J. Path. Bact.* 77. 511-521.
2. Willis A. T. (1977) *Anaerobic Bacteriology* 3rd Edn. Butterworths London.

HORSE SERUM

Code: SR0035

A extract of horse blood for addition to culture media.

LAKED HORSE BLOOD

Code: SR0048

Haemolysed blood for addition to culture media. Recommended for Corynebacterium media.

TOMATO JUICE

Code: SR0032

Description
This product is the juice of ripe tomatoes, clarified by filtration. It has a pH of 4.1 (approx) and 100ml of SR0032 is equivalent to 227 grams of tomato. Tomato juice can be added to nutrient media as a specific growth stimulant for lactic acid bacteria.

LACTIC ACID 10%

Code: SR0021

A solution for addition to culture media, primarily to lower the pH.

POTASSIUM LACTATE

Code: SR0037

A solution for addition to culture media, Lysine Medium CM0191 for example.

POTASSIUM TELLURITE 3.5%

Code: SR0030

A solution for addition to culture media.

TTC SOLUTION (1%)

Code: SR0229

A solution for the addition to culture media.

Description
TTC Solution is supplied as 2 ml of a filtered aqueous solution of tri-phenyltetrazolium chloride. It is used to supplement K-F Streptococcus Agar CM0701, one vial for 500 ml medium.

TTC SOLUTION (5%)

Code: SR0211

A solution for the addition to Tergitol-7 Agar CM0793 or K-F Streptococcus Agar CM0701.

UREA 40%

Code: SR0020

A solution for addition to culture media.

BROAD SPECTRUM BETA-LACTAMASE MIXTURE

Code: SR0113

Broad Spectrum Beta-Lactamase Mixture SR0113 is a mixture of beta-lactamase (E.C.3.5.2.6.) from *Bacillus cereus*[1] 569/ H9.

The enzymes are presented as a freeze-dried powder containing buffer and zinc salts. Each vial contains minima of 500 units beta-lactamase I and 50 units of beta-lactamase II. 1 unit of enzyme activity will hydrolyse 1 m mol of substrate per minute at pH 7.0 and at 25°C; beta-lactamase I is assayed using benzyl penicillin in the presence of EDTA, and beta- lactamase II using cephalosporin C in the presence of Zn^{2+}.

Definition of Units of Enzyme Activity
The scientific literature describes a number of methods which are used to measure and define a unit of penicillinase of beta-lactamase activity[2]. Note that 1 IU of activity = 600 Levy units of activity.

Application
There are four major uses of this preparation of enzymes.

1. Inactivation of beta-lactam antibiotics in blood or other tissue samples prior to routine microbiological examination[2,3,4].
2. Inactivation of beta-lactam antibiotics in blood and other tissue samples prior to the microbiological estimate of aminoglycosides or other non-lactam antibiotics[4,5].
3. The inactivation of beta-lactam antibiotic preparations to enable sterility testing to be carried out before the administration of such preparations to patients undergoing therapy with immuno-suppressants, or who have a naturally low level of immunity[6].
4. Assessment of the susceptibility of new beta-lactam antibiotics to inactivation by lactamase.

Methods

1. Blood Culture Procedures
Inject 5 ml of sterile distilled water into a vial of enzyme mixture and mix gently. Add 1 ml of this solution aseptically to the blood culture bottle, preferably before or immediately after inoculation with the blood sample (5-10 ml).

2. Microbiological Assay of Non-Lactam Antibiotics
1 ml of the beta-lactamase enzyme solution should be added aseptically to 1 ml of blood sample or serum. This should be incubated at 30°C for a period of time depending on the beta-lactam antibiotic present. A minimum time would be 5 minutes and a maximum 60 minutes. After incubation, the blood or serum samples should be applied to wells in previously seeded antibiotic assay plates in the normal manner.

Stability of Reagents
Solutions of the enzyme will remain active for several days when stored at 4°C or several weeks when stored at minus 20°C.

Repeated freezing and thawing should be avoided. However, it is not advisable to store the solution for long periods because of the possibility of contamination.

References

1. Davis R. B., Abraham E. P. and Melling J. (1974) *Biochem. J.* 143. 115-127.
2. Waterworth P. M. (1973) *J. Clin. Path.* 26. 596-598.
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5. Sabath L. D., Casey J. I., Ruch P. A., Stumpf L. L. and Finland M. (1971) *J. Lab. Clin. Med.* 78. 457-463.
6. Code of Federal Regulations, Title 21, Part 436, Sec.436.20 U.S. Govt. Printing Office, Washington, D.C.

NITROCEFIN (GLAXO RESEARCH 87/312)

Code: SR0112

For the rapid chromogenic detection of beta-lactamase activity.

Reagents
SR0112 Vial of lyophilised Nitrocefin, containing 1 mg Nitrocefin.

SR0112A Rehydration fluid. The vial contains 1.9 ml of phosphate buffer (0.1M, pH 7.0) and 0.1 ml of dimethylsulphoxide.

Directions
Reconstitute the contents of one vial of lyophilised Nitrocefin SR0112 by adding the entire contents (2 ml) of one vial of rehydration fluid SR0112A. This yields a working Nitrocefin solution of 500 mg/ml, (approx 10-3 M) suitable for most applications.

Precautions
Nitrocefin, particularly in solution, is very light sensitive. The solution may be stored at –20°C for up to two weeks. INGESTION OR INHALATION, OR CONTACT WITH THE SKIN AND EYES SHOULD BE AVOIDED.

General Introduction and Intended Uses
Nitrocefin is the chromogenic cephalosporin developed by Glaxo Research Limited.

(Coded 87/312; 3-(2,4 dinitrostyrl) - (6R,7R-7-(2-thienylacetamido)-ceph-3-em-4-carboxylic acid, E-isomer)[1].

This compound exhibits a rapid distinctive colour change from yellow (max at pH 7.0 = 390nm) to red (max at pH 7.0 = 486nm) as the amide bond in the beta-lactam ring is hydrolysed by a beta-lactamase (E.C 3.5.2.6); it is sensitive to hydrolysis by all known lactamases produced by Gram-positive and Gram-negative bacteria. This characteristic reaction forms the basis of a number of methods suitable for diagnostic use.

Apart from its use in giving rapid indication of beta-lactamase potential, the reagent has been found extremely useful for the detection of beta-lactamase patterns from bacterial cell extracts on iso-electric focusing[2,3,4] and has been used in inhibition studies in development work on beta-lactamase resistant antibiotics5.

Description of Use

Demonstration of beta-lactamase activity in bacterial cells
Nitrocefin degradation should be used to give a rapid indication of beta-lactam inactivating systems and the result so obtained will, in most cases, predict the outcome of susceptibility tests with beta-lactam anti-microbials. However, it should not entirely replace conventional susceptibility testing as other factors also influence the results of such tests, and on occasion intrinsic resistance to beta-lactam antimicrobials has not been correlated with production of beta-lactamase[6].

Nitrocefin degradation has been found to be highly efficient in detecting beta-lactamase producing isolates of Neisseria gonorrhoeae[7,8], Haemophilus influenzae[7,9,10,11] and staphylococci[10,11].

Excellent results have also been obtained with certain anaerobic bacteria, notably with Bacteroides species[13,14,15]. It should be emphasised that the efficacy of the Nitrocefin tests in predicting the beta-lactam susceptibilities of other micro- organisms is at present unproven.

Another chromogenic cephalosporin, PADAC (Hoechst-Roussel) was not as effective as Nitrocefin in detecting staphylococcal beta-lactamase[12].

Technique
Rehydrate the Nitrocefin as directed, and use this solution in the following ways:

1. Direct Plate Method[1]
Add one drop of the Nitrocefin solution on to the surface of the colony. If the isolate is a high beta-lactamase producer then the colony and the surrounding area will quickly turn red.

To detect a weak beta-lactamase producer the plate should then be incubated for 30 minutes before being reported as negative.

2. Slide Method[1]
Add one drop of the Nitrocefin solution on to a clean glass slide. Using a sterile loop, pick one colony from the plate and emulsify into the Nitrocefin drop. Report as positive if the colour changes from yellow to red within 30 minutes (protect the slide from desiccation during the waiting period).

3. Broth Method[1]
Add four drops of Nitrocefin solution to 1 ml of the grown culture. Report as positive if the colour changes to red within 30 minutes.

4. Broken Cell Method[1]
Sonicate 1 ml of the culture in order to break open the cells. Add 4 drops of Nitrocefin solution. Report as positive if the colour changes to red within 30 minutes.

5. Paper Disc Spot Test[10]
A Whatman No.1 filter paper disc (diameter 7 cm) is placed in a petri dish and impregnated with Nitrocefin solution (0-5 ml). This impregnated paper is generally usable for one day, but should be kept away from light to avoid spontaneous degradation. An isolated colony is applied to the impregnated paper with a loop; a pink to red reaction developing within 15 minutes indicates beta-lactamase presence.

Detection of beta-lactamase activity on gels
Methods for preparing extracts containing the beta-lactamase activities from bacterial cells and the technique for analytical iso-electric focusing have been described by Matthew *et al*.[2] The developed gels are stained by applying Whatman No. 54 paper impregnated with the Nitrocefin solution[2]. Focused bands in the gel with beta-lactamase activity appear pink on a yellow background.

Determination of beta-lactamase activity by spectrophotometric assay
The working solution of Nitrocefin (500 mg/ml) is diluted tenfold in buffer (0.1M phosphate; 1mM EDTA, pH 7.0). Spectrophotometric assays for beta-lactamase are carried out measuring changes in wavelength at 486 nm. The molar extinction coefficient of Nitrocefin at this wavelength is 20,500.

Test samples of the finished product for performance with control cultures.

References

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15. Bourgault A. M. and Rosenblatt J. E. (1979) *J. Clin. Micro.* 9. 654-656.

PENASE

Code: SR0129

569/H9 Lactamase active against a range of penicillins.

Materials Supplied
Penase SR0129 is a *Bacillus cereus* 569/H9 lactamase (E.C.5.2.6) presented as a freeze-dried powder containing buffer salts. Each vial contains 3,300 IU of activity (1 unit of enzyme activity will hydrolyse 1.0 µ mol of benzylpenicillin to benzylpenicilloic acid per minute pH 7.0 and at 25°C). The preparation will successfully inactivate a range of penicillins[1].

Definition of Units of Enzyme Activity
The scientific literature describes a number of methods which are used to measure and define a unit of penicillinase activity[2]. Note that 1 IU of activity = 600 Levy units of activity.

Application
The major use of this enzyme preparation is for the inactivation of susceptible beta-lactam antibiotic preparations to enable sterility testing to be carried out[3].

The preparation may also be used for inactivation of susceptible antibiotics in blood or other tissue samples prior to routine microbiological examination[2,4,5], and inactivation of susceptible antibiotics in blood and other tissue samples prior to the microbiological estimation of aminoglycosides or other non beta-lactam antibiotics[6].

Methods

1. Sterility Testing of Penicillin Products[3]
The product is rehydrated by adding 5 ml of sterile distilled water to a vial of enzyme with gentle mixing. The resulting solution will contain 660 IU of activity/ml.

The sterility of penicillin products with respect to bacterial contamination is determined by adding 300 mg or less of the test sample to sterile Fluid Thioglycollate Medium CM0173 or other prescribed media to which a suitable amount of Penase solution has been added aseptically when the temperature has fallen below 50°C. Tubes (35 mm x 200 mm) containing 90-100 ml medium are incubated for 7 days at 30°C.

The sterility of penicillin products with respect to yeast and moulds is accomplished by adding the test sample to Sabouraud Liquid Medium CM0147 and incubating for 7 days at 20-25°C.

2. Blood Culture Procedures
0.1 ml of the reconstituted solution should be added aseptically to blood culture broths, preferably before but otherwise immediately after inoculation with the blood sample.

Stability of Reagents
Solutions of the enzyme will remain active for several days when stored at 4°C or several weeks when stored at minus 20°C. Repeated freezing and thawing should be avoided. However, it is not advisable to store the solution for long periods because of the possibility of contamination.

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SPUTASOL (LIQUID)

Code: SR0233

Formula	***per vial***
Dithiothreitol	0.1 g
Sodium chloride	0.78 g
Potassium chloride	0.02 g
Disodium hydrogen phosphate	0.112 g
Potassium dihydrogen phosphate	0.02 g
Water 7.5 ml	pH 7.4 ± 0.2

Directions
Aseptically add the contents of one vial (7.5 ml) to 92.5 ml of sterile distilled water. Use the working solution immediately or store at 2-8°C for upto 48 hours only.

Description
Sputum generally consists of inflammatory exudate from the lower respiratory tract mixed with saliva.

Mulder[1] recognised the problem of interpreting the significance of growth from sputum and suggested. rinsing it in saline before culture to remove the saliva. May[2] showed that bacteria are often unevenly distributed in the sputum of patients suffering from chronic bronchitis and that single cultures may fail to reveal all the bacterial species present.

The introduction by Rawlins[3] of a method for the homogenisation of sputum before culture overcame the variations present in any method that is based on the examination of small proportions of heterogeneous material. It enables the bacteria in the sputum to be distributed evenly throughout the specimen after digestion. Dixon and Muller[4] in an attempt to distinguish between contaminants and bronchial pathogens, suggested a semi-quantitative analysis by diluting the digested sputum down to10^{-4}.

Dithiothreitol, Cleland's Reagent[5], has been evaluated as a sputum liquefying agent[6]. It was found the most effective of a group of agents tested containing a sulphydryl group.

A 0.1 m solution of dithiothreitol was found to achieve a significantly greater decrease in sputum viscosity than 1.2M N- acetyl cysteine for use prior to sputum culture.

The use of dithiothreitol instead of N-acetyl cysteine to digest sputum before decontamination has been shown[7] to yield a higher number of acid-fast bacilli when smears are stained by the Ziehl-Neelsen method. After culture and incubation for three weeks it was reported that in general the number and size of colonies isolated using dithiothreitol as a liquefying agent was greater than that using N-acetyl cysteine.

Technique

The procedure for the routine liquefaction of sputum is as follows:

1. The sputum is expectorated into a sterile Universal container or other wide mouthed screw-capped bottle.
2. Add approximately 5 times the volume of 0.85% saline and agitate to free the sputum from adherent saliva. Remove the saline with a sterile Pasteur pipette.
3. To the washed sputum, add an equal volume of Sputasol solution.
4. Shake the mixture well, place in a 37°C water bath and incubate, with periodic shaking, until liquifaction is complete.
5. Inoculate on to a suitable culture medium. For the total cell count, place a drop of the liquefied sputum in a haemocytometer for enumeration. For a differential cell count, fix a dried smear in methyl alcohol and stain with haematoxylin and eosin or with Lieshmann stain.

The working solution of Sputasol, if kept sterile, will remain stable for at least 48 hours stored at 2-8°C.

An investigation into the survival of respiratory pathogens in specimens that had been stored for 48 hours at 4°C following homogenisation using Sputasol, showed that the organisms remained viable and, when necessary, treated specimens could be succesfully re-cultured[8].

References

1. Mulder J. (1938) *Acta. Med. Scand.* 94. 98.
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SINGLE ANTIBIOTIC SUPPLEMENTS

Vial contents	*Per vial*	*Per litre*	*Code*
Ampicillin	2.5 mg	5.0 mg	SR0136
Cefixime	0.025 mg	0.050 mg	SR0191
Cephalexin	20.0 mg	40.0 mg	SR0082
Chloramphenicol	50.0 mg	100 mg	SR0078
Cycloheximide*			SR0222
D-cycloserine	200 mg	400 mg	SR0088
Gentamicin	256 mg	512.0 mg	SR0185
Kanamycin Sulphate	10.0 mg	20.0 mg	SR0092
Meropenem	1.0 mg	2.0 mg	SR0184
Neomycin	150 mg	75 mg	SR0163
Nitrocefin**			SR0112
Novobiocin	10.0 mg	20.0 mg	SR0181
Oxacillin	18.0 mg	36.0 mg	SR0193
Oxtetracycline	50 mg	100 mg	SR0073
Polymyxin B	50,000 IU	100,000 IU	SR0099
Vancomycin	3.0 mg	6.0 mg	SR0186

*SR0222 100 ml bottle at 0.1% solution; ** SR0112 See separate entry for details.

4
LABORATORY PREPARATIONS

2006

LABORATORY PREPARATIONS

Oxoid Laboratory Preparations are Culture Media reagents which are either:

(i) manufactured within Oxoid to specified quality performance standards

(ii) manufactured outside for Oxoid to the same high standards

(iii) selected from screened buying samples by extensive laboratory testing.

The L-P range includes peptones, protein hydrolysates, biological extracts, agars and the critical culture media chemicals such as selective agents, dyes etc.

Products are provided for users who wish to create their own media or who wish to supplement existing formulae. It should be stressed, however, that the use of these products will not necessarily reproduce the performance of listed Oxoid Culture Media, even used in identical formulae. This is because it is impossible to produce peptones or hydrolysates which can be universally applied to any formulae.

3.1 Peptones, hydrolysates and biological extracts.

3.2 Agars.

3.3 Bile, bile salts and derivatives.

3.4 Chemicals for culture media.

For bulk users Oxoid can manufacture laboratory preparations to meet special requirements.

1. INTRODUCTION

The first time the term ‘peptone’ appeared was in papers published in 1880 and 1882 by Nageli. He has been credited as the first bacteriologist to discover that chemo-organotrophic organisms grow best in culture media containing a partially digested protein. The problems associated with the production of protein hydrolysates were quickly recognised and their manufacture became the concern of commercial suppliers. In fact protein hydrolysate was the first complex culture medium ingredient to be supplied commercially. This was the fore-runner of the large range of commercial culture media now available.

Oxoid (then the Medical Division of Oxo) started its investigation into the manufacture of peptone in 1924. The variety of peptones and extracts available reflects the differing demands of micro-organisms for amino acids, peptides and other nutrients. Substrates used by Oxoid for hydrolysis include: meat, casein, lactalbumin, milk, gelatin, soya and yeast cells.

2. BASIC INFORMATION

Biochemistry of Proteins

Proteins are macro-molecules and are fundamental to the structure and function of all living organisms. Chemically, proteins are made up of one or more chains of alpha-amino-carboxylic acids (amino acids), consecutively linked covalently between the alphaamino group of one moiety and the alpha carboxylic group of the next with the elimination of water. This linkage is termed the ‘peptide bond’. Chains of three or more amino acids are termed ‘polypeptides’, whilst larger structures, with an arbitrarily determined lower molecular weight limit of 5,000 are the proteins. An example of a Peptide and Amino Acid is shown in Figure A.

Only 20 amino acids commonly occur in proteins. They can occupy any position in the protein chain which can be at least 80 units long with molecular weights of several millions. The chains are folded in a variety of complex forms and the structures may incorporate other macro-molecules such as carbohydrates and lipids.

Hydrolysis of Proteins

The hydrolysis of proteins, which breaks them down to their constituent amino acids and peptides can be achieved by the use of strong acids, strong bases or proteolytic enzymes such as pepsin, papain and pancreatin (which contains trypsin)[1]. Hydrolysis with strong mineral acids, often at high temperatures and pressures is much used in the food industry to produce food flavourings. The most commonly used product in microbiology is based on the hydrolysis of casein. In this process all peptide bonds are attacked and in theory, complete breakdown into component parts could be obtained.

However, because the reaction conditions are so severe, some of the amino acids produced are themselves destroyed by the process, notably tryptophan which is totally lost. Cystine, serine and threonine are partially broken down but asparagine and glutamine are converted to their acidic forms. Any vitamins present are largely destroyed. A series of reactions may also take place between carbohydrates and amino acids (such as the Maillard reactions) which give rise to very dark products often toxic to the growth of micro-organisms[2]. For microbiology the amount of hydrolysis is controlled to produce a suitable nitrogen source for bacteria.

Proteolytic enzymes act on proteins under less severe conditions. They will function at much lower temperatures and at normal pressures and are usually specific to the peptide bond they will attack. This means that the protein is not completely hydrolysed to its constituent amino acids but into polypeptides of varying lengths, depending on the frequency of the specific amino acid linkage. Also, since proteins have a very consistent primary structure, the mixture of peptides produced after proteolytic digestion by a specific enzyme is also consistent. Enzymes commonly used are papain, pepsin and pancreatin, Figure A.

Pepsin will cut the chain anywhere there is a phenylalanine or leucine bond[3]. Papain cuts adjacent to arginine, lysine, phenylalanine and glycine[4].

Pancreatin has its action at arginine, lysine, tyrosine, tryptophan, phenylalanine and leucine bonds[4]. Raw materials may vary considerably in composition and the extent to which the protein components have been denatured during any processing procedures, therefore the conditions of manufacture must be carefully controlled to minimise the variations inherent in biological materials and so maintain quality.

More defined protein sources, such as casein and gelatin will give more consistent mixtures of peptides when treated with enzymes or acid. In practical terms, total breakdown of a protein to its individual component amino acids is difficult even with a mixture of enzymes; the result, even with well defined proteins such as casein, is a peptone containing a chemically undefined mix of peptides and amino acids.

Manufacture of Peptones
The manufacturing process is illustrated diagrammatically in Figure B and the syrup formed can be stored for long periods at room temperature because the high dissolved solid content inhibits bacterial contamination. This syrup can be used in fermentation processes without drying to a powder.

Quality Assurance
It is essential that the quality of these products is maintained at the highest level and lot to lot variation reduced to a minimum by closely following codes of Good Manufacturing Practice (GMP)[5]. In order to achieve this several types of analysis are carried out and strict quality control specifications must be met for a lot to be accepted. A list of average analyses of hydrolysed products is shown on page 3±12.

To ensure that the product conforms to predetermined specifications tests are carried out and the following criteria are routinely monitored: clarity and colour, moisture content, pH value, ash residue, chloride, nitrogen content and microbiology.

Clarity and pH Value
These tests are performed on an autoclaved 2% solution of the final product and are controlled by comparison with reference materials.

Moisture Content
The level of moisture should be below 5% to ensure no chemical changes occur if the product is stored at high ambient temperatures.

Ash Residue
The ash residue consists mainly of inorganic material and is estimated after ignition.

Chloride
Chloride content is determined using the Volhard titration method on the ash residue.

Metal Analysis
The presence of cations, such as calcium and magnesium, is often of value to organism growth, since they contribute significantly through their roles as co-factors in key metabolic pathways. Consequently, these are routinely measured by atomic absorption spectroscopy, to ensure control and consistency in the final products.

Total Nitrogen
An important measure of any hydrolysate or extract is its nitrogen content. Investigations are carried out to ascertain the total nitrogen (TN) which is measured by the Kjeldhal digestion and titration method[6]. To calculate the % protein, peptide or amino acid present multiply %TN by 6.25. This is approximate because of the other sources of nitrogen in peptones such as nucleotides.

Amino Nitrogen
A second investigation of nitrogen content measures the amino nitrogen (AN) also by a titration method which reacts only with amino groups of peptides and amino acids[7].

The amino nitrogen titration shows the extent of hydrolysis by measuring the increase in free amino groups from the protein. The greater the percentage of AN the greater the degree of digestion.

Degree of Hydrolysis
The degree of hydrolysis (D_H)[8] is measured by the number of peptide bonds cut, divided by the total number of peptide bonds, multiplied by a hundred and is calculated by the formula:

$$\%D_H = \frac{\text{AN Peptone} \pm \text{AN Protein} \times 100}{\text{TN Protein}}$$

An approximate chain length of the hydrolysate can be derived by dividing 100 by the D_H, e.g. if the D_H of Casein Hydrolysate (Acid) LP0041 is 22% then 100/22 = 4.55 which is the average peptide chain length (ACL).

Total Amino Acids
Another indication of the potential nitrogen availability is the total amino acid profile, which is determined by High Pressure Liquid Chromatography (HPLC). This data is the result of the complete breakdown of the polypeptides to their constituents and their subsequent analysis. If a microorganism was able to repeat this reaction biochemically, then it would have the spectrum of amino acids recorded in the Table of Analysis available for assimilation and utilisation. In reality, it is the spectrum of peptides which are of more value to the organism than the amino acids and these can be analysed by different techniques.

Molecular Weight Profile
Moelcular size information can be obtained from analytical data and gives a useful indication of the amount of hydrolysis the substrate underwent, or degree of digestion. Using HPLC, the method of Size Exclusion Chromatography reveals the distribution of polypeptides and amino acids present in the peptone. Peptides of high molecular weight are eluted first and the smaller amino acids elute later. In the examples of the profiles below, the X axis represents elution time, or volume of mobile phase eluted and the Y axis represents the detection wavelength. This gives an indication of the amount and type of component present. At 280nm only those peptides containing aromatic amino acid residues are observed, whereas at 214nm a wider range of peptides are detected.

Casein Hydrolysate (Acid) has a high D_H as acid breaks peptide bonds indiscriminately. Tryptone is casein hydrolysed with pancreatin and as this enzyme has its action at specific bonds, less hydrolysis is the result. Proteose peptone is specially digested to contain higher molecular weight peptides and so has the lowest DH of all. From work by Adler-Nissen (ref 8) during the course of a digest the D_H achieved depends on a number of factors such as enzyme concentration or hydrolysing agent used. Other variables that affect D_H include type of substrate, temperature and pH. Size exclusion is perhaps one of the most useful analyses of protein hydrolysates and assists in the development of new products while helping to maintain quality and reproducibility of existing processes. The peptone profiles show the affect of hydrolysis time on the molecular profile. Thus a range of peptones can be made with a wide variety of chemical and bacteriological properties to different specifications.

How the Test can Help the End User
Molecular profiles can give valuable information about the user's application and particularly shows how to improve yields or growth of organisms. Profiles can be run on peptones or complete fermentation media before and after microbial growth. By making a comparison of the profiles before and after use, an indication of the efficiency of the peptone for growing a particular organism is obtained. These peptones can then be modified to maximise organism growth or product yield.

3. MEAT PEPTONES
Oxoid manufactures a comprehensive range of Meat Peptones derived from different animal tissues to suit a range of nutritional requirements, using a number of proteolytic enzymes and manufacturing processes. Since the origin of these animal materials is important, Oxoid only source from countries whose disease status is acceptable and only use tissues from selected portions of the animal. The tissues are hydrolysed to produce straw coloured peptones which are highly nutritious and clearly soluble in water. The product reaches the consumer as an easily handled, spray dried powder, although for some applications the product can be used in syrup form.

BILE SALTS

Code: LP0055

A standardised bile extract, consisting mainly of sodium glycocholate and sodium taurocholate, for use as a selective inhibitory agent in bacteriological culture media such as MacConkey Agar (CM0007) and MacConkey Broth (CM0005). Bile Salts (LP0055) conforms to bacteriological requirements and batches are standardised, with respect to inhibitory properties, by the method of Burman[1]. It is generally employed in

culture media at a concentration of 5 grams per litre. Bile Salts (LP0055) meets the following specification:

Appearance – a 2% aqueous solution is light straw coloured, clear and free from extraneous matter.

Reaction – (2% aqueous solution) – pH 6 ± 0.2.

Minimal Effective Concentration (Burman[1]) – 0.25% – 0.5%.

Bacteriological Performance – MacConkey Broth (CM0005) and MacConkey Agar (CM0007) made up with Bile Salts (LP0055) at the minimal effective concentration (MEC) support satisfactory growth of bile-tolerant organisms, including pathogenic *Staphylococci*. Gas production by *Clostridia* in broths containing 0.5% of Bile Salts (LP0055) is inhibited when the concentration is raised to 2%, as in Brilliant Green Bile (2%) Broth (CM0031) thus eliminating false positives in the 448C test for *Escherichia coli*. During the testing of milk for *Escherichia coli* with Brilliant Green Bile (2%) Broth (CM0031), false positives due to milk *Lactobacilli* do not occur if the medium contains Bile Salts (LP0055).

Reference

1. Burman N. P. (1955) *Proc. Soc. Water Treat. Exam.* 4. 10.

BILE SALTS No. 3

Code: LP0056

Oxoid Bile Salts No. 3 (LP0056) was developed to meet the demand for a refined bile salt for use as a selective inhibitory agent in bacteriological culture media. It consists of a specially modified fraction of bile acid salts which is effective at less than one-third of the concentration of bile salts normally quoted. In selective media such as MacConkey Agar No. 3 (CM0115, SS Agar (CM0099) and Violet Red Bile Agars (CM0107, CM0978, CM0485) the optimum concentration of Bile Salts No. 3 (LP0056) is 0.15% w/v. In such media there is a very sharp differentiation between lactose-fermenters and non-lactose-fermenters of enteric origin – permitting the detection of scanty non-lactose-fermenters in the presence of numerous coliforms.

CASEIN HYDROLYSATE (ACID)

Code: LP0041

An hydrolysate prepared by the reaction of casein with hydrochloric acid at high temperature and pressure, followed by neutralisation with sodium hydroxide. The aggressive hydrolysis conditions require specialised processing to decolorise, to achieve a light coloured peptone. The high availability of amino acids in their native form is advantageous in many culture media formulations and the molecular profile shows a definite shift to the lower molecular spectrum. It has particular characteristics which make it compatible for use in sensitivity media and those applications where salt tolerant organisms are used.

Typical analysis	***(% w/w)***
Total Nitrogen	8.2
Amino Nitrogen	5.3
Sodium chloride	30.2
pH (2% solution)	7.0

GLUCOSE (DEXTROSE)

Code: LP0071

A special bacteriological grade of anhydrous glucose for use in culture media. Each batch is tested chromatographically to ensure purity and correct identity.

GELATIN

Code: LP0008

Gelatin is a collagenous protein used for the solidification of culture media and for the detection and differentiation of certain proteolytic bacteria. Oxoid-Gelatin is a bacteriological grade which has been

manufactured and selected specifically for use in culture media. It is readily soluble in water to give a clear solution, free from sulphite and other preservatives. A satisfactory firm gel is obtained from a 15% solution, and media containing this proportion of gelatin will withstand short-term autoclaving for 15 minutes at 121°C without significant loss of gel strength.

SOLUBLE HAEMOGLOBIN POWDER

Code: LP0053

Prepare a 2% w/v solution of Soluble Haemoglobin Powder by adding 250 ml of distilled water at 50°C to 5 g of Haemoglobin Powder. Continually stir the solution during the addition of water. Sterilise by autoclaving at 121°C for 15 minutes.

LAB-LEMCO

Code: LP0029

Lab-Lemco (LP0029) is a meat extract made from specially selected raw materials, adjusted to neutrality and dried to a fine powder. The product has considerable advantages over conventional meat extracts. Being a refined and clarified extract it can be used with other refined ingredients to make culture media which require no filtration. Being only slightly hygroscopic this product is very easy to handle. Its use eliminates the troublesome procedures associated with handling conventional meat extracts which have a paste-like consistency.

It will enhance the growth of many bacteria and is therefore incorporated into a wide range of culture media as a solid foundation material.

It is used in fermentation processes.

Typical analysis	***(% w/w)***
Total Nitrogen	13.3
Amino Nitrogen	2.5
Sodium chloride	1.1
pH (2% solution)	7.2 ± 0.2

LACTALBUMIN HYDROLYSATE

Code: LP0048

After removal of casein from milk, lactalbumin is a protein extracted from the resulting whey. LP0048 is a pancreatic digest of this protein and contains high levels of essential amino acids.

It is most commonly used in media for tissue culture and therefore production of vaccines of viral origin, including foot and mouth disease, polio, dengue, coxsackie B3 and many other viruses.

Other uses include growth of *Lactobacilli*, spore growth of *Clostridia* and in fermentation procedures for hormone production.

Typical analysis	***(% w/w)***
Total Nitrogen	12.5
Amino Nitrogen	5.4
Sodium chloride	0.2
pH (2% solution)	7.5 ± 0.5

LACTOSE BACTERIOLOGICAL

Code: LP0070

A special grade for inclusion in microbiological media. Each batch is tested chromatographically to ensure purity and correct identity.

LIVER DESICCATED

Code: LP0026

Dehydrated whole liver, specially manufactured for the preparation of infusion media. Liver Desiccated is prepared by the dehydration of fresh ox livers under carefully controlled conditions designed to ensure maximum retention of nutritive properties, and is equivalent to five times its weight of fresh liver.

To prepare a liver infusion medium, add 50 grams of Liver Desiccated (LP0026) to 1 litre of distilled water and allow to infuse (with frequent agitation) for 1 hour at 50°C. Boil the mixture for a few minutes to coagulate protein, strain through 60-mesh stainless steel gauze, add 10 grams of Peptone (L0034) and 5 grams of Sodium chloride (LP0005). Adjust the reaction to pH 7.2, boil, strain through gauze as above, and sterilise by autoclaving at 121°C for 15 minutes.

LIVER DIGEST NEUTRALISED

Code: LP0027

A biologically standardised papaic digest of liver for use as a source of nutrients in microbiological culture media.

The digest is water soluble and compatible with other culture media ingredients and may be sterilised by filtration or autoclaving; thus it is suitable for use as an integral part of many culture media or as a valuable supplement. Being derived from liver this product contains relatively high levels of iron. The profile shows the characteristic even spread of peptides obtained from papaic digests.

Typical Analysis	***(% w/w)***
Total Nitrogen	11.0
Amino Nitrogen	3.6
Sodium chloride	1.6
pH (2% solution)	7.0 ± 0.2

MALT EXTRACT

Code: LP0039

This is prepared by extracting the soluble products from sprouted grain, followed by low temperature evaporation to dryness which conserves the nitrogenous and carbohydrate constituents.

It is recommended for use in media for the growth of yeast and moulds.

Typical analysis	***(% w/w)***
Total Nitrogen	1.1
Amino Nitrogen	0.6
Sodium chloride	0.1
pH (2% solution)	5.4 ± 0.4

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PEPTONE BACTERIOLOGICAL

Code: LP0037

PEPTONE BACTERIOLOGICAL NEUTRALISED

Code: LP0034

Oxoid Peptone Bacteriological (LP0037) and its neutralised form are very nutritious all-purpose peptones prepared by the enzymatic digestion of selected animal protein sources. They are specially prepared to provide a solid foundation in culture media formulations and are compatible with other refined culture media ingredients. The combination of pancreatin and papain enzyme systems ensures that these bacteriological peptones contain a wide spectrum of polypeptides, reflected in their broad molecular profiles.

The neutralised form evolved from the original to meet those occasions when a slightly higher pH is required. Both, when reconstituted, give a solution free of haze, cloudiness or precipitation.

LP0037 Typical analysis	***(% w/w)***
Total Nitrogen	15.2
Amino Nitrogen	2.9
Sodium chloride	1.0
pH (2% solution)	6.2 ± 0.2
LP0034 Typical analysis	***(% w/w)***
Total Nitrogen	13.9
Amino Nitrogen	2.4
Sodium chloride	3.2
pH (2% solution)	7.0 ± 0.2

Either may be used wherever a high quality bacteriological peptone is called for. Both products are found in a wide range of culture media in routine diagnostic and research bacteriology.

The above products are used in industry to produce antibiotics, interferon, pasteurella vaccine and as a stabiliser for other vaccines.

MYCOLOGICAL PEPTONE

Code: LP0040

Mycological Peptone (LP0040) was developed specifically for incorporation in solid media used for the isolation and diagnosis of pathogenic and non-pathogenic fungi. It rapidly gives a luxuriant growth with typical morphology and pigmentation. Since it does not encourage bacterial growth because of its acidity, media containing this peptone are useful for the isolation of pathogenic fungi from material heavily infected with bacteria.

It is a blend of peptones with a pH of 5.3.

Typical analysis	***(% w/w)***
Total Nitrogen	9.5
Amino Nitrogen	2.9
Sodium chloride	1.3
pH (2% solution)	5.3 ± 0.2

PEPTONE P

Code: LP0049

A peptic digest of meat proteins used in culture media which is bacteriologically tested to the USP Specification for peptic digest of animal tissue. The molecular profile shows the characteristics of peptic hydrolysates, having a shift to higher molecular peptides and the salt content reflects the low pH required for the optimum activity of the enzyme during processing.

It has been used as a replacement for bovine serum in a medium on which Baby Hamster Kidney (BHK) cells were grown. Also incorporated in media to produce interferon.

Typical analysis	***(% w/w)***
Total Nitrogen	12.8
Amino Nitrogen	2.8
Sodium chloride	9.3
pH (2% solution)	7.0 ± 0.2

SPECIAL PEPTONE

Code: LP0072

A specially designed mixture of peptones, including meat, plant and yeast digests designed to encourage the growth of the most demanding organisms. It contains a wide spectrum of peptide sizes together with those minerals, vitamins, nucleotides and other carbon compounds present in the individual peptones.

Special Peptone (LP0072) is an ingredient of media where a wide range of fastidious organisms are to be cultured such as, Columbia Blood Agar Base (CM0331) or Schaedler media (CM0437, CM0497), and GC Agar Base (CM0367).

Typical analysis	***(% w/w)***
Total Nitrogen	12.2
Amino Nitrogen	3.5
Sodium chloride	3.5
pH (2% solution)	7.3 ± 0.2

PEPTONISED MILK

Code: LP0032

This is a pancreatic digest of high grade skimmed milk powder. It constitutes a source of nitrogen more readily available than milk or milk powder and has a high level of carbohydrate. As with milk powder, the calcium level is relatively high.

The product may be used on its own or in conjunction with other ingredients in media for isolation of *Lactobacilli* and bacteriological examination of dairy products.

It has a high tryptophan content and is therefore used in media for testing the indole reaction.

Typical analysis	***(% w/w)***
Total Nitrogen	5.3
Amino Nitrogen	1.8
Sodium chloride	1.6
Tryptophan	0.53
pH (2% solution)	6.0-6.5

PROTEOSE PEPTONE

Code: LP0085

A specialised peptone prepared from a mixture of peptones. This product contains proteoses as defined in the United States Pharmacopoeia[9]. This has been achieved by carefully controlling manufacturing conditions to achieve a product rich in the higher molecular weight peptides, (e.g. 4000 plus).

Proteose Peptone is especially suitable in media for *Corynebacterium diphtheriae* toxin, including that for the Elek reaction for the recognition of toxigenic strains, as well as in the media for the production of toxins from *Staphylococci*, *Clostridia* and *Salmonellae*. Media incorporating this peptone are suitable for the cultivation of different bacteria with a wide range of nutritional requirements, e.g. *Neisseria*, *Staphylococcus*, *Haemophilus*, *Salmonella*, *Pasteurella*, *Corynebacterium* and *Histoplasma* species.

It is the peptone used to manufacture diptheria toxoid, pertussis vaccine and measles vaccine.

Typical analysis	***(% w/w)***
Total Nitrogen	13.0
Amino Nitrogen	2.2
Sodium chloride	8.0
pH (2% solution)	7.0 ± 0.2

SKIM MILK POWDER

Code: LP0031

The use of ordinary skim milk powder is undesirable in bacteriological media, because of the presence of heat-resistant organisms which give rise to erroneous cultural results. Oxoid Skim Milk Powder (LP0031) is a special bacteriological grade of spray-dried skim milk free from thermophilic organisms.

Average analysis:	
Moisture	5.0%
Ash 8.0%	8.0%
Total Nitrogen	5.3%
Reducing Sugars (as lactose monohydrate)	48.0%
Ether Soluble Extract	0.25%

Mix the powder to a smooth paste with a small quantity of distilled water, then gradually add more distilled water until a 10% w/v mixture is obtained. This is equivalent to fresh milk, and may be sterilised by autoclaving for 5 minutes at 121°C. Care should be taken not to overheat during sterilisation, otherwise caramelisation will occur. This product may be used alone or as a constituent of more complex bacteriological culture media. A 10% 'solution' of Skim Milk Powder (LP0031), containing 0.001% of bromo-cresol purple forms a highly satisfactory purple milk which may be employed for the cultivation of dairy organisms or for the differentiation of *Clostridium* species, etc. Media containing skim milk powder are of particular value for diagnostic cultural tests involving the fermentation of lactose and digestion or coagulation of casein.

This product is not always free from antimicrobial residues. Where antibiotic-free milk powder is specified in a formulation, tests must be carried out to determine if it is satisfactory.

SODIUM BISELENITE

(Sodium hydrogen selenite)

Code: LP0121

For use in Oxoid Selenite Broth Base (CM0395/CM0396) and Mannitol Selenite Broth Base (CM0399).

SODIUM CHLORIDE

Code: LP0005

See also Saline Tablets BR0053.

This product is prepared from analytical grade salt to avoid problems associated with additives.

SOYA PEPTONE

Code: LP0044

Soya Peptone is obtained by the hydrolysis of soya flour and complies with the USP specification (ref 9). In additon to its nitrogen constituents, this peptone has a high carbohydrate content and is suitable for many purposes. The presence of the sugars stachyose, raffinose, sucrose and various reducing sugars may be of importance in certain applications. It is widely used in culture media and is often used for the cultivation of many fastidious organisms and where rapid, luxuriant growth is required.

Typical analysis	***(% w/w)***
Total Nitrogen	9.1
Amino Nitrogen	2.3
Sodium chloride	0.4
pH (2% solution)	7.2 ± 0.2

TRYPTONE

Code: LP0042

Tryptone (LP0042) is a pancreatic digest of casein. It can be used in any formulation where a pancreatic or tryptic digest of casein is specified and complies with the specification for pancreatic digest of casein in the U.S. Pharmacopoeia[10]. Casein is the main protein of milk and is a rich source of amino acid nitrogen. The profile shows a broad spread of peaks throughout the molecular weight range characteristic of a pancreatic digest. This hydrolysate is often mentioned in published works, either as a constituent of culture media for metabolic or growth studies, or for other purposes where high performance and uniformity of composition are of paramount importance. It has a high tryptophan content and is therefore used in media for testing the indole reaction.

Tryptone can detect 'flat-sour' or 'sulphide' spoilage organisms in the canning industry and is also used in sterility testing media.

It is a constituent of media used in fermentation processes to produce antibiotics, extra-cellular protein, interferon and diphtheria toxoid.

Typical analysis	***(% w/w)***
Total Nitrogen	13.3
Amino Nitrogen	3.7
Sodium chloride	0.4
pH (2% solution)	7.3 ± 0.2

TRYPTONE T

Code: LP0043

This product was developed from Oxoid Tryptone (LP0042) by controlled enzymatic hydrolysis and modified by the method of Meuller and Miller[11]. This produces a lower level of calcium, magnesium and iron than in Tryptone (LP0042) which makes it ideal for the production of toxin by *Clostridium tetani*.

Typical analysis	***(% w/w)***
Total Nitrogen	13.3
Amino Nitrogen	3.5
Sodium chloride	3.5
Calcium	280ppm
Magnesium	24ppm
Iron	3ppm
pH (2% solution)	6.9-7.4

TRYPTOSE

Code: LP0047

Tryptose is a mixed enzymatic hydrolysate with unique nutritional properties.

The digest conditions are such that it contains many different peptides, including those of higher molecular weight (proteoses). It is used to grow the most fastidious of organisms especially when a rapid or profuse growth is required e.g. in blood culture media. Tryptose is also recommended to demonstrate haemolytic reactions on a blood agar base.

It is used in the production of foot and mouth disease vaccine.

Typical analysis	***(% w/w)***
Total Nitrogen	13.7
Amino Nitrogen	3.2
Sodium chloride	1.0
pH (2% solution)	7.2

YEAST EXTRACT

Code: LP0021

This is a dried yeast autolysate which is a good source of amino-nitrogen and vitamins, particularly the water soluble B-complex vitamins. Its addition to many media or fermentation broths increases the yield of organisms and is recommended where rapid and luxuriant growth is required.

Typical analysis	***(% w/w)***
Total Nitrogen	10.9
Amino Nitrogen	5.3
Sodium chloride	0.3
pH (2% solution)	7.0 ± 0.2

OXOID AGARS

Agar is a complex mixture of polysaccharides extracted from species of the red algae known as agarophytes (*Gelidium*, *Gracilaria*, *Pterocladia*, *Acanthopeltis* and *Ahnfeltia* species). It is a sulphuric acid ester of a linear galactan, soluble in hot water but insoluble in cold water. A 1.5% w/v aqueous solution should set at 32±39°C and not melt below 85°C.

There are two dominating polysaccharides in agar which particularly affect its performance in culture media.

1. A virtually neutral polymer, agarose – (1±4) linked 3,6-anhydro-α-L-galactose alternating with (1-3) linked β-D-galactose.
2. A charged polymer, agaropectin, having the same repeating unit as agarose but with some of the 3,6-anhydro-L-galactose residues replaced with L-galactose sulphate residues, together with partial replacement of the D-galactose residues with pyruvic acid acetal 4,6-0-(1-carboxyethylidene)-D-galactose.

Agarose is the component responsible for the highstrength gelling properties of agar, whereas agaropectin provides the viscous properties. The proportion of agarose to agaropectin in agar varies according to the algae of origin but it can be as high as 75% agarose to 25% agaropectin.

The characteristic property of agar to form high-strength gels which are reversible with a hysteresis cycle over a range of 40°C is due to three equatorial hydrogen atoms on the 3,6-anhydro-L-galactose residues which constrain the molecule to form a helix with a threefold screw axis. It is the interaction of these helices which causes gel formation.

Agar is hydrolysed with heat at acid pH values because the 3,6-anhydro-α-L-galactoside linkage is very susceptible to acid cleavage.

Agar is manufactured in many parts of the world, although it is essential to locate the industry near suitable beds of algae and have efficient low-cost methods of harvesting the weed. It requires 100 tons of fresh water to produce one ton of dried agar, therefore the quality of the local water will influence the quality of the processed agar.

The presence of ‘free’ metal ions of Ca, Mg and Fe in agar which can react with phosphate salts in culture media to form insoluble precipitates or hazes is undesirable. Equally undesirable is the presence of chelating compounds which can bind these cations and make them unavailable to the organisms. Lowering the phosphate level of the culture medium to overcome its interaction with the metals usually results in poor growth-promoting properties. Compatibility tests between agar and the various culture media formulae are essential.

The agars used in such tests vary as follows:

1. **Bacteriological agar Clear**, colourless products in which the mineral/metal components may be reduced making them satisfactory for most formulae.
2. **Processed bacteriological agar** Clear, colourless products in which the mineral/metal components have been reduced to low levels, making them compatible with all formulations. A further advantage of chemical processing to reduce divalent cations is that it overcomes the antagonism of certain agars to amino-glycoside antimicrobials and tetracycline. It also considerably improves the diffusion of antimicrobials in the disc-diffusion assay.
3. **Technical grade agar** Less clear and colourless products in which the higher mineral/metal components may have advantages in certain lowphosphate formulations.

All such agars must be free from toxicity to microorganisms and free from impurities such as non-agar gums, nitrogenous compounds, insoluble salts, free sugar compounds, dead micro-organisms and live thermophilic organisms.

The process of agar production has been fully described by Whistler[1], Chapman[2] and Bridson & Brecker[3] further details on the properties and testing of bacteriological agar can be found in Bridson[4].

References

1. Whistler R. L. (1973) *Industrial Gums*, 2nd Edn., Academic Press, New York, pp. 29-48.
2. Chapman V. J. (1970) *Seaweeds and their Uses*. 2nd Edn., Methuen & Co. London. pp. 151-193.
3. Bridson E. Y. and Brecker A. (1970) *Methods in Microbiology*. Vol. 3A, Academic Press, London, pp. 257-266.
4. Bridson E. Y. (1978) *Natural and Synthetic Culture Media for Bacteria*. In: *Handbook series in nutrition and food.* Section G. Vol III. Ohio. CRC Press. 91-281.

AGAR BACTERIOLOGICAL (AGAR No. 1)

Code: LP0011

A processed bacteriological agar of very high working gel strength (1% w/v) which has low Ca and Mg levels. It is compatible with all culture media and it enables broth and agar formulations of the same medium to have very similar metal values. This characteristic is especially valuable in antimicrobial MIC studies where differences in mineral/metal content can profoundly influence the results. It is also a highly satisfactory agar for antimicrobial diffusion studies (disc diffusion susceptibility tests) because its low mineral/metal content allows free diffusion of antimicrobial substances.

AGAR TECHNICAL (AGAR No. 3)

Code: LP0013

A technical grade, high working gel strength agar (1.2% w/v) suitable for purposes where clarity and compatibility are not of prime importance or where the high mineral/metal content has cultural advantages.

PURIFIED AGAR

Code: LP0028

An agar that has been extensively processed to give a low electroendosmosis factor (m_r) enabling the product to be used in electrophoresis studies without the high expense of using agarose preparations. It can also be used for bacteriological culture media where its special properties are required. An agar recommended for immuno-electrophoresis and gel diffusion studies.

VEGGIETONES

COLD FILTERABLE VEGETABLE PEPTONE BROTH

Code: VG0104

A gamma-irradiated cold filterable vegetable peptone broth suitable for microbiological media fill trials (MFT) in the pharmaceutical industry.

Formula	***gm/litre***
Vegetable Peptone	18.0
Dextrose	2.5
Di-potassium hydrogen phosphate	2.5
Yeast Extract	3.0
Sodium chloride	5.0
pH 7.1 ± 0.2 at 25°C	

Directions

Suspend 31 g of dehydrated powder in 1 litre of distilled water. Mix well to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. 1 kg of Cold Filterable VPB dehydrated powder will make 32.2 litres of medium.

Incubation of media fills is usually carried out for 14 days[6] at both 20-25°C and 30-35°C. Where possible visual inspection of the units should be carried out on a daily or every second day basis. Micro-organisms from any contaminated units should be sub-cultured, purified and identified to species level. Refer to the appropriate regulatory body for full guidelines[2,3,4,5].

Description

Cold Filterable VPB is a highly nutritious, general purpose medium which can support the growth of a wide range of bacteria, yeasts and fungi when incubated under the appropriate conditions. The peptone in this medium is derived from the kernel of the split yellow pea which is digested using fungal enzymes.

Each component of this medium has been specially screened and selected to give a highly filterable solution. The performance of the medium is tested according to the specifications for growth of control micro-organisms in Tryptone Soya Broth (CM0129) laid down in the European Pharmacopoeia 5th Edition 2005[2], the British Pharmacopoeia 2004[3], the United States Pharmacopoeia USP 28 2005[4] and the Japanese Pharmacopoeia JP 14 2001[5].

Packs of Cold Filterable VPB have been given a sterilising dose of gammairradiation (minimum 25 KGy) validated as a lethal dose for all yeasts, moulds and bacteria including bacterial spores and mycoplasmas.

Technique

A medium completely free from all animal-derived materials, particularly suitable for use in Media Fill Trials (MFT) for the pharmaceutical industry. Dehydrated Cold Filterable VPB can be substituted for the powdered components that go into making sterile aqueous drugs or added as a sterile liquid downstream of processing a placebo of sterile solid dosage form. After carrying out MFT the medium is incubated under appropriate conditions for the recovery of any bacteria, yeasts and moulds.

Oxoid pre-screen and select the raw materials that go into Cold Filterable VPB so that every batch of product will have a high Vcap value. Vcap is the theoretical maximum volumetric throughput for the filter under test. With this information the maximum filterable volume of VPB may be calculated before starting a MFT1. At Oxoid a filter management system is used with a test filter to determine Vcap values for each batch of Cold Filterable VPB. The final filterable volume will depend on the membrane type, pore size and area of the process filter used.

Vcap is the extrapolation to a ‘flow = zero’ point; the time to this point may be very long. Therefore Vcap is good for comparative analysis but is not practical for MFT where time for a process is limited. A more useful value is V90 which is calculated as 68% of Vcap and is the point at which flow has decayed to 10% of the initial rate. Contact your filter manufacturer for guidance.

N.B. Cold Filterable VPB should not be used to validate the suitability of the chosen filtration system for its ability in providing a sterile drug product. The components will be quite different to those found in an aqueous drug formulation and validation for this purpose should be carried out on the drug preparation itself.

Typical Vcap values for Oxoid Cold Filterable VPB:

Filter Membrane	***Vcap (ml) 47 mm Disc (Area 14 cm²)***	***Vcap (Litres/m²)***
Polyvinylidene fluoride (PVDF)	913	652
Polyethersulfone (PES)	1,274	950
Nylon (NR)	1,817	1,298

Storage conditions and Shelf Life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Prepared medium should only be used fresh.

Appearance

Dehydrated Medium: Straw-coloured free flowing powder

Prepared medium: Clear to straw-coloured solution.

Quality control

Positive controls:	***Expected results***
Staphylococcus aureus ATCC®6538*	Turbid growth
Pseudomonas aeruginosa ATCC®9027*	Turbid growth
Bacillus subtilis ATCC®6633*	Flocculent/surface growth
Aspergillus niger ATCC®16404*	White mycelia, black spores or no spores
Candida albicans ATCC®10231*	Flocculent/surface growth
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®.

Precautions

Cold Filterable Vegetable Peptone Broth must only be used for *in vitro* diagnostic purposes. Do not use beyond the expiry date or if the product shows any sign of deterioration.

References

1. Badmington F., Wilkins R., Payne M. and Nonig E. S. (1995) Vmax Testing for Practical Microfiltration Train Scale-Up in Biopharmaceutical Processing, Pharmaceutical Technology, September, p64-76.
2. European Pharmacopoeia 5th Edition 2005.
3. British Pharmacopoeia 2004.
4. United States Pharmacopoeia USP 28 2005.
5. Japanese Pharmacopoeia JP 14 2001.
6. Halls N., (2002) Microbiological Media Fills Explained. Sue Horwood Publishing Ltd, UK.

VEGGIETONE SOYA PEPTONE

Code: VG0300

A GMO-free alternative to traditional Soya peptones. A highly nutritious general purpose peptone for the growth of bacteria and fungi.

Since the emergence of genetically modified crops in the 1990s and subsequent concerns over the use of GMOs in the Pharmaceutical industry there has been a growing need for products which can be certified to be GMO free. Oxoid have responded to this need by offering peptones which are made from GMO free raw materials under the Veggietone name. These products are also manufactured without using any materials of animal origin, thus reducing concerns over BSE and prion transmission.

This product has been certified as free of genetically modified material. Peptone & Enzymes No raw materials of animal origin have been used in this product. Soya flour has been used as a protein source for the peptone and fungal enzymes have been used to make the peptone. Excellent growth of micro-organisms. Formulated to give a good nutritional base to allow luxuriant growth of fastidious organisms. Quick and easy filtration. Designed to give a clear straw coloured broth which has good filtration rates on both cellulose acetate and cellulose nitrate filters.

Oxoid can now offer a choice of Veggietone Soya Peptone (VG0300) or Vegetable Peptone No. 1 (VG0100) as GMO and meat-free alternatives to traditional peptones.

Veggietone Soya Peptone contains a wide distribution of peptides including low molecular weight di- and tri-peptides as well as individual amino acids.

TYPICAL AMINO ACID ANALYSIS

	Total Amino Acids n.Mol/mg
ASP	500
THR	180
SER	310
GLU	800
PRO	250
GLY	310
ALA	250
VAL	200
MET	46
ILE	180
LEU	310
TYR	85
PHE	130
HIS	120
LYS	220
ARG	140

TYPICAL CHEMICAL ANALYSIS

Metals	
Ca	320 ppm
Mg	1755 ppm
Cu	3 ppm
Fe	80 ppm
Zn	18.5 ppm
Nitrogen	
Formal Nitrogen	9.30%
Total Nitrogen	8.75%
Ash	13.10%

TYPICAL ANION ANALYSIS

Anions	
Bromate	<0.01%
Chloride	1.1% w/w
Phosphate	0.2% w/w
Sulphate	0.4% w/w

TYPICAL CARBOHYDRATE ANALYSIS

Carbohydrate	
Glucose	0.14%
Fructose	0.20%
Sucrose	5.61%
Maltose	0.14%
Mannose	<0.05%
Galactose	<0.05%

Storage and stability
Veggietone Soya Peptone is a straw coloured, free flowing powder. Veggietone Soya Peptone (VG0300) should be stored tightly capped in the original container at 10-30°C. When stored as directed, the medium will remain stable until the stated expiry date.

Quality control testing
For quality control of the medium the organisms named below can be used:

Staph. aureus ATCC®9144*
Staph. aureus ATCC®25923*

*This organism is available as a Culti-Loop®

Precautions
Veggietone Soya Peptone (VG0300) must only be used for *in vitro* diagnostic purposes. Do not use beyond expiry date or if the product shows any sign of deterioration.

MYCOPEPTONE PEPTONE

Code: VG0500

MycoPeptone is a nutritious peptone rich in B vitamins, with a high carbohydrate content. It is an animal-free alternative to traditional peptones.

Since the emergence of Bovine Spongiform Encephalopathy (BSE) in the 1980s and subsequent worries about transmissible spongiform encephalopathies (TSEs) in other species, there has been a growing concern over the use of meat and animal derived products in microbiology. Despite a strict policy of sourcing from countries where BSE is not known, and tight regulation and certification of all raw materials, Oxoid have recognised the need for a range of meat-free products for use within the pharmaceutical industry.

No raw materials of animal origin are used in this product. A fungal protein isolate is the raw material base for the peptone and fungal enzymes are used in the manufacture.

All raw materials used to manufacture MycoPeptone are certified as free from genetically modified material.

Formulated to give a nutritious base to allow luxuriant growth of fastidious organisms. This peptone has been shown to give particularly good growth of *Streptococcus* species.

MycoPeptone (VG0500) contains a wide distribution of peptides including low molecular weight di and tri-peptides and as individual amino acids.

TYPICAL AMINO ACID ANALYSIS

	Total Amino Acids Free g/100 g	***Amino Acids g/100 g***
Aspartic acid	1.34	<0.02
Serine	0.69	0.26
Glutamic acid	4.73	2.03
Glycine	0.75	0.15
Histidine	0.26	<0.02
Arginine	1.08	0.62
Threonine	0.6	<0.02
Alanine	1.82	1.07
Proline	0.66	<0.02
Cystine	0.36	<0.02
Tyrosine	0.5	<0.02
Valine	0.54	0.15
Methionine	0.24	<0.02
Lysine	0.79	0.68
Isoleucine	0.34	<0.02
Leucine	0.63	0.12
Phenyalanine	0.48	0.12

TYPICAL IONIC ANALYSIS

Chloride %w/w	0.2
Calcium ppm	66
Magnesium ppm	797
Copper ppm	11.9
Iron ppm	28.9

TYPICAL PHYSICAL AND CHEMICAL CHARACTERISTICS

Characteristic	Level
Total nitrogen (TN) %	6.0
Formol nitrogen (FN) %	2.0
FN % : TN %	0.3
Ash %	25.0
pH of 2% solution @ 25°C	7.2 ± 0.2
Moisture	< 7.0%
Clarity 2% solution	Clear with no sediment

TYPICAL VITAMIN ANALYSIS

Vitamin	*Concentration mg/kg*
B1 (as HCl)	28.3
B2	62.0
B3	351.0
B6 (as pyridoxine)	7.65
B12	0.014
Pantothenic acid	150.0
Folic acid	27.0
Biotin	0.89
Niacin	13.7
Niacinamide	337.0
Choline chloride	421.0
Free Inositol	<0.05 g/100g

Storage and stability
MycoPeptone is a dark straw coloured, free flowing powder. Store tightly capped in the original container at 10-30°C. When stored as directed, the medium will remain stable until the stated expiry date.

Quality control testing
For quality control of the medium the organisms named below can be used:

Bacillus cereus ATCC®11778*
Escherichia coli ATCC®25922*

*This organism is available as a Culti-Loop®

Precautions
Mycopeptone Peptone must only be used for *in vitro* diagnostic purposes. Do not use beyond the expiry date or if the product shows any sign of deterioration.

VEGETABLE PEPTONE No. 1

Code: VG0100

A meat-free alternative to traditional peptones. A highly nutritious general purpose peptone for the growth of bacteria and fungi.

Since the emergence of Bovine Spongiform Encephalopathy (BSE) in the 1980s and subsequent worries about transmissible spongiform encephalopathies (TSEs) in other species, there has been a growing concern over the use of meat and animal derived products in microbiology.

Despite a strict policy of sourcing from countries where BSE is not known, and tight regulation and certification of all raw materials, Oxoid have recognised the need for a range of meat-free products for use within the pharmaceutical industry. This has led to the development of Vegetable Peptone No. 1 a meat-free alternative to traditional peptones.

No raw materials of animal origin have been used in this product. Pea flour has been used as a protein source for the peptone and fungal enzymes have been used to make the peptone.

All our raw materials have been certified as free of genetically modified material.

Vegetable Peptone No. 1 contains a wide distribution of peptides including low molecular weight di and tri-peptides as well as individual amino acids.

AMINO ACID ANALYSIS

	Total Amino Acids n.Mol/mg	***Free Amino Acids n.Mol/mg***
ASP	700	36
THR	280	65
SER	410	69
GLU	1000	150
PRO	310	39
GLY	450	21
ALA	400	82
CYS (h+)	23	4.6
VAL	300	110
MET	50	11
ILE	230	68
LEU	360	160
TYR	150	56
PHE	200	130
HIS	180	200
LYS	390	130
ARG	390	130
TRP	24	–

VITAMIN ANALYSIS

Vitamin	***Concentration mg/kg***
B1 (as HCL)	<0.2
B2	0.55
B6	0.31
B12	0.1
Nicotinic Acid	3.8
Pantothenic Acid	3.0
Folic acid	0.07
Biotin	0.1
Choline Chloride	493
Free Inositol	<0.05

ANION ANALYSIS

Anion	***% w/w***
Fluoride	0.02
Chloride	7.41
Nitrate	0.10
Phosphate	2.54
Sulphate	0.48

PHYSICAL AND CHEMICAL CHARACTERISTICS

Clarity of 2% solution	Clear
pH of 2% solution	7.2 ± 0.2
Formal Nitrogen	3–5%
Total Nitrogen	10–14%

Storage and stability
Vegetable Peptone No. 1 should be stored tightly capped in the original container at 10-30°C. When stored as directed, the medium will remain stable until the stated expiry date.

Quality Control
For quality control of the medium the organisms named below can be used:
Staphylococcus aureus ATCC® 6538*
Clostridium sporogenes ATCC® 19404*
Clostridium perfringens ATCC® 13124*
Candida albicans ATCC® 10231*
Bacillus subtilis ATCC® 6633*
Pseudomonas aeruginosa ATCC® 9027*
Micrococcus luteus ATCC® 9341*
Escherichia coli ATCC® 8739*
Aspergillus niger ATCC® 16404*

*This organism is available as a Culti-Loop®

Precautions
Vegetable Peptone No. 1 must only be used for *in vitro* diagnostics purposes. Do not use beyond expiry date or if the product shows any sign of deterioration.

VEGETABLE PEPTONE BROTH

Code: VG0101

A meat-free alternative to Tryptone Soya Broth. A highly nutritious general purpose medium for the growth of bacteria and fungi, which is particularly useful for sterility testing.

Formula	***gm/litre***
Vegetable Peptone	18.0
Dextrose	2.5
Di-potassium hydrogen phosphate	2.5
Yeast Extract	3.0
Sodium chloride	5.0
PH 7.0 ± 0.2	

Directions
Suspend 31 grams of Vegetable Peptone Broth in 1 litre of distilled water and distribute into final containers. Sterilise by autoclaving at 121°C for 15 minutes.

Description
Since the emergence of Bovine Spongiform Encephalopathy (BSE) in the 1980s and subsequent worries about transmissible spongiform encephalopathies (TSEs)in other species there has been a growing concern over the use of meat and animal derived products in microbiology.

Despite a strict policy of sourcing from countries where BSE is not known, and tight regulation and certification of all raw materials, Oxoid have recognised the need for a range of meat-free products for use within the pharmaceutical industry.

This has led to the development of Vegetable Peptone Broth, a meat-free alternative to traditional Tryptone Soya Broth (CM0129).

A pea peptone has been developed, and incorporated into Vegetable Peptone Broth (VG0101). This peptone uses pea flour as a base, and fungal enzymes for digestion.

Vegetable Peptone Broth is an excellent nutritional base for the growth of a wide range of organisms, including many fastidious species.

To ensure that it meets the needs of pharmaceutical companies for sterility testing, the performance of Vegetable Peptone Broth is tested according to the specifications for growth of micro-organisms laid down in the British, US, European, and Japanese Pharmacopoeia[1-4], and the NCCLS.

Vegetable Peptone Broth is also tested to ensure that it gives quick and easy filtration through both cellulose acetate and cellulose nitrate filters.

No raw materials of animal origin have been used in this product. Pea flour has been used as a protein base for this medium and fungal enzymes have been used to make the peptone.

Technique

Excellent growth of microorganisms
Formulated to give a good nutritional base with optimal buffering to allow luxuriant growth of fastidious organisms.

Quick and easy filtration
Designed to give a clear straw coloured broth which has good filtration rates on both cellulose acetate and cellulose nitrate filters.

Performance tested
The medium is quality control tested to meet US, British, European and Japanese Pharmacopoeia performance standards,

Storage and stability
Vegetable Peptone Broth should be stored tightly capped in the original container at 10-30°C. When stored as directed, the medium will remain stable until the stated expiry date.

Prepared medium may be stored for up to two weeks at 2-8°C in the dark.

Quality control
For quality control of the medium the organisms named below can be used:

Staphylococcus aureus ATCC® 6538P*
Clostridium sporogenes ATCC® 19404*
Clostridium perfringens ATCC® 13124*
Candida albicans ATCC® 10231*
Bacillus subtilis ATCC® 6633*
Pseudomonas aeruginosa ATCC® 9027*
Micrococcus luteus ATCC® 9341*
Escherichia coli ATCC® 8739*
Aspergillus niger ATCC® 16404*

*This organism is available as a Culti-Loop®

Precautions
Vegetable Peptone Broth must only be used for *in vitro* diagnostic purposes.

Do not use beyond the expiry date or if the product shows any sign of deterioration.

References
1. British Pharmacopoeia, Volume No.2 1993
2. Japanese Pharmacopoeia, 12th Edition, Supplement II
3. European Pharmacopoeia, 3rd Edition 1998
4. United States Pharmacopoeia, 23 NF 18 1995

VEGETABLE PEPTONE PHOSPHATE BROTH

Code: VG0200

A meat-free alternative to Tryptose Phosphate Broth CM0283. Suitable for use as a component of tissue culture media and for the cultivation of fastidious organisms.

Formula	***gm/litre***
Vegetable Peptone No. 1	15.0
Dextrose	2.0
Di-basic sodium phosphate	2.5
Yeast Extract	5.0
Sodium chloride	5.0
pH 7.3 ± 0.2 at 25°C	

Directions
Suspend 29.5 g of dehydrated powder in 1 litre of distilled water distribute into final containers. Mix well to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. If the reconstituted medium has been stored prior to use, remove dissolved oxygen by placing the tubes in a boiling water bath for 15 minutes and cool without agitation before inoculation.

Description
Vegetable Peptone Phosphate broth has been designed to be comparable to Tryptose Phosphate Broth, a buffered dextrose broth for the growth of fastidious microorganisms.

Tryptose Phosphate Broth is also used for tissue culture. Vegetable Peptone Phosphate Broth has been tested using Baby Hamster Kidney 21 (BHK 21) cells and is suitable for use in place of standard Tryptose Phosphate Broth.

Vegetable Peptone Phosphate Broth is made from raw materials that are free from any animal derived products and are GMO free.

Technique
For details of specific techniques please refer to Tryptose Phosphate Broth CM0283.

Storage conditions and Shelf Life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.

Prepared medium at 2-8°C.

Appearance
Dehydrated Medium: Straw-coloured free flowing powder

Prepared medium: Clear to straw-coloured solution.

Quality control

Positive controls:	***Expected results***
Streptococcus pyogenes ATCC® 19615*	Turbid growth
Staphylococcus aureus ATCC® 25923*	Turbid growth
Negative control:	
Uninoculated medium	No change

*This organism is available as a Culti-Loop®.

5
ANAEROBIC SYSTEMS

2006

ANAEROBIC BACTERIOLOGY

THE ROLE OF OXYGEN

The fact that life almost certainly evolved in an environment which lacked oxygen was not originally appreciated. At the time of Pasteur there was no precedent that life could exist in the absence of oxygen. When, in 1861, Pasteur declared that some organisms could exist without oxygen and appeared to die in its presence, the theory of the toxicity of oxygen was born.

The beginning of this theory was Pasteur's observation, looking down a microscope, that rapidly moving bacteria in a fermenting sugar solution ceased moving at the periphery of the drop, whilst remaining actively motile in the centre. Such organisms he called 'anaerobes' as opposed to 'aerobes' who depended on oxygen for their survival.

ANAEROBIC BACTERIA

Pasteur's fermentation studies on yeasts showed that these organisms could multiply with or without air and changed their metabolism of sugar to suit the gaseous environment. Thus, facultative anaerobes, which could adapt and survive, were distinguished from strict anaerobes and from strict aerobes[2]. In 1893, Veillon[3] isolated a micrococcus which was strictly anaerobic (obligate anaerobe). Later, in 1897, Veillon and Zuber[4] described various pathological conditions of which anaerobic bacteria could be the cause of the infective condition.

Further work between 1898–1902, linked anaerobes to several human pathologies including empyaema, female genital infections, gangrene of the lung etc. The anaerobic bacteriology of botulism, gas gangrene and tetanus soon became familiar to clinicians because the causative organisms, members of the *Clostridium* genus, were relatively easy to cultivate. Other anaerobes remained obscure even though they were responsible for common supperative conditions. The reason for their obscurity was the cumbersome and difficult techniques required for their cultivation.

ANAEROBIC METHODS

Methods to cultivate anaerobic organisms in liquid media were quickly developed because simple boiling expelled most of the dissolved oxygen. The addition of a heated iron strip or meat granules to the broth maintained the low oxygen levels. A small amount of agar (0.2% w/v) in the broth was also effective by increasing the viscosity of the liquid and slowing down the re-absorption of air in the cooled medium.

THEORY OF OXYGEN TOXICITY

The addition to media of Eh reducing agents, such as thioglycollate or cysteine which contain sulphydral groups, is protective to anaerobic organisms under certain circumstances. The oxidation-reduction potential (Eh) of an environment or medium is measured in mV and expressed as the tendency either to accept electrons (become reduced) or donate electrons (become oxidised). The Eh of normal tissue is +150mV but in the colon it can be as low as –250mV.

However it appears that the presence of oxygen is of greater importance because it has been shown that the growth of anaerobes was inhibited in the presence of air, even though the Eh was –50mV. In the absence of air, anaerobic organisms grew in spite of positive Eh values[6]. The toxicity of oxygen occurs because of the radicals formed by electron capture along reduction pathways: superoxide O_2-, singlet oxygen O_2/2 as well as hydroxyls and peroxides. The enzymes catalase, peroxidase and superoxide dismutase, are protection systems against these toxic radicals[7]. Aerobic organisms and facultative anaerobes have efficient enzyme systems which can neutralise such toxic radicals; obligate anaerobes lack such protective systems.

Furthermore, the addition of protective substances to culture media can overcome oxygen toxicity produced biologically by the organisms or chemically in the medium and help protect vunerable organisms. Examples of such substances are blood, charcoal, iron salts, pyruvate and cysteine.

Anaerobic methods for solid media

Anaerobic bacteria can be grown in solid agar media by making deep stabs in tubes of agar or adding a suspension of the organism to molten, cooled agar before pouring butts or plates of agar media. The latter method usually requires an agar overlay to ensure anaerobic growth. However, submerged colonies of bacteria are difficult to access and investigate further. It would not have been possible to make good progress in anaerobic bacteriology without the ability to produce separate colonies of organisms on the surface of agar plates. To achieve this goal, systems of anaerobic cultivation for agar plates had to be created.

Metal or glass jars with gas-tight lids that had valves fitted to them were developed. Air could be evacuated from the jar through a valve and oxygen-free gas flushed through the jar. A more efficient method came with

the use of a palladium catalyst fixed to the underside of the lid and hydrogen gas being added to the jar. The necessity to heat the catalyst and the potentially violent combination of oxygen and hydrogen to form water, gave an ever present risk of explosions.

It was the development of the 'cold' catalyst, in a flameproof capsule, and the replacement of glass with polycarbonate plastic which made the process much safer. A further development was the replacement of gas cylinders by foil sachets of chemicals which, when activated with water, produced hydrogen in sufficient quantities to reduce the oxygen level in the jars below 1% v/v. These sachets also supply carbon dioxide at a level of 5-8% in the jar to improve the growth of many anaerobes. Further modifications of the chemicals used in the sachets enabled atmospheres to be created which would support micro-aerophilic organisms (about 6% oxygen in the jar) or capnoeic organisms (about 10% CO_2).

Anaerobic indicators will show whether the redox potential (Eh) in the jar has been reduced below –50mV, which is the level required to change the resazurin indicator from pink to white.

Collection and transport of anaerobic specimens It must be emphasised that, in spite of every care taken in cultivation, poorly collected and transported samples will yield poor or negative results. Samples for anaerobic investigation should be collected with care and protected from air. Swabs will not be suitable, unless they are specially treated[8] or placed in a suitable Transport Medium[9]. Generous samples of pus or fluids do not need anaerobic transport but they should not be unduly delayed before examination[10].

It was Dack who said, 50 years ago, that it should be possible to isolate and study anaerobes in a relatively convenient, routine fashion using 'modern apparatus'[11]. Much later and armed with the components described below, anaerobic bacteriology is within reach of all microbiological laboratories.

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ANAEROBIC SYSTEMS

For some time now, Oxoid has been marketing two distinctly different systems for growing bacteria which require special atmospheres including anaerobic organisms, microaerophilic organisms and CO_2 dependent organisms.

The original Oxoid system consisted of an anaerobic jar of advanced design (Code HP0011) together with a gas generating kit for anaerobes (BR0038), a CO_2 gas generating kit (BR0039) and gas generating kits for Campylobacter (BR0056 and BR0060). In the case of BR0038, BR0056 and BR0060, water is added to the sachets to initiate a reaction and the production of hydrogen and CO_2. A low temperature catalyst (BR0042) is required to be used in the anaerobic jar on each occasion.

PRODUCT LISTINGS – HP0011 – CD0020

NOTE

In addition, Oxoid manufacture, a wide range of media for the transport, culture, selective isolation and susceptibility testing of anaerobic bacteria. The Microbiology Laboratory that is equipped with Oxoid Anaerobic Systems will have:

- Highly flexible systems that can cope equally well with Gas Generating Envelopes or Gas Cylinders.
- Effective systems that will provide rapid production of the atmosphere within the jar and ensure growth even of those anaerobes which may have been damaged in transit to the laboratory.
- The safest Anaerobic Systems.
- Advanced systems which contain all the facilities recommended by leading microbiologists in the field of anaerobic bacteriology.
- The best systems to ensure high isolation rates of even the most fastidious anaerobes.

THE OXOID ANAEROBIC JAR

Codes: HP0011 & HP0031

A 3.4 litre capacity Anaerobic Jar of advanced design that gives great flexibility in use by coping equally well with Gas Generating Envelopes or Gas Cylinders.

Both Jar and Lid are of robust construction and used with the Low Temperature Catalyst BR0042 to ensure protection of operator and equipment.

The Oxoid Anaerobic Jar has a number of design features, for greater convenience in use it is supplied with a corrosion-resistant plate carrier that greatly reduces the time and effort needed to load the jar. This jar will hold up to 15 Plates. A test tube carrier is available as an optional extra to minimise the risk of spillage of broth cultures.

Anaerobiosis is achieved rapidly, safely and efficiently using the Gas Generating Kit BR0038 or hydrogen obtained from cylinders.

The Oxoid Anaerobic Jar may also be used for the isolation of microaerophilic and CO_2-dependent organisms by using the Campylobacter Gas Generating Kit BR0056 or the CO_2 Gas Generating Kit BR0039.

Catalytic activity may be checked both by the pressure gauge, for an immediate indication of efficiency, and by the Anaerobic Indicator BR0055 which changes colour, from pink to white, to indicate anaerobiosis has been achieved. These checks ensure that any absence of growth does not reflect poor anaerobic incubations.

The Oxoid Anaerobic Jar HP0011 is part of the complete Oxoid Anaerobic System consisting of:

(i) 3.4 Litre Anaerobic Jar of advanced design.

(ii) Gas Generating Kit which is superior in design to any other on the market.

(iii) A new safe low temperature catalyst.

(iv) An Anaerobic Indicator that is reliable and reacts faster than any other equivalent products.

OXOID GAS GENERATING KIT

Code: BR0038

The Oxoid Gas Generating Kit BR0038 is a laminated foil envelope presented with an inner container holding tablets of sodium borohydride, sodium bicarbonate and tartaric aci in a pourous membrane container. The addition of water to the envelope activates the system causing hydrogen and carbon dioxide to be produced.

Gas production takes place smoothly and reproducibly because the porous membrane of the inner container regulates the passage of water inwards and gas outwards.

Each individual Gas Generating Kit, when activated with water, evolves sufficient hydrogen for the catalytic removal of oxygen present in the jar and leaves the final internal pressure approximating to that of the atmosphere.

Carbon dioxide is also evolved to give a final concentration of 10% v/v in the jar.

Gas generation is completed within 30 minutes and because the resultant solution is acidic it does not reabsorb the carbon dioxide so necessary for the growth of fastidious anaerobes.

GAS GENERATING KIT CARBON DIOXIDE SYSTEM

Code: BR0039

Description

The Oxoid Carbon-Dioxide Generating Kit is a reliable and convenient method for producing suitable conditions, in standard jars, for organisms requiring an enhanced CO_2 atmosphere.

Each sachet contains two tablets, both of which are composed of tartaric acid and sodium bicarbonate. When used as directed, they will, together, produce 350 ml carbon dioxide, which in the Oxoid Anaerobic Jar will give a final carbon dioxide level of approximately 10% (v/v) within 1-1.5 hours.

The requirement of CO_2 by gonococci is well documented[1,2] although strains vary widely in their requirement for this gas.

Chapin[3] introduced the candle-jar which produced approximately 2.5% (v/v) CO_2. However, this is below the optimum level for the growth of carbon dioxide requiring gonococci, particularly if the number of bacteria is small[4].

The Carbon-Dioxide Gas Generating Kit may also be used to provide the enhanced CO_2 atmosphere required for growth of meningococci.

A 10% (v/v) CO_2 atmosphere is required for isolation of *Brucella* species[5,6]. Plates of Blood Agar Base No. 2, CM0271, or Brucella Medium Base, CM0169, supplemented with 5-10% (v/v) inactivated horse serum and Brucella Selective Supplement, SR0083, should be incubated at 35°C in a carbon dioxide enriched atmosphere for ten days and examined every two days.

Hale[7] recorded the isolation of a dwarf-colony *Staphylococcus aureus* from an abscess which was dependent on CO_2 for characteristic growth.

Thomas and Cowlard[8] reported strains of *Staph. aureus* that grew normally in 1-2% of CO_2 but which on aerobic culture plates grew as minute unpigmented colonies that were coagulase and catalase negative.

Other reports[9,10,11] also concern dwarf variants of differing phage types of Staph. aureus that grow normally in a CO_2 enriched atmosphere.

Barker *et al*[12] have identified strains of *Klebsiella* species that are CO_2 dependent and Eykyn and Phillips[13] reported the isolation of a CO_2 dependent *Escherichia coli* from a urine specimen.

It can be clinically important that CO_2 dependent strains of such commonly occurring organisms are recognised and routine incubation of all specimens in CO_2 is recommended.

Disposal
After opening the jar, the exhausted sachet should be removed without spilling the contents. The solution remaining in the sachet is mildly acidic and may be poured away into a sink or flushed with running water. The empty sachet can then be discarded with normal laboratory litter.

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13. Eykyn S. and Phillips I. (1978) *B.M.J.* 1. 576.

GAS GENERATING KITS FOR CAMPYLOBACTER

Code: BR0056 & BR0060

Description
The Oxoid Gas Generating Kits for Campylobacter isolation, BR0056 and BR0060 constitute a reliable and convenient method for producing suitable gaseous conditions, in standard jars, for organisms such as *Campylobacter* species which require a reduced oxygen atmosphere.

BR0056 and BR0060 are disposable gas generating kits that produce hydrogen and carbon dioxide in sufficient quantity that, after reaction with a palladium catalyst in an anaerobic jar, will produce an optimal gaseous atmosphere for the growth of campylobacters and other microaerophilic organisms. BR0056 is designed for 3.0-3.5 litre jars, and is suitable for the Oxoid Anaerobic Jar HP0011 and for many other jars currently in use in laboratories. When used as directed, each sachet will produce about 1,000 ml hydrogen and 350 ml carbon dioxide.

BR0060 is designed for 2.5-3.0 litre jars, and is for use with the small lightweight plastic anaerobic jar. When used as directed, each sachet will produce about 700 ml hydrogen and 250 ml carbon dioxide. In each case a residual concentration of about 6% oxygen and 10% carbon dioxide in the jar is obtained in under 30 minutes.

Some variation in the oxygen level in each jar will occur, depending on the number of inoculated plates.

Use of an Anaerobic Indicator to check the efficiency of catalysis is inappropriate, but an active catalyst will produce pressure changes which can be observed from the gauge fitted to the Oxoid Anaerobic Jar. After an initial pressure increase of approximately 0.1 bar, catalytic activity will lead to a pressure reduction to zero or even – 0.05 bar. Should the catalyst be inactive, the pressure will rise to approximately 0.2 bar, and be maintained.

Campylobacter species require some oxygen yet are inhibited by the amount in air. The preferred level of oxygen required for growth has been reported to be between 5 and 7%. This exacting level, together with a carbon dioxide requirement, has made isolation of these organisms from human and animal sources a complicated procedure[1].

Alternative methods for achieving the special atmosphere may be hazardous and give a wide variation in the residual oxygen level in the jar, leading to erratic recovery of *Campylobacter* species.

The Gas Generating Kit for Campylobacter provides a reproducible atmosphere containing approximately 6% of oxygen and 10% of carbon dioxide.

An evaluation which compared different methods for producing microaerobic atmospheres confirmed the effectiveness of gas generating envelopes[2].

By using media made selective by the addition of Campylobacter Selective Supplements maximum recovery of the majority of Campylobacter strains will be achieved.

Directions

1. Cut off the corner of a sachet as indicated by the broken line. Avoid folding or crushing the sachet.

2. Add a measured 10 ml of water.

3. Immediately stand the sachet upright in the anaerobic jar **fitted with an active catalyst** and close the lid according to the manufacturer's instructions. (If preferred, water may be added to sachets already located in the jar.) The use of the Oxoid catalyst is recommended, because of its high efficiency, combined with inbuilt safety features. Activity of the catalyst may be prolonged by ensuring that it is dry on each occasion it is used. Heating to 160°C for 90 minutes after each use is recommended.

4. Disposal. After opening the jar, the exhausted sachet should be removed without spilling the contents. The solution left in the sachet is mildly acid and may be poured away into a sink and flushed with running water. The empty sachet can then be discarded with normal laboratory litter.

Warning

Gas Generating Kits activated but not in gas jars should be kept away from unguarded flames and sparks. Once the reaction has subsided (after about 30 minutes) the sachet can be discarded as above.

Reference

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THE OXOID ANAEROBIC CATALYST

Code: BR0042

The Oxoid low temperature Catalyst BR0042 is of patented design[1] and is a safer and more efficient version of the cold catalytic devices which are used, universally, to create low oxygen atmospheres within anaerobic gas jars.

Each Oxoid Catalyst contains 4 grams of palladium-coated pellets and is suitable for use in anaerobic jars up to 3.5 litres effective volume. This follows the recommendation of the United Kingdom Department of Health and Social Security[2] that there should be not less than 1 gram of catalyst for each litre volume of anaerobic jar. The extra large charge of activated palladium in the Oxoid Catalyst is wrapped in a special foil and enclosed in a large surface area capsule woven from very fine stainless steel wire.

The graph shows the difference in operating temperature between a conventional catalyst (unprotected pellets enclosed in wire gauze) and the Oxoid Catalyst. The description 'safe' applied to the Oxoid Catalyst refers to the reduction in risk of an explosion in the Oxoid Anaerobic Jar. Explosions in Anaerobic Systems although rare, can occur when hydrogen and oxygen are present in critical proportions. Such explosions are triggered by extremely hot catalyst envelope temperatures or by glowing particles of catalyst which have become detached. The Oxoid Catalyst design protects the customers from both of these possibilities.

How the Oxoid Anaerobic Catalyst BR0042 helps the microbiologist

Features

1. Maximum working temperature is approximately half that of other catalysts.
2. Double wrapped in perforated aluminium foil and fine stainless steel mesh.
3. Four grams of catalyst (more than 1 gram/litre airspace) is provided.

Benefits

1. Safety in use because the maximum operating temperature is lower than the flash point of hydrogen gas.
2. Acts as an efficient heat sink
3. Method of wrapping minimises the likelihood of small particles falling into jars, thus ensuring extra safety in use.
4. Helps to create anaerobic conditions quickly.

THE OXOID ANAEROBIC INDICATOR

Code: BR0055

The Oxoid Anaerobic Indicator BR0055 consists of a cotton strip impregnated with a redox indicator solution, enclosed in a laminated foil envelope. This formulation and a pure cotton strip gives a reproducible redox colour change in a shorter time than similar products that are available. Use of the Oxoid Anaerobic Indicator will support the evidence of pressure changes which occur with active catalysts and ensure that any absence of growth does not reflect poor anaerobic incubation.

How the Oxoid Anaerobic Indicator BR0055 helps the microbiologist

Features

1. Changes from red to white.
2. Improved sensitivity to detect lower levels of oxygen than has previously been achievable.

Benefits

1. Indicates when true anaerobiosis has been achieved.
2. Indicates better anaerobic conditions.

References

1. Patent application 54354/7 developed by Don Whitley Scientific Limited.
2. United Kingdom Department of Health and Social Security. February 1979.

ANAEROGEN

Code: AN0025 & AN0035

Description

When an AnaeroGen sachet is placed in a sealed jar, the atmospheric oxygen in the jar is rapidly absorbed with the simultaneous generation of carbon dioxide. This novel method differs from others commonly used in that the reaction proceeds with no evolution of hydrogen, and therefore, does not require a catalyst. Furthermore, water is not required to activate the reaction.

When used as directed, the AnaeroGen sachet will reduce the oxygen level in the jar to below 1% within 30 minutes. The resulting carbon dioxide level will be between 9% and 13%.

AnaeroGen was used in methodology for detecting bifidobacteria in meat and meat products in an investigation into the suitability of these organisms as indicators of faecal contamination.

Components

Each box contains:

10 AnaeroGen paper sachets which are individually foil packed.

1 Product Insert.

The active component within each AnaeroGen sachet is ascorbic acid.

Precautions
This product is for *in vitro* use only.

As soon as the AnaeroGen paper sachet is exposed to the air, the reaction will start. It is therefore essential that the paper sachet is placed in the jar and the jar sealed within one minute.

The reaction of the ascorbic acid with oxygen is exothermic. However, the temperature of the AnaeroGen paper sachet will not exceed 65°C.

Storage
Store at 2-25°C. Under these conditions, the AnaeroGen sachets will retain their reactivity until the expiry date given on the outer box and on the foil sachet.

Directions
AN0035 is designed for use in 3.5 litre jars. It is also suitable for use in the Oxoid Anaerobic Jar HP0011 and for other jars of similar capacity.

AN0025 is designed for use in 2.5 litre jars, such as the new Oxoid AnaeroJar AG0025 and other jars of similar capacity.

1. Place the inoculated media plates in the appropriate anaerobic jar. Disposable plastic Petri dishes should be of the vented variety to aid gas transfer between the interior and exterior of the plates.
2. Tear open an AnaeroGen foil sachet at the tear-nick indicated, and remove the AnaeroGen paper sachet from within.
3. Immediately place the AnaeroGen paper sachet in the appropriate clip on the plate carrier within the jar. **N.B.** The AnaeroGen paper sachet will become warm to touch on exposure to air.
4. Close the jar lid immediately.
   N.B. The time taken between opening the foil sachet and sealing the jar should not exceed 1 minute. Extended exposure will result in loss of reactivity, and full anaerobic conditions may not be achieved in the jar.
5. After the appropriate incubation period remove the plates and examine for the presence of anaerobes. If the plates require re-incubation then a fresh AnaeroGen sachet must be used following steps 2-5 described above.
6. After incubation, the exhausted AnaeroGen sachet should be discarded with the appropriate laboratory waste.

Control Testing
It is recommended that an OXOID Anaerobic Indicator (BR0055) is used in the jar as a visual check that anaerobic conditions have been achieved and maintained.

The user should check their Anaerobic system periodically for its ability to provide adequate conditions for the growth of appropriate bacteria.

The following strains are recommended:

Clostridium novyii ATCC® 9690 growth

Kocuria rhizophila ATCC® 9341 no growth.

Disposal
On removal from the jar after incubation, the AnaeroGen paper sachet will retain a small amount of reactivity and will warm up. The sachets should be allowed to cool at room temperature prior to disposal alongside the appropriate laboratory waste.

Reference
1. Beerens H. (1998) *Int. J. Food Microbiol.* 40. 203-207.

ANAEROGEN™ COMPACT

Code: AN0010 & AN0020

AnaeroGen Compact is a simple system for the anaerobic incubation of up to 4 Petri dishes or an identification panel.

Description
The system consists of a plastic pouch and a paper gas generating sachet. The paper sachet contains

ascorbic acid and activated carbon which reacts on with air. Oxygen is rapidly absorbed and carbon dioxide is produced. When the paper sachet is placed in a sealed plastic pouch, this reaction will create ideal atmospheric conditions for the growth of anaerobes. It proceeds with **no evolution of hydrogen**, and therefore does **not require a catalyst**. **No water** is required to activate the reaction. This gives the system many advantages over the commonly used borohydride systems including increased safety and convenience.

When used as directed, the AnaeroGen Compact sachet will reduce the oxygen content in the pouch to below 1% within 30 minutes. The resulting carbon dioxide content will be between 8% and 14%. The level of carbon dioxide will depend on how many plates are placed in the pouch. AnaeroGen Compact has been designed for use with 1-4 plates.

The active components within each AnaeroGen Compact sachet are ascorbic acid and activated carbon.

Other Components

Each box of AN0010 contains:

10 AnaeroGen Compact paper sachets, individually wrapped in aluminium foil

10 Plastic Pouches

1 Product Insert

Each box of AN0020 contains:

20 AnaeroGen Compact paper sachets, individually wrapped in aluminium foil

1 Product Insert

Materials available separately

Sealing Clips (AN0005).

Plastic Pouches (AG0020)

W-Zip Pouches (AG0060)

Precautions

This product is for *in vitro* use only.

The AnaeroGen Compact paper sachet will become active on contact with air. It is therefore essential that the paper sachet is placed into the pouch and the pouch sealed within one minute.

The reaction of the ascorbic acid with oxygen is exothermic. However, the temperature of the AnaeroGen Compact paper sachet will not exceed 65°C.

Once the oxygen in the pouch has been absorbed, the temperature within the pouch will return to ambient temperature.

Storage

Store at 2-25°C. Under these conditions, the AnaeroGen Compact sachets will retain their activity until the expiry date given on the outer box and on the foil wrapped sachet.

Directions

1. Place the inoculated media plates or identification panel in the plastic pouch provided. Disposable plastic Petri dishes should be of the vented variety to aid gas transfer between the interior and exterior of the plates.
2. Tear open an AnaeroGen Compact foil sachet at the tear-nick indicated. Remove the AnaeroGen Compact paper sachet from within.
3. Immediately place the AnaeroGen Compact paper sachet in the plastic pouch. N.B. The AnaeroGen Compact paper sachet will become warm to the touch on exposure to air.
4. Expel excess air from the plastic pouch. Seal the plastic pouch immediately with the AnaeroGen Compact clip (AN0005). **N.B.** the time taken between opening the foil sachet and sealing the plastic pouch should not exceed 1minute. Extended exposure will result in loss of reactivity, and full anaerobic conditions may not be achieved in the pouch.
5. Incubate appropriately.
6. After the incubation period remove the plates or ID panel and examine for the presence of colonies or biochemical reaction. If the plates require re-incubation then a fresh AnaeroGen Compact sachet must be used following steps 2-5 described above. **N.B.** The plates may be initially inspected through the transparent plastic pouch. If the bag is opened, a fresh AnaeroGen Compact sachet is required for re-incubation.

7. After incubation, the exhausted AnaeroGen Compact paper sachet and plastic pouch should be sterilised and discarded with the non-hazardous laboratory waste.

Control Testing
It is recommended that OXOID Anaerobic Indicator (BR0055) is also used in the plastic pouch as a visual check that anaerobic conditions have been achieved and maintained.

The user should check their anaerobic technique, periodically, for its ability to provide adequate conditions for the growth of anaerobic bacteria.

The following strains are recommended:

Clostridium novyii ATCC® 9690 growth

Kocuria rhizophila ATCC® 9341 no growth

Disposal
On removal from the pouch after incubation, the AnaeroGen Compact paper sachet will retain a small amount of activity and become warm. The sachets should be allowed to cool to room temperature prior to sterilisation and disposal with the non-hazardous laboratory waste.

ANAEROJAR

Code: AG0025

Description
The 2.5 litre Oxoid AnaeroJar is an important addition to the Oxoid range of Atmosphere Generation Products. The jar is designed for use with the 2.5 litre AnaeroGen/CampyGen sachet and will hold up to 12 plates.

Important features include:
- No catalyst required.
- Polycarbonate base which is secured to the lid by 4 clips. These clips are designed to allow venting in the unlikely event of a positive pressure build-up occurring i.e. by allowing lid to lift and reseal to maintain correct conditions.
- A carrying handle for the safe transportation of the jar from bench to incubator.
- Vacuum Relief Screw to overcome any vacuum which may occasionally occur.

Operating Instructions

Note

Before use check:
a. 'O' ring is correctly seated

b. The vacuum relief screw is in the closed position.

1. Place inoculated plates into the plate carrier. Disposable plastic Petri dishes should be of the vented variety to aid gas transfer between interior and exterior of the dishes.

2. When using the anaerobic system (i.e. AN0025), prepare the Oxoid Anaeraobic Indicator (BR0055) by cutting and exposing 10 mm of the fabric strip, insert into the smaller, upper clip on the dish carrier.

3. Lower the carrier into the polycarbonate base.

4. Tear open an AnaeroGen/CampyGen/CO_2Gen sachet at the tear-nick indicated, and remove the paper sachet from within.

5. Immediately place the paper sachet in the appropriate clip in the plate carrier within the jar (see Technical insert).

6. Having inserted the sachet into the carrier immediately place the lid on the jar, making sure the 'O' ring is in place. Secure the clips with fingers shown in figure[1]. Repeat this process with each of the four clips to properly secure the lid.

7. Use carrying handle to transport jar to the incubator.

8. The anaerobic indicator will change from pink to white giving a visual indication of anaerobiosis.

9. Remove jar after the appropriate incubation period and open lid by carefully depressing the clips to release the jar lid from the base. Excessive downward pressure on the clips should be avoided.

10. Occasionally, a slight vacuum may occur after anaerobiosis, producing a negative pressure and resulting in resistance to the removal of the lid (after release of the clips). This is overcome by using an appropriate object such as a small coin to turn the vacuem relief screw anticlockwise allowing an inflow of air. It is important, however, to ensure the valve is resealed, by turning clockwise, prior to further use.

Precautions

1. **THE JAR IS DESIGNED TO BE USED WITH ANAEROGEN/CAMPYGEN/CO_2GEN AND MUST NOT BE USED WITH GAS GENERATING SYSTEMS THAT REQUIRE THE USE OF CATALYST (BR38), WHICH, WITHOUT A CATALYST WOULD RESULT IN A POTENTIALLY EXPLOSIVE H_2/O_2GAS MIXTURE.**
2. The jar should not be autoclaved.

Cleaning and Disinfection

Note

Disposable gloves should be worn throughout the following operations. The internal surface should be cleaned and disinfected with a compatible, proprietary disinfectant made up to manufacturer's recommended instructions. Disinfectants such as sodium hypochlorite, phenolic compounds, methyl alcohol and chloroform should be avoided as they will damage the surface of the jar. It is imperative that the jar is properly disinfected if it is necessary to return it to Oxoid.

Routine Maintenance and Checking

1. Lid and outer surface can be cleaned and dried with a soft tissue.
2. Regularly check integrity of the 'O' ring. Replace if there are any signs of deterioration such as splitting. Do not allow grease/organic solvents to come into contact.
3. Ensure that the jar is dry before use. Store in a suitable environment as excess moisture may quench reaction. The appearance of condensation during use is normal.

CAMPYGEN

Code: CN0025 & CN0035

Description

When a CampyGen sachet is placed in a sealed jar, the atmospheric oxygen in the jar is rapidly absorbed with the simultaneous generation of carbon dioxide, producing the appropriate microaerobic conditions. This novel method differs from others commonly used in that the reaction proceeds with no evolution of hydrogen, and therefore, does not require a catalyst. Furthermore, water is not required to activate the reaction.

An evaluation which compared CampyGen with the evacuation/replacement method and gas generating envelopes showed CampyGen to be effective.

Components

Each box contains:

10 CampyGen paper sachets which are individually foil packed.

1 Product Insert

The active component within each CampyGen sachet is ascorbic acid.

Precautions

This product is for *in vitro* use only

As soon as the CampyGen paper sachet is exposed to air, the reaction will start. It is therefore essential that the paper sachet is placed in the jar and the jar sealed within one minute.

The reaction of the ascorbic acid with oxygen is exothermic. However, the temperature of the CampyGen paper sachet will not exceed 65°C.

Storage

Store at 2-25°C. Under these conditions, the CampyGen sachets will retain their reactivity until the expiry date given on the outer box and on the foil sachet.

Directions

CN0025 is designed for use in 2.5 litre jars, including the new Oxoid AnaeroJar.

CN0035 is designed for use in 3.5 litre jars.

1. Place the inoculated media plates in the appropriate jar. Disposable plastic Petri dishes should be of the vented variety to aid gas transfer between the interior and exterior of the plates.
2. Tear open the CampyGen foil sachet at the tear-nick indicated, and remove the CampyGen paper sachet from within.
3. Immediately place the CampyGen paper sachet in the appropriate clip on the plate carrier within the jar. **N.B.** The CampyGen paper sachet will become warm to the touch on exposure to air.
4. Close the jar lid immediately. **N.B.** The time taken between opening the foil sachet and sealing the jar should not exceed 1 minute. Extended exposure will result in loss of reactivity, and microaerobic conditions may not be achieved in the jar.
5. After the appropriate incubation period, remove the plates and examine for the presence of Campylobacter. If the plates require re-incubation, a fresh CampyGen sachet must be used, following steps 2-5 described above.
6. After incubation, the exhausted CampyGen sachet should be discarded with the appropriate laboratory waste.

Control Testing

The user should check their microaerobic system periodically for its ability to provide adequate conditions for the growth of appropriate bacteria.

The following strain can be used for this purpose.

Campylobacter jejuni ATCC® 33291

Disposal

On removal from the jar after incubation, the CampyGen paper sachet may retain a small amount of reactivity and will warm up. The sachets should be allowed to cool to room temperature on an inert surface prior to disposal with the laboratory waste.

Reference

1. Bolton F. J., Wareing D. R. A. and Sails A. D. (1997) Eur. *J. Clin. Microbiol. Inf. Dis.* 16. 839-842.

CAMPYGEN COMPACT

Code: CN0020

Description

CampyGen Compact for 1 or 2 Petri dishes, is a simple system for generating microaerobic conditions. The system consists of a plastic pouch and sealing clip and a paper gas generating sachet. The paper sachet contains ascorbic acid which reacts on contact with air to produce the microaerobic conditions for the growth of microaerophilic organisms.

Components

20 CampyGen Compact paper sachets, individually wrapped in foil

1 product leaflet

Materials Required but not Provided

Sealing Clips (AN0005)

Plastic Pouches (AG0020)

W-Zip Plastic Pouches (AG0060)

Precautions

This product is for *in vitro* use only. The CampyGen Compact paper sachet will become active on contact with air. It is essential that the plastic pouch is sealed within one minute of exposing the paper sachet to the air. The reaction of ascorbic acid with oxygen is exothermic. However, the temperature of the CampyGen Compact paper sachet will not exceed 65°C.

Storage

Store at 2-25°C. Under these conditions, the CampyGen Compact sachets will retain their activity until the expiry date given on the outer box and on the foil wrap of the sachets.

Directions

1. Place 2 inoculated plates in a plastic pouch. Disposable plastic Petri dishes should be of the vented variety to aid gas transfer between the interior and exterior of the plates. If only one plate is to be inoculated, an uninoculated plate should also be placed in the plastic pouch to prevent further activity as the volume of O_2 and CO_2 is critical.
2. Tear open a CampyGen Compact foil sachet at the tear-nick indicated. Remove the CampyGen Compact paper sachet from within.
3. Immediately place the paper sachet in the plastic pouch with the plates. **N.B.** The paper sachet will become warm to the touch on exposure to air.
4. Expel excess air from the plastic pouch. Seal the plastic pouch immediately with a sealing clip or w-zip closure as appropriate. The time taken between opening the foil sachet and sealing the plastic pouch should not exceed 1 minute.
5. Incubate appropriately.
6. After the incubation period, remove the plates and examine for the presence of colonies. If the plates require re-incubation, a fresh CampyGen Compact sachet must be used, following steps 2-5 described above. **N.B.** The plates may be, initially inspected through the transparent plastic pouch. If the bag is opened, a fresh CampyGen Compact sachet is required for re-incubation.
7. After incubation, the exhausted CampyGen Compact paper sachet and plastic pouch should be sterilised and discarded with the non-hazardous laboratory waste.

Control Testing

The user should check their technique periodically for the ability to provide adequate conditions for the growth of microaerophilic bacteria. *Campylobacter jejuni* ATCC® 33291 may be used for this purpose.

Disposal

On removal from the pouch after incubation, the CampyGen Compact paper sachet will retain a small amount of activity and become warm. The sachets should be allowed to cool to room temperature prior to sterilisation and disposal with the non-hazardous laboratory waste.

CO_2GEN

Code: CD0025

Description

CO_2Gen is designed for the generation of a carbon dioxide-rich atmosphere within a gas jar. The paper sachet contains ascorbic acid which reacts with air to produce a level of approximately 6% carbon dioxide within a 2.5 litre gas jar such as the Oxoid AnaeroJar (AG0025). The final concentration of oxygen is 15%.

Components

10 CO_2Gen paper sachets, individually wrapped in foil

1 product leaflet

Materials Required but not Provided

2.5 litre gas jar (Oxoid AnaeroJar AG0025).

Precautions

This product is for in vitro use only. The CO_2Gen paper sachet will become active on contact with air. It is therefore essential that the paper sachet is placed in the jar and the jar sealed within one minute. The reaction of the ascorbic acid with oxygen is exothermic. However, the temperature of the CO_2Gen paper sachet will not exceed 65°C.

Storage

Store at 2-25°C. Under these conditions, the CO_2Gen sachets will retain their activity until the expiry date given on the outer box and on the foil wrap of the sachets.

Directions

1. Place inoculated media plates in a 2.5 litre gas jar. Do not use a 3.5 litre jar. Disposable plastic Petri dishes should be of the vented variety to aid gas transfer between the interior and exterior of the plates.
2. Tear open a CO_2Gen foil sachet at the tear-nick indicated. Remove the sachet from within.

3. Immediately place the paper sachet in the 2.5 litre gas jar. **N.B.** The paper sachet will become warm to the touch on exposure to air.
4. Seal the jar immediately. The time taken between opening the foil sachet and sealing the jar should not exceed 1 minute.
5. Incubate appropriately.
6. After the incubation period, remove the plates and examine for the presence of colonies. If the plates require re-incubation, a fresh CO_2Gen sachet must be used, following steps 2-5 described above.
7. After incubation, the exhausted CO_2Gen paper sachet should be sterilised and discarded with the non-hazardous laboratory waste.

Disposal
On removal from the jar after incubation, the CO_2Gen paper sachet will retain a small amount of activity and become warm. The sachets should be allowed to cool to room temperature prior to sterilisation and disposal with the non- hazardous laboratory waste.

CO_2GEN COMPACT

Code: CD0020

Description
CO_2Gen Compact is a simple system for the generation of a carbon dioxide-enriched atmosphere for the incubation of 2 Petri dishes. The system consists of a plastic pouch and sealing clip (alternatively a w-zip pouch can be used) and a paper gas generating sachet. The paper sachet contains ascorbic acid which reacts with air to produce an atmosphere which contains approximately 6% carbon dioxide. The final concentration of oxygen is 15%.

Components
20 CO_2Gen Compact paper sachets, individually wrapped in foil

1 product leaflet

Materials Required but not Provided
Sealing Clips (AN0005)

Plastic Pouches (AG0020) or

W-Zip Pouches (AG0060)

Precautions
This product is for *in vitro* use only. The CO_2Gen Compact paper sachet will become active on contact with air. It is therefore essential that the paper sachet is placed in the pouch and the pouch sealed within one minute. The reaction of the ascorbic acid with oxygen is exothermic. However, the temperature of the CO_2Gen Compact paper sachet will not exceed 65°C.

Storage
Store at 2-25°C. Under these conditions, the CO_2Gen Compact sachets will retain their activity until the expiry date given on the outer box and on the foil wrap of the sachets.

Directions
1. Place 2 inoculated media plates in a plastic pouch. Disposable plastic Petri dishes should be of the vented variety to aid gas transfer between the interior and exterior of the plates. If only one plate is to be inoculated, an uninoculated plate should also be placed in the plastic pouch.
2. Tear open a CO_2Gen Compact foil sachet at the tear-nick indicated. Remove the CO_2Gen Compact paper sachet from within.
3. Immediately place the paper sachet in the plastic pouch. N.B. The paper sachet will become warm to the touch on exposure to air.
4. Expel excess air from the plastic pouch. Seal the plastic pouch immediately with a sealing clip or w-zip closure as appropriate. The time taken between opening the foil sachet and sealing the plastic pouch should not exceed 1 minute.
5. Incubate appropriately.
6. After the incubation period, remove the plates and examine for the presence of colonies. If the plates

require re-incubation, a fresh CO_2Gen Compact sachet must be used following steps 2-5 described above. N.B. The plates may be initially inspected through the transparent plastic pouch. If the bag is opened, a fresh CO_2Gen Compact sachet is required for re-incubation.

7. After incubation, the exhausted CO_2Gen Compact paper sachet and plastic pouch should be sterilised and discarded with the non-hazardous laboratory waste.

Disposal
On removal from the pouch after incubation, the CO_2Gen Compact paper sachet will retain a small amount of activity and become warm. The sachets should be allowed to cool to room temperature prior to sterilisation and disposal with the non-hazardous laboratory waste.

THE ATMOSPHERE GENERATION SYSTEM

In 1993, Oxoid launched a new range of innovative products under the title of Atmosphere Generation System (AGS). These novel products are safer (no hydrogen produced) and more convenient (no water to add). They include a new jar of advanced design ± Anaerojar – in which it is not necessary to use a catalyst.

This range has, subsequently, been extended and now consists of the following products:

Anaerobic Indicator	Code BR0055	Anaerobic Atmosphere Generation System
AnaeroGen™ (for 2.5 litre jar)	Code AN0025	
AnaeroGen™ (for 3.5 litre jar)	Code AN0035	
AnaeroGen Compact™ (for use with plastic pouches	Code AN0010 & AN0020	
CampyGen™ (for 2.5 litre jar)	Code CN0025	Atmosphere Generation for Microaerophilic organisms
CampyGen™ (for 3.5 litre jar)	Code CN0035	
CampyGen Compact (for use with plastic pouches	Code CN0020	
CO_2Gen™ (for 2.5 litre jar)	Code CD0025	Atmosphere Generation for CO_2dependent organisms
CO_2Gen Compact (for use with plastic pouches	Code CD0020	
AnaeroJar™	Code AG005	

A range of accessories for the Compact products and the AnaeroJar completes the Atmosphere Generation System.

Plastic pouches	Code AG0020
Sealing clips (for use with AN0020)	Code AN0005
W-Zip plastic pouches (no clips required)	Code AG0060

6
BLOOD CULTURE

OXOID SIGNAL BLOOD CULTURE SYSTEM

The following section describes briefly the Oxoid SIGNAL Blood Culture System, the principles of its function and the equipment required for its optimal performance. For full details of the usage of the System the product insert should be consulted.

Principle of the Test

Blood samples are collected from patients, using strict aseptic technique and sterile equipment. The samples are inoculated into the blood culture bottles and mixed with the medium.

The formulation of the medium encourages the growth of aerobic, anaerobic and micro-aerophilic organisms. The medium is also designed to create pressure in the sealed bottle when organisms are growing.

The detection of positive pressure is by means of a growth indicator device which is connected to the bottle after the blood sample is added. A positive pressure in the bottle displaces a quantity of blood/broth mixture into the chamber as a sign of microbial activity.[2,3,4,5]

A positive result is indicated when the blood/broth mixture rises above the green locking sleeve of the growth indicator device.

Medium Composition

Typical formulation (European Patent 0124193 Al)

	gm/litre
Tryptone Soya Broth	10.0
Gelatin peptone	10.0
Yeast extract	5.0
Meat extract	5.0
Sodium chloride	8.0
Potassium nitrate	2.0
Glucose	1.0
L-arginine	1.0
Sodium pyruvate	1.0
Gelatin	1.0
Sodium thioglycollate	0.5
Cysteine HCl	0.4
Sodium bicarbonate	0.4
Phosphate buffer	0.3
Sodium polyanethol sulphonate	0.3
Dithiothreitol	0.2
Adenine sulphate	0.01
Sodium succinate	0.01
Ammonium chloride	0.008
Magnesium sulphate	0.008
Menadione	0.005

Sodium polyanethol sulphonate (SPS), 0.03% is added because it inhibits clotting[6], neutralises the bactericidal effect of human serum[7], prevents phagocytosis[8] and partially inactivates certain antibiotics (streptomycin, kanamycin, gentamicin and polymyxin B)[9,10]. SPS may be inhibitory to some strains of *Peptostreptococcus anaerobius*, *Neisseria meningitidis* and *N. gonorrhoeae*; therefore gelatin is added to the medium to neutralise this inhibition[11,12]. When human blood is added to this medium, CO_2 produced can be detected at 2.5 to 5% v/v in the bottle head-space.[5]

Materials required but not provided

1. Sterile syringe or other means of obtaining blood.
2. Alcohol solutions, or other suitable skin disinfection material.
3. Culture media and other equipment for subcultures.
4. Incubator equipment to maintain 36 ± 1°C.
5. Orbital shaker (for optimal results)

Components of the System

1. A sealed blood culture bottle containing 84 ml of broth medium. 20 bottles per pack. a sterile growth indicator device which is vented through a 0.2 micron hydrophobic membrane. 20 per pack Product Code BC0100.

Method of Use (for full details see current product insert)

A. Inoculation Procedure

1. Examine the bottle of broth before taking the blood sample and discard it if any evidence of contamination can be seen.

2. Prepare the bottle for inoculation before taking the blood sample. Remove the green plastic 'flip-off' cap and disinfect the exposed part of the rubber stopper.

3. Aseptically inject a maximum volume of 10 ml of blood through the central ring of the rubber stopper. (The partial vacuum in the bottle will accept 12 ml of blood.)

4. Thoroughly mix the blood with the broth in the bottle.

5. Write the patient's name and identification details on the bottle label.

6. Immediately transfer the inoculated blood culture bottle to the laboratory. In the event of the laboratory being closed or transportation being delayed, the bottle should be incubated at 36 ± 1°C, and the 'Laboratory Procedure', detailed below, carried out at the earliest opportunity (within 24 hours).

B. Laboratory Procedure

1. Place the inoculated bottle in an incubator at 36 ± 1°C for approximately 1 hour.

2. Remove from the incubator and place the bottle in an incubation tray.

3. Disinfect the rubber stopper of the bottle by swabbing, e.g. with alcohol.

4. Remove the growth indicator device from its sterile package and ensure that the needle and cap are fully tightened. (Hold the clear plastic body of the device with the covered needle pointing downwards. Tighten the needle by turning the needle cover anti-clockwise. Tighten the cap by turning it clockwise.)

5. Slide the plastic shield from the needle. Do not touch the needle.

6. Aseptically insert the needle through the centre of the rubber stopper. Push the needle shaft as far as it will go through the rubber stopper.

7. Slide the green locking sleeve of the growth indicator device downwards until it fully locks on to the neck of the blood culture bottle. Press down the chamber to ensure full contact with the rubber seal of the bottle.

8. For optimal results shake the system for approximately 24 hours at 150 orbits/minute, using a shaker placed in the incubator, or a bench top integrated shaker/incubator, at 36 ± 1°C. (If use of a shaker in the first 24 hours is impossible the system should be manually shaken as often as possible (at least 4 times) during this period.)

9. Examination of the system for a positive result should be carried out at least twice daily.

10. At the end of the 24 hour period, remove the system from the shaking apparatus and place on the shelf of an incubator preset at 36 ± 1°C.

11. Examine the system on the incubator shelf twice daily and if positive remove for further examination. Vigorously agitate the negative systems to resuspend the erythrocytes in the broth and return to the incubator shelf. A total incubation period of at least 7 days is recommended concluding with a terminal subculture.

12. POSITIVES – mix the contents of the chamber, unscrew the green cap and aseptically remove a sample of blood/broth mixture for subculture, microscopy and susceptibility testing. The vent in the cap contains a 0.2 micron hydrophobic membrane which ensures that the chamber is not under pressure. After sampling replace the cap on the chamber.

A positive blood culture, indicating growth of micro-organisms is recognised by the appearence of the blood/broth mixture in the transparent growth indicator device above the level of the locking sleeve. A visual inspection is also recommended for lysis, turbidity or the appearence of colonies on the interface of the blood layer as these may become apparent before the blood/broth mixture appeares above the locking sleeve.

Quality Assurance

The following organisms are used by Oxoid as part of the quality assurance of the product. The total inoculum challenge for each test organism per bottle is 10 to 50 colony forming units (CFU's).

	ATCC® Number	***NCTC Number***
Bacillus cereus	10876	7464
Bacteroides fragilis	25285	9343
Clostridium novyi	27606	
Clostridium perfringens 13124		
Escherichia coli	25922	
Fusobacterium nucleatum	10953	10562
Haemophilus influenzae	19418	4560
Klebsiella pneumoniae	29665	11228
Neisseria meningitidis	13077	10025
Peptostreptococcus anaerobius	27337	11460
Prevotella bivia 29303		
Pseudomonas aeruginosa	27853	
Staphylococcus aureus	25923	
Staphylococcus epidermidis	14990	
Streptococcus pneumoniae	6303	
Streptococcus mutans	25175	10449
Candida albicans	10231	(NCPF 3179)

User Quality Assurance

1. Examine the bottles of broth for turbidity and/or change of colour before adding any blood. Discard any bottles showing abnormal characteristics.
2. If further user quality control is required, it is recommended that 3 aerobes and 1 anaerobe from the above list be used.

References

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7
ANTIMICROBIAL SUSCEPTIBILITY TESTING

2006

ANTIMICROBIAL SUSCEPTIBILITY TESTING

ANTIMICROBIAL SUSCEPTIBILITY TESTING (AST)

The Oxoid system of AST is the disc diffusion method which although developed in 1947[1] remains as the most widely used test. Performed with care, using adequate controls, it is as accurate as more costly and complicated tests.

In common with all other methods it cannot mimic the *in vivo* environment but it does uniquely show the effect of a changing antimicrobial concentration (first rising and then falling) on an increasing microbial population. The minimum inhibitory concentration (MIC) of the antimicrobial appears at the edge of the zone of inhibition which represents the interaction of a critical concentration of antimicrobial at a critical time on a critical microbial population[2].

In October 1997 Oxoid *aura* was launched. This enhances the AST system by using an accurate and objective electronic zone size measurement facility linked to unique species specific interpretative software, and other recognised suscepability databases.

Oxoid *aura* ensures high reproducibility with the facility to store and process data for future comparison and epidemiological studies.

The methods are simple but the above interactions are very complex and the accuracy and precision of the test is based on the following critical components:

CULTURE MEDIUM

The medium chosen for the test must have been manufactured and tested specifically for AST. Oxoid manufactures and recommends the following agar media:

i Diagnostic Sensitivity Test Agar (DST Agar) CM0261 which was the first medium specifically designed for this test and is still very popular.

ii HR Medium CM0845 – a chemically defined medium for susceptibility testing with antifungal agents.

iii HTM (Haemophilus Test Medium) CM0898 was specifically formulated for the susceptibility testing of *Haemophilus influenzae*. The medium is based on Mueller-Hinton Agar to which a supplement (SR0158) containing NAD and Haematin is added.

iv Iso-Sensitest Agar CM0471 was developed as a semi-synthetic AST medium in which undefined protein hydrolysates were reduced to the minimum level which would allow optimum growth of a wide range of organisms and the ‘free’ cation strength adjusted to give correct MIC results. Its popularity rests on its reproducible performance.

v Mueller-Hinton Agar CM0337 was originally formulated to grow pathogenic neisseria and it was adopted for use in the Bauer-Kirby test[4]. Its variable performance was strongly criticised but following agreement between culture media manufacturers and representatives of the Clinical Laboratory Standards Institute and the FDA a uniform medium is now produced which must meet the CLSI specifications[5]. Therefore Mueller-Hinton medium suitable for AST will carry a statement on the label that it meets CLSI standards (M6).

vi Sensitest Agar CM0409 the first semi-defined AST medium which remains popular with laboratories which have designed methods around this specific formulation[3].

vii Wilkins-Chalgren Anaerobic Agar CM0619 was developed as a medium for the growth of anaerobic bacteria. Its relative lack of inhibitory action towards anaerobes made it the medium of choice for anaerobic agar dilution tests[6].

Anaerobic disc diffusion tests can be carried out but it is essential to include adequate controls which can monitor the effect of anaerobiosis on antimicrobials.

All the above media have broth versions of their formulations (except DST and HTM.). This allows dilution or pre- enrichment to be carried out in the same medium. It is also helpful when carrying out MIC tests in broth to standardise disc diffusion zones to establish the breakpoint categories of S,I,R.

Many of the constituents used in culture media affect the precision and accuracy of AST results. The agar must allow free diffusion of the antimicrobial from the disc. Variations in pH and ionic strength will cause differences in zone sizes. Blood can reduce the zone size of highly protein-bound antimicrobials e.g. fusidic acid. Changes in ‘free’ electrolyte content will affect aminoglycosides, tetracyclines and polymyxins. Glucose will enlarge the zones of antimicrobials against organisms which are adversely affected by a fall in pH following fermentation of the sugar. Thymidine and thymine levels have to be monitored and reduced, if necessary, to prevent antagonism of trimethoprim and sulphonamides. All AST media must be specifically tested with critical `drug-bug’ combinations to measure their performance and to ensure that they meet the

quality specifications. The latter tests are carried out by the manufacturer, to ensure that the medium conforms to regulatory standards, they should also be carried out in an abbreviated form in the user laboratory to monitor the performance of all of the components of AST.

ANTIMICROBIAL DISCS

The paper discs used in the diffusion method are made from paper which conforms to the WHO[7] and FDA[8] standards.

Impregnation of the discs ensures that prepared solutions of antimicrobials are accurately appliedacross the paper. The drying procedures used do not affect this uniform distribution or cause deterioration in activity of the antimicrobial.

When each cartridge of 50 discs is sealed together with a molecular sieve in a foil-covered see-through blister, the discs contain less than 2% moisture and are stored at low temperatures. Shelf-life studies of the discs in their packaging demonstrate that they meet the stated storage life printed on the labels.

Finally, samples are taken from every batch/lot manufactured and the discs tested microbiologically to confirm that the antimicrobial content lies within 90-125 % of the stated content on the label.

Storage

Discs must be stored at –20°C if kept for long periods. Storage at 2-8°C is suitable for discs currently being used or to be used very soon. Discs should be returned to the refrigerator as quickly as possible after use. The most common cause of moisture reaching the discs and causing destruction of labile antimicrobials is condensation of warm laboratory air on cold discs removed from the refrigerator.

It is important to allow the cartridge blister pack to reach room temperature before exposing the discs, a period of one hour is generally sufficient. Use discs in order of expiry date, which is valid only for unopened blisters stored under proper conditions. Once a cartridge has been opened it needs to be stored in a dessicated environment at 2-8°C for no more than one week.

INOCULUM

One of the very critical factors for accuracy and precision in disc diffusion tests is the inoculum preparation. It is therefore important to use a technique which will always yield a uniform suspension of the correct number of organisms.

Various techniques are described in which suspensions of pre-grown organisms are prepared or small inocula are incubated for fixed periods of time. It is important that more than one colony is sampled (4-10 cols) to ensure a representative sample of the organism has been taken.

Some form of standardisation of the final suspension is necessary and it should be noted that different organisms will display different opacities of solution to yield a dense but not confluent growth.

To complement the Oxoid aura System Oxoid have the Oxoid Turbidometer. This instrument provides the inoculum density standardisation for 0.5 McFarland necessary to ensure accurate reproducible results.

OTHER FACTORS INFLUENCING THE RESULTS

Temperature of incubation – the incubators should be checked for satisfactory performance and their recording thermometers should show air temperatures of 35-37°C with fluctuations of not more than 2°C. Agar plates should not be placed in high stacks because the middle plates will take longer to reach the incubator temperature and this delay could cause overlarge zones.

Pre-incubation and pre-diffusion conditions – a routine procedure should be established so that inoculated plates have discs applied not later than 15 minutes after inoculation. This prevents a pre-incubation of organisms before the antimicrobial discs are applied. Similarly once the discs have been applied, plates should be placed in the incubator within a 15 minute interval, to prevent pre-diffusion of the antimicrobial at room temperature.

Uniformity of agar depth – plates should be poured on levelled surfaces, using dishes with flat bottoms, to ensure a uniform depth of agar (3-4 mm in depth).

Application of discs – it is essential that discs are in intimate contact with a moist agar surface. Therefore either use dispensers which have a tamping action on the discs or press them separately after application. Do not overdry the agar surface before applying the discs.

Incubation period – ensure uniform times of incubation for the plates, 16-18 hours at 35-37°C is usually satisfactory.

Interpretation of zone sizes – after incubation the plates are removed and the zones of inhibition noted and measured. The diameter of the zone (including the diameter of the disc) is measured to the nearest millimetre, using calipers and compared to the appropriate standard method.

Where other systems are used the zone size breakpoints should have been determined using MIC/zone comparative tests, following the particular methodology chosen.

Control cultures – it is essential that each laboratory maintains adequate control over AST methods by testing reference cultures at regular intervals. Daily tests may be required if new media and discs are constantly being used.

The following reference strains are used in the CLSI test method.

Staphylococcus aureus ATCC® 25923
Escherichia coli ATCC® 25922
Pseudomonas aeruginosa ATCC® 27853
Haemophilus influenza ATCC® 49247
Neisseria gonorrhoeae ATCC® 49226
Streptococcus pneumonia ATCC® 49619

Escherichia coli ATCC® 35218 for testing compounds containing beta lactamase inhibitors.

Records of the results obtained with these reference strains should be maintained in log books and any deviation of zone sizes from the range accepted should be investigated.

References

1. Bondi A., Spauling E. H., Smith E. D. & Dietz C. C. (1947) *Amer. J. Med. Sci.* 214. 221-225.
2. Cooper H. E. (1964) *The theory of antibiotic zones.* In Kavanagh F. (Ed) *Analytical Microbiology*. Academic Press. New York. 1-86.
3. Bell S. M. (1975) *Pathology.* 7. I-48 (Suppl).
4. Bauer A. W., Kirby W. M. M., Sherris J. C. & Turck M. (1966) *Amer. J. Clin. Path.* 45. 493-496.
5. Pollock H. M. et al. (1986) *J. Clin. Microbiol.* 24. 1-6.
6. Nat. Comm. Clin. Lab. Standards (1979) Proposed reference dilution procedure for AST of anaerobic bacteria. NCCLS PSM-11. Villanova. Pa. USA.
7. WHO (1977) *Tech. Rep. Ser.* No. 610.
8. FDA (1978) *Codes of Fed. Rebs.* 21. Part 460.

Note

The list of antimicrobial discs manufactured, the generic names, the symbols and range of levels available are supplied in the Oxoid Product List and the list is updated annually.

ANTIMICROBIAL SUSCEPTIBILITY DISCS

Discs in routine use should be stored at 2-8°C. Longer term storage should be at –20°C. After cold storage allow discs to reach room temperature before opening storage containers. Discs are presented in cartridges for dispensing either individually with Oxoid ejectors or with the Oxoid Dispenser System. There are 50 discs per cartridge, 5 cartridges per pack.

Each cartridge is individually sealed together with a molecular seiveto maintain low moisture levels. The cartridge is packed in a foil-covered see-through blister designed to allow the microbiologist better control and storage of the discs in use.

THE OXOID DISC DISPENSER MKIII

An enhanced system for antimicrobial susceptibility testing incorporating the new ergonomically designed Disc Dispenser. It has a simple one-handed operation and is fully height-adjustable to cater for various depths of media. The cover and base of the dispenser are fully interlocked to prevent the ingress of moisture. Cartridges ‘click’ positively into their correct locations and a plastic skirt ensures that each agar plate is precisely centred every time the dispenser is used.

Product	*Pack size*	*Order Code*
Disc Dispenser (90 mm) for 6 cartridges	1	ST6090
Disc Dispenser (90 mm) for 8 cartridges	1	ST8090
Disc Dispenser (100 mm) for 6 cartridges	1	ST6100
Disc Dispenser (100 mm) for 8 cartridges	1	ST8100
Disc Dispenser (150 mm) for 12 cartridges	1	ST1215
Ejector 6 HP0053 for using cartridges individually	6	HP0053
Replacement Dessicant Pack	1	ST9100

ANTIMICROBIAL SUSEPTIBILITY TESTING MEDIA

Media for Antimicrobial Susceptibility Testing (coded CM) should be stored in their closed containers at an even temperature in a cool, dry place, away from direct light.

Product	*Pack Size*	*Order Code*
Diagnostic Sensitivity Test Agar Base (DST Agar)	500 g	CM0261
HR Medium	100 g	CM0845
Haemophilus Test Medium Base (HTM)	500 g	CM0898
HTM Supplement	10 vials	SR0158
'Iso-Sensitest' Agar	500 g	CM0471
'Iso-Sensitest' Broth	500 g	CM0473
Mueller-Hinton Agar	500 g	CM0337
Mueller-Hinton Broth	500 g	CM0405
'Sensitest' Agar	500 g	CM0409
Wilkins-Chalgren Anaerobe Agar	500 g	CM0619
Wilkins-Chalgren Anaerobe Broth	500 g	CM0643

DIAGNOSTIC DISCS

Code: DD

These products are, as the name implies, 6 mm paper discs impregnated with compounds which aid the identification of micro-organisms when placed on agar surfaces or into suspensions of organisms.

Storage
Diagnostic Discs should be stored at 2-8°C. They may also be stored in the freezer (–20°C). DD0004 and DD0005 are best stored between –10 to –20°C.

Moisture Protection
Cartridges of discs should be kept in moisture-proof containers with desiccant sachets and allowed to reach room temperature before opening. Discs in cartridges are protected from moisture but care should be taken not to open them before they have reached room temperature. This is especially important when cartridges are taken from –20°C storage.

Quality Control
Routine tests should be made using known strains of positive and negative-reaction organisms to confirm the activity of the discs and to control the other factors in the test.

'AN-IDENT' DISCS

Code: DD0006

To provide a rapid and simple method for presumptive identification of anaerobic gram negative bacteria.

Disc Contents

The set consists of one cartridge of each of the antibiotic discs (see Table) packed with a desiccant sachet in a securitainer. Each cartridge contains 50 discs.

Store at 2-8°C. May be stored in the freezer (–20°C).

Antibiotic levels on the discs are as follows:

		Coded
Erythromycin	60 µg	E60
Rifampicin	15 µg	RD15
Colistin	10 µg	CT10
Penicillin	2 IU	P2
Kanamycin	1000 µg	K
Vancomycin	5 µg	VA5

The set should be arranged in the Oxoid Disc Dispenser (ST6090, ST6100) in the above order to coincide with the order given in the identification table. It is primarily designed to allow easy identification of *Bacteroides fragilis*, the anaerobe species most commonly isolated in the routine hospital laboratory.

Description

'An-ident' discs (DD0006) provide a rapid and simple method for presumptive identification of anaerobic Gram negative bacteria. **They are not suitable for antibiotic susceptibility testing to assess the clinical effectiveness of the various antibiotics**.

Sutter and Finegold[1] proposed a method for the identification of Gram negative *anaerobic bacilli*, based on differences in response to a number of antibiotics. Using carefully chosen levels of colistin, erythromycin, kanamycin, neomycin, penicillin and rifampicin, with disc diffusion sensitivity testing techniques, they showed that these bacteria could be placed fairly consistently into five groups. Other simple biochemical tests and cultural characteristics were used for further identification. In later work, summarised by Finegold[2] 5 ug vancomycin discs were substituted for neomycin.

The Oxoid 'An-ident' (DD0006) set makes available these discs in a cartridge presentation, suitable for use, either in the Oxoid Disc Dispenser (ST6090, ST6100) or with Oxoid Ejectors ((HP0053). Ejectors (HP0053) can be used independently of the dispenser.

Technique

The technique as recommended by Leigh and Simmons[3] is as follows:

An actively growing culture in Thioglycollate Medium (CM0173) or Schaedler Broth (CM0497) is used as an inoculum. Using a sterile swab, spread the organism uniformly across the surface of a blood agar plate. Alternatively a flood-plate may be prepared from a suspension of several colonies in Thioglycollate Broth (CM0391).

Discs of each antibiotic are then applied and the plate incubated anaerobically at 35°C for 24-48 hours. The Oxoid Gas Generating Kit provides a convenient way of achieving anaerobiosis and ensuring that adequate levels of CO_2 are present in the anaerobic jar. Strains of *Bacteriodes fragilis*, *Fusobacterium fusiforme*, *Sphaerophorus necrophorus* and *S. varius* are used as controls. The diameters of inhibition zones are measured in millimetres; less than 10 mm is considered resistant; equal to or greater than 10mm considered sensitive.

Quality Control

See under Technique.

References

1. Sutter V. L. and Finegold S. M. (1971) *Applied Microbiology* **21.** 13-20.
2. Finegold S. M. (1977) *Paper presented at the IMLS meeting, Liverpool, August 1977*.
3. Leigh D. A. and Simmons K. (1977) *J. Clin. Path.* **30.** 991-992.

BACITRACIN DISCS

Code: DD0002

For the differentiation of Lancefield Group A Streptococcus from other beta-haemolytic Streptococci.

Contents
Two pack formats are available, a single cartridge containing 50 discs, or a five pack version containing five individually sealed cartridges each with 50 discs.

Store at 2-8°C. May also be stored in the freezer (–20°C).

Description
Sterile paper discs each containing 0.04 units of bacitracin, for the differentiation of Lancefield Group A from other beta- haemolytic streptococci. When used as a screening test, prior to serological grouping, Bacitracin Discs (DD0002) save time, labour and materials.

Maxted[1] showed that Group A streptococci were more sensitive to bacitracin than beta-haemolytic strains of other groups, and that bacitracin might, therefore, be used as a rapid diagnostic agent. He found that only 1.7% of 2,386 bacitracin sensitive strains proved not to be Group A *Streptococci*, and only 2.5% of 851 resistant strains were Group A. Levinson and Frank[2] who employed impregnated paper discs for the same purpose, observed that 93.2% of 866 sensitive beta-haemolytic streptoccoci belonged to Group A. Streamer *et al*.[3] compared bacitracin disc, fluorescent antibody and Lancefield precipitin techniques for the identification of Group A Streptococci. The bacitracin disc technique was considered to be the simplest and most practical for the routine clinical laboratory.

The use of bacitracin sensitivity testing is not restricted to the differentiation of beta-haemolytic Streptococci. Guthof[4] found sensitivity to optochin, bacitracin, and furacin to be a useful test for distinguishing *Aerococcus viridans* and *Streptococcus milleri* from enterococci and mitis streptococci.

Technique

Pure Cultures
1. Evenly inoculate the surface of a blood agar plate (Blood Agar Base No. 2 (CM0271) with 7% Sterile Oxalated Horse Blood (SR0049)) with a pure culture of the beta-haemolytic *Streptocccus* to be tested.
2. Aseptically place a Bacitracin Disc (DD0002) on the inoculated surface.
3. Incubate for 18 -24 hours at 35°C.
4. Examine for the presence of a zone of inhibition around the Bacitracin Disc (DD0002). A zone indicates that the streptococcus is presumptively Group A – if desired, further confirmation is obtained by serological grouping.

Clinical Material
1. Inoculate a Blood Agar plate with the throat swab or other material. Streak the inoculum to ensure that discrete colonies occur on some portion of the plate - so that the species in a mixed growth may be determined.
2. Aseptically place a Bacitracin Disc (DD0002) on the secondary area of inoculation.
3. Incubate for 18-24 hours at 35°C.
4. Examine for zones of inhibition. Organisms other than beta-haemolytic *Streptococci* are sensitive to bacitracin, so that the presence of a zone of inhibition does not necessarily indicate Lancefield Group A *Streptococci*. It is possible to select presumptive Group A *Streptococci* if attention is given to colonial morphology. Further confirmation is obtained by serological grouping. Bacitracin Discs (DD0002) are not recommended for routine testing of the sensitivity of organisms to bacitracin. For this purpose it is advisable to employ Bacitracin Sensitivity Discs (CT0005B) at a level of 10 units.

Quality Control
Use known Group A and non-Group A Streptococci to monitor the accuracy of the discs and inoculum.

References
1. Maxted W. R. (1953) *J. Clin. Path.* **6.** 224-226.
2. Levinson M. L. and Frank P. F. (1955) *J. Bact.* **69.** 284-287.
3. Streamer C. W. *et al.* (1962) *Amer. J. Dis. Children* **104.** 157-160.
4. Guthof O. (1960) *Ztschr. f. Hyg. u. Infektionskr.* **146.** 425-432.

CEFOXITIN DISCS

Code: DD0025

Code: DD0026

Two diagnostic discs have been made available for the detection of mecA resistance.

Contents
5 cartridges individually sealed, each containing 50 discs.

Disc Content

	DD0025	***DD0026***
Cefoxitin	5 µg	10 µg

Description
Cefoxitin is a cephamycin which is bactericidal through inhibition of cell wall synthesis.

A number of recent studies have indicated that the use of Cefoxitin has a higher sensitivity and specificity than other compounds traditonally recommended for detecting mecA resistance in Staphylococci.

Quality control
It is recommended that known mecA positive a nd negative strains are used to monitor performance.

KANAMYCIN 1000 µg DISCS

Code: DD0027

Contents
1 cartridge containing 50 discs.

Description
Kanamycin is an aminoglycoside which in high concentration can be used in combination with routine susceptibility discs to presumtively identify Gram negative anaerobic bacilli.

	VA5	***K1000***	***CT10***
Bacteroides fragilis Group	R	R	R
Bacteroides ureolyticus Group	R	S	S
Fusobacterium spp.	R	S	S
Porphyromonas spp.	S	R	R
Veillonella spp.	R	S	S

METRONIDAZOLE DIAGNOSTIC DISCS (50 µg)

Code: DD0008

1 cartridge containing 50 discs.

SULPHONAMIDE DIAGNOSTIC DISCS (1000 µg)

Code: DD0011

An aid in the identification of Gardnerella vaginalis.

Contents
1 cartridge containing 50 discs.

Disc Contents
DD0008 Metronidazole 50 µg

DD00011 Sulphonamide 1000 µg

Description

Smith and Dunkelberg[1] reported that metronidazole, previously thought to inhibit obligate anaerobic bacteria only, was found *in vitro*, to inhibit the growth of facultatively anaerobic strains of *Gardnerella vaginalis*, when using the agar diffusion method.

Small pleomorphic Gram negative rods associated with 'non-specific' bacterial vaginitis were recognised by Gardner and Dukes[2] as the aetiologic agent. They named the organism *Haemophilus vaginalis*. However, in obvious conflict with the accepted definition of the genus *Haemophilus*, it does not have a requirement for Haemin (X factor), Nicotinamide Adenine Dinucleotide (NAD or V factor), or any other co-enzyme-like growth factor. On the basis of microscopic morphology, Zinnemann and Turner[3] argued that it should be reclassified in the genus *Corynebacterium* and suggested the name *Corynebacterium vaginale*.

Taxonomic studies[4,5] have led to the naming of a new genus *Gardnerella* for inclusion of the organisms previously classified as *H. vaginalis* or *C. vaginale* with the type species *G. vaginalis*.

Bailey *et al*.[6] have recommended that the susceptibility and resistance to metronidazole and sulphonamide in conjunction with fermentation tests should be used as an aid in the separation of *G. vaginalis* from other possibly unrecognised biotypes of *G. vaginalis* or other vaginal bacteria that presumptively resemble the organism. They recommended a content of 50 µg metronidazole per disc (DD0008).

Bacterial group or species susceptibility to discs of:

	sulphonamide (1 mg)	***metronidazole (50 µg)***
G. vaginalis	R	S
Bifidobacteria and *G. vaginalis*-like organisms	S	S
Streptococci	R	R
Lactobacilli	R	R

S: Susceptible R: Resistant

Technique

Inoculate the isolation medium with the specimen and place a 50 µg metronidazole disc (DD0008) and a 1000 µg sulphonamide (DD0011) disc on an area of the plate where heavy, but not confluent, growth can be expected. Smith and Dunkelberg[1] incubated at 35°C in air containing approximately 8% carbon dioxide, but more recently Ralph *et al*.[9] in a study of MICs of a number of antibiotics for *G. vaginalis* reported that susceptibility to metronidazole was significantly increased by incubation in an anaerobic atmosphere and more reliable results may be expected by incubation under these conditions.

G. vaginalis is best isolated on Columbia Agar (CM0331) with Gardnerella vaginalis Supplement (SR0119).

Quality Control

Use known strains of *G. vaginalis* to monitor the performance of the discs.

References

1. Smith R. F. and Dunkelberg W. E. (1977) *Sex. Trans. Dis*. **4.** 20-21.
2. Gardner H. L. and Dukes C. D. (1955) *Am. J. Obstet. Gynecol.* **60.** 962-976.
3. Zinnemann K. and Turner G. C. (1963) *J. Pathol. Bacteriol.* **85.** 213-219.
4. Piot P., Van Dyke E., Goodfellow M. and Falkow S. (1980) *J. Gen. Microbiol.* **119.** 373-396.
5. Greenwood J. R. and Pickett M. J. (1980) *Int. J. Syst. Bacteriol.* **30.** 170-178.
6. Bailey R. K., Voss J. L. and Smith R. F. (1979) *J. Clin. Microbiol.* **9.** 65-71.
7. Ralph E. D. and Amatnieks Y. E. (1980) *Sex. Trans. Dis.* **7.** 157-160.
8. Clay J. (1981) *J. Antimicrob. Chemotherapy* **7.** 501-504.
9. Ralph E. D., Austin T. W., Pattison F. L. M. and Schieven B. C. (1979) *Sex. Trans. Dis.* **6.** 199-202.

ONPG DISCS

Code: DD0013

For the rapid detection of beta-galactosidase activity in micro-organisms.

Contents
1 cartridge. Each contains 50 discs.

Disc Contents
Each disc is impregnated with phosphate buffered O-nitrophenyl-β-D-galacto-pyranoside (ONPG).

Description
Lactose fermentation is a classical identification test for many organisms. It is normally demonstrated by acid production, after the disaccharide has been cleaved into galactose and glucose by the enzyme beta-galactosidase. However, it is essential for the lactose to be conveyed into the cell by a specific galactoside-permease enzyme before such cleavage.

The role of these two essential enzymes is important in the classification of organisms into:

1. Active lactose fermenters (taking 18-24 hours), possessing both permease and galactosidase P+G+.

2. Delayed lactose fermenters (taking longer than 24 hours), lacking permease but possessing galactosidase: P-- G+.

3. Non-lactose fermenters, lacking both permease and galactosidase: P-- G–.

For the ONPG test[1] a synthetic galactoside (ortho-nitrophenyl-β-D-galacto-pyranose) is substituted for lactose. It is hydrolysed in the same way as lactose but the ortho-nitrophenol is chromogenic and when cleaved off in alkaline solution, it produces a yellow solution:

Bacterium + ONPG (colourless)	hydrolysed β-galactosidase	O-nitrophenol (yellow)

This test is independent of an induced or constitutive permease enzyme and can be very rapid.

The ONPG test can be used to:

1. Differentiate late lactose fermenters (P- G+) from non-lactose fermenters (P- G-).
(a) *Citrobacter* (+) and *Salm. arizonae* (+) from *Salmonella* (-).
(b) *Escherichia coli* (+) from *Shigella sonnei* (-).

2. Aid in species differentiation.
(a) *Burkholderia cepacia* (+) and *Sterotrophemonas maltophila* (+) from other *Pseudomonas* species.
(b) *Neisseria lactamica* (+) from other *Neisseria* (-).

Technique

1. Place one disc (DD0013) into a sterile tube.

2. Add 0.1 ml of sterile 0.88% sodium chloride (physiological saline).

3. Pick the colony under test with a sterile loop and emulsify it in the tube containing the disc and physiological saline.

4. Incubate at 35°C.

5. Examine at hourly intervals, for up to 6 hours, to detect active lactose fermenters.

6. Organisms that are negative after 6 hours should be incubated for up to 24 hours to detect the late lactose fermenters.

Interpretation of Results

Colourless	ONPG Negative
Yellow	ONPG Positive

The reaction speed depends on the size of inoculum.

Quality Control
Use known positive and negative beta-galactosidase producing organisms to monitor the disc reactions.

Reference
1. Lowe G. H. (1962) *J. Med. Lab. Technol.* **19.** 21-25.

OPTOCHIN DISCS

Code: DD0001

For the differentiation of Streptococcus pneumoniae from other alpha haemolytic streptococci.

Contents
Two pack formats are available, a single cartridge containing 50 discs and a five pack version containing five individually sealed cartridges each with 50 discs.

Description
Bowers and Jeffries[1] have shown that there is complete correlation between bile-solubility and full 'Optochin' susceptibility for the differentiation of *S. pneumoniae* from other streptococci. Oxoid 'Optochin' Discs (DD0001) are paper discs ready impregnated with 'Optochin' (ethylhydrocuprein hydrochloride) which provide a convenient and reliable alternative to the bile-solubility test. *Pneumococci* are sensitive to 'Optochin' so that a culture shows a zone of inhibition around the impregnated disc, whilst streptococci either grow right up to the edge of the disc or, occasionally, show a very small zone of inhibition.

Technique
Streak a pure culture of the organism to be tested across one half of a blood agar plate (Blood Agar Base (CM0055) with 7% sterile blood) and apply an 'Optochin' Disc (DD0001) immediately before incubation. At the same time, apply a second `Optochin' Disc (DD0001) to the other half of the plate, previously streaked with a known pneumococcus, in order to provide a positive control. After incubation, pneumococci show a zone of inhibition at least 5 mm from the edge of the disc, *Streptococci* are completely resistant or show a small zone of inhibition, extending not more than 2 mm from the edge of the disc.

Oxoid 'Optochin' Discs (DD0001) may be placed on the primary culture plate, before incubation, to provide rapid indication of the presence of large numbers of pneumococci.

Quality Control
See under Technique.

Reference
1. Bowers E. F. and Jeffries L. R. (1955) *J. Clin. Path*. **8.** 58.

SPS DISCS

Code: DD0016

Sodium polyanethol sulphonate discs for the presumptive identification of Peptostreptococcus anaerobius.

Contents
1 cartridge containing 50 discs.

Disc Content

SPS	1 mg

Description
Wideman *et al*.[1] reported that *Peptostreptococcus anaerobius* may account for one-fifth to one-third of all Gram-positive cocci encountered in clinical specimens and confirmed that all strains of *Peptostreptococcus anaerobius* are totally inhibited by sodium polyanethol sulphonate (SPS) as described previously by Graves *et al*.[2]. It has been recommended[1] that the SPS disc (DD0016) method should be used as a rapid and simple method for the presumptive identification of *Peptostreptococcus anaerobius*. By this method all strains of *Peptostreptococcus anaerobius* give inhibition zones of 12 to 30 mm. *Peptostreptococcus micros* and *Peptococcus prevotii* may give zones that overlap the zone size range obtained with *Peptostreptococcus anaerobius*.

SPS Disc Sensitivity of Clinical Isolates[1]

Organism	***SPS Disc Inhibition Zone Diameter (Range mm)***
Peptostreptococcus anaerobius	18-27
P. micros	6-12[a]
P. parvulus	No zone
P. productus	No zone
Peptococcus asaccharolyticus	No zone
P. magnus	No zone[b]
P. prevotii	6/16[c]
P. saccharolyticus	No zone
P. variabilis	No zone
Peptococcus species	No zone
Acidaminococcus fermentans	No zone
Gaffkya anaerobia	No zone
Streptococcus intermedius	No zone
S. morbillorum	No zone
Streptococcus species	No zone
Veillonella alcalescens	No zone
V. parvula	No zone

[a] Two isolates had zone diameters of 10 mm and one isolate had a 12 mm zone diameter.

[b] One isolate had a 17 mm zone of diminished growth.

[c] One isolate had a zone of 16 mm.

Technique

1. Prepare Wilkins-Chalgren Anaerobe Agar (CM0619) plates as directed.

2. Adjust the 18-24 hour culture of the test organism to a 0.5 McFarland.

3. Evenly inoculate the surface of a Wilkins-Chalgren Anaerobe Agar (CM0619) plate with the culture under test.

4. Place one DD0016 on the plate.

5. Incubate anaerobically at 35°C for 48 hours.

6. Observe zones of inhibition.

Quality Control

Use known strains of *P. anaerobius* to monitor the performance of the discs.

References

1. Wideman P. A., Vargo V. L., Citronbaum D. and Finegold S. M. (1976) *J. Clin. Micro*. **4.** 330-333.

2. Graves M. H., Morello J. A. and Kocka F. E. (1974) *Appl. Microbiol.* **27.** 1131-1133.

0129 DISCS

Codes: DD0014 and DD0015

For the differentiation of vibrios from other Gram-negative rods.

Contents

DD0014 1 cartridge containing 50 discs.

DD0015 1 cartridge containing 50 discs.

Disc contents

DD0014 0129 10 µg.

DD0015 0129 150 µg.

Description

The sensitivity of vibrios to the vibrio static agent 0129 (2,4-Diamino-6,7-di-iso-propylpteridine phosphate) has long been recognised[1]. This test is of great value in differentiating vibrios from other Gram-negative rods and particularly from aeromonads, which are characteristically resistant to 0129. The degree of sensitivity of vibrios to 0129 can also be used as a diagnostic feature in differentiation of *Vibrio* species (see Table 1).

Negative 0129 tests must be interpreted with caution. Strains of *V. cholerae* resistant to 0129 and trimethoprim have been reported[2]. Both plasmids and transposons appear to be responsible[3].

Specialised sensitivity testing media which contain low levels of sodium chloride should not be used as these may give poor growth of halophilic vibrios and may also give false positive results with *Enterobacteriaceae*[4]. Nutrient Agar CM0005 can be recommended for such testing.

Table 1* Sensitivity of Vibrio Species to 0129

	10	***150***
V. parahaemolyticus	R	S
V. alginolyticus	R	S
V. fluvialis	R	S
V. furnissii	R	S
Aeromonas	R	R
V. cholerae 0139	R	R
V. cholerae 01	R	R
V. cholerae	S	S
V. cholerae spp	S	S
V. vulnihicus	*S*	*S*

Technique

1. Evenly incubate the surface of a Nutrient Agar plate (CM0003).
2. Place one DD0014 and one DD0015 disc on each plate.
3. Incubate at 35°C for 24 hours.
4. Observe for zones of inhibition.

Quality Control

Use known strains of 0129 sensitive and resistant organisms to monitor the performance of the discs.

References

1. Shewan J. M. and Hodgkiss W. (1954) *Nature* **63.** 208-209.
2. *The Lancet*, August 8th 1992, **340.** 366-367.
3. Gerbaud G., Dodin A., Goldstein F. and Courvain P. (1985) *Ann. Inst. Past./Microbiol.* **136B.** 265-273.
4. Furniss A. L., Lee J. V. and Donovan T. J. (1978) '*The Vibrios*'. *Public Health Laboratory Service, Monograph Series. Number 11*.
5. BSOP ID 19 Standards Unit, Evaluations and Standards Laboratory. Identification of Vibrio species 2005.

X FACTOR DISCS
V FACTOR DISCS
X + V FACTOR DISCS

Code: DD0003

Code: DD0004

Code: DD0005

Discs impregnated with growth factors for the differentiation of the Haemophilus group of bacteria.

Contents
1 cartridge containing 50 discs.

Description
Paper discs impregnated with growth factors for the differentiation of the *Haemophilus* group of bacteria.

Haemophilus and *Bordetella* species may be identified according to whether basal media require the addition of 'X' and 'V' growth factors before growth will occur. X factor (haemin) and V factor (coenzyme I) may be directly incorporated into a basal medium or, more conveniently, are impregnated into paper discs which are placed upon the surface of an inoculated basal medium. Colonies of those species which show growth only in the vicinity of a disc impregnated with the particular growth factor are unable to synthesise it in optimal amounts[1].

Technique
Evenly inoculate the surface of a Blood Agar Base plate (CM0055 without blood) with the organism to be tested and aseptically apply the Diagnostic Discs in the following positions around the periphery of the plate (approximately 1 or 2 cm in from the edge of the medium):

X Factor Disc	12 o'clock
V Factor Disc	4 o'clock
X+V Factor	8 o'clock

Incubate overnight at 35° C or for 48 hours if necessary, and observe for growth or no growth at the edge the disc. If the organism requires X factor alone, it will grow only at the edge of the X and X + V factor discs; if it requires V factor alone, it will grow at the edge of the V and the X + V factor discs; if both X and V factors are required, it will grow only in the vicinity of the X + V factor disc.

Growth of Bacterial Species with and without X and V factors

	No Growth Factors	*With X Factor*	*With Y Factor*	*With X+Y Factor*
Haemophilus influenzae	-	-	-	+
Haemophilus aegyptius (Koch-Weeks bacillus)	-	-	-	+
Haemophilus parainfluenzae	-	-	+	+
Haemophilus ducreyi	-	+	-	+
*Bordetella pertussis**	+	+	+	+

+ Growth - No growth

*Requires special media for initial isolation, e.g. Bordet-Gengou medium, but laboratory strains show adaptation.

N.B. V and X + V FACTOR DISCS MUST BE STORED AT -10 to -20° C.

Quality Control
Use known strains of *Haemophilus* species to monitor the performance of the discs and the medium.

Reference
1. Kilian M. (1980) *Haemophilus*. In *Manual of Clinical Microbiology*. Eds. Lennette *et al. Amer. Soc. for Microbiol.* 3rd Edn. Washington.

X + V FACTOR DISCS

Code: DD0005

Discs impregnated with growth factors for the differentiation of the Haemophilus group of bacteria.

Contents

Two pack formats are available, a single cartridge containing 50 discs, or a five pack version containing five individually sealed cartridges each with 50 discs.

Store at –10°C to –20°C.

Description

Paper discs impregnated with growth factors for the differentiation of the *Haemophilus* group of bacteria.

Haemophilus and *Bordetella* species may be identified according to whether basal media require the addition of 'X' and 'V' growth factors before growth will occur. X factor (haemin) and V factor (coenzyme I) may be directly incorporated into a basal medium or, more conveniently, are impregnated into paper discs or strips which are placed upon the surface of an inoculated basal medium. Colonies of those species which show growth only in the vicinity of a disc impregnated with the particular growth factor are unable to synthesise it in optimal amounts[1].

Technique

1. Evenly inoculate the surface of a blood agar base plate (CM0055 without blood) with the organism to be tested.
2. Aseptically apply one each of the Diagnostic Discs (DD0003, DD0004, DD0005) in the following positions around the periphery of the plate (approximately 1 or 2 cm in from the edge of the medium):

X Factor Disc	12 o'clock
V Factor Disc	4 o'clock
X+V Factor	8 o'clock

3. Incubate overnight at 35°C or for 48 hours if necessary, and observe for growth or no growth in the edge of the discs. If the organism requires X factor alone, it will grow only in the vicinities of the X and X + V factor discs; if it requires V factor alone, it will grow around the edge of the V and the X + V factor discs; if both X and V factors are required, it will grow only around the edge of the X + V factor disc.

Growth of Bacterial Species with and without X and V factors

	No Growth Factors	*With X Factor*	*With Y Factor*	*With X+Y Factor*
H. influenzae	–	–	–	+
H. aegyptius (Koch-Weeks bacillus)	–	–	–	+
H. parainfluenzae	–	–	+	+
H. ducreyi	–	+	–	+
*B. pertussis**	+	+	+	+

+ Growth – No growth

*Requires special media for initial isolation, e.g. Bordetella Selective medium (Charcoal Agar Base (CM0119), Bordetella Selective Supplement (SR0082)), but laboratory strains show adaptation.

N.B. V and X + V FACTOR DISCS MUST BE STORED AT –10 to –20° C.

Quality Control

Use known strains of *Haemophilus* species to monitor the performance of the discs and the medium.

Reference

1. Kilian M. (1980) *Haemophilus. In Manual of Clinical Microbiology*. Eds. Lennette *et al*. *Amer. Soc. for Microbiol.* 3rd Edn. Washington.

8
BIOCHEMICAL IDENTIFICATION

MICROBACT

The Microbact Manual Identification system is a standardised micro substrate system designed to simulate conventional biochemical substrates. Organism identification is based on colour changes in the test due to pH and/or substrate utilisations as established by published reference methodologies.

The test results generate an octal code which is entered into the Microbact Computer Aided Identification package. This will give an identification profile for the organism with a probability figure shown against the organism name. The percentage figure is the percentage share of the probability for that organism as part of the total probability of all choices.

Test kits are available for Gram Negative Bacilli, Listeria species and Staphylococcal species.

The Microbact Gram Negative system consists of two separate strips, 12A and 12B, each with 12 different biochemical substrates. The 12A strip may be used alone for identification of oxidase negative, nitrate positive glucose fermenters. This can be used for screening pathogenic Enterobacteriaceae from enteric and urine specimens or identification of other common isolates. The 12B strip can be used in conjunction with the 12A strip for the identification of oxidase positive, nitrate negative, and glucose non fermenters as well as Enterobacteriaceae. Other formats of this test are available with a microplate format.

The Microbact Listeria 12L strip consists of 12 tests, 11 of which are sugar utilisation tests plus a rapid haemolysis test. This system can be used as a rapid 4 hour incubation test using a heavier inoculum or as an 18-24 test when using an individual colony.

For Staphylococcal testing, the Microbact Staph 12S system will identify 22 Staphylococcal species. The strips consist of 12 tests. The sugar utilisation tests rely on a pH indicator colour change, while the enzyme detection substrates produce a coloured end product or react with an added indicator.

GRAM-NEGATIVE IDENTIFICATION SYSTEM

MICROBACT™ 12A (12E) AND 24E (12A (12E) + 12B)

GRAM-NEGATIVE IDENTIFICATION SYSTEM

Codes: MB1073, MB1074, MB1076, MB1077, MB1130, MB1131, MB1132, MB1133

Intended use

The Microbact™ Gram-negative system is to be used for the identification of aerobic and facultatively anaerobic Gram-negative bacteria (Enterobacteriaceae and miscellaneous Gram- negative bacteria)[2,3,4,5,6,7].

Principle of the test

The Microbact™ Gram-negative system is a standardised micro-substrate system designed to simulate conventional biochemical substrates used for the identification of Enterobacteriaceae and common miscellaneous Gram-negative bacilli (MGNB). Organism identification is based on pH change and substrate utilisations as established by published reference methodologies[4,6,8,9]. Refer to the Table of Reactions below for the substrates contained in each well, the specific reaction principle, and colour changes.

The Microbact™ Gram-negative product consists of two separate substrate strips, 12A and 12B. Each strip consists of 12 different biochemical substrates. The 12A strip may be used alone for identification of oxidase-negative, nitrate-positive glucose fermenters comprising 15 genera and may be useful for screening pathogenic Enterobacteriaceae from enteric and urine specimens or identification of other common isolates. The 12B strip can be used in conjunction with the 12A strip for the identification of oxidase-positive, nitrate-negative, and glucose-nonfermenters (MGNB) as well as the Enterobacteriaceae.

Note: The 12 substrates contained in the 12A strips are available in a solid microplate format, referred to as 12E. The 12B strips can be used alongside the 12E, but in a separate tray. The 24E solid microplate format contains the 24 substrates contained in the combination of both the 12A and 12B strips.

THE FOLLOWING SPECIES OF OXIDASE-NEGATIVE, GRAM-NEGATIVE BACILLI CAN BE IDENTIFIED USING THE 12A (12E) ALONE:

Enterobacteriacea

***Acinetobacter* spp.**	***Shigella* spp.**	*P. stuartii*
A. baumannii	*Shigella serogp. AB&C*	*P. alcalifaciens*
A.lwoffii	*S. sonnei*	***Salmonella* spp.**
A. haemolyticus	***Hafnia* sp.**	*Salmonella spp.*
***Citrobacter* spp.**	*H. alvei*	*S. typhi*
C. freundii	***Klebsiella* spp.**	*S. cholerae-suis*
C. diversus	*K. pneumoniae*	*S. paratyphi A*
***Enterobacter* spp.**	*K. oxytoca*	*S. arizonae*
E. aerogenes	*K. ozaenae*	***Serratia* spp.**
E. cloacae	*K. rhinoscleromatis*	*S. marcescens*
E. agglomerans	***Morganella* sp.**	*S. liquefaciens*
E. gergoviae	*M. morganii*	*S. rubidaea*
E. sakazakii	*Proteus spp.*	***Tatumella* sp.**
***Escherichia* spp.**	*P. mirabilis*	*T. ptyseos*
E. coli	*P. vulgaris*	***Yersinia* spp.**
E. coli-inactive	***Providencia* spp.**	*Y. enterocolitica*
E. vulneris	*P. rettgeri*	*Y. pseudotuberculosis*

ADDITIONAL OXOIDASE-NEGATIVE, GRAM-NEGATIVE BACILLI CAN BE IDENTIFIED USING THE 12A (12E) + 12B COMBINED OR BY USING 24E:

***Acinetobacter* spp.**	***Ewingella* sp.**	*Salmonella subsp.3B*
A. baumannii	*E. americana*	*Salmonella subsp.4*
A. lwoffii	***Hafnia* sp.**	*Salmonella subsp.5*
A. haemolyticus	*H. alvei*	*Salmonella subsp.6*
***Budvicia* sp.**	*H. alvei biogp 1*	***Serratia* spp.**
B. aquatica	***Klebsiella* spp.**	*S. marcescens*
***Buttiauxella* sp.**	*K. pneumoniae*	*S. marcescens biogp 1*
B. agrestis	*K. oxytoca*	*S. liquefaciens*
***Cedecea* spp.**	*K. ornithinolytica*	*S. rubidaea*
C. davisae	*K. planticola*	*S. odorifera biogp 1*
C. lapagei	*K. ozaenae*	*S. odorifera biogp 2*
C. neteri	*K. rhinoscleromatis*	*S. plymuthica*
Cedecea sp 3	*K. terrigena*	*S. ficaria*
Cedecea sp 5	*Klebsiella gp 47*	*S. entomophila*
***Citrobacter* spp.**	*K. ascorbata*	*S. fonticola*
C. freundii	*K. cryocrescens*	***Shigella* spp.**
C. diversus	***Leclercia* sp.**	*Shigella serogp.AB&C*
C. amalonaticus	*L. adecarboxylata*	*S. sonnei*
C. amalonaticus biogp 1	***Leminorella* spp.**	***Trabulsiella* sp.**
C. farmeri	*L. grimontii*	*T.guamensis*
C. youngae	*L. richardii*	***Xenorhabdus* spp.**
C. braakii	***Moellerella* sp.**	*X. luminescens(25C)*
C.werkmanii	*M. wisconsensis*	*X. luminescens gp 5*
C. sedlakii	*Morganella sp.*	*X. nematophilis (25C)*
Citrobacter sp 9	*M. morganii*	***Xanthomonas* sp.**
Citrobacter sp 10	*M. morganii ssp morganii*	*X. (S.)maltophilia*
Citrobacter sp 11	*M. morganii biogp 1*	***Yersinia* spp.**
***Edwardsiella* spp.**	*M. morganii ssp. Siboni 1*	*Y. enterocolitica*

Table continues on page 8-3

ADDITIONAL OXOIDASE-NEGATIVE, GRAM-NEGATIVE BACILLI CAN BE IDENTIFIED USING THE 12A (12E) + 12B COMBINED OR BY USING 24E: *continued*

E. tarda	***Obesumbacterium* sp.**	*Y. frederiksenii*
E. tarda biogp 1	*O. proteus biogp 2*	*Y. intermedia*
E. hoshinae	***Pragia* sp.**	*Y. kristensenii*
E. ictaluri	*P. fontium*	*Y. rohdei*
***Enterobacter* spp.**	***Proteus* spp.**	*Y. aldovae*
E. aerogenes	*P. mirabilis*	*Y. bercovieri*
E. cloacae	*P. vulgaris*	*Y. mollaretii*
E. agglomerans	*P. penneri*	*Y. pestis*
E. gergoviae	*P. myxofaciens*	*Y. pseudotuberculosis*
E. sakazakii	***Providencia* spp.**	*Y. ruckeri*
E. taylorae	*P. rettger*	***Yokenella* sp.**
E. amnigenus biogp 1	*P. stuartii*	*Y. regensburgei*
E. amnigenus biogp 2	*P. alcalifaciens*	**Enteric Gp17**
E. asburiae	*P. rustigianii*	**Enteric Gp41**
E. hormaechei	*P. heimbachae*	**Enteric Gp45**
E. intermedium	***Rahnella* sp.**	**Enteric Gp58**
E. cancerogenus	*R. aquatilis*	**Enteric Gp59**
E. dissolvens	***Salmonella* spp.**	**Enteric Gp60**
E. nimipressuralis	*Salmonella subsp.1*	**Enteric Gp63**
***Escherichia* spp.**	*S. typhi*	**Enteric Gp64**
E. coli	*S. cholerae-suis*	**Enteric Gp68**
E. coli-inactive	*S. paratyphi A*	**Enteric Gp69**
E. fergusonii	*S. gallinarum*	
E. hermannii	*S. pullorum*	
E. vulneris	*Salmonella subsp.2*	
E. blattae	*S. arizonae subsp.3A*	

For further information refer to the Help File in the Microbact™ Computer Aided Identification Package.

AND THE FOLLOWING OXIDASE-POSITIVE BACTERIA CAN BE IDENTIFIED USING THE 12A (12E) + 12B COMBINED OR BY USING 24E:

***Pseudomonas* spp.**	***Flavobacterium* spp.**	***Moraxella* spp.**
Ps. aeruginosa	*F. meningosepticum (Chryseobacterium meningosepticum)*	*Moraxella spp.*
Ps. fluorescens-25	*F. odoratum (Myroides odoratus)*	***Plesiomonas* sp.**
Ps. fluorescens-35	*F. breve (Empedobacter brevis)*	*P. shigelloides*
Ps. putida	*F. indologenes (Chryseobacterium indologenes)*	***Aeromonas* spp.**
Ps. stutzeri	***Vibrio* spp.**	*A. hydrophila*
Ps. diminuta	*V. fluvialis*	*A. veronii bio sobria*
***Burkholderia* spp.**	*V. furnissii*	*A. veronii bio veronii*
B. cepacia	*V. mimicus*	*A. caviae*
B. pseudomallei	*V. vulnificus*	***Weeksella* spp.**
***Shewanella* sp.**	*V. hollisae*	*W .virosa*
S. putrefaciens	*V. cholerae*	*W. zoohelcum*
***Alcaligenes* spp.**	*V. parahaemolyticus*	***Pasteurella* spp.**
A. faecalis type 11	*V. alginolyticus*	*P. multocida*
A. faecalis		*P. haemolytica*
A. xylosoxidans spp. xylos (Achromobacter xylosoxidans)		***Actinobacillus* spp.** *Actinobacillus sp.*

For further information refer to the Help File in the Microbact™ Computer Aided Identification Package.

Warning and precautions

1. These strips are intended for *in vitro* use only; for use by qualified laboratory personnel using aseptic techniques and established precautions against microbiological hazards.
2. Used materials should be autoclaved, incinerated, or immersed in germicide before disposal.
3. DO NOT incubate strips in a CO_2 incubator as substrates and/or enzyme reactions could be adversely affected, giving false reactions.

Storage instructions

The test strips are stable if kept unopened in the foil envelopes until the expiry date specified when stored at 2-8°C. Once the foil pouch has been opened, unused strips must be placed back in the foil pouch, and the foil pouch taped closed. Strips stored in this manner must be used within 7 days.

Kit presentation

Each kit contains the following:

1 Holding Tray.
Technical Product Insert.
Organism ID Report Forms including Colour Interpretation Chart.

Small Kits
MB1130A Microbact 12E (10 pouches, 80 identifications)
MB1131A Microbact 24E (10 pouches, 40 identifications)
MB1132A Microbact 12A (10 pouches, 60 identifications)
MB1133A Microbact 12B (10 pouches, 60 identifications)
Large Kits
MB1073A Microbact 12E (20 pouches, 160 identifications)
MB1074A Microbact 24E (20 pouches, 80 identifications)
MB1076A Microbact 12A (20 pouches, 120 identifications)
MB1077A Microbact 12B (20 pouches, 120 identifications)

Materials Required but not provided

The following materials may be required but are not provided:

Order Code	*Product Name*
MB0209	Indole
MB0181	VP I
MB0184	VP II
MB0180	TDA
MB0186	NIT A
MB0187	NIT B
MB1093	Mineral Oil
MB0266	Oxidase Strips

These can be purchased individually or as a set by ordering product code MB1082:

Reagent Set D (Indole, VPI, VPII, TDA, NIT A/B)
MB1244 Microbact™ Computer Aided Identification Package
MB0266A Oxidase reagent
Motility medium
2.5/5.0 ml suitable saline solution, 0.85%
5.0 ml peptone water (for use with selective media)
SR0035 Sterile serum
Zinc powder
Inoculating loop
Sterile pipettes
Fanless Incubator (or use container in Incubator with fan) (35° ± 2°C), Non - CO_2

Set up procedure

Isolation

An 18-24 hour pure culture of the organism to be identified must be obtained. Appropriate agar media, for example MacConkey (CM0007), Eosin Methylene Blue (CM0069), Blood or Chocolate (CM0331 and SR0050), may be used to grow organisms.

Qualified Personnel should collect specimens according to standards routinely required for specimen handling[10].

Before use, perform an oxidase test on the organism to be identified.

Note: Oxidase positive organisms cannot be identified using the Microbact™ 12A (12E) alone and should be examined with the Microbact™ 24E (12A (12E) + 12B).

Refer to the procedure chart for a condensed version of the following procedures.

Procedure chart:

	12A (or 12E) Enterobacteriaceae	24E (12A (or 12E) + 12B) Enterobacteriaceae	24E (12A (or 12E) + 12B) Miscellaneous Gram-Negative Bacilli
Oxidase	Negative	Negative	Positive
Preparation of Inoculum	1-2 colonies 2.5 ml saline	2-3 colonies 5.0 ml saline	2-3 colonies 5.0 ml saline When Actinobacillus or Pasteurella spp. are suspected, add 1 drop sterile serum per ml of saline suspension
Strip Inoculation	4 drops to each well	4 drops to each well	4 drops to each well
Oil Overlay	Well 1 (lysine) Well 2 (ornithine) Well 3 (H2S)	12B well 8 (arabinose) 24E well 20 (arabinose) 12B well 12 (arginine) 24E well 24 (arginine)	12B well 12 (arginine) 24E well 24 (arginine)
Incubation Temperature	35° ± 2°C	35° ± 2°C	35° ± 2°C (25°C for Ps. fluorescens)
Test Reading: **Read and record all positive test results**	**Reagent Addition:** **Well 8 – Indole** = 2 drops Kovacs, read within 2 minutes **Well 10 – VP** = 1 drop of each, VPI and VPII, read at 15 – 30 minutes **Well 12 – TDA** = 1 drop TDA, read immediately	**Reagent Addition:** See 12A/12E **Well 1 (12B) Well 13 (24E)** Interpret Gelatin at 24-48 hours **Well 12 (12B) Well 24 (24E)** Arginine Yellow - Negative Green-blue - Positive	**Reagent Addition:** See 12A/12E **Well 1 (12B) Well 13 (24E)** Interpret Gelatin at 48 hours **Well 12 (12B) Well 24 (24E)** Arginine Yellow-green - Negative Blue - Positive

Preparation of inoculum

Pick 1-3 isolated colonies from an 18-24 hour culture and emulsify in 2.5 ml of sterile saline solution if 12A/E alone is being used or 5.0 ml of sterile saline if 24E (12A/E and 12B) are being used. Mix thoroughly to prepare a homogeneous suspension.

If the organism has been grown on a selective medium and the colony is small or inhibited, it may be necessary to emulsify the colony in 5.0 ml of peptone water and incubate at 35° ± 2°C for four hours. Using a sterile Pasteur pipette, transfer one drop of the peptone water culture into the appropriate volume (see Procedure Chart) of sterile saline solution (0.85%).

Inoculation

The wells of individual substrate sets can be exposed by cutting the end tag of the sealing strip and slowly peeling it back.

Place the strip or plate in the holding tray and using a sterile Pasteur pipette add 4 drops (approximately 100 µl) of the bacterial suspension, or half fill each well in the set. When *Actinobacillus* or *Pasteurella* sp. are suspected (no growth on media containing bile salts or on media deficient in blood or serum) add one drop of sterile serum (SR0035) per ml of saline suspension.

Using a sterile pipette or dropper bottle, overlay the substrates underlined on the holding tray with sterile mineral oil, i.e. wells 1, 2 and 3 for 12A (12E) or 24E and wells 8 and 12 for 12B or wells 20 and 24 for 24E. (Well 8 for 12B and 20 for 24E is not overlayed with oil for oxidase- positive, miscellaneous Gram-negative bacilli.)

Incubation

Reseal the inoculated rows with the adhesive seal and write the specimen identification number on the end tag with a marker pen. Incubate at 35° ± 2°C for 18-24 hours. When Ps. *fluorescens* appears as the organism of choice, repeat the test at an incubation temperature of 25° ± 2°C.

To determine the purity of the inoculum, it is advisable to inoculate a solid non-selective medium with the test suspension to act as a culture purity check.

Reading the test strip

1. The 12A (12E) strip should be read at 18-24 hours. The 12B/24E strip is read at 24 hours when identifying Enterobacteriaceae. All systems should be read after 48 hours for the identification of Miscellaneous Gram-negative bacilli.

2. Remove the strips or tray from the incubator, peel back the sealing tape. Record all positive results. The reactions are evaluated as positive or negative by comparing them with the colour chart. Record the results under the appropriate heading on the report form. For aid in interpreting reactions, refer to the Table of **Reactions**.

1. 12A (12E) or 24E

Add the following reagents: Well 8 (Indole production) – add 2 drops of Indole (Kovacs) reagent. Evaluate within 2 minutes of the addition of the reagent.

Well 10 (Voges-Proskaüer reaction) – add 1 drop each of VPI reagent and VPII reagent. Evaluate 15 to 30 minutes after the addition of reagents.

Well 12 (Tryptophan Deaminase) – add 1 drop of TDA reagent. Test can be evaluated immediately after the addition of the reagent.

2. 12B/24E

The gelatin well (well 1 for 12B and well 13 for 24E) must be read at 24-48 hours for Enterobacteriaceae and at 48 hours for miscellaneous Gram-negative bacilli (MGNB). Hydrolysis of gelatin is indicated by dispersal of the black particles throughout the well.

The arginine reaction (well 12 for 12B and well 24 of 24E) is interpreted differently at 24 hours and 48 hours incubation.

24 Hours Incubation (Enterobacteriaceae):
Yellow – Negative
Green-blue – Positive

48 Hours Incubation (MGNB):
Yellow-green – Negative
Blue – Positive

Additional Test

Nitrate Reduction Test (o-nitrophenyl-β-d-galactopyranoside (ONPG))
This test is performed in well 7 (ONPG) AFTER reading the ONPG reaction. One drop of Nitrate reagent A and 1 drop of Nitrate reagent B is added to the well. Production of a red colour within a few minutes of the addition of the reagent indicates that nitrate reduction to nitrite (NO_2) has occurred. A small amount of zinc powder should be added to those wells which exhibit a yellow colour after the addition of the nitrate reagents. This will determine whether nitrate has been reduced completely to nitrogen gas (N2). The results should be interpreted as follows:

After the addition of Nitrate reagents A and B:

Red colour	Positive	NO_2 Positive
Yellow colour	Negative	NO_2 Negative
On the addition of zinc powder:		
Yellow colour	Positive	(N2+)
Red colour	Negative	(N2-)

All organisms belonging to the family Enterobacteriaceae reduce nitrates to nitrites and give a positive reaction.

Note: Gram-negative bacilli which do not reduce nitrates can only be identified by utilising the 24E (12A (12E) + 12B) system.

TABLE OF SUBSTRATES AND REACTIONS (12A/12E/24E):

Well No.	Designation	Reaction Principle	Reaction colours Negative	Positive	Comments
1	**Lysine**	Lysine decarboxylase	Yellow	Blue-green	Green or blue is positive reaction. Bromothymol blue indicates formation of the specific amine cadaverine.
2	**Ornithine**	Ornithine decarboxylase	Yellow-green	Blue	Green should be regarded as a negative reaction. The pH shift indicated by bromothymol blue caused by formation of the specific amine putrescine is greater than that caused by lysine decarboxylation.
3	**H2S**	H2S production	Straw colour	Black	H2S is produced from thiosulphate. H2S reacts with ferric salts in the medium to form a black precipitate.
4 5 6	**Glucose** **Mannitol** **Xylose**	Glucose fermentation Mannitol fermentation Xylose fermentation	Blue-green Blue-green Blue-green	Yellow Yellow Yellow	Bromothymol blue indicator changes from blue to yellow when the carbohydrate is utilised to form acid.
7	**ONPG**	Hydrolysis of o-nitrophenyl-β-d-galactopyranoside (ONPG) by action of β-galactosidase	Colourless	Yellow	β-galactosidase hydrolysis of the colourless ONPG releases yellow orth-onitrophenol.
8	**Indole**	Indole production from tryptophan	Colourless	Pink-red	Indole is formed from metabolism of tryptophan. Indole Kovacs reagent forms a pink-red complex with indole.
9	**Urease**	Urea hydrolysis	Straw colour	Pink-red	Ammonium released from splitting of urea causes the pH to rise - indicated by phenol red changing from yellow to pink-red.
10	**VP**	Acetoin production (Voges-Proskaüer reaction)	Straw colour	Pink-red	Acetoin is produced from glucose indicated by the formation of a pink-red complex after the addition of alpha-naphthol and creatine.
11	**Citrate**	Citrate utilization (citrate is the only source of carbon)	Green	Blue	Citrate is the sole carbon source, which if utilized results in a pH rise, indicated by bromothymol blue, with a colour change from green to blue.
12	**TDA**	Production of indolepyruvate by deamination of tryptophan	Straw colour	Cherry red	Tryptophan deaminase forms indolepyruvic acid from tryptophan which produces a brown colour in the presence of ferric ions. Indole positive organisms may produce a brown colour. This is a negative reaction.

TABLE OF SUBSTRATES AND REACTIONS (12B/24E):

Well No.	Designation	Reaction Principle	Reaction colours Negative	Positive	Comments
1/13	**Gelatin**	Gelatin liquefaction	Colourless	Black	Liquefaction of gelatin by proteolytic enzymes diffuses the black pigment. Solid gelatin particles which may drift across the well after rehydration should be considered as a negative reaction.
2/14	**Malonate**	Malonate inhibition	Green	Blue	Sodium malonate is the sole carbon source and this inhibits the conversion of succinic acid to fumaric acid. An organism unable to utilize this substrate results in the accumulation of succinic acid and the organism cannot grow. Bromothymol blue is the indicator. Yellow-green is indicative of a negative result. Utilisation of Na malonate at the same time that ammonium sulphate is utilised as the nitrogen source produces sodium hydroxide resulting in increased alkalinity and a blue colouration.
3/15	**Inositol**	Inositol fermentation	Blue-green	Yellow	Bromothymol blue indicator changes from blue to yellow when the carbohydrate is fermented.
4/16	**Sorbitol**	Sorbitol fermentation	Blue-green	Yellow	
5/17	**Rhamnose**	Rhamnose ermentation	Blue-green	Yellow	
6/18	**Sucrose**	Sucrose fermentation	Blue-green	Yellow	
7/19	**Lactose**	Lactose fermentation	Blue-green	Yellow	
8/20	**Arabinose**	Arabinose fermentation	Blue-green	Yellow	
9/21	**Adonitol**	Adonitol fermentation	Blue-green	Yellow	
10/22	**Raffinose**	Raffinose fermentation	Blue-green	Yellow	
11/23	**Salicin**	Salicin fermentation Arginine dihydrolase	Blue-green	Yellow	
12/24	**Arginine**	24 hours 48 hours	Yellow Yellow-green	Green-blue Blue	Argine dihydrolase converts arginine into ornithine, ammonia and carbon dioxide. This causes a pH rise as indicated by bromothymol blue. Green reactions occurring at 48 hours should be interpreted as negative.

Interpretation
An octal coding system has been adopted for Microbact™[1]. Each group of three reactions produces a single digit of the code. Using the results obtained, the indices of the positive reactions are circled. The sum of these indices in each group of three reactions forms the code number. This code is entered into the computer package.

Computer aided identification Package
The Microbact™ Computer Aided Identification Package should be consulted for the identification choices. The percentage figure shown against the organism name is the percentage share of the probability for that organism as a part of the total probabilities for all choices.

Note: Miscellaneous Gram-negative bacilli - Weakly positive reactions are recorded as negative results. The results of tests for oxidase, nitrate reduction and motility are included as part of the reaction pattern. Using the results obtained, from each group of three reactions a 9 (nine) digit code number is produced.

Quality control
The overall performance of the system should be monitored by testing appropriate control strains. The following organisms are recommended for independent laboratory assessment.

Proteus mirabilis ATCC® 12453	*Escherichia coli* ATCC® 25922
Klebsiella pneumoniae ATCC® 13883	*Acinetobacter baumannii* ATCC®19606

The following chart gives the expected results on the Microbact System after an 18-24 hour incubation:

			Escherichia coli ATCC® 25922	*Klebsia pneumoniae* ATCC® 13883	*Proteus mirabilis* ATCC® 12453	*Acinetobacter baumannii* ATCC® 19606
1		LYS	+	+	–	–
2		ORN	+	–	+	–
3		H2S	–	–	+	–
4		GLU	+	+	+	+
5		MAN	+	+	–	–
6		XYL	+	+	–	+
7	**12E/A 24E**	ONP	+	+	–	–
8		IDN	+	–	–	–
9		URE	–	+	+	–
10		VP	–	+	–	–
11		CIT	–	+	+	+
12		TDA	–	–	+	–
7		NIT	+	+	–	–
1		GEL	–	–	–	–
2		MAL	–	+	–	+
3		INO	–	+	–	–
4		SOR	+	+	–	–
5		RHA	+	+	–	–
6	**24E/12B**	SUC	–	+	–	–
7		LAC	+	+	–	–
8		ARA	+	+	–	+
9		ADO	–	+	–	–
10		RAF	–	+	–	–
11		SAL	–	+	–	–
12		ARG	–	–	–	–

Note 1: *Serratia marcescens* (ATCC® 43861) can be run as supplemental QC to test the sensitivity of the GEL reaction. The expected results is positive.

Note 2: *Flavobacterium multivorum* (ATCC® 35656) may be used to show a negative glucose result.

Limitations

1. Some bacterial strains may have atypical biochemical reactions due to unusual nutritional requirements or mutations and may be difficult to identify.
2. Reactions obtained using the Microbact System may differ from published results using other substrate formulations. Prolonged incubation, insufficient incubation, improper filling of wells, or inadequate inoculum may lead to false results.
3. Species with low frequency of occurrence require additional testing.
4. *Acinetobacter calcoaceticus var*. *anitratus* will include those strains that have been designated as A. calcoaceticus, A. baumannii, and unnamed genospecies 3; most clinical isolates that are glucose-positive and nonhemolytic are *A. baumannii*.
5. The interpretation of mathematically calculated identification results requires trained clinical personnel who should use judgement and knowledge in conjunction with the following information before accepting the ID of an organism: Gram-stain, colonial morphology, source of isolate, percent probability (degree of separation), tests against, additional test indications and results, frequency of ID choice and antibioGram.
6. A Gram-stain and oxidase test should be performed prior to set-up of tests. In addition, motility and nitrate test should be performed for miscellaneous Gram-negative bacilli.

7. When using the 12E/A strip alone, *Klebsiella* spp., *Enterobacter* spp. and *Serratia* spp. should be reported as Klebsiella/Enterobacter/Serratia group. Twelve substrates provide insufficient data to speciate within this group as a single aberrant reaction may result in an incorrect identification. The lysine and ornithine decarboxylase reactions should be carefully interpreted. Motility and DNase tests are recommended for further speciation of this group. The inclusion of a 12B strip is strongly advised.
8. If further speciation is required for *Yersinia* spp. (i.e. other than *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*), additional testing is required.

References

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5. Evaluation of the API20E and Microbact 24E Systems for the Evaluation of *Pseudomonas pseudomallei*. A. D. Thomas, (1983) *Veterinary Microbiology*. 8, 611-615.
6. Biochemical Characterisics of Enterotoxigenic *Aeromonas* sp. V. Burke, J. Robinson, H. M. Atkinson, and M. Gracey, (Jan. 1982) *Journal of Clinical Microbiology*. 48-52.
7. Comparison of five commercial methods for the Identification of Non-fermentative and Oxidase Positive Fermentative Gram-Negative Bacilli. Bilkey, Mary K., *et al.* (1988) *N.Z.J. Med. Lab Technol.*, 8-12,
8. S. T. Cowen, K. J. Steel (1977) *Manual for the Identification of Medical Bacteria*, 2nd Edition Cambridge University Press.
9. A. Balows, W. J. Hausler, K. L. Herrmann, J. D. Isengerg, H. Jean Shadomy (eds). (1991) *Manual of Clinical Microbiology*, 5th Edition, American Society of Microbiology, Washington, D.C.

LISTERIA IDENTIFICATION SYSTEM 12L

Code: MB1128

Intended use

The Microbact™ Listeria 12L system is intended to be used for the identification of *Listeria* spp. isolated from clinical, food and food related samples.

The Microbact™ Listeria 12L system is a standardised micro substrate system designed to simulate conventional biochemical substrates used for the identification of *Listeria* spp[1,2]. Each identification strip consists of 12 tests, (11 sugar utilisation tests plus a rapid haemolysis test). The reactions occurring during the incubation period are demonstrated through either a colour change in the sugar utilisation tests or in the lysis of sheep red blood cells in the haemolysis test.

The Microbact™ Listeria 12L may be used as a rapid 4 hour incubation test when an inoculum equivalent to a MacFarland 0.5 Standard is prepared, or as an 18-24 hour incubation test when inoculated from a single colony.

After incubation, the reactions are read visually and interpreted against the tables provided in this product insert or by using the Microbact™ Computer Aided Identification Package.

Principle of test

Organism identification is based on pH change and substrate utilisation as established by published reference methodologies[1,2]. Refer to the table of reactions below for the substrates in each well, specific reaction principles and colour changes. Tests can be read at 4 and 24 hours.

Well No.	Designation	Reaction Principle	Reaction colours Negative	Positive	Comments
1	**Esculin**	Hydrolysis of Esculin	Yellow	Black	
2	**Mannitol**		Purple	Yellow	
3	**Xylose**		Purple	Yellow	
4	**Arabitol**		Purple	Yellow	
5	**Ribose**		Purple	Yellow	Bromocresol Purple indicator changes
6	**Rhamnose**		Purple	Yellow	colour when appropriate sugar is
7	**Trehalose**	Utilisation of specific sugars	Purple	Yellow	utilised producing acid
8	**Tagatose**	resulting in the production	Purple	Yellow	IMPORTANT: After 4 hours incubation,
9	**Glucose-1-**	of acidic end products			positive reactions may exhibit a brown/
	Phosphate		Purple	Yellow	straw colour indicating the transition to a
10	**Methyl-D-**				positive yellow
	Glucose		Purple	Yellow	
11	**Methyl-D-**				
	Mannose		Purple	Yellow	
12	**Haemolysis**	Haemolysis of red blood cells	Red cell deposit	Brown	If haemolysin is present, red cells lyse, resulting in the production of a brown colour throughout the well. In the absence of haemolysins, intact red cells rapidly settle to the bottom of the reaction well resulting in the formation of a discreet red deposit

The following species can be identified using the Microbact™ Listeria 12L System

L. monocytogenes	*L. ivanovii*
L. innocua	*L. welshimeri*
L. seeligeri	*L. grayi*

Warnings and precutions

1. These strips are intended for in vitro use only; for use only by qualified laboratory personnel using aseptic techniques and established precautions against microbiological hazards.

   Qualified laboratory personnel shall:

   – be employed to work in a laboratory capable of handling Category 2 pathogens

   – understand basic microbiological procedures including sterile and aseptic techniques

   – be trained on handling the strips, preparing them and reading the results. The level of academic qualification is not paramount as the kit is designed to be simple to use.
2. Used materials must be autoclaved, incinerated or immersed in germicide before disposal.
3. DO NOT incubate 12L strips in a CO_2 incubator as as substrates and/or enzyme reactions could be adversely affected, giving false reactions.
4. DO NOT use the Haemolysin Reagent if it appears to be contaminated (very dark or haemolysed).

Storage instructions

Store at 2–8°C. The test strips are stable until the expiry date specified if kept unopened in the foil envelopes at this temperature.

Once opened the Haemolysin Reagent may be used, using aseptic technique, until the expiry date shown on the bottle.

Kit presentation

Each kit contains the following:

1 x Holding Tray
20 x Report Forms
20 x Test strips
20 x Suspending Media
1 x Haemolysin Reagent

Materials required but not provided.
The following material may be required but is not provided:

MicrobactTM Computer Aided Identification Package.
Inoculating Loop.
Incubator (35°C ± 2°C).
Sterile Pipettes.

Set up procedure
This test may be performed on colonies isolated from a wide range of selective and non-selective media.

Preparation of the inoculum
Before testing, isolates should be checked to ensure that they belong to the genus Listeria (Oxoid Listeria Test Kit, motile at 25°C, non-motile at 37°C, catalase-positive, oxidase-negative, short Gram-positive bacilli)

1. 4 Hour test
Pick 4 5 colonies from an 18-24 hour culture and emulsify in suspending medium. Mix thoroughly to prepare a homogeneous suspension.

2. 18- 24 Hour test
Pick a single isolated colony from an 18-24 hour culture and emulsify in suspending medium. Mix thoroughly to prepare a homogeneous suspension.

Inoculation
1. Remove a test strip from its foil pouch and place in the holding tray provided.
2. Warm the haemolysin reagent to room temperature.
3. Remove the lid from the test strip.
4. Using a sterile Pasteur pipette place 4 drops (approximately 100 µl) of the bacterial suspension into each well.
5. Add 1 drop of haemolysin reagent to well number 12.
6. Replace the lid.
7. Place 1 drop of the inoculum onto an appropriate non-selective medium for a purity check. Incubate at 35°C ± 2°C for 24 hours. Check for purity.

Incubation
Incubate inoculated strip(s) at 35°C ± 2°C for 4 hours or 18-24 hours depending on the inoculum prepared. (See section entitled Preparation of the Inoculum).

Note: If reactions cannot be interpreted with confidence after 4 hours incubation, replace the strip(s) in the incubator. Reactions may be read after further incubation.

Reading of the test strip(s)
Remove the strip(s) from the incubator.

Remove the lid.

Record all test results onto the report form. For assistance in interpreting the results refer to the Table of Reactions.

Interpretation
Compare the test reactions obtained with the predicted results in the Data Table provided.

Example:

	Result	***Reaction Index***	***Sum of Positive reactions***
Oxidase	–		
Catalase	+		
Latex Agglut	+		
Esculin	+	4	
Mannitol	–	2	5
Xylose	+	1	
Arabitol	+	4	
Ribose	+	2	6
Rhamnose	–	1	
Trehalose	+	4	
Tagatose	–	2	5
Gluc-1-Phos	+	1	
M-D-Gluc	+	4	
M-D-Man	+	2	7
Haemolysis	+	1	

Microbact™ code = 5657

Computer aided identification package
A four digit octal code has been adopted for the Microbact™ Listeria 12L, each group of 3 reactions producing a single digit of the four digit code. Using the results obtained, the indices of the positive reactions are circled. The sum of these indices in each group of three reactions forms the code number.

The Microbact™ Computer Aided Identification Package or the Listeria Profile Register provided may then be consulted for the identification choices.

Quality control
The overall performance of the system should be monitored by testing appropriate control strains. The following organisms are recommended for independent laboratory assessment.

	L. monocytogenes **ATCC® 15313**	***L. grayi*** **ATCC® 25400**
Esculin	+	+
Mannitol	–	+
Xylose	–	–
Arabitol	+	+
Ribose	–	+
Rhamnose	+	–
Trehalose	+	+
Tagatose	–	–
Gluc-1-Phos	–	–
M-D-Gluc	+	–
M-D-Man	+	+
Haemolysis	+	–

Limitations

1. The Microbact™ Listeria 12L, Listeria identification system is designed to identify bacteria belonging to the genus *Listeria*. It cannot be used to identify organisms belonging to other genera.
2. Specimens or samples may contain a mixture of species. This may result in mixed cultures being used to prepare the inoculum when 4 hour identifications are attempted. If the results achieved using this heavy inoculum do not produce a reaction pattern clearly representative of a single species:

Check the purity plate. A horse blood agar plate may be of assistance.

Check catalase (+), oxidase (–) and Gram morphology (Gram-positive bacillus).

Repeat the test using a single colony.

Note: All *Listeria* spp. should be positive for esculin, arabitol and trehalose.

Performance characteristics

In house studies have shown that identification to the species level can be achieved with 92% of isolates after 4 hours incubation and 100% of isolates after 24 hours incubation when results are interpreted using the Computer Aided Identification Package. (see below)

	Isolates Tested	4 Hours	24 Hours
L. monocytogenes	45	40	45
L. innocua	27	27	27
L. welshimeri	2	2	2
L. grayi	1	1	1
Totals	75	70	75
Agreement		92%	100%

Compiled data from various studies[3]:

Listeria spp	No. of isolates	No. Correctly identified by Microbact 12L (24 h)	% Correctly identified
L. monocytogenes	86	85	99
L. innocua	98	97	99
L. ivanovii	5	4(a)	80
L. welshimeri	7	7	100
L. seeligeri	6	5(a)	83
L. grayi	8	6(b)	75
TOTAL	210	204	97

(a) One strain identified as *L. ivanovii* by genotypic methods yielded a biochemical profile for *L. seeligeri* using biochemical tests including another commercial identification kit and traditional biochemical tests.

(b) Two strains deposited as *L. grayi* yielded biochemical profiles for *L. innocua* using the 12L and another commercial biochemical identification system.

REPRODUCIBILITY CHARACTERISTICS (Data on file at Oxoid):

Organism Tested	No. of batches tested	No. of correct identifications	Reproducibility within batch	Reproducibility between batches
L. monocytogenes	10	20	100%	100%
L. innocua	10	20	100%	100%
L. seeligeri	10	20	100%	100%
L. ivanovii	10	20	100%	100%
L. grayi	10	20	100%	100%
L. welshimeri	10	20	100%	100%

Over a 14 month period 10 batches of Microbact™ 12L strips were tested for reproducibility using 6 Listeria species. As the table shows, identification both within and between batches was 100% reproducible for all Listeria species.

References

1. USDA, FSIS Method for the isolation and identification of *Listeria monocytogenes*, from processed meat and poultry products.

2. AS 1766.1.15(INT) 1991, TC 34/SC5 N307, *FDA Bacteriological Analytical Manual*, 7th Edn (1992) 141 162.

3. CCFRA report on file at Oxoid Ltd.

STAPHLOCOCCAL 12S IDENTIFICATION SYSTEM

Code: MB1561

Introduction
Staphylococci remain an important component of the human commensal flora, with coagulase-negative staphylococci representing the major proportion[1]. Despite their frequency as contaminants, coagulase-negative staphylococci have become important nosocomial pathogens, in part because of the increased use of indwelling medical devices. Because of their increased importance it has now become imperative for microbiology laboratories to identify these coagulase-negative staphylococci to the species level[2].

Intended use
Microbact™ Staph 12S system is based upon conventional identification systems[3,10]. Using a combination of sugar utilisation and colorimetric enzyme detection substrates, the 12S is able to identify 22 of the most important clinical species of staphylococci[2]. This includes both coagulase-negative and coagulase-positive staphylococci.

The Microbact™ Staph 12S system is intended for the identification of *Staphylococcus* species only. Staphylococci are Gram-positive cocci (0.5 to 1.5 µm in diameter) they are non-motile, non-spore forming, catalase-positive facultative anaerobes.

Micrococcus and related genera can be eliminated by their inability to grow anaerobically and resistance to Furazolidone (100 µg disc [5].

Principle of the Test
The Microbact™ Staph 12S system is a standardised micro-substrate system designed to emulate conventional biochemical substrates used for the identification of *Staphylococcus* species. Each identification strip consists of 12 tests. Reactions that occur during the incubation period are demonstrated by an easily interpreted colour change. The sugar utilisation tests rely on a pH indicator colour change, whilst the enzyme detection substrates produce a coloured end product or react with an added indicator.

Organism identification is based on pH change and substrate utilisation as established in published references[3,4,6,7,8,9]. Refer to the table of substrates/reactions for the specific reaction principles and colour changes.

Each species of *Staphylococcus* produces a different pattern of reactions. When the results of the reactions are entered into the Microbact™ software, using a numeric code, a probable identification of the Staphylococcus species in question is produced. Oxoid is continually extending and improving its independent database of reactions for use with the Microbact™ Staph 12S.

TABLE OF SUBSTRATES AND REACTIONS:

Well No.	Designation	Reaction Principle	Reaction colours Negative	Positive	Comments
1	**Maltose**	Utilisation of specific sugars resulting in the production of acidic end products	Red	Yellow	Phenol red indicator changes colour when appropriate sugar is utilised producing acid.
2	**2 Mannitol**		Red	Yellow	
3	**Mannose**		Red	Yellow	
4	**Sucrose**		Red	Yellow	
5	**Trehalose**		Red	Yellow	
6	**N-acetyl glucosamine**		Red	Yellow	
7	**Arginine utilisation**	Detection of arginine dihydrolase at 24 hours	Yellow/ Olive green	Green/Blue	Arginine dihydrolase converts arginine into ornithine, ammonia and carbon dioxide. The pH rise is indicated by bromothymol blue. Green reactions occurring at 48 hours should be interpreted as negative.
8	**Urease test**	Urea hydrolysis	Straw/Yellow	Pink/Red	Urease splits urea into carbon dioxide and ammonia. The pH rise is indicated by phenol red.
9	**Beta-glucosidase**	Hydrolysis of p-nitrophenyl-β-D-glucopyranoside by action of β-glucosidase	Pale Yellow/ Colourless	Bright Yellow	Hydrolysis of colourless p-nitrophenyl-β-d-glucopyranoside releases yellow p-nitrophenol.
10	**Alkaline phosphatase**	Hydrolysis of p-nitrophenyl-phosphate by action of alkaline phosphatase	Pale Yellow/ Colourless	Bright Yellow	Hydrolysis of colourless p-nitrophenyl-phosphate releases yellow p-nitrophenol.
11	**Beta-glucuronidase**	Hydrolysis of p-nitrophenyl-β-D-glucuronide by action of β-glucuronidase	Pale Yellow/ Colourless	Bright Yellow	Hydrolysis of colourless p-nitrophenyl-β-d-glucuronide releases yellow p-nitrophenol.
12	**Beta-galactosidase**	Hydrolysis of β-naphthyl-β-d-galactopyranoside by action of β-Glucosidase	Pale Yellow/ Colourless	Plum Purple	Hydrolysis of colourless β-naphthyl-β-d-galactopyranoside releases β-naphthol which is detected by Fast blue BB salt which turns purple.

THE FOLLOWING SPECIES CAN BE IDENTIFIED USING THE MICROBACT™ STAPH 12S SYSTEM.

S. aureus subsp. aureus	*S. saprophyticus*
S. epidermidis	*S. cohnii subsp. cohnii*
S. capitis subsp. capitis	*S. cohnii subsp. urealyticum*
S. capitis subsp. ureolyticus	*S. xylosis*
S. caprae	*S. simulans*
S. warneri	*S. carnosus*
S. haemolyticus	*S. intermedius*
S. hominis	*S. hyicus*
S. lugdunensis	*S. chromogenes*
S. schleiferi	*S. sciuri*
S. auricularis	*S. lentus*

Warning and precautions

1. These strips are intended for *in vitro* use only, for use by qualified laboratory personnel using aseptic techniques and established precautions against microbiological hazards.
2. Used materials should be autoclaved, incinerated or immersed in germicide before disposal.
3. DO NOT incubate 12S strips in a CO_2 incubator as substrates and/or enzyme reactions could be adversely affected, giving false reactions.

Storage Instructions
Store at 2–8°C. The test strips are stable until the expiry date specified if kept unopened in the foil envelopes at this temperature.

Kit Presentation
Each kit contains the following:

20 Microbact™ Staph 12S test strips consisting of 12 wells each containing a different dehydrated substrate as described in the table of substrates.

21 x 3mL vials of Microbact™ Staph 12S suspending media consisting of buffering agents and peptones for preparing the inoculum.

1 Holding Tray.

Technical Product Insert.

Organism ID Report Form including Colour Interpretation Chart.

Report Forms.

Materials not provided
The following material may be required but are not provided:

MicrobactTM Staph Fast Blue Reagent 2 x 10 mL (code MB1588)

Microbact™ Computer Aided Identification Package (code MB1244)

Sterile Mineral Oil (code MB1093)

Inoculating Loops

Sterile Pipettes

Staphylococcal latex (codes DR0850, DR0850)/Coagulase reagent

DNase plates and 1N HCL

Incubator (35 +/ 2°C)

Procedure

Isolation
The basic procedure for culture and isolation of bacteria from either *Staphylococcus* spp. or staphylococci clinical specimens has been well documented. When isolating *Staphylococcus*, every specimen should be plated onto blood agar and other media appropriate for the specimen.

On blood agar, abundant growth of *Staphylococcus* spp. or staphylococci occurs within 18-24 hours. Since most species cannot be distinguished from one another during this time period, colonies should be picked at this time only for preliminary identification testing. Colonies should be allowed to grow for an additional 2-3 days before the primary isolation plate is confirmed for species composition.

Failure to hold the plates for this length of time can lead to mixed cultures being used in the identification process, producing false results. Specimens from heavily contaminated sources should also be streaked onto media selective for *Staphylococcus* spp. or staphylococci, such as Mannitol-Salt Agar or Columbia Colistin-Nalidixic Acid Agar. These media inhibit the growth of Gram-negative organisms but allow *Staphylococcus* spp., staphylococci and certain other Gram-positive cocci to grow.

Additional Tests
Before using the Microbact™ Staph 12S system some additional testing will need to be performed. These results will be recorded on the report form and entered into the computer software to aid in identification.

Test results required:

1. Coagulase/Staphylococcal Latex results
There are many systems available for detecting coagulase, but the slide agglutination test using human or rabbit plasma is one of the most common. Staphylococcal latex is another fast and convenient way of detecting coagulase. Oxoid Staphytect Plus is an accurate and highly specific test which can be used in conjunction with the 12S system for efficient Staphylococcal identification.

2. DNase Detection
DNase detection is an easy and useful test that can help greatly in the identification of various Staphylococcus species.

Staphylococcal species other than S.aureus that can produce DNase include: *S. caprae*, *S. hyicus*, *S. chromogens*, *S. intermedius*.

3. Pigment

Pigment production can be a useful adjunct to the Staphylococcal identification process. Positive pigment is defined as the visual detection of carotenoid pigments (e.g., yellow, yellow-orange or orange) during colony development at normal incubation or room temperatures.

Pigmented colonies are often hard to visualise. To aid visualisation a white swab can be used to remove one or more colonies from the agar plate. The swab can then be checked for pigment colour, using the white background for contrast. Pigment production on blood containing media is often poor. Pigments may be enhanced by the addition of milk, fat, glycerol monoacetate or soap to media such as Tryptone Soya Agar (TSA).

Colony colour on this agar is more easily visualised than on blood containing agar.

Staphylococcus species that can produce pigment include: *S. aureus*, *S. chromogens*, *S. scuiri*, *S. lugdunensis.*

Identification

On non-selective agars, isolated colonies of *Staphylococcus* are 1-3 mm in diameter after 24 hours and 3-8 mm in diameter by 3 days of incubation in air at 35 ± 2°C. Some species such as *S. auricularis* can require 24–36 hours of incubation before detectable colony development.

The Microbact™ Staph 12S system is to be used for the identification of *Staphylococcus* spp. only. Before testing, isolates should be checked to ensure that they belong to the *Staphylococcus* genus.

Staphylococci are Gram-positive cocci (0.5 to 1.5 µm in diameter) that occur singly and in pairs, tetrads, short chains (three or four cells) and irregular grape-like structures. They are non-motile, non-spore forming, catalase- positive facultative anaerobes.

Micrococcus and related genera can be eliminated by their inability to grow anaerobically and resistance to Furazolidone (100 µg disc)[5].

Preparation of inoculum

Pick 2-5 isolated colonies (depending on colony size) from an 18–24 hour pure culture and emulsify in 3 ml of staphylococcal suspending medium. Mix thoroughly to prepare a homogeneous suspension.

Inoculation

1. Remove a test strip from its foil pouch and place in the holding tray. Label appropriately.
2. Remove the lid from the test strip.
3. Using a sterile Pasteur pipette add 4 drops (100 µl) of the bacterial suspension to each well.
4. Overlay well No. 7, arginine (indicated by a black circle on the test strip) with 2 drops of Mineral Oil (code MB1093). Replace the lid.
5. Place 1 drop of the inoculum onto an appropriate non-selective medium (e.g. TSA or Columbia Agar) for a purity check and incubate at 35 ± 2°C for 24 hours. If growth on the plate indicates that the suspension was not pure, then the test must be considered invalid and repeated.

Incubation

Incubate the inoculated test strips at 35 ± 2°C aerobically for 24 hours. If the arginine result cannot be interpreted confidently after 24 hours the strips can be replaced in the incubator and reread after further incubation.

Reading the test strip

1. If necessary, reconstitute the Fast Blue reagent (code MB1588) by adding the entire contents of the diluent bottle to the reagent bottle. Mix thoroughly and write the date of reconstitution on the bottle. Once reconstituted Fast Blue reagent has a shelf life of 8 weeks.
2. Remove test strip from the incubator.
3. Remove the lid.
4. Add one drop of Fast Blue reagent to well No. 12, indicated by a green circle on the test strip (Beta-galactosidase). A colour change within 5-10 seconds to plum purple will occur if the test is positive.
5. Record all test results onto the Microbact™ Organism ID Report Forms provided in the kit. Use the colour chart on the inside flap of the pad to aid in the interpretation of the colour changes in each well of the 12S strip. Page 2 of this booklet contains a table of substrates/reactions that can be used as a guide for interpreting reactions. Positive reactions are indicated by a + and negative reactions by a –.

Interpretation
Once each reaction is recorded on the report form, convert each block of three reactions into a numeric value. If the reaction in the well is considered positive the numeric value below the result (reaction index number) will be included. Add the three numbers together to obtain each digit of the Microbact™ code which is either compared to the profile register or entered into the computer package.

Example:

	Result	Reaction Index	Sum of Positive reactions
Maltose	+	4	
Mannitol	–	2	4
Mannose	–	1	
Sucrose	+	4	
Trehalose	–	2	4
N-Acetyl glucosamine	–	1	
Arginine	+	4	
Urease	+	2	6
Beta-Glucosidase	–	1	
Alkaline Phosphatase	+	4	
Beta-Glucuronidase	–	2	4
Beta-Galactosidase	–	1	
Latex/Coagulase	–	4	
Dnase	–	2	0
Pigment	–	?	

Microbact™ code = 44640
Results of the test reactions, via the five digit code number, are entered into the Microbact™ Computer Aided Identification Software producing a probable species ID of the *Staphylococcus* in question. Results can also be entered individually into the software if preferred. Please consult the Microbact™ software help for full details.

The software also allows Individual reactions to be changed once entered. In cases where reaction results are hard to interpret or not known a question mark (?) can be used. Of the 12 reactions a limit of 2 can be entered as question marks. For full details please consult the Microbact™ software help.

The Microbact™ Computer Aided Identification Software should be consulted for the identification choices. The percentage figure shown against the organism name is the percentage share of the probability for that organism as part of the total probabilities for all choices.

Quality control
The overall performance of the system should be monitored by testing the appropriate control strains. The following organisms are recommended for independent laboratory assessment.

Staphylococcus aureus ATCC® 25923	Oxoid Cultiloops® C7010L
Staphylococcus epidermidis ATCC® 12228	Oxoid Cultiloops® C6500L
Staphylococcus saprophyticus ATCC® 15305	Oxoid Cultiloops® C7014L

The following chart gives the expected results on the Microbact™ 12S system after 18–24 hours of incubation.

	S. aureus ATCC® 25923	*S. epidermidis* ATCC® 12228	*S. saprophyticus* ATCC® 15305
Maltose	+	+	+
Mannitol	+	–	+
Mannose	+	–	–
Sucrose	+	+	+
Trehalose	+	–	+
N-Acetyl glucosamine	+	–	–
Arginine	+	+	–
Urease	+	+	+
Beta-Glucosidase	+	–	–
Alkaline Phosphatase	+	+	–
Beta-Glucuronidase	–	–	–
Beta-Galactosidase	–	–	+

Limitations

1. Some Staphylococcal strains may have atypical biochemical reactions due to unusual nutritional requirements and may be difficult to identify.
2. Prolonged incubation, insufficient incubation, improper filling of wells or inadequate inoculum may lead to false results.
3. Reactions obtained using the Microbact™ Staph 12S System may differ from published results using other substrate formulations.
4. Species with a low frequency of occurrence may require additional testing.
5. The interpretation of mathematically calculated identification results requires trained personnel who should use judgement and knowledge in conjunction with the following information before accepting the identification of an organism: Gram-stain, colonial morphology, source of isolate, percentage probability, tests against, additional tests, frequency of ID choice and the antibiogram.

References

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9. McTaggart L., Elliot T. S. J. Is resistance to novobiocin a reliable test for confirmation of the identification of *Staphylococcus saprophyticus*? *J. Med. Microbiol.* 1989 30: 253-266
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BARBITONE CFT DILUENT TABLETS

Code: BR0016

Formula	*gm/litre*
Barbitone	0.575
Sodium chloride	8.5
Magnesium chloride	0.168
Calcium chloride	0.028
Barbitone soluble	0.185
pH 7.2 ± 0.2	

Directions
Dissolve 1 tablet in 100 ml of warm distilled water.

Description
Complement fixation tests are extensively employed in the diagnosis of many different diseases, including infections due to viruses as well as bacteria.

These tablets are intended for the simple and convenient preparation of the special diluent for use in complement fixation tests. This diluent, a modification of that described by Whillans[1], is similar to the diluent employed by Mayer *et al.*[2] It is superior to the physiological saline formerly used because it contains calcium and magnesium, and is of the correct pH for optimum results.

Mayer *et al*.[2] investigated the effect of cations on the haemolytic function of complement, by the addition of a number of substances to the veronal buffered saline base. They were able to conclude:

(i) Some divalent cations, especially Mg^{++}, are essential for the haemolytic action of complement.

(ii) The haemolytic system does not contain sufficient Mg^{++} for optimal haemolytic activity, so that a marked enhancement can be obtained by the addition of extra Mg^{++}. The enhancing effect of tissue fluids can be ascribed to their contribution of Mg^{++}.

(iii) The anticomplementary effect of some substances can be overcome by the addition of extra Mg^{++}.

(iv) Ca^{++} may also be essential to the haemolytic process but its action is much less pronounced than that of Mg^{++}.

The use of Oxoid Complement Fixation Test Diluent Tablets in a description of complement fixation tests for virus diseases was recommended[3,4].

Precaution
Barbitone is a registered narcotic drug and subject to control regulations on transport, storage and records. See Hazchem precaution.

References
1. Whillans D. (1950) *J. Clin. Path.* 3. 57.
2. Mayer M. M. et al (1946) *J. Exp. Med.* 84. 535-548.
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4. Fulton F. and Dumbell K. R. (1949) *J. Gen. Microbiol.* 3. 97.

(BASIC) FUCHSIN

Code: BR0050

Description
This dye has been selected for use in Endo Agar Base CM0479. It should be dissolved at 10% w/v in 95% ethyl alcohol. For each litre of medium use 8 ml of a 10% w/v solution of this dye dissolved in 95% ethyl alcohol. The magenta dyes are closely related to known carcinogenic substances; therefore basic fuchsin should be handled with care, avoiding inhaling the powder or staining the skin with dye.

MUG REAGENT

Code: BR0071

A fluorescent agent for the detection of Escherichia coli.

Vial content

4-methylumbelliferyl-β-D-glucuronide	50 mg

Directions
Add 2 ml of distilled water to a vial and invert gently until completely dissolved. Add the vial contents to the following volumes of suggested media, before sterilisation.

Medium	***Final conc. of MUG per litre***	***Number of vials per litre***
Violet Red Bile Agar CM0107	100 mg	2
MacConkey Agar No. 3 CM0115	100 mg	2
Brilliant Green Bile (2%) Broth CM0031	50 mg	1
MacConkey Broth Purple CM0005a	50 mg	1
Lauryl Tryptose Broth CM0451	50 mg	1

Other media can be used, consult Oxoid for further advice.

Description
Oxoid MUG reagent is a lyophilised presentation of the substrate 4-methylumbelliferyl-β-D-glucuronide in 50 mg quantities. The incorporation of MUG reagent into culture media is reported to improve the sensitivity and specificity of *Escherichia coli* detection[1,2,3,4,5]. This improved sensitivity is mainly due to the detection of anaerogenic strains of *Escherichia coli* when present in mixed cultures. The sensitivity reported for various media varies from 59% to 85.8%[6].

MUG reagent is cleaved by the enzyme glucuronidase to release an end product 4-methylumbelliferone which produces a visible green/blue fluorescence under long wave ultra-violet light (366nm). The addition of MUG reagent to culture media provides another criterion by which to determine the presence of *Escherichia coli* in food and environmental samples.

Alkaline pH increases the intensity of fluorescence. Maddocks and Greenan[7] adjusted the pH of their cultures with sodium hydroxide to maximise light output in their investigations of MUG hydrolysis as an alternative to conventional biochemical tests for identifying bacteria. The acidification of the agar surrounding *Escherichia coli* colonies on lactose-based media diminishes the discrimination of MUG-hydrolysing colonies[8]. Freir and Hartman[9] exposed membrane filter cultures to ammonia vapour to enhance fluorescence. A phosphate-buffered MUG agar was used by Entis and Boleszczuk to minimise pH fall in an improved 24 hour hydrophobic grid membrane filter method for coliform and *Escherichia coli* enumeration[10].

MUG has been included in media used with membrane filters in methods for enumeration of E. coli in foods, water and sewage[11,12]. Conditions that affect the fluorescence intensity of MUG were investigated by Villari, Iannuzzo and Torre and recommendations made for its optimum use[13].

Technique
Follow the method and procedure relevant to the sample and the selected medium. Uninoculated tubes or agar plates should be used as controls. (See Precaution.)

After incubation detect glucuronidase activity by examining the microbial growth under UV light (366nm).

The presence of blue/green fluorescence indicates glucuronidase activity.

Report fluorescence as presumptive presence of *Escherichia coli* and confirm by further biochemical tests.

Storage and stability
Should be stored at 2-8°C. When stored as directed the unopened vial is stable until the expiry date on the label.

Quality control
Positive control:
Escherichia coli ATCC® 25922

Negative control:
Proteus mirabilis ATCC® 10975

Precautions
The presence of endogenous glucuronidase in shellfish samples may result in false positive fluorescence. Test tubes used in the MPN method should be checked under UV light to ensure the glass does not fluoresce. To avoid false positive flourescence the source of long wave UV light must not exceed 6 watts.

References

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2. Harsen W. and Yourassowsky (1984) *J. Clin. Microbiol.* 20. 1177-1179.
3. Le Uinor L., Buissieve J., Novel G. and Novel M. (1978) *Ann. Microbiol.* (Paris) 129B. 155-165.
4. Kilan M. and Bulow P. (1976) *Acta Pathol. Microbiol. Scand.* sect B. 84. 245-251.
5. Kilan M. and Bulow P. (1979) *Acta Pathol. Microbiol. Scand.* sect B 87. 271-276.
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12. Shadix L. C., Dunningan M. E. and Rice E. W. (1993) *Can. J. Microbiol.* 39. 1066-1070.
13. Villari P., Iannuzzo M. and Torre I. (1997) *Lett. Appl. Microbiol.* 24. 286-290.

PHOSPHATE BUFFERED SALINE

Code: BR0014
(Dulbecco ‘A’ Tablets)

Formula	*gm/litre*
Sodium chloride	8.0
Potassium chloride	0.2
Disodium hydrogen phosphate	1.15
Potassium dihydrogen phosphate	0.2
pH 7.3	

Directions
Dissolve 10 tablets in 1 litre of distilled water and autoclave for 10 minutes at 115°C. The solution will be quite free from insoluble matter.

Description
This balanced salt solution is issued to meet the requirements of those tissue culture workers who use the Dulbecco Solution with and without calcium and magnesium. Oxoid Dulbecco ‘A’ Solution corresponds to the original formulation of Dulbecco and Vogt[1] except that calcium and magnesium are omitted and may be added separately.

An example of the use of Solution ‘A’ is in the suspension of cells from a monolayer culture[2]. Cells growing in monolayer on a glass surface may be brought into suspension by a number of methods, including the application of chelating agents such as Versene (diamino-ethane-tetra-acetic acid) or its salts. Chelating agents bind divalent cations, such as calcium and magnesium ions, and it is probably this effect which causes the cells to become detached from the glass. Dulbecco ‘A’ Solution is therefore used to rinse the sheet of cells free from growth medium before application of the chelating agent – this latter solution may consist of Dulbecco ‘A’ Solution containing 0.02% w/v of sodium versenate and 0.02% w/v of glucose.

Molarity
The molarity of a solution of phosphate-buffered saline is expressed as the number of moles of solute in 1 litre and is associated with each species in a mixture.

Molarity figures given are for the individual components of the formula.

Sodium choloride	0.16 mol
Potassium chloride	0.003 mol
Disodium hydrogen phosphate	0.008 mol
Potassium dihydrogen phosphate	0.001 mol

References
1. Dulbecco and Vogt (1954) *J. Exp. Med.* 99. 167-182.
2. Paul J. (1965) '*Cell and Tissue Culture*' 3rd ed, Livingstone Ltd., London.

'CALGON' RINGER TABLETS

Code: BR0049

Formula	*gm/litre*
Sodium chloride	2.25
Potassium chloride	0.105
Calcium chloride 6H2O	0.12
Sodium bicarbonate	0.05
Sodium hexametaphosphate ('Calgon')	10.0

One tablet makes 10 ml of quarter-strength Ringer Solution containing 1% of `Calgon'. (Sodium hexametaphosphate.)

Directions
To prepare a solution containing 1% sodium hexametaphosphate in quarter-strength Ringer Solution, dissolve 1 tablet in 10ml of distilled water. Sterilise by autoclaving at 121°C for 15 minutes.

Description
Higgins[1] showed that bacteriological swabs prepared from calcium alginate wool will dissolve completely in 'Calgon' Ringer Solution, thus releasing all the organisms taken up on the swab and giving a more accurate quantitative recovery. She concluded that the use of this material in place of cotton-wool for the preparation of swabs, seemed justified in quantitative work since the recovery of organisms was much greater.

This principle was successfully applied to the bacteriological examination of tableware[2] and also of crockery and kitchen utensils[3].

Trimarchi[4] found that calcium alginate swabs were superior to raw cotton swabs for the bacteriological examination of eating utensils.

Technique
Prepare swabs, using approximately 25-50 milligrams of alginate wool to each wooden applicator. Sterilise, in plugged tubes, by autoclaving at 121°C for 15 minutes. After swabbing, aseptically break off the swab end and drop into a screw- top bottle containing 10 ml of sterile 'Calgon' Ringer Solution. Close the bottle and shake vigorously for 10-30 minutes to dissolve the alginate wool completely. The suspension may then be examined quantitatively or qualitatively in the usual manner.

References
1. Higgins M. (1950) *Mon. Bull. Min. Hlth Pub. Hlth Lab Serv*. 9. 50-51.
2. Higgins M. and Hobbs B. C. (1950) *Ibid.* 38-49.
3. Higgins M. (1950) *Ibid.* 52-53.
4. Trimarchi G. (1959) *Igiene Moderna* 52. 95-111.

RINGER SOLUTION 1/4 STRENGTH RINGER SOLUTION TABLETS

Code: BR0052

Formula	*gm/litre*
Sodium chloride	2.25
Potassium chloride	0.105
Calcium chloride $6H_2O$	0.12
Sodium bicarbonate 0.05	0.05
pH 7.0	

One tablet makes 500 ml of quarter-strength Ringer solution.

Directions
To prepare quarter-strength Ringer Solution, dissolve 1 tablet in 500 ml of distilled water. Sterilise by autoclaving at 121°C for 15 minutes.

Note
It is our experience that providing the quality of the water and glassware used in preparation conform to the specifications laid out in the Oxoid Manual (Section 2 page 4), under 'General Guide to the use of Oxoid culture media'; a pH of 7.0 ± 0.2 prior to autoclaving can be considered confirmation of the integrity of the product. Variation of pH out of specification after autoclaving is most likely to be caused by factors originating from sources other than the product e.g. water, glassware and autoclaving.

Description
These tablets are prepared according to the formula in the Dept. of Health & Social Security publication Memo. 139/ Foods[1]. They dissolve readily in water to give a solution which does not precipitate when sterilised by autoclaving.
The sterile solution is used as an isotonic diluent for both bacterial cells and bacteriological specimens, or as a rinse during the bacteriological examination of plant and apparatus.

Technique
Oxoid quarter-strength Ringer Solution may be used for the decimal dilution of milk and dairy product samples, e.g. prior to inoculation into MacConkey Broth for the presumptive coliform test, the maceration and suspension of solid food and other specimens before bacteriological examination; the preparation of suspensions and serial dilutions from pure cultures of bacteria; the examination of dairy plant and apparatus by the swab or rinse method[2].

See also Thiosulphate Ringer Tablets BR48 and 'Calgon' Ringer Tablets BR49.

References
1. Dept. of Health & Social Security (1937) *Memo. 139/Foods*.
2. Davis J. G. (1956) '*Laboratory Control of Dairy Plant*'. Dairy Industries Ltd., London.

THIOSULPHATE RINGER TABLETS

Code: BR0048

Formula	*gm/litre*
Sodium chloride	2.15
Potassium chloride	0.075
Calcium chloride $6H_2O$	0.12
Sodium thiosulphate $5H_2O$	0.5
pH.*	

*Due to the low ionic content of this medium no pH is specified.

One tablet makes 500 ml of quarter-strength Ringer containing 0.05% of sodium thiosulphate.

Directions
To prepare quarter-strength Ringer Solution containing thiosulphate, dissolve 1 tablet in 500 ml distilled water. Sterilise by autoclaving at 121°C for 15 minutes.

Description
Oxoid Thiosulphate Ringer Tablets are formulated to provide, after the addition of distilled water, a Ringer Solution (British Pharmacopoeia[1]) containing 0.05% of sodium thiosulphate hydrate. Use of the B.P. Ringer instead of the Ministry of Health Ringer ensures chemical stability without altering the physiological properties of the diluent. The purpose of the sodium thiosulphate is to neutralise residual chlorine present in bacteriological samples and rinses as a result of the previous use of gaseous chlorine or chlorine-containing disinfectants. The thiosulphate immediately terminates the bactericidal action of the chlorine in the sample – so that subsequent bacteriological examination more accurately indicates the pollution of the sample at the time of collection. Sterile Thiosulphate Ringer Solution is consequently employed for rinse water samples taken for sanitary tests (from equipment and pipe lines, etc.) where a chlorine-containing cleansing fluid has been used. Davis used a Ringer Solution with added sodium thiosulphate, as a rinse after the use of sodium hypochlorite[2]. Egdell *et al*.[3] employed a Thiosulphate Ringer Solution in their bacteriological studies of immersion cleaned milking equipment.

Technique
100 ml of prepared solution will completely neutralise 7 mg of chlorine. Oxoid Thiosulphate Ringer Solution may be used for assessing the cleanliness and sterility of dairy plant, after hypochlorite solution has been used for disinfection purposes (see Davis[2]). The methods described by Davis rely on swabbing or rinsing of apparatus or milk bottles. In reference to the swab method, see 'Calgon' Ringer Tablets.

References
1. British Pharmacopoeia (1953) p 501.
2. Davis J. G. (1956) '*Laboratory Control of Dairy Plant*'. Dairy Industries Ltd., London.
3. Egdell J. W., Lomax K. L., Adams R. P. and Aitken M. J. (1958) *J. Appl. Bact.* 21(1). 109-117.

SALINE TABLETS (FOR LABORATORY USE ONLY)

Code: BR0053

Dissolve 1 tablet in 500 ml of distilled water in order to obtain 0.85% ('normal', physiological, or isotonic) saline.

O.B.I.S.

(Oxoid Biochemical Identification System)

A range of rapid biochemical tests for the differentiation of bacterial species direct from culture media. The tests reveal the presence of specific enzymes with each card including the key biochemical tests to differentiate the target organism.

OXOID BIOCHEMICAL IDENTIFICATION SYSTEM - MONO

Code: ID0600

A rapid colorimetric test for the differentiation of Listeria monocytogenese from other Listeria species.

Intended use

The Oxoid Biochemical Identification System (O.B.I.S.) mono is a rapid colourimetric test for the determination of D-alanyl aminopeptidase (DALAase). It has been designed for the differentiation of presumptive *Listeria* species that have been isolated from selective media and plated on to a secondary medium for further biochemical testing.

Principle of the Test

The O.B.I.S. mono test offers a rapid screening method for differentiation of *Listeria monocytogenes* from other *Listeria* species. This reduces the need for full biochemical identification of all suspect colonies.

Listeria species, with the exception of Listeria monocytogenes, possess the enzyme D-alanyl aminopeptidase[1,2,3].

Oxoid has developed a new system for aminopeptidase testing which uses a non-carcinogenic substrate. This is in response to health concerns associated with amino acid conjugates of β naphthylamine[4,5] as these are potent carcinogens[6].

D-alanyl-7-amido-4-methylcoumarin (DALA) is provided as a suspension. An acidic solution of dimethylaminocinnamaldehyde is used as a colour developer. If the substrate is hydrolysed by DALAase, free 7-amino-4-methylcoumarin (7AMC) combines with the developer to produce a purple Schiff's base[7].

Components of the O.B.I.S mono kit (ID600M)

Each O.B.I.S mono kit contains the following reagents with enough material for 60 tests.

ID090 O.B.I.S. Reaction Sleeves

One pouch containing 30 plastic bags (76 mm x 102 mm).

ID120 O.B.I.S. mono Buffer

One white capped bottle containing 4.5 ml of a 0.5% w/v D-alanyl-7-amido-4-methylcoumarin suspension in distilled water.

ID220 O.B.I.S. mono Developing Solution

One purple capped bottle containing 4.5 ml of a 0.5% w/v dimethylamino-cinnamaldehyde in 1 M hydrochloric acid.

ID601M O.B.I.S. mono Test Cards

One pouch containing 10 cards. There are six reaction areas per card, labelled 'DALA'.

Materials required but not included

Sterile plastic disposable inoculating loops.

Positive and negative quality control organisms.

37 ± 2ºC incubator

Precautions

This product is for *in vitro* diagnostic use.

Do not use O.B.I.S. mono reagents beyond the stated expiry date.

Specimen material may contain pathogenic organisms, handle with appropriate precautions.

The O.B.I.S mono Developing Solution contains acid. Wear suitable personal protective equipment. If the reagents come in to contact with the skin, mucous membranes or eyes, immediately flush the area with water.

Used O.B.I.S. Test Cards and inoculating loops should be disposed of as biohazardous waste. This should be incinerated, or autoclaved at 121°C for at least 15 minutes.

Storage and Opening
The O.B.I.S. mono kit must be stored at 2°C to 8°C. Allow the pouches to reach room temperature before use to prevent the formation of condensation on the test cards.

Open the pouches by cutting at the notch between the end seal and the clip-lock opening.

Remove the number of Test Cards required and reseal the pouch.

If fewer tests are required than the number on the Test Card, cut the card and return the unused portion to the pouch. Do not return used Test Cards to the pouch.

When stored as described, O.B.I.S. mono reagents will retain their activity until the expiry date shown on the box.

Quality Control Procedure
Each day the kit is used the following procedure should be performed:

Positive control – Use a known DALAase positive strain such as Listeria innocua ATCC® 33090 (Culti-loop® C9005L). Follow the method given in the test procedure. Ensure that a purple colour forms within 20 seconds.

Negative control – Use a known DALAase negative strain such as Listeria monocytogenes ATCC® 7644 (Culti-loop® C3970L). Follow the method given in the test procedure. Ensure that no purple colour forms within 20 seconds.

Specimens
The test is designed for use from purity plates, not from primary isolation media, as the colonies on primary isolation media are too small to carry out an effective test.

Pick colonies which have typical Listeria morphology from selective Listeria isolation media such as Oxford Agar (CM0856), PALCAM Agar (CM0877) and chromogenic Listeria Media, and streak onto a purity plate.

O.B.I.S. mono tests can be performed from purity plates recommended by international standards, such as Tryptone Soya Agar (CM0131), Tryptone Soya Yeast Extract Agar (TSA-YE)[8,9] or a recognised chromogenic Listeria Medium8.

Test procedure and interpretation of results
Important notes:

Before use shake the ID120 O.B.I.S. mono Buffer (white capped bottle) well to suspend the reagent.

Ensure that the culture to be tested is a Gram-positive, catalase-positive, oxidase-negative bacterium.

1. Remove one of the O.B.I.S. mono Test Cards (ID601M) from the pack.
2. Using a plastic loop, take colonial material equivalent to five colonies (each greater than 1 mm in diameter) from the purity plate.
3. Touch the sample to the reaction zone on the O.B.I.S. mono Test Card. Drag the loop across the diameter of the reaction zone.
4. Shake the ID120 O.B.I.S. mono Buffer well and dispense one drop on to the inoculated reaction zone.
5. Place the Test Card in a plastic Reaction Sleeve (ID090), so that the reaction zones are within the sleeve and the portion of the Test card with the Oxoid logo is outside the sleeve.
6. Place the Test Card and Reaction Sleeve into a 37 ± 2ºC incubator for 10 minutes.
7. Remove from incubator. Dispense one drop of ID220 O.B.I.S. mono Developing Solution onto each of the inoculated reaction zones.
8. The appearance of a purple colour within 20 seconds, is a positive D alanyl aminopeptidase reaction. A positive reaction indicates the organism is not a Listeria monocytogenes.

If no colour develops within 20 seconds this is a negative reaction, and indicates the organism is a presumptive *Listeria monocytogenes*.

Limitations of the Test
O.B.I.S. mono is intended for the detection of DALAase in Gram-positive, catalase-positive, oxidase-negative short rod shaped bacteria, capable of growing on selective Listeria primary isolation media. It can be used as a screen to differentiate *Listeria monocytogenes* from other *Listeria*.

Occasionally aesculin positive Bacillus species may grow on Listeria isolation media. Bacilli are DALAase positive, but the colonies are different from Listeria and bacilli appear as large rods upon Gram staining.

Due to the small size of Listeria colonies on primary isolation media it is not possible to carry out the test directly from these plates. Use of multiple colonies from the primary isolation plate is not recommended as this may lead to a mixed culture and an incorrect result. International standards recommend sub-culturing presumptive *Listeria* species on to purity plates TSA (CM131), TSA-YE[8,9] or a recognised chromogenic Listeria Medium[8].

Occasionally certain Listeria when grown on non-selective media form colonies that are difficult to remove with an inoculating loop. Ensure that there is sufficient colonial material on the inoculating loop before inoculating the reaction area. Failure to pick up sufficient material on the loop will result in a negative DALA test.

O.B.I.S. mono provides presumptive identification of *Listeria monocytogenes*, but does not replace full biochemical testing.

References

1. Kämpfer P., Böttcher S., Dott W., Rüden H. (1991) Physiological characterization and identification of Listeria species. *Zentralblatt für Bakteriologie* 275, 423 435.
2. Kämpfer P. (1992) Differentiation of Corynebacterium spp., *Listeria* spp., and related organisms by fluorogenic substrates. *Journal of Clinical Microbiology* 30, 1067 1071.
3. Clark A. G., McLauchlin J. (1997) Simple color tests based on an alanyl peptidase reaction which differentiate *Listeria monocytogenes* from other *Listeria* species. *Journal of Clinical Microbiology* 35, 2155-2156.
4. Morgan J. W. (1987) Evaluation of a rapid method for the determination of L pyrrolidonyl-β-naphthylamide hydrolysis. *Laboratory Medicine* 18, 682-683.
5. Bascomb S. and Manafi M. (1998) Use of enzyme tests in characterization and identification of aerobic and facultatively anaerobic Gram-positive cocci. *Clinical Microbiology Reviews* 11, 318-340.
6. Anonymous (2000) 2-Naphthylamine. In Croner's *Hazardous Substances: Carcinogens Guide.* pp. 2-291. Kingston-upon-Thames: Croner.CCH Group Limited.
7. Druggan P., Roberts P. B. and Swaine D. (1999) A rapid chromogenic method for the differentiation of *Citrobacter* spp. and *Salmonella* spp. directly from enteric media. Abstract of the Annual Meeting of the American Society for Microbiology 1999, C444, p.71. Washington: ASM Press.
8. International Standards Organisation (1996) ISO 11290-1, *Microbiology of food and animal feeding stuffs – horizontal method for the detection and enumeration of Listeria monocytogenes* - part 1: detection method. London: British Standards Institute.
9. FDA (1998) Appendix 3: Trypticase Soy Agar with 0.6% Yeast Extract (TSAYE). In *Food and Drug Administration Bacteriological Analytical Manual*, 8th edition, revision A ed. Jackson G. J. pp. 54-55. Gaithersburg: AOAC international.

OXOID BIOCHEMICAL IDENTIFICATION SYSTEM – PYR

Code: ID0580

The Oxoid Biochemical Identification System (O.B.I.S.) – PYR is a rapid colorimetric test for the determination of PYRase activity in Streptococci and Citrobacter spp.

Principal of the Test

PYRase activity distinguishes Group A *streptococci* and *enterococci* from other streptococcal groups including Group D *streptococci* (previously called faecal *streptococci*)[1,2], Thus offering a rapid diagnostic alternative to time-consuming culture methods. In addition, the same enzymatic activity may be used to aid differentiation of *Salmonella* spp. from *Citrobacter* spp. and other Enterobacteriaceae[3,4].

The PYRase test has traditionally been based on the use of a β-naphthylamide peptide. This is a potent carcinogen[5]. Oxoid has developed a new system using a non-carcinogenic substrate, in response to associated health concerns.

The O.B.I.S. – PYR test uses Test Cards impregnated with L-pyroglutamic acid 7-amino-4-methyl-coumarin (7AMC) and dimethylamino-cinnamaldehyde for the detection of PYRase activity. The enzymatic hydrolysis of this substrate by enterococci, Group A streptococci and Citrobacter spp. produces a purple colour following the addition of the Developing Solution[6].

Components of the Kit

Each O.B.I.S. PYR Kit contains the following reagents sufficient for 60 tests:

ID581M O.B.I.S. Test Cards:
1 Pouch containing 10 Cards and a moisture absorbent sachet. There are 6 reaction areas on each card. 60 tests in total. Each Test Card is impregnated with 7AMC.

ID200M Developing Solution:
1 dropper bottle containing 7 ml of 1 M hydrochloric acid 0.5% w/v dimethylamino-cinnamaldehyde.

ID100M Buffer Solution:
1 dropper bottle containing 7 ml of Phosphate Buffered Saline (PBS).

Instruction Leaflet.

Materials required but not provided
Mixing sticks or microbiological Loop.

Precautions
This product is for *in vitro* diagnostic use only.

Do not use O.B.I.S. PYR reagents beyond the stated expiry date.

Specimen material may contain pathogenic organisms, handle with appropriate precautions.

The Developing Solution (ID200M) contains an acid. Avoid direct contact by wearing suitable protective equipment. If the reagents come into contact with the skin, mucous membranes or eyes immediately wash the area thoroughly with plenty of water.

Used Test Cards and mixing sticks should be disposed of as biohazardous waste and incinerated or autoclaved for 15 minutes at 121°C.

Storage and Opening
This Kit must be stored at 2-8°C. Allow the pouches to reach room temperature before use to prevent the formation of condensation on the Test Cards. Open the pouches by cutting at the notch between the end seal and the zip lock opening. Once opened, remove the number of Test Cards required for immediate testing (testing within 10 minutes) and reseal the pouch straight away. If fewer tests are reqiured, cut the Test Card and return the unused portions to the pouch. Do not return used portions to the pouch as they will be contaminated. When stored as indicated, reagents will retain their activity until the expiry date shown on the box.

Quality Control Procedure
Each day the Kit is used the following control procedures should be performed.

Positive control – Use a known pyroglutamyl aminopeptidase positive strain such as *Enterococcus faecalis* ATCC® 29212 (Culti-Loop® C7030L) Streptococcus pyogenes ATCC® 19615 (Culti-Loop® C7000L) or *Citrobacter freundii* ATCC® 8090 (Culti-Loop® C1800L). Follow the method given in the test procedure. Ensure that a purple colour forms within 20 seconds.

Negative control – Use a known PYRase negative strain such as Streptococcus agalactiae ATCC® 13813 *Salmonella enteriditis* ATCC® 13076 (Culti-Loop® C8200L). Follow the method given in the test procedure. Ensure that no purple colour forms within 20 seconds.

Do not use the reagents if the reactions with control organisms are incorrect.

Specimen Collection and Preparation
For details of specimen collection and treatment a standard text book should be consulted[7].

When identifying enterococci and Group A streptococci, fresh primary or secondary cultures grown overnight on non selective media such as blood agar give best results. Colonies tested must be Gram-positive cocci and catalase-negative. In case of insufficient growth, a subculture should be performed.

When identifying Citrobacter spp. from Salmonella spp. and Enterbacteriaceae, colonies from non-selective media such as (XLD Medium, MLCB Agar, Desoxycholate Citrate Agar, Salmonella Shigella Agar, Brilliant Green Agar or Hektoen Enteric Agar may be tested. Colonies should be Gram-negative, oxidase-negative and urease-negative[7].

Test Procedure
1. Apply one suspect colony (0.5 mm or larger) onto the test area (enough to make a visible smear).
2. Moisten test area with 1 drop of Buffer.
3. Incubate the inoculated Test Card at room temperature (15-30°C) for 5 minutes.
4. Dispense 1 drop of Developing solution onto the test area. Development of a vivid purple colour on and around the colonies within 20 seconds confirms PYRase activity.

Reading and Interpretation of Results

Positive Result
A positive result is indicated by the development of a vivid purple colour in the inoculated portion of the test area within a 20 second period following addition of Developing Solution.

Negative Result
A negative result is indicated by lack of colour development in the inoculated portion of the test area within a 20 second period following addition of Developing Solution.

Expected Results

Organism	***Lancefield Group***	***O.B.I.S. PYR***
Streptococcus pyogenes	A	+
Streptococcus agalactiae	B	–
Streptococcus Group C	C	–
Entercoccus faecalis	D	+
Enterococcus faecium	D	+
Streptococcus Group F	F	–
Streptococcus Group G	G	–
Citrobacter	–	+
Salmonellae	–	–
Escherichia coli	–	–

Limitations of the Test
O.B.I.S.-PYR is intented for the detection of PYRase activity in Gram-positive, catalase negative cocci and Citrobacter spp. Less commonly encountered isolates of lactococci and aerococci may be PYRase positive. The confirmation of enterococci or Group A streptococci can be achieved by serological grouping with a suitable test, e.g. Oxoid Streptococcal Grouping Kit (DR 595), Oxoid Strep Plus (DR 575) or Oxoid Dryspot Streptococcal Grouping Kit (DR 400).

Some strains of Enterobacter cloacae are PYRase negative.

The reactions with O.B.I.S. – PYR are a marker for enzyme activity and atypical strains may occasionally occur.

A slight colour change may develop with negative reactions. This is restricted to the immediate site of inoculation. Refer to the positive and negative controls to aid interpretation.

Incubation beyond 20 seconds following addition of the Developing Solution may produce non-specific colour reactions. It is important therefore that the test is read as indicated.

References

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7. Faklam R. R. and Washing J. A. (1991). Streptococci and Aerococci pp. 238-258, In Lunette I. H., Balles A., Hausler (Jr), W. J. and Shadomy H. J. (eds) Manual of Clinical Microbiology, 5th edition. *Amer. Soc. For Microb*., Washington D.C.

OXOID BIOCHEMICAL IDENTIFICATION SYSTEM (O.B.I.S.) SALMONELLA

Code: ID0570

The Oxoid Biochemical Identification System (O.B.I.S.) Salmonella Test is a rapid colorimetric test for the determination of pyroglutamyl aminopeptidase (PYRase) and nitrophenylalanine deaminase (NPA) activity.

Principal of the Test

The O.B.I.S. salmonella Test offers a rapid screening method to distinguish *Salmonella* spp. From those organisms exhibiting similar colonial appearance on common selective *Salmonella* media. This reduces the need for a full biochemical identification of suspect colonies.

Identification of *Salmonella* relies on primary isolation of the organism on selective enteric media. However there are several other genera among the Enterobacteriaceae also capable of growth on such media and which can have similar colony morphology to salmonellae.

The lack of PYRase and NPA activity in *Salmonella* spp. Can be used to differentiate them from Citrobacter spp. Which possess PYRase activity[1,2,3] and *Proteus*, *Morganella* and *Providencia* spp. Which have NPA activity[4].

Oxoid has developed a new system for PYRase testing[5], using a non-carcinogenic substrate in response to health concerns associated with the use of β-naphthylamide (a potent carcinogen)[6].

The PYRase area on the O.B.I.S. salmonella Test Card is impregnated with L-pyroglutamic acid 7-amino-4-methylcoumarin (7AMC)[5]. Dimethylaminocinnamaldehyde is used as a colour development reagent. The enzymatic hydrolysis of the substrate produces a purple colour on addition of the O.B.I.S. PYR Developing Solution[5].

The NPA area on the O.B.I.S. Salmonella Test Card is impregnated with nitrophenylalanine. Deaminatin of the reagent is shown by an orange-brown colour when the O.B.I.S. NPA Developing Soloution (0.25 M sodium hydroxide) is added.

Components of the O.B.I.S. Salmonella Kit

Each O.B.I.S. Salmonella Kit contains the following reagents sufficient for 60 tests:

ID571M O.B.I.S. Salmonella Test Cards:

2 Zip Lock Pouches, each containing 10 Cards and a desiccant. There are 3 tests on the card each consisting of 2 reaction areas.

Each reaction area designated as PYR is impregnated with 1% w/v L-pyroglutamic acid 7-amino-4-methylcoumarin (7 AMC).

Each reaction area designated as NPA is impregnated with 5% w/v nitrophenylalanine.

ID200M O.B.I.S. PYR Developing Solution

1 dropper bottle containing 7 ml of 1 M hydrochloric acid 0.5% w/v dimethylamino-cinnamaldehyde.

ID210M O.B.I.S. NPA Developing Solution

1 dropper bottle containing 7 ml of 0.25 M sodium hydroxide

ID100M O.B.I.S. Buffer Solution

1 dropper bottle containing 7 ml of Phosphate Buffered Saline (PBS).

Instruction Leaflet.

Materials required but not provided

Microbiological Loop.

Positive and negative quality control organisms.

Precautions

This product is for *in vitro* diagnostic use only.

Do not use O.B.I.S. salmonella reagents beyond the stated expiry date.

Specimen material may contain pathogenic organisms, handle with appropriate precautions.

The O.B.I.S. PYR Developing Solution (ID200M) contains an acid. The O.B.I.S. NPA Developing Solution (ID210M) contains a weak base. Avoid direct contact by wearing suitable protective equipment. If the reagents come into contact with the skin, mucous membranes or eyes immediately wash the area thoroughly with plenty of water.

Used O.B.I.S. Test Cards and microbiological loops should be disposed of as biohazardous waste and incinerated or autoclaved for 15 minutes at 121°C.

Storage and Opening
The O.B.I.S. Salmonella Kit must be stored at 2-8°C. Allow the pouches to reach room temperature before use to prevent the formation of condesation on the Test Cards. Open the pouches by cutting at the notch between the end seal and the zip lock opening. Once opened, remove the number of Test Cards required for immediate testing (testing within 10 minutes) and reseal the pouch straight away. If fewer tests are reqiured, cut the Test Card and return the unused portions to the pouch. Do not return used portions to the pouch as they will be contaminated. When stored as indicated, O.B.I.S. Salmonella reagents will retain their activity until the expiry date shown on the box.

Quality Control Procedure
Each day the Kit is used the following control procedures should be performed.

PYRase Test
Positive control – Use a known pyroglutamyl aminopeptidase positive strain such as *Citrobacter freundii* ATCC® 8090 (Culti-Loop® C1800L). Follow the method given in the test procedure. Ensure that a purple colour forms within 20 seconds.

Negative control – Use a known pyroglutamyl aminopeptidase negative strain such as *Salmonella enteriditis* ATCC® 13076 (Culti-Loop® C8200L). Follow the method given in the test procedure. Ensure that no purple colour forms within 20 seconds.

NPA Test
Positive control – Use a known phenylalanine deaminase positive strain such as *Proteus mirabilis* ATCC® 43071 (Culti-Loop® C7073L). Follow the method given in the test procedure. Ensure that an orange-brown colour forms within 20 seconds.

Negative control – Use a known phenylalanine deaminase negative strain such as *Citrobacter freundii* ATCC® 8090 (Culti-Loop® C1800L) or *Salmonella enteriditis* ATCC® 13076 (Culti-Loop® C8200L). Follow the method given in the test procedure. Ensure that no orange-brown colour forms within 20 seconds.

Do not use the reagents if the reactions with control organisms are incorrect.

Specimens
Colonies with typical Salmonella morphology on the following media may be tested: Brilliant Green Agar (Modified) CM0329, MLCB Agar CM0783, XLD Medium CM0469, SS Agar CM0099, SS Agar Modified CM0533, Hektoen Enteric Agar CM0419, Desoxycholate Citrate Agar CM0035 of Bismuth Sulphite Agar CM0201. Colonies tested should be Gram-negative and Oxidase negative.

Plates should be tested a maximum of one hour after being removed from the incubator.

Test Procedure and Interpretation of Results
Important Note: nitrophenylalanine impregnated on the NPA Test area is light sensitive. Exposure to light for more than 1 hour must be avoided.

1. Select one suspect colony (0.5 mm or larger). Apply the colony to the PYR and NPA Test areas (sufficient colony should be applied to each area to make a visible smear)
2. Moisten both Test areas with 1 drop of Buffer solution (ID100M).
3. Incubate the inoculated Test Card at room temperature (15-30°C) for 5 minutes.
4. Dispense 1 drop of O.B.I.S. PYR Developing Solution (ID200M) to the PYR Test area. Development of a vivid purple colour confirms PYRase activity.
5. Dispense 1 drop of the O.B.I.S. NPA Developing Solution (ID210M) to the test area. Development of an orange-brown colour on and around the smeared colony within 20 seconds confirms NPA activity.

Interpretation chart

Typical organism reactions

Organism	***PYRase***	***NPA***
Salmonella spp.	–	–
Citrobacter spp.	+	–
Proteus, Morganella and *Providencia* spp.	–	+

Trial Results

Results from pure cultures

Organism.	***No. of specimens tested***	***Positive PYRase Reactions***	***Positive NPA Reactions***
Salmonella spp.	150	0	0
Citrobacter spp.	56	55*	0
Proteus, Morganella and *Providencia* spp.	50	0	50

*One Citrobacter strain showing a negative PYRase reaction. This was confirmed as a *Citrobacter youngae* thus showing a truly negative result.

In trail of 470 faecal samples O.B.I.S. Salmonella gave the following results:

Sensitivity 100%
Specificity 81.5% – 95.4%*

*Depending on the plating medium used.

Limitations of the Test

O.B.I.S. Salmonella is intented for the detection of PYRase and NPA activity in Gram -negative, Oxidase negative micro-organisms. It can be used as a screen to differentiate *Salmonella* spp. from Citrobacter, *Proteus*, *Providencia* and *Morganella* spp. from the following selective enteric media: Brilliant Green Agar (Modified) CM0329, MLCB Agar CM0783, XLD Medium CM0469, SS Agar CM0099, SS Agar Modified CM0533, Hektoen Enteric Agar CM0419, Desoxycholate Citrate Agar CM0035 of Bismuth Sulphite Agar CM0201.

A few isolates of H_2S-positive *Escherichia coli* may appear as salmonellas on MLCB Agar. These organisms will show the same results as *Salmonella* spp. on the O.B.I.S. Salmonella Test as they lack both PYRase and NPA activity.

The neutral red in Desoxycholate Citrate Agar CM0035 may produce a pink colour on the O.B.I.S.Test Card, to avoid any confusion with the positive purple reaction in the PYR test the result should be compared to a positive control strain.

Escherichia coli and indole-positive *Proteus* spp. obtained from media with a high tryptophan content may generate a blue-green colour development on PYR Test area. This is a negative result.

Citrobacter youngae is PYRase-negative and will show the same result as *Salmonella* spp. in the Test.

O.B.I.S. Salmonella provides a reliable presumptive identification of *Salmonellae*, but does not replace the need for full biochemical testing.

References

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2. Mulczyk M. and Szewczuk A. (1970). Pyrrolionyl Peptidase in Bacteria: A New Colorimetric Test for Differentiation of Entertobacteriaceae. *J. Gen. Microbiol*. 61 9-13.
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OXOID BIOCHEMICAL IDENTIFICATION SYSTEM - ALBICANS

Code: ID0700

A rapid colorimetric test for the differentiation of Candida albicans from other yeast species.

Intended use
The Oxoid Biochemical Identification System (O.B.I.S.) albicans is a simple, non-hazardous colorimetric test for the differentiation of Candida albicans from other yeast species.

Principle of the Test
The O.B.I.S. albicans is a two-stage biochemical test that detects the presence of two enzymes, β-galactosaminidase and L-proline aminopeptidase, using chromogenic substrates. Both these enzymes are produced by *Candida albicans* whereas one or both enzymes are absent in other yeast species[1,2]. The test offers a rapid diagnostic alternative to traditional, time-consuming and subjective physiological methods such as the germ-tube test[3].

Other biochemical test kits have traditionally been based on the use of a β-naphthylamide peptide, which is a potent carcinogen[4]. Oxoid has developed a new system using a non-carcinogenic substrate, in response to associated health concerns. The use of chromogenic rather than fluorogenic substrates eliminates the need for UV detection, providing both safety and ease of use.

O.B.I.S. albicans uses Test Cards impregnated with p-nitrophenyl-N-acetyl-β-D-galactosaminide (pNP-NAGal) and L-prolinyl 7-amido-4-methylcoumarin (PRO-AMC). Following addition of the O.B.I.S. NaOH Developer the presence of β-galactosaminidase is indicated by the formation of a yellow colour. The addition of O.B.I.S. DMAC Developer indicates the presence of L-proline aminopeptidase by the formation of a magenta colour. Absence of either enzyme (indicated by no colour change) confirms that the culture is **not** *Candida albicans*.

Components of the O.B.I.S albicans kit (ID700M)
Each O.B.I.S mono kit contains the following reagents with enough material for 60 tests:

ID0703M Test Cards: 1 Pouch containing 10 cards and a moisture absorbent sachet. There are 6 test reaction areas on each card, providing a total of 60 tests. Each Test Card is impregnated with pNP-NAGal and PRO-AMC.

ID0701M O.B.I.S. Rehydration Solution – 1 dropper bottle containing 7 ml of 0.1% Tween 80® solution.

ID0702M O.B.I.S. NaOH Developer – 1 dropper bottle containing 7 ml of 0.1M sodium hydroxide.

ID0221M O.B.I.S. DMAC Developer – 1 dropper bottle containing 7 ml of 0.5% w/v dimethylaminocinnamaldehyde dissolved in 1M hydrochloric acid.

ID0090M O.B.I.S. Sleeves – 1 pouch containing 30 plastic reaction sleeves.

ID0898 60 Plastic Paddle Pastettes.

Instruction leaflet.

Materials required but not included
37ºC incubator

Precautions
This product is for *in vitro* diagnostic use.

Do not use the O.B.I.S. albicans Test Cards or reagents beyond the expiry date.

Specimen material may contain pathogenic organisms, take appropriate precautions when handling.

The O.B.I.S. NaOH Developer is a weak base and the O.B.I.S. DMAC Developer contains an acid. Avoid direct contact by wearing suitable personal protective equipment. If the material comes into contact with the skin, mucous membranes or eyes, immediately rinse the affected area with plenty of water.

Used Test Cards and paddle pastettes must be disposed of as bio-hazardous waste and incinerated or autoclaved for 15 minutes at 121°C.

Storage and Opening
The O.B.I.S. mono kit must be stored at 2-8°C. Allow the pouches to reach room temperature before use to prevent the formation of condensation on the test cards.

Open the pouches by cutting at the notch between the end seal and the clip-lock opening.

Once opened, remove the number of Test Cards required for testing within the next 60 minutes and reseal the pouch immediately.

If fewer tests are required, cut the Test Card and return the unused portions to the pouch. Do not return used portions to the pouch as they will be contaminated.

When stored as directed, reagents will retain their activity until the expiry date shown on the box.

Quality control procedure
Each day the kit is used the following control procedures should be performed:

1. **Positive Control** – *Candida albicans* ATCC® 10231. Follow the method given in the test procedure. Ensure that a yellow colour forms immediately after addition of O.B.I.S. NaOH Developer (ID0702) AND a magenta colour forms within 10 seconds after addition of O.B.I.S. DMAC Developer (ID0221).
2. **Negative Control** – *Candida kefyr* ATCC® 8555 or *Saccharomyces cerevisiae* ATCC® 9763. Follow the method given in the test procedure. Ensure that no yellow colour forms after addition of O.B.I.S. NaOH Developer (ID0702M) **and** no magenta colour forms within 10 seconds after addition of O.B.I.S. DMAC Developer (ID0221).

Do not use the reagents if reactions with control organisms are incorrect.

Specimens
For details of specimen collection and processing, standardmethods or procedures should be consulted.

When identifying *Candida albicans*: Fresh primary or secondary cultures grown on Sabouraud Dextrose Agar (SDA) (CM0041) for 24 to 48 hours give best results. Cultures older than 96 hours should not be used as enzymic activity may be impaired. In the case of insufficient growth, a subculture (passage) should be performed.

Test procedure
1. Using the paddle end of an unused plastic Paddle Pastette, transfer approximately three to five 1 mm colonies, or equivalent, onto the circular test area, ensuring the material is smeared thinly and evenly within the circular test area.
2. Moisten the inoculated test area with 1 drop of O.B.I.S. Rehydration Solution (ID0701 – white cap).
3. Place the card into a plastic Reaction Sleeve ensuring the test area is covered and incubate at 37°C for 60 minutes.
4. After incubation, remove the Test Card from the plastic Reaction Sleeve and dispense 1 drop of O.B.I.S. NaOH Developer (ID0702 – yellow cap) onto the inoculated test area. Development of a yellow colour on and around the smear of inoculum indicates presence of β-galactosaminidase.

Record the result on the Test Card before progressing to step 5.
No colour change indicates absence of this enzyme, i.e. the organism is not Candida albicans. If there is no colour development no further steps are required.

5. Dispense 1 drop of O.B.I.S. DMAC Developer (ID0221 – purple cap) onto the test area. Development of a magenta colour on and around the smear of inoculum within 10 seconds indicates presence of L-proline aminopeptidase. No colour change after 10 seconds indicates absence of this enzyme, i.e. the organism is not *Candida albicans*. Record the result on the Test Card.

Reading and interpretation of results
Positive Result: A positive result is indicated by the development of a yellow colour in the inoculated portion of the test area following addition of O.B.I.S. NaOH Developer (ID0702) **and** the development of a magenta colour in the inoculated portion of the test area within the 10 second period following addition of O.B.I.S. DMAC Developer (ID0221).

Negative Result: A negative result is indicated by lack of colour development in the inoculated portion of the test area within the 10 second period following addition of either the O.B.I.S. NaOH Developer (ID0702) or the O.B.I.S. DMAC Developer (ID0221).

Following addition of O.B.I.S. NaOH Developer (ID0702), it is important that the result is recorded immediately, before the addition of the O.B.I.S. DMAC Developer (ID0221), as the reactions are non-reversible.

The inoculum itself may appear as a straw-yellow coloured smear. To aid interpretation of positive and negative results after addition of the O.B.I.S. NaOH Developer (ID0702), refer to the positive and negative control reactions for comparison.

Following addition of the O.B.I.S. DMAC Developer (ID0221), a pale yellow background will be apparent. This should not be confused with the yellow colouration observed for positive reactions after addition of the O.B.I.S. NaOH Developer (ID0702).

Some yeast species express L-proline-aminopeptidase, but not β-galactosaminidase. If a negative result is

recorded following addition of O.B.I.S. NaOH Developer (ID0702), there is no need to add the O.B.I.S. DMAC Developer (ID0221). Presumptive identification of Candida albicans is only achieved by a positive result for the presence of both enzymes.

Limitations of the Test

O.B.I.S. albicans is intended for the presumptive identification of *Candida albicans* from pure culture.

Less commonly encountered isolates of *Candida dubliniensis* (a recently described species, closely related to Candida albicans) are also positive for this test.

For best results only use colonies grown Sabouraud Dextrose Agar (CM0041). Other media may interfere with enzymic activity or cause false positive cross-reactions[3].

Continuous passaging may affect enzyme expression in the target organism. Therefore, it is preferable to use cultures that have not been passaged more than three times.

Only Paddle Pastettes should be used for the transfer of material from the culture media to the Test Card. Use of microbiological loops for this purpose may result in weak positive reactions.

The reactions with O.B.I.S. albicans are markers for enzyme activity and atypical strains may occasionally occur.

Incubation beyond 10 seconds following addition of the DMAC Developer (ID0221) may produce non-specific colour reactions. Therefore, it is important that the test is read as directed.

Trial Results

Results from organisms grown on Sabouraud Dextrose Agar (SDA). Isolates were incubated at 30°C for 48 hours.

Comparison of O.B.I.S. albicans with Germ Tube

		Germ Tube		
		+	–	
O.B.I.S. albicans	+	83	0	Sensitivity 100%
	–	0	133	Specificity 100%

References

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TOUCH STICKS

BETA-LACTAMASE IDENTIFICATION STICKS

Code: BR0066

Oxoid Identification Sticks, Beta-Lactamase are used for the detection of beta-lactamases produced by Gram-positive and Gram-negative bacteria. The tips of the sticks are impregnated with Nitrocefin, a chromogenic cephalosporin (Glaxo Research 87/312). The sticks are convenient to use and overcome the necessity for preparing fresh reagents daily.

Formula
One end of each stick is impregnated with a solution of Nitrocefin, phosphate buffer and dimethylsulphoxide. The opposite end is coloured black to identify the correct end for handling. The colour also identifies the product.

General Introduction and Intended Uses
Nitrocefin is the chromogenic cephalosporin developed by Glaxo Research Limited coded 87/312; 3 – (2,4 dinitrostyrl) - 6R, 7R) - 7 - (2 thienylacetamido)-ceph-3-em-4 carboxylic acid, E-isomer. This compound exhibits a rapid distinctive colour change from yellow to red as the amide bond in the beta- lactam ring is hydrolysed by a beta-lactamase (E.C. 3.5.2.6.); it is sensitive to hydrolysis by all known lactamases produced by Gram-positive and Gram-negative bacteria.

Demonstration of Beta-Lactamase Activity by Bacterial Cells
Nitrocefin hydrolysis has been found to be highly efficient in detecting beta-lactamase producing isolates of *Neisseria gonorrhoeae*[1,2,3], *Haemophilus influenzae*[2,4,5,6], staphylococci[5,6] and bacteroides[7,8,9,10,11]. It should be emphasised that the efficiency of Nitrocefin tests in predicting the beta-lactam susceptibilities of other micro-organisms is at present unproven. In a positive reaction the beta-lactamase enzymes hydrolyse the amide bond in the beta-lactam ring. This is detected by a colour change on the end of the stick from yellow to pink/red.

Lactamase Induction
It should be noted that some organisms will not exhibit lactamase unless the enzyme has been induced by exposure to a beta-lactam antimicrobial. In such circumstances the organism should be tested from growth adjacent to beta-lactam antimicrobial discs or from agar containing beta-lactams.

Nitrocefin hydrolysis will give a rapid indication of beta-lactamase activity and the result so obtained will, in most cases, predict the outcome of susceptibility tests with these antimicrobials. However, it should not entirely replace conventional susceptibility testing as other factors also influence the results of such tests.

Technique

1. Remove the container from the freezer and allow it to reach room temperature.
2. Select a well separated representative colony from the primary isolation medium.
3. Remove one stick (colour coded black) from the container and holding the coloured end, touch the colony with the impregnated tip of the stick, rotate the stick, picking up a small mass of cells.
4. Place the inoculated tip of the stick between the lid and the base of the inverted plate.
5. **The reaction requires moisture. The inoculated tip of the stick should be placed in the moisture condensate in the lid. If condensate is not available in the inverted lid add one or two drops of distilled water to the lid and moisten the tip of the stick.**
6. **Examine the reagent impregnated tip of the stick for up to five minutes and, if negative re-examine after fifteen minutes.**

Note: Some staphylococci may take up to 1 hour before reaction shows a colour change.

7. A positive reaction is shown by the development of a pink/red colour. No colour change is observed with organisms that do not produce beta-lactamase. To ensure correct reading the colour of the stick should be compared to an unused stick.

Caution
Organisms producing pigmented colonies i.e. *Staph. citreus*, may give false positive results. It is recommended therefore that when pigmented colonies are to be tested Nitrocefin in solution – code SR112, should be used.

Quality Control
Reference strains should be tested to control the product at appropriate intervals.

Positive control: Beta-lactamase producing staphylococcus

Negative controls: Non beta-lactamase producing staphylococcus

Storage Temperature
Store below –10°C.

References

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OXIDASE IDENTIFICATION STICKS

Code: BR0064

A convenient and stable presentation of oxidase reagent for the detection of oxidase-positive bacteria. The enzyme cytochrome oxidase is produced by many organisms including *Neisseria* and *Pseudomonas* species and the 'Oxidase Test' is an important and commonly used reaction for the screening and presumptive identification of microbial cultures. Unfortunately, the reagent used is unstable in solution and needs frequent preparation for reliable results to be obtained.

Oxidase Identification Sticks utilise a dry reagent specially stabilised to give it a long life. They therefore overcome the necessity for daily preparation of fresh reagent and are very convenient to use.

Formula
The tip of each stick is impregnated with a solution of N,N-dimethyl-p-phenylenediamine oxalate, ascorbic acid and a-napthol. The other end is coloured red to ensure that the correct end is held.

Description
The Oxidase test is an important differential procedure which should be performed on all Gram-negative bacteria that are to be identified. The Oxidase reaction, based upon the ability of certain bacteria to produce indophenol blue from the oxidation of dimethyl-p-phenylenediamine anda-napthol was introduced by Gordon and McLeod[1] to aid in the identification of gonococci. Its wider use originated with the test devised by Kovacs[2], to distinguish *Pseudomonas* species from enteric bacteria. Kovacs smeared bacterial growth on filter-paper impregnated with 1% w/v aqueous tetramethyl-p-phenylenediamine dihydrochloride solution. Steel[3] found Kovacs' method too sensitive, with some staphylococci giving weak or delayed reactions. More useful results were obtained by the method described by Gaby and Hadley[4] using N,N-dimethyl-p-phenylenediamine oxalate where all staphylococci were oxidase negative.

Barry and Bernsohn[5] confirmed the observations of Carpenter *et al*.[6] that dried crystals of the oxalate salt dimethyl-p-phenylenediamine have a longer shelf life than the tetra-methyl-p-phenylenediamine dihydrochloride. The loss of activity of the oxidase reagents is caused by autoxidation which may be retarded by the addition of 0.1% ascorbic acid[3]. Filter paper strips impregnated with the oxidase reagents and their use have been described by Rogers[7] and by Barry and Bernsohn[5]. However, the Oxoid oxidase stick, impregnated with oxidase reagents described by Gaby and Hadley[4], is a much more convenient technique to use. The colony under test is touched with the impregnated end of the stick so that some microbial mass is picked from the colony. The use of the stick also overcomes the problems of iron oxidation of the reagent associated with nichrome wire loops.

In a positive reaction the enzyme cytochrome oxidase combines with N,N-dimethyl-p-phenylenediamine oxalate and a-napthol to form the dye indophenol blue.

Technique

1. Remove the container from the refrigerator and allow it to stand for approximately 5 minutes at room temperature before opening.
2. Choose a well separated representative colony on the primary isolation medium or purity plate.
3. Remove one stick (colour coded red) from the container and holding it by the coloured end, touch the colony with the impregnated end of the stick. **Rotate the stick**, picking off a small mass of cells.
4. Place the stick between the lid and the base of the inverted plate.
5. Examine the impregnated end of the stick after 30 seconds. If a colour change has not occurred examine again after 3 minutes.
6. A positive reaction is shown by the development of a blue-purple colour. No colour change is observed with organisms that are oxidase negative.

Main Uses

To differentiate *Neisseria* (oxidase positive) from other Gram-negative diplococci.

To differentiate strains of *Pseudomonas* species (pigmented and non-pigmented) from other enteric organisms.

To differentiate *Aeromonas hydrophila* (oxidase positive) from *Escherichia coli* (negative).

To differentiate *Plesiomonas shigelloides* (oxidase positive) from Shigella sonnei (negative).

Quality Control

Reference strains should be tested to control the product at appropriate intervals.

Positive Control: *Pseudomonas aeruginosa* ATCC® 27853

Negative Control: *Staphylococcus aureus* ATCC® 25923

Precaution

Cytochrome oxidase production may be inhibited by acid production. False negative reactions may be given by *Vibrio*, *Aeromonas* and *Plesiomonas* species when growing on a medium containing a fermentable carbohydrate e.g. MacConkey Agar and Cholera Medium TCBS. However, well isolated non-fermenting colonies can be tested. Colonies picked from media containing nitrate may give unreliable results.

Storage Temperature

Store at 2-8°C.

References

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9
RAPID FOOD TESTS

OXOID LISTERIA RAPID TEST

Code: FT0401

Intended Use
The Oxoid Listeria Rapid Test is designed for the detection of *Listeria* species in foods. (Dairy samples, dessert and confectionery foods, raw vegetables, dry foods and additives, raw and processed food, meats, poultry and fish) and environmental samples. (Stainless steel, Plastic (polythene, polypropylene or polycarbonate) ceramic, glazed earthen material and glass, Rubber, Food grade painted surfaces, wood, concrete, Cast Iron and airfilter materials) within 43 hours. The test protocol allows for the availability of the result two working days after the sample is received in the testing laboratory. The procedure uses two carefully selected enrichment steps for the maximum recovery and growth of *Listeria* followed by an immunoassay using a test device. This simple system gives a clear visual result 20 minutes after the addition of the heated and cooled sample to the test device with no further manipulations being required. The Listeria device was reviewed by AOAC Research Institute (USA) and was found to perform according to the specifications mentioned in this insert.

Introduction
Listeria is a genus of Gram positive, non-sporing bacilli with a DNA G+C content of 36–38%. They have up to 6 peritrichous flagella and are motile when grown at 30°C or below. They are aerobic and facultatively anaerobic, catalase positive and oxidase negative. The genus comprises six species, *Listeria monocytogenes*, *Listeria ivanovii*, *Listeria innocua*, *Listeria welshimeri*, *Listeria seeligeri* and *Listeria grayi* Subsp. grayi and *Listeria grayi* Subsp. murrayi. All Listeria, except *Listeria grayi*, variously share 4 flagella antigens A, B, C, and D, of which flagella antigen B is common[1].

Pathogenic and non-pathogenic Listeria are ubiquitous in nature and can be isolated from soil, vegetables and natural waters as well as from healthy animals and man. They are able to grow over a temperature range of 1–45°C. Consequently, *Listeria monocytogenes* is a food poisoning risk to susceptible individuals if present in foods that are subsequently stored at these temperatures for sufficient time for the organism to grow to infectious levels before ingestion. Clinical symptoms include flu like illness, spontaneous abortion, still birth, meningitis, pneumonitis, septicaemia and endocarditis. *Listeria monocytogenes* infections mainly occur in neonates, pregnant women, the elderly and immunocompromised individuals.

Test Principle

Enrichment Broth System
Culture of the test sample is in two sequential enrichments, taking 42 hours. Any Listeria organisms present in the food or environmental sample are selectively enriched using growth conditions which are optimal for flagella expression.

Antigen Extraction
The second enrichment media is heated at 80°C for 20 minutes to extract the flagella antigen.

Listeria Device
The *Listeria* device contains specific monoclonal antibodies to the B flagella antigen[2] that is common to the Listeria species indicated earlier.

The extracted antigen is added onto a pad in the Sample Window. This contains blue latex labelled with antibody. The extract rehydrates the complex and the specific antigen reacts, if present, with the antibody.

The complex moves through the pad by capillary action to a test strip containing an immobilised line of antibody midway along the Result Window.

A further reaction between antigen/latex complex and the fixed antibody results in a blue line in the Result Window.

If no flagella antigen is present the Result Window will remain clear.

The Listeria device also provides an integral control feature. The appearance of a blue line in the Control Window shows the test has been carried out correctly.

Precautions
Pregnant women should not be in contact with this organism in the laboratory.

Listeria screening and the use of this kit should be restricted to persons with a basic knowledge of microbiology and associated hazards.

For *in vitro* use only.

Standard guidelines for the safe handling and the disposal of infectious micro-organisms should be observed throughout all procedures.

Do not mix kit components from different lots.

Do not use after the expiry date stated on the kit.

Components of the Kit
Oxoid SR0166 Half Fraser Supplement: 50 vials

Half Fraser Supplement is used in conjunction with Fraser Broth (CM0895), it is modified by the addition of only half the level of selective agents normally found in Fraser Supplement. Each vial is sufficient for 225 ml of broth.
Listeria Test Units: 50
Positive Control: 3 vials
Non-viable *Listeria monocytogenes* suspension.
Instruction leaflet

Materials Required but not Provided
Oxoid Fraser Broth, (FB) CM0895
Oxoid Buffered Listeria Enrichment Broth, (B.L.E.B.) CM0897
B.L.E.B. Selective Enrichment Supplement, SR0141
Incubator at 30 ± 2°C
Water bath at 80 ± 2°C
Glass test tubes of 5-8 ml capacity
Sterile water/ethanol mixture 1:1, (v/v).

Storage
This kit must be stored at 2–8°C. Under these conditions the components of the kit will retain activity until the date shown on the box. Do not freeze.

Test Protocol

(A) Media Preparation
Primary Enrichment – Half Fraser Broth

FB CM0895 should be prepared in accordance with the manufacturer's instructions and dispensed into appropriate volumes prior to sterilisation. Reconstitute the Half Fraser supplement (SR0166) by adding 4 ml of the 1:1 ethanol/water mixture to a vial. This is sufficient for 225 ml of broth.

Add an appropriate amount of supplement to the dispensed volumes of F.B. immediately prior to use.

Secondary Enrichment Broth (BLEB)

BLEB – CM0897 should be prepared in accordance with the manufacturer's instructions and dispensed into 10 ml volumes prior

to sterilisation. Reconstitute the BLEB supplement (SR0141) in accordance with the manufacturer's instructions.

Add 40 µl of the reconstituted BLEB supplement to 10 ml of BLEB immediately prior to use.

(B) Sample Culture System
Food samples are prepared by making an homogenate of the food in the Half Fraser Broth, as described opposite (Table 1).

Environmental swabs should be quenched in 10 ml of an appropriate neutralising agent and the whole added to 90 ml of Half Fraser Broth (Table 1).

1. Dilute the test sample in 1–10 weight/volume of the prepared Half Fraser Broth and stomach for the appropriate time according to the sample type.
2. Incubate the mixture at 30°C for a minimum of 21 but not exceeding 24 hours.
3. Transfer 0.1 ml of the Half Fraser Broth into 10 ml of prepared BLEB.
4. Incubate the BLEB at 30°C for a further 21 but not exceeding 24 hours.

Table 1
Protocol for Oxoid Listeria Rapid Test

Foods 25 g + 225 ml 1/2FB*		***Swabs Swab + 10 ml neutralising agent + 90 ml 1/2FB***
30°C	0.1 ml 10 ml BLEB	21–24 h
30°C	2 ml glass tube (bijou)	21–24 h
80°C	Cool 135 µl	20 mins
	Listeria Device (20 mins at RT)	

*Half FB Supplement – 1 vial per 225 ml FB. Supplement reconstitute in 4 ml 1:1 ethanol:water.

(C) Preparation of the BLEB sample for testing

1. Carefully remove the incubated BLEB from the incubator without disturbing any food debris present. Transfer 2 ml of the upper region of the broth to a small glass tube.
2. Place the glass tube in the water bath at 80°C for 20 minutes.
3. Allow the extract to cool to room temperature.

(D) Listeria Device Test Protocol

1. Ensure that the device is at room temperature before beginning the assay.
2. Remove a test unit from the foil wrapper and place on a level surface. Label with the identity of the test sample.
3. Pipette 135 µl of the BLEB extract onto the Sample Window. If any precipitate or viscous material is present after heating avoid pipetting this into the device.
4. After 20 minutes, examine for the presence of a blue line of any intensity in the Result Window.

Interpretation of the Results

A blue line in the Result Window indicates the presence of Listeria flagella antigen in the BLEB. This is a positive result. If identification at species level is required the Listeria organisms may be sub-cultured from the BLEB broth onto a selective agar, e.g. Oxford Agar. Colonies resembling Listeriashould be purified on non-selective media and appropriate confirmation and specification tests carried out.

A blue line in the Control Window indicates that the device has worked correctly.

Differences in the intensity of the blue lines in the Result and Control Windows may occur but this does not affect the interpretation of the result. A very strong blue line in the Result Window with no line in the Control Window indicates an excess of flagella antigen. This is an uncommon event (typically less than 0.1%). If reassurance that the test has worked properly is required, the sample may be retested with a dilution step. The heated extract should be diluted 1 in 10 in fresh BLEB and retested on another device.

If no line appears in the Result or Control Window within 20 minutes a further Listeria device should be set up using the same culture extract, providing this is not more than 1 hour old.

Positive and Negative Controls

A positive (non-viable) flagella freeze-dried antigen control is included in the kit. Reconstitute a vial with 2 ml of sterile distilled water. The reconstituted positive control should be stored at 2–8°C. Under these conditions it will retain its activity for 6 months. Add 135 µl of positive control reagent onto the Sample Window of a separate device.

If a negative control is require, add 135 µl of uninoculated BLEB onto a separate device. Do not use any other reagents as a negative control.

Limitations of the Listeria Device

The Listeria device is only recommended for use with the Oxoid enrichment broths and supplements that are described in the above protocol[3].

Negative results may occur if the amount of antigen extracted is below the minimum sensitivity of the test, or if incubation temperatures above 30°C are used.

False negative results may be obtained when testing concentrated yeast materials by this method. This can be improved by extending the incubation times to 24–26 hours for both primary and secondary enrichment broths. The Listeria device has demonstrated not to detect Listeria at lower concentration in raw beef samples. *Listeria grayi* is not detected.

Performance Characteristics

The Oxoid Listeria Rapid Test has been evaluated in a wide variety of foods, comparing the device with isolation on Oxoid Oxford agar from the secondary enrichment broth. More than 1000 tests have given >99% correlation[4].

Results of an independent evaluation of 62 artificially contaminated samples comprised of a wide variety of surface types demonstrated a sensitivity rate of approximately 80%. Even though the sensitivity rate for Listeria is lower for surfaces, it is comparable to results obtained when using BAM.

The antibodies in the device have been tested for crossreactivity against a panel of organisms listed below. The organisms were at a minimum level of 1 x 108/ml. No cross-reactivity was observed with any of the organisms.

Organism

Klebsiella pneumoniae	*Enterococcus faecalis*
Acinetobacter anitratus	*Aerococcus viridans*
Pseudomonas aeruginosa	*Bacillus licheniformis*
Escherichia coli	*Bacillus circulans*
Bacillus cereus	*Staphylococcus aureus*
Arizona sp.	*Streptococcus faecalis*
Salmonella sp.	*Bacillus subtilis*
Enterobacter aerogenes	*Bacillus macerans*
Citrobacter diversus	*Edwardsiella tarda*
Citrobacter koseri	*Hafnia alvei*
Enterobacter cloacae	*Proteus stuartii*

Non-Listeria organisms – 7 aesculin positive isolates from Oxford agar.

Limit of Detection

Independent studies (AFNOR, preliminary study, 1994; EMMAS Assessment, 1998) have shown that *Listeria monocytogenes* and *Listeria innocua* were consistently detected at levels of <10 cells/25 grams of food from a variety of food samples, including raw milk, cheese, cured meat, smoked fish and raw vegetables.

Further studies showed *Listeria monocytogenes* was consistently detected at the lowest challenge levels of 2–7 cells/gram of food. *Listeria innocua* was detected at the lowest level of 10 cells/gram of food and *Listeria seeligeri* was detected at levels of 3 cells/gram of food. These levels meet the minimum detectable limits as defined by AOAC Research Institute. This data was verified by an independent testing laboratory.

Validation

These changes are being made to meet the requirements for Nordval. The Oxoid Listeria Rapid Test has been validated and approved by Nordval for all types of foods.

References

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2. Parry S. H., Briggs T., Blades J. A., Garni M. and Piron J. (1993) *A rapid Clearview Immunoassay for detection of Listeria*. 7th. International Congress on Rapid Methods and Automation in Microbiology and Immunology.
3. Holbrook R., Briggs T. A., Anderson J. A. and Sheard P.N. (1993) *Detection of Listeria species in foods in 43 hours using enrichment and the Listeria Clearview™ Immunoassay*. 7th. International Congress on Rapid Methods and Automation in Microbiology and Immunology.
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SALMONELLA RAPID TEST

Code: FT0201

The Oxoid Salmonella Rapid Test is for the presumptive detection of motile salmonella in all food materials, finished food products and factory environmental samples.

Principle
Pre-enrichment of a homogenised sample in a suitable medium is followed by inoculation of the culture vessel containing a salmonella elective medium with pre-enriched culture. The culture vessel is equipped with two tubes. Each tube contains a selective medium and an upper indicator medium separated by a porous partition. Salmonella migrate actively through the lower selective medium to the upper indicator media where their presence is indicated by a colour change.

Components of the Kit
FT0201 – 50 culture vessels

Each culture vessel contains 2 tubes :

Tube A (blue cap) contains Modified Rappaport- Vassiliadis medium as a selective medium, and Modified Lysine Iron Cystine Neutral Red Medium as the indicator medium.

Tube B (red cap) contains Modified Lysine Deoxycholate Medium as the selective medium and Modified Brilliant Green Medium as the indicator medium.

50 Novobiocin Discs (FT0207) each containing 1.8 mg of novobiocin.

2 syringes and needles

1 spanner (FT0202)

50 labels

1 instruction leaflet

Materials required but not supplied
Pre-enrichment Medium

Sterile Distilled Water

Salmonella Rapid Test Elective Medium (SRTEM) Code CM0857

Pipettes

Salmonella Latex Test (FT0203)

Vortex Mixer

Incubators, 35, 37 and 41ºC

Preparation of the Test Material
Test samples are pre-enriched in a suitable medium for example by homogenisation of a 1:10 dilution of the sample in Buffered Peptone Water (CM0509). Incubate the pre-enrichment culture at 35ºC or 37ºC for 18 hours.

Procedure
Preparation of the culture vessel

Use good microbiological techniques throughout. Each vessel must be prepared to stage six before preparing the next one.

1. Tap the culture vessel at an angle to loosen any medium which may have become compacted in the tubes. Unscrew the lid of the culture vessel.
2. Add sterile distilled water up to the lower line (line 1) marked on the side of the culture vessel (approximately 27 ml). Check that the base of broth tubes A and B is below the level of the liquid.
3. Expose the head of the needle but do not remove the needle from its safety sheath. Attach the head of the needle to the syringe. Remove the safety sheath and carefully push the needle through the central wall in the top of the blue cap (Tube A) ensuring the needle is visible below the cap. Smoothly withdraw the syringe plunger until the liquid level in the tube reaches the line (line 3) marked on the culture vessel. The rate of withdrawal should be such that approximately 5 seconds is taken for the operation. Remove the syringe and needle and replace the safety sheath. Take care that the tube is not lifted from its position in the container and that the tube cap is not loosened.

4. Immediately repeat step 3 for tube B that has the red cap.
5. Replace the culture vessel lid. Press the side of the culture vessel containing the 2 tubes firmly to a vortex mixer and operate the mixer. It is important that the liquids in the tubes are agitated **vigorously** for approximately 5 seconds. After mixing, leave the container to stand for approximately 5 minutes. The vessel may now be left for up to 4 hours before moving on to stage 6.
6. Carefully remove the lid of the culture vessel. Pour sterile, cooled Salmonella Rapid Test Elective Medium (SRTEM) into the culture vessel container until the liquid level reaches the upper line (line 2) on the culture vessel wall (prepare the medium as directed on the label of the SRTEM container CM0857).
7. Aseptically add 1 Novobiocin disc to the culture vessel.

WARNING: Take care that the culture vessel is maintained in an upright position throughout the remainder of the test.

8. Use the spanner provided to remove the red and blue caps from the tubes. Avoid touching the tubes themselves or the inner surface of the culture vessel by hand. Discard the red and blue caps.
9. Replace the culture vessel lid. The culture vessel is now ready for use

Use of the culture vessel

1. The incubated pre-enrichment culture should be shaken and then allowed to stand until the coarse particles have settled. Identify the sample using one of the labels provided and attach the label to the culture vessel.
2. Remove the culture vessel lid and add 1ml of the pre-enrichment culture to the vessel.
3. Replace the lid on the culture vessel.
4. Place the culture vessel in an incubator controlled to 41ºC (± 0.5ºC) and incubate for 24 hours. The culture vessel must be kept in an upright position at all times.

Reading and Interpretation

1. After 24 hours incubation, remove the culture vessel from the incubator and in good light examine the upper indicator sections of the tubes for colour changes. Examine the tubes through the container wall. Do not remove the tubes from the container!

   Blackening may occur in the lower sections of the tubes. Colour reactions should be noted in the upper indicator sections only.
2. The possible presence of salmonella is shown by changes in colour of the upper indicator medium in either one or both of the tubes.

POSITIVE:
Tube A – ANY DEGREE OF BLACK COLOURATION
Tube B – ANY DEGREE OF RED OR BLACK COLOURATION

NEGATIVE:
Tube A – ABSENCE OF BLACK COLOURATION
Tube B – ABSENCE OF RED OR BLACK COLOURATION

Tube A
Colour reactions of the indicator media in the upper compartments:

(1) Prepared unit before incubation
(2) Negative result – no blackening
(3) Positive result – some blackening
(4) Positive result – Strong blackening

Tube B
Colour reactions of the indicator media in the upper compartments:

(1) Prepared unit before incubation
(2) Negative result – little change
(3) Negative result – yellow, green top
(4) Negative result – yellow, green top
(5) Positive result – reddening only. Reddening may be limited to a very narrow band at the meniscus.
(6) Positive result – weak reddening and blackening.
(7) Positive result – red and black
(8) Positive result – red and black

NOTE: THESE COLOUR DESCRIPTIONS ARE INTENDED AS GENERAL GUIDE ONLY. IF WEAKER POSITIVE REACTIONS ARE SEEN, THE CULTURES SHOULD BE TESTED WITH THE SAMONELLA LATEX TEST.

3. Tubes which show positive reactions must be tested further with the Oxoid Salmonella Latex Test (Code FT0203). Those giving positive results with the Salmonella Latex Test may be reported as presumptively containing Salmonella. Results should then be confirmed using traditional culture and serological techniques by subculturing through a selective enrichment from the upper indicator compartment in the tube giving a positive latex test result.

Limitations

The detection method used in this test is not appropriate for the detection of non-motile strains of Salmonella (incidence <0.1%). Salmonella growth in this system may be inhibited at or above 43ºC. The system is designed to be compatible with the Oxoid Salmonella Latex Test (FT0203). It has not been validated with other serological confirmation tests.

Precautions

Good microbiological techniques must be used at all times.

Do not pipette by mouth.

Autoclave all used culture vessels before disposal.

It is recommended that at the start of each new batch of tests the syringe and needle should be decontaminated by flushing through the 70% ethyl alcohol at least twice. Expel any residual alcohol from the syringe and needle before use. When not in use store the syringe and needle in a closed container.

Storage Conditions

Store the kit at 18-25ºC in a dry place.

Once the full pouches containing the culture vessels are opened, any unused culture vessel should be kept with the silica gel pack in the resealed foil pouches.

All kit components should be used before the expiry date shown on the box.

Performance Characteristics

The Oxoid Salmonella Rapid Test was compared against traditional culture techniques in a variety of foods. The study involved over 800 samples assessed at nine independent food laboratories throughout Europe and in the United States .

The trial laboratories were asked to use the Oxoid Salmonella Rapid Test in parallel with their traditional culture technique.

Food Product	*No. Samples*		*Total Positive‡Sample*		*No. Positive Results Oxoid*		*Traditional*	
Poultry	116	(10)†	48	(8)	47	(7)	43	(8)
Raw Meats	236	(45)	87	(34)	87	(34)	76	(30)
Cooked Meats	23	(6)	6	(6)	6	(6)	6	(6)
Offal	10		6		5		3	
Vegetables	25	(1)	1	(1)	1	(1)	1	(1)
Pastry Products	35	(24)	18	(18)	18	(18)	18	(18)
Herbs and Spices	38	(15)	15	(15)	15	(15)	15	(15)
Whey	9		1		1		1	
Milk Powders	28	(10)	10	(10)	10	(10)	10	(10)
Ice Cream	19	(6)	6	(6)	6	(6)	6	(6)
Egg/Milk Mix	21		2		2		2	
Environmental Swabs	37		2		1		2	
Animal Feeds	100		8		7		5	
Others								
TOTAL	820	(117)	223	(98)	216	(97)	201	(94)

Traditional method sensitivity: 201/223 = 90.13%; Oxoid sensitivity: 216/223 = 96.8%

‡ Samples positive by traditional methods, or Oxoid with confirmation or both

† The figures in brackets represent artificially inoculated samples.

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10
DIAGNOSTIC REAGENTS

DRYSPOT *E. COLI* O157 TEST KIT

Code: DR0120

The Oxoid Dryspot Escherichia coli O157 test is a latex agglutination test for the identification of Escherichia coli serogroup O157.

Principle of the Test.
Enterohaemorrhagic *Escherichia coli* belong to a number of O serogroups with O157 being most significant in human disease.

The potentially verocytotoxin (VT) producing strains are associated with a range of symptoms from non-bloody diarrhoea, fever and vomiting to cases of haemorrhagic colitis (HC) and haemolytic uraemic syndrome (HUS).

The Dryspot *Escherichia coli* O157 latex test will demonstrate by slide agglutination

Escherichia coli strains possessing the O157 serogroup antigen. The test is best used in conjunction with Sorbitol MacConkey Agar (Oxoid CM813) The majority of *Escherichia coli* O157: H7 strains do not ferment sorbitol and therefore give colourless colonies on this medium. The majority of non O157 *Escherichia coli* isolates do ferment sorbitol and give characteristic pink colonies

Sorbitol MacConkey Agar should be used as the primary screen. Non-sorbitol fermenting colonies can then be tested with the latex reagents, to determine whether the isolate belongs to the O157 serogroup and therefore is a potential VT-producing strain.

Production of verotoxin may be confirmed with the Oxoid VTEC-RPLA kit TD0960.

Components of the kit
DR0121. Dryspot *Escherichia coli* O157 Test Reagent Cards Blue latex particles coated with antibody specifically reactive with the *Escherichia coli* O157 serogroup antigen. 4 pouches each containing 10 cards and a moisture absorbent sachet. There are 3 test and 3 control reaction areas on each card – 120 tests in total.

DR0122. Positive control strips (10 Sticks – pink spots) Pink-dyed inactivated antigenic extract of *Escherichia coli* O157.

DR0123. Negative control strips (10 sticks – green spots) Green-dyed inactivated antigenic extract of *Escherichia coli* 0116.

Instruction leaflet.

Materials required but not provided
Saline (0.9% NaCl prepared using distilled or deionised water)
Microbiological loop and bunsen burner.
A suitable laboratory disinfectant.
A timer.

Storage and opening
This kit must be stored between 2-25°C. If stored in a cold environment, allow pouches to reach room temperature before opening to prevent condensation of moisture on the cards. The dry reagents will deteriorate and give false results if they are allowed to absorb moisture.

Once opened, remove the number of cards required for immediate testing (testing within the next 10 minutes) and re-seal the pouch immediately by clamping the open end of the bag between the two halves of the plastic clip.

The kit should not be used after the expiry date printed on the carton.

DRYSPOT E. COLI SEROCHECK AND SEROSCREEN

Code: DR0300

Introduction

Enterohaemorrhagic *Escherichia coli* belong to a number of O antigen serotypes. Serotype O157 is the most significant in human disease[1, 2 ,3] and these strains are often Verocytotoxin producers. However a number of non-O157 serotypes have been shown to produce Verocytotoxin.

Infection with Verocytotoxin (VT) producing strains is associated with a range of symptoms from non-bloody diarrhoea, fever and vomiting, to cases of Haemorrhagic Colitis (HC) and Haemolytic Uraemic Syndrome (HUS). Transmission of these organisms occurs primarily through the consumption of contaminated foods. The major foods implicated in outbreaks include cheese made from unpasteurised milk, raw vegetables, undercooked ground beefburgers and fermented meats[4,5].

The Oxoid *Escherichia coli* Seroscreen and Serocheck kits can be used for the detection and identification of 6 non-O157 serotypes – O26, O91, O103, O111, O128 and O145. These are among the serotypes most commonly associated with Verocytotoxin production.

Six Serocheck kits are available, one for each non-O157 serotype named above. The Oxoid *Escherichia coli* Seroscreen kit is a single kit which detects the presence of any of the 6 serogroups named above.

Strains for testing should be grown on Enterohaemolysin[6] or MacConkey Agar and should have typical non-O157 *Escherichia coli* appearance. When insufficient material is available for testing, a non-selective purity plate should be prepared.

The tests are not intended for direct testing of faecal specimens.

Principle of the Tests

The Oxoid *Escherichia coli* Serocheck and Seroscreen Tests use antibody-sensitised blue latex particles dried onto cards. The latex will agglutinate in the presence of specific *Escherichia coli* cell wall antigens to form visible clumps. The test provides a fast and simple screening procedure for common non-O157 serogroups which may produce Verocytotoxin.

Positive and negative controls are presented as dried spots on strips of 10 tear-off, single use card sticks.

These latex agglutination tests do not indicate the presence or absence of Verocytotoxin. Production of Verocytotoxin may be confirmed with the Oxoid VTEC-RPLA TD0960 or VTEC Screen Kit TD0965.

Components of the kits

DR0300 Seroscreen
DR0310 Serocheck O26
DR0320 Serocheck O91
DR0330 Serocheck O103
DR0340 Serocheck O111
DR0350 Serocheck O128
DR0360 Serocheck O145

Each of the kits;contain the following components:

Test Cards

Blue latex particles sensitised with specific rabbit antibody reactive with the relevant serogroup of *Escherichia coli* (as indicated on test card) dried onto cards (Test Reaction Area).

Blue latex particles sensitised with non-reactive rabbit globulin (Control Reaction Area).

2 pouches each containing 10 cards and a moisture absorbent sachet. There are 3 Test and 3 Control Reaction Areas on each test card – 60 tests in total.

Positive Control Sticks (10 sticks-pink spots)
Pink-dyed inactivated antigenic extract of the relevant *Escherichia coli* (as indicated on stick).

Negative Control Strips (10 sticks-green spots).
Green–dyed inactivated antigenic extract of *Escherichia coli* O116.

Phosphate Buffered Saline (PBS)
pH 7.3 (± 0.1). Contains 0.095% sodium azide as a preservative.

Mixing Paddles
Plastic Pouch Clips for storage of opened pouch
Instruction Leaflet

Materials required but not provided
Sterile microbiological loops
Laboratory disinfectant, eg sodium hypochlorite solution >1.3% w/v

Storage and opening
The kits should be stored between 2-25°C. If stored in a cold environment, allow pouches to reach room temperature before opening to prevent condensation of moisture on the cards. The Dryspot reagents will deteriorate and may give false results if they are allowed to absorb moisture.

Once opened, remove the number of cards required for immediate testing (testing within the next 10 minutes) and re-seal the pouch immediately by clamping the open end of the bag between the two halves of the plastic clip provided.

The Control Sticks are also provided in a moisture-impermeable pouch. Ensure that the same techniques are used to avoid moisture damage.

The kit should not be used after the expiry date printed on the carton.

References
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DRYSPOT IM TEST

Code: DR0180

Intended use
The Oxoid Dryspot IM Test is a latex agglutination screening test for the detection of infectious mononucleosis (IM) heterophile antibody in serum and in plasma.

Introduction
Acute infectious mononucleosis is a self-limiting clinical syndrome that most commonly occurs in teenagers and young adults in developed nations[1]. In developing countries, IM can occur much earlier in life. This assay is intended for use as an aid in the rapid diagnosis of IM.

The IM syndrome is caused by Epstein-Barr virus (EBV) and usually presents symptoms including fatigue, pharyngitis, malaise, fever, jaundice, lymphadenopathy and splenomegaly[2,3]. Since these symptoms are not specific for IM, serological evidence is necessary to confirm all suspected IM cases[4,5].

The detection of heterophile antibodies to infectious mononucleosis by the agglutination of sheep red blood cells was first reported by Paul and Bunnell[6].Subsequent work by Davidsohn[4,7] Lee[7], and Beer[8] showed the need for differential absorption of sera to remove non-infectious mononucleosis, heterophile antibodies. Fletcher and Woolfolk[9] showed that antigens obtained from the membrane of bovine erythrocytes were more effective in combining with the IM heterophile antibodies than those antigens obtained from either sheep or horse erythrocytes.

The Dryspot IM Test is a simple two minute latex agglutination test for the detection of the specific heterophile antibody associated with IM in serum and plasma. The purified specific heterophile antigen from bovine red cell membranes is used to coat latex particles. The purity and potency of the antigen used in the Dryspot IM Test leads to improved sensitivity and specificity and eliminates the need to perform differential absorptions on test samples. When a drop of serum or plasma containing the heterophile antibody associated with IM is mixed with a drop of the latex, visible agglutination of the latex occurs within 2 minutes. Agglutination will not occur when such an antibody is absent.

Positive and Negative controls are presented as dry spots on strips of 10 tear-off, single use card sticks.

If required, the latex reagent may be used in a semi-quantitative assay for the antibody.

A final diagnosis of IM however, should only be made when clinical and haematological findings as well as the results from the Dryspot IM Test have been taken into consideration.

Components of the kit

DR0181 Dryspot IM Test Reagent Cards. Blue latex particles sensitised with purified bovine antigen.

2 pouches each containing 10 cards and a moisture absorbent sachet. There are 3 test reaction areas on each card.

60 tests in total.

DR0182 Positive control strips (10 sticks – pink spots). Pink-dyed rabbit antiserum containing specific antibody reactive with the test reagents.

DR0183 Negative control strips (10 sticks – green spots). Green-dyed rabbit serum not reactive with the test latex.

DR0699 Paddle Pastettes. These maybe used to apply a drop of serum or plasma to the test card and to mix the serum or plasma and latex together. Plastic pouch clip for storage of open pouches. Instruction leaflet

Materials required but not provided

Timer.

A suitable laboratory disinfectant

Additional items required for the optional semi-quantitative assay.

Test tube (12 x 75 mm).Pipettes (for delivery of 0.5 ml). Saline (0.9% NaCl prepared using distilled or de-ionised water).

Storage and opening

This kit must be stored between 2-25°C. If stored in a cold environment, allow the pouches to reach room temperature before use to prevent condensation of moisture on the cards. The dry reagents will deteriorate and give false results if they are allowed to absorb moisture.

Once opened, remove the number of cards required for immediate testing (testing within the next 10 minutes) and re-seal the pouch immediately by clamping the open end of the bag between the two halves of the plastic clip.

The control sticks are also provided in a moisture impermeable pouch. Ensure that the same techniques are used to avoid moisture damage.

The kit should not be used after the expiry date printed on the carton.

References

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DRYSPOT PNEUMO TEST

Code: DR0420

The Oxoid Dryspot Pneumo Test is a latex agglutination test for the detection of capsular antigen from Streptococcus pneumoniae to provide rapid identification of *Strep. pneumoniae* isolated from culture plates and blood culture.

Introduction

Strep. pneumoniae is a primary cause of bacterial pneumonia, meningitis and otitis media. The anti-phagocytic properties of the polysaccharide capsule are the key to the organism's virulence[1]. The organism may harmlessly inhabit the upper respiratory tract but may also gain access to the lungs by aspiration where it may establish an acute pneumonia. In addition, this organism also accesses the blood stream and the meninges to cause acute, purulent life-threatening infections[2].

The Oxoid Dryspot Pneumo Test uses antibody sensitised blue latex particles dried onto cards covering most of the recognised serological types of pneumococci[3,4]. The latex will agglutinate in the presence of sufficient antigen to form visible clumps. This test provides a fast and simple screening procedure for *Strep. pneumoniae.*

Components of the kit

DR0421 Dryspot Pneumo Test Reagent Cards

Blue latex particles coated with rabbit antibodies specifically reactive with the recognised serological types of pneumococci and dried onto cards (Test Reaction Area).

Blue latex particles sensitised with non-reactive globulin (Control Reaction Area).

Two pouches each containing 10 cards and a moisture absorbent sachet. There are 3 Test Reaction Areas and 3 Control Reaction Areas on each card – 60 tests in total.

DR0422 Positive Control Strips (20 sticks – pink spots)

Pink dyed inactivated antigenic extract of *Strep. pneumoniae*.

DR0423 Negative Control Strips (20 sticks – green spots)

Green dyed inactivated extract of Aerococcus viridans.

Phosphate Buffered Saline (PBS)

pH 7.3 ± 0.1. Contains 0.095% sodium azide as a preservative.

Mixing paddles.

Instruction leaflet.

2 x Clips for sealing pouches.

Materials required but not provided

Sterile microbiological loop.

Laboratory disinfectant e.g. Sodium hypochlorite solution >1.3% w/v.

Laboratory Centrifuge.

Precautions

This product is for *in vitro* diagnostic use only.

Specimen materials may contain pathogenic organisms, handle with appropriate precautions.

Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. If reagents containing sodium azide are disposed of in a sink, they should be flushed with plenty of water to prevent build up of metal azides.

Storage and opening

The kit must be stored between 2-25°C. If stored in a cold environment, allow pouches to reach room temperature before opening to prevent condensation of moisture on the cards. The Dryspot reagents will deteriorate and may give false results if they are allowed to absorb moisture.

Once opened, remove the number of cards required for immediate testing (testing within the next 10 minutes) and re-seal

the pouch immediately by clamping the open end of the bag between the two halves of the plastic clip provided.

The Control Sticks are also provided in a moisture-impermeable pouch. Ensure that the same techniques are used to avoid moisture damage.

The kit should not be used beyond the expiry date printed on the outside of the carton.

References

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DRYSPOT STAPHYTECT PLUS

Code: DR0100

Introduction

Dryspot Staphytect Plus is a latex slide agglutination test[1] for the differentiation of *Staphylococcus aureus* by detection of clumping factor, Protein A and certain polysaccharides found in methicillin resistant *Staphylococcus aureus* (MRSA) from those Staphylococci that do not possess these properties.

Principle of the test

Traditionally, differentiation between coagulase-positive and coagulase-negative staphylococci has been performed with the tube coagulase test that detects extracellular staphylocoagulase or the slide coagulase test that detects the clumping factor (bound coagulase) present on the bacterial cell surface. Several other differentiation tests are also available including the passive haemagglutination test (Oxoid Staphylase DR0595) and the DNAase test.

It has been reported that approximately 97% of human strains of *Staphylococcus aureus* possess both bound coagulase and extracelluar staphylocoagulase.

Protein A is found on the cell surface of about 95% of human strains of *Staphylococcus aureus* and has the ability to bind the Fc portion of immunoglobulin G (IgG)[2].

It has been observed that certain methicillin-resistant strains of *Staphylococcus aureus* (MRSA) may express undetectable levels of clumping factor and Protein A[3,4,5]. It has been shown however that these strains all possess capsular polysaccharide[6]. The capsule can mask both Protein A and the clumping factor thereby preventing agglutination.

Dryspot Staphytect Plus uses blue latex particles coated with both porcine fibrinogen and rabbit IgG including specific polyclonal antibodies raised against capsular polysaccharides of *Staphylococcus aureus*[7,8].

The reagent is dried onto the reaction card. When the reagent is mixed on the card with colonies of *Staphylococcus aureus* emulsified in saline rapid agglutination occurs through the reaction between (i) fibrinogen and clumping factor, (ii) Fc portion of IgG and Protein A (iii) specific IgG and capsular polysaccharide. Agglutination may also occur with other species which possess clumping factor or Protein A such as *Staphylococcus hyicus* and *Staphylococcus intermedius*. If neither clumping factor, Protein A nor specific capsular polysaccharides are present, agglutination will not occur and the result will be regarded as negative. The most frequent coagulase and Protein A negative isolates of staphylococci are *Staphylococcus epidermidis*.

Components of the kit

Dryspot Staphytect Plus Reagent cards.

Blue latex particles coated with both porcine, fibrinogen and rabbit IgG together with specific polyclonal antibodies raised against capsular polysaccharide of *Staphylococcus aureus*. (Test Reaction Area)

Blue latex particles sensitised with non-reactive globulin (Control Reaction Area)

4 pouches each containing 10 cards and a moisture absorbent sachet. There are 3 test and 3 control reaction areas on each card. 120 tests in total.

Plastic pouch clip for storage of opened pouches

Instruction Leaflet

Materials required but not provided

Saline (0.85%)
Timer
Pipette or dropper (50 µl)
Sterile Microbiological Loops
A suitable laboratory disinfectant

Positive Control: *Staphylococcus aureus* strain such as ATCC 25923
Negative Control: *Staphylococcus epidermidis* strain such as ATCC 12228

References

1. Essers L. and Radebold K. (1980). 'Rapid and Reliable Identification of *Staphylococcus aureus* by a Latex Agglutination Test'. *J. Clin. Microbiol.* 12: 641-643.
2. Taussig M. J. (1984). *Processes in Pathology and Microbiology* 2nd Ed. 520-530. Blackwell, Oxford.
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4. Roberts J. I. S. and Gaston M. A. (1987). 'Protein A and coagulase expression in epidemic and non-epidemic *Staphylococcus aureus*'. *J. Clin. Pathol*. 40: 837-840.
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OXOID DRYSPOT STREPTOCOCCAL GROUPING KIT

Code: DR0400

The Oxoid Dryspot Strep Grouping is a Streptococcal grouping kit which can be used in conjunction with nitrous acid or enzyme extraction procedures for rapid identification of β-haemolytic streptococci of Lancefield types A, B, C, D, F and G.

Introduction

β-haemolytic streptococci can be differentiated into Lancefield groups based on specific carbohydrate antigens1. Differentiation is necessary for clinical treatment and for epidemiological purposes[2]. For extraction of the group specific antigen before grouping, a variety of methods have been used including hot acid[1], hot formamide[3] and enzyme extraction methods[4,5]. The Dryspot Strep Grouping kit can be used with an enzyme extraction technique DR0593. This requires a 10 minute incubation period and efficiently extracts antigens from streptococci of Lancefield groups A, B, C, D, F and G[6]. As an alternative method the Dryspot Strep Grouping Kit may be used with nitrous acid reagents which will rapidly extract the group antigens without the need for any incubation. However, Group D antigen is not extracted efficiently by nitrous acid and an alternative method must be used to confirm suspected Group D streptococci.

The test is intended for use with streptococcus colonies that are β-haemolytic on blood agar. The group specific antigens are extracted from streptococci by either using enzyme extraction or an instant room temperature nitrous acid extraction procedure.

Components of the kit

Blue latex particles sensitised with rabbit antibody to appropriate group specific antigen (A, B,C,D, F and G).

6 Pouches each containing 6 strips of 10 Test Reagent sticks. In each pouch are sticks with tips spotted with Dryspot latex reagent which reacts with the indicated Lancefield group of Streptococci. There are 60 tests in total for each group.

DR0407 Positive Control 3 strips (10 Sticks – pink spots).

DR0500 White disposable reaction cards.

DR0593 Oxoid Extraction Enzyme Reagent

Plastic pouch clip for storage of open pouches.

Instruction leaflet.

Materials required but not provided

Microbiological loop
12 x 35 mm test tubes

Pasteur Pipettes
Suitable laboratory disinfectant
Saline (0.9% NaCl prepared using distilled or de-ionised water)
Distilled or deionised water

Storage and opening
On arrival immediately remove the Extraction Enzyme and store as indicated. The remaining reagents may be stored as indicated below.

The kit should be stored between 2-25°C. If stored in a cold environment, allow the pouches to reach room temperature before use to prevent formation of condensation on the cards. The dry reagents will deteriorate and may give false results if they are allowed to absorb moisture.

Once opened, remove the number of sticks required for immediate testing (test within the next 10 minutes) and re-seal the pouch immediately by clamping the opened end of the bag between the two halves of the plastic clip.

The control sticks are provided in a moisture impermeable pouch. Do not store the control sticks in the same pouch as the test sticks/**reagent cards** and ensure that the same techniques are used to avoid moisture damage.

The kit should not be used after the expiry date printed on the carton.

References

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6. Data on file, Oxoid.

DRYSPOT LEGIONELLA LATEX TEST

Codes: DR0200, DR0210, DR0220

Introduction
The Oxoid Dryspot Legionella Latex Test is a latex agglutination test for the identification of *Legionella* species isolated either from patients with suspected legionellosis or from environmental sources. The Oxoid Dryspot Legionella Latex Test allows separate identification of *Legionella pneumophila* serogroup[1], serogroups 2-14 and seven other *Legionella* species that have been implicated in human disease.

Principle of the test
Legionella pneumophila has been shown to be a major cause of both pneumonia and an acute self limiting febrile disease called Pontiac Fever. *Legionella pneumophila* strains and other *Legionella* species are isolated predominantly from patients with pneumonia and from the environment (mainly water).

Legionella pneumophila is the most common cause of Legionnaires' disease. At present, 14 different serotypes are known of which serogroup 1 accounts for 90% of cases. Rare isolations have also been made from sites other than the respiratory tract such as wound abscesses. The major reservoirs of *Legionella* species appear to be fresh water sites, air-conditioning units, cooling towers and water plumbing fixtures. The Oxoid Dryspot Legionella Latex Test uses antibody-sensitised blue latex particles dried onto cards. The latex will agglutinate in the presence of specific Legionella cell wall antigens to form visible clumps. The tests provide a fast and simple screening procedure for pathogenic *Legionella* species and serotypes[1,2].

Components of the kits

DR0200 Legionella pneumophila serogroup 1 Test Kit

DR0201 *Legionella pneumophila* serogroup 1 Test Cards. Blue latex particles sensitised with specific rabbit antibody reactive with *Legionella pneumophila* serogroup 1 antigen and dried onto cards (Test reaction area). Blue latex particles sensitised with non-reactive rabbit globulins (Control reaction area). Two pouches each

containing 10 cards and a moisture-absorbent sachet. There are 3 Test and 3 Control reaction areas on each test card – 60 tests in total.

DR0205 Positive Control Strips (10 Sticks – pink spots). Pink-dyed inactivated polyvalent antigenic extract of *Legionella pneumophila* 1 strains dried onto strips.

DR0240 Negative Control Strips (10 Sticks – green spots). Green-dyed inactivated antigenic extract of *L. moravica* and *L. birminghamensis* dried onto strips.

DR0230 Dryspot Legionella Buffer Mixing Paddles Plastic Pouch Clip for storage of opened pouches Instruction Leaflet.

DR0210 Legionella pneumophila serogroups 2–14 Test Kit

DR0211 *Legionella* serogroups 2–14 Test Cards. Blue latex particles sensitised with specific rabbit antibody reactive with *Legionella* serogroups 2–14 antigen and dried onto cards (Test reaction area). Blue latex particles sensitised with non-reactive rabbit globulins (Control reaction area). Two pouches each containing 10 cards and a moisture-absorbent sachet. There are 3 Test and 3 Control reaction areas on each test card – 60 tests in total.

DR0215 Positive Control Strips (10 Sticks – pink spots). Pink-dyed inactivated polyvalent antigenic extract of *Legionella* 2–14 strains dried onto strips.

DR0240 Negative Control Strips (10 Sticks – green spots). Green-dyed inactivated antigenic extract of *L. moravica* and *L. birminghamensis* dried onto strips.

DR0230 Dryspot Legionella Buffer Mixing Paddles Plastic Pouch Clip for storage of opened pouches Instruction Leaflet.

DR0220 Legionella species Test Kit

DR0221 Legionella species Test Cards. Blue latex particles sensitised with specific rabbit antibody reactive with the following species and serotypes: *L. longbeachae* 1 and 2, *L. bozemanii* 1 and 2, *L. dumoffii*, *L. gormanii*, *L. jordanis*, *L. micdadei*, *L. anisa* dried onto cards (Test reaction area). Blue latex particles sensitised with non-reactive rabbit globulins (Control reaction area). Two pouches each containing 10 cards and a moisture-absorbent sachet. There are 3 Test and 3 Control reaction areas on each test card – 60 tests in total.

DR0225 Positive Control Strips (10 Sticks – pink spots). Pink-dyed inactivated polyvalent antigenic extract of *Legionella* species dried onto strips.

DR0240 Negative Control Strips (10 Sticks – green spots). Green-dyed inactivated antigenic extract of *L. moravica* and *L. birminghamensis* dried onto strips.

DR0230 Dryspot Legionella Buffer Mixing Paddles Plastic Pouch Clip for storage of opened pouches Instruction Leaflet.

Materials required but not provided

Sterile microbiological loop.

Suitable laboratory disinfectant e.g. sodium hypochlorite solution >1.3% w/v.

Storage and opening

These kits must be stored between 2-25°C. If stored in a cold environment, allow pouches to reach room temperature before opening to prevent condensation of moisture on the cards. The Dryspot reagents will deteriorate and may give false results if they are allowed to absorb moisture. Once opened, remove the number of cards required for immediate testing (testing within the next 10 minutes) and re-seal the pouch immediately by clamping the open end of the bag between the two halves of the plastic clip provided. The Control Sticks are also provided in a moisture-impermeable pouch. Ensure that the same techniques are used to avoid moisture damage. The kits should not be used after the expiry date printed on the label.

Precautions

The Buffer DR0230 contains a mild corrosive agent. Avoid direct contact by wearing suitable protective equipment. If the material comes into contact with the skin, mucous membranes or eyes immediately wash the areas by rinsing with plenty of water.

References

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STREPTOCOCCAL GROUPING KIT

Code: DR0585

A latex agglutination test for the identification of streptococcal groups A, B, C, D, F and G.

Introduction

Lancefield showed that the majority of pathogenic streptococci possess specific carbohydrate antigens, which permit the classification of streptococci into groups. These streptococcal group antigens can be extracted from the cells and their presence demonstrated with latex particles previously coated with group-specific antibodies. These latex particles will agglutinate in the presence of homologous antigen, but will remain in smooth suspension in the absence of such antigen. The Oxoid Streptococcal Grouping Kit is such a latex agglutination test for the identification of the streptococcal group and reagents are provided for groups A, B, C, D, F and G. The use of a enzymatic extraction procedure* considerably shortens the time required for antigen extraction and much improves the antigen yield, partially for Group D streptococci.

*British Patent Application 8414273.

Components of the kit

DR0586 Latex Group Reagent A
DR0587 Latex Group Reagent B
DR0588 Latex Group Reagent C
DR0589 Latex Group Reagent D
DR0590 Latex Group Reagent F
DR0591 Latex Group Reagent G
DR0592 Polyvalent Positive Control
DR0593 Extraction Enzyme
DR0500 Disposable reaction cards

Storage

The Oxoid Streptococcal Grouping Kit should be stored at 2-8°C. Do not freeze. The kit should not be used after the expiry date printed on the outside of the carton.

Precautions

Each latex reagent contains 0.1% sodium azide.

The positive control reagent contains 0.1% sodium azide.

After reconstitution the extraction enzyme solution contains 0.01% thiomersal.

STREPTOCOCCAL GROUPING KIT ANTIGEN EXTRACTION BY NITROUS ACID

Code: DR0575

This kit is a streptococcal grouping test utilising nitrous acid extraction for the rapid identification of b haemolytic streptococci of Lancefield types A, B, C, F & G.

Introduction

β-haemolytic streptococci can be differentiated into Lancefield groups based on specific carbohydrate antigens[1]. Differentiation is necessary for clinical treatment and for epidemiological purposes[2]. For extraction of the group specific antigen prior to grouping a variety of methods have been used including hot acid[1], hot formamide[3] and enzyme extraction methods[4,5]. The Oxoid Streptococcal Grouping Kit (DR585) utilises an enzyme extraction technique. This requires a 10 minute incubation period and efficiently extracts streptococci of Lancefield Groups A, B, C, D, F and G[6]. The Oxoid Nitrous Acid Kit is based on modified nitrous reagents[6,7], which will rapidly extract the group antigens without the requirement for any incubation. However, the group D antigen is not extracted as efficiently and therefore an alternative method must be used to confirm suspected group D streptococci.

Principal of the test

The test is intended for use with streptococcal colonies that are β-haemolytic on blood agar. The group specific antigens are extracted from streptococci by using an instant room temperature nitrous acid extraction procedure. The extract is then neutralised and the antigens are identified by agglutination.

Components of the kit

DR0576 Extraction Reagent 1
One bottle containing 8 ml of sodium nitrate solution with a pH indicator. The reagent contains 0.095% sodium azide.

DR0577 Extraction Reagent 2
One bottle containing 8ml of 0.4N hydrochloric acid.

DR0578 Extraction Reagent 3
One bottle containing 8 ml of a neutralising solution with 0.095% sodium azide.

DR0586 Latex Grouping Reagent A
DR0587 Latex Grouping Reagent B
DR0588 Latex Grouping Reagent C
DR0590 Latex Grouping Reagent F
DR0591 Latex Grouping Reagent G
DR0592 Polyvalent Positive Control
DR0500 Disposable Reaction Cards
Instruction Leaflet

Material required but not provided
Microbiological loop
12 x 75 mm test tubes
Pasteur pipette
Suitable laboratory disinfectant
For full proceedure please see product insert

Precautions
Reagents contain 0.095% sodium azide as a preservative. Sodium axide is toxic and may react with lead or copper plumbing to produce metal azides which are explosive by contact detonation. To prevent azide accumulation in plumbing flush with copious amounts of water immediately after waste disposal.

Specimen materials may contain pathogenic organisms, handle with the appropriate precautions. The extraction procedure may not kill bacteria therefore the extract must be handled with the same precautions.

Extraction Reagents 1, 2 contain a mild irritant and a weak acid respectively. Avoid direct contact by wearing suitable protective equipment. If the material comes into contact with the skin, mucous membranes or eyes immediately wash the area by rinsing with plenty of water.

Storage
This kit should be stored 2-8°C. Do not freeze. The kit should not be used after the expiry date printed on the outside of the carton.

References

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DRYSPOT CAMPYLOBACTER TEST KIT

Code: DR0150

The Oxoid Dryspot Campylobacter test is a latex agglutination test for the identification of enteropathogenic Campylobacter spp. from solid culture media. *Campylobacter jejuni*, *C. lari*, *C. col*i, *C. fetus* and *C. upsaliensis* will give positive results. Isolates of other *Campylobacter* spp. such as *C. fetus* subsp. *fetus* will give variable results.

Introduction

Campylobacters are helical, or curved, Gram-negative, oxidase positive rod-shaped bacteria.[1]

They have been isolated from the environment as well as from humans and animals. The adoption of *Campylobacter* culture in laboratory routines for investigating enteritis has shown *Campylobacter* spp. to be the leading cause of diarrhoeal disease. Infection has been associated with the consumption of contaminated water and foods, particularly poultry and unpasteurised milk.

Current methods for the isolation and culture of *Campylobacter* have been reviewed.[2]

The Dryspot Campylobacter test reagent consists of blue latex particles sensitised with rabbit antibody reactive with selected Campylobacter cell surface antigens. The control reagent consists of blu latex particles sensitised with rabbit antibody not reactive with Campylobacters.

The latex reagents are dried onto reaction cards. When a Campylobacter extract is mixed with the test reagent, agglutination occurs due to cross-linking of latex-bound antibody and Campylobacter antigens. If the extract does not contain recognised Campylobacter antigens, agglutination will not occur and the result is negative.

The Oxoid Dryspot Campylobacter Test includes antigen extraction reagents and a positive control antigen preparation.

Components of the Kit (DR0150)

DR0151 Dryspot Campylobacter Reagent Cards

Test areas: Blue latex particles sensitised with rabbit antibody reactive with selected Campylobacter cell surface antigens.

Control areas: Blue latex particles sensitised with rabbit antibody not reactive with selected Campylobacters.

10 foil-sealed plasic trays each containing five reaction cards and a desiccant pouch. Each card has a test and a control area. 50 tests in total.

DR0152 Extraction Reagent[1]

A solution of acetic acid (1.2M)

DR0153 Extraction Reagent[2]

A neutralising reagent of Tris buffer containing 0.09% sodium azide as a preservative.

DR0154 Campylobacter Dryspot Positive Control Reagent

Consists of a neutralised acid extract of appropriate Campylobacter organisms in buffer containing 0.09% sodium azide as a preservative.

DR0699 Paddle Pastettes

DR0155 Storage bag

Materials required required but not provided

Timer
Sterile loop (5 ml calibrated)
12 x 75 mm test tubes
A suitable laboratory disinfectant
For full proceedure please see product insert.

Storage

The Oxoid Dryspot Campylobacter Test Kit should be stored at 2-8°C. Do not freeze. The kit should not be used after the expiry date printed on the outside of the carton.

Precautions

Extraction Reagent 1 contains acid. Avoid direct contact by wearing suitable protective equipment. If the material comes into contact with the skin, mucous membranes or eyes immediately wash the area by rinsing with plenty of water.

Some reagents contain sodium azide which may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent build-up.

References

1. Cowan, S. T. and Steel, K. J. (1965) Charecters of Gram-negative bacteria. In *Manual for Identification of Medical Bacteria*. Barrow, G. I. and Felton, R. K. A. (ed.) Third Edition. Cambridge University Press. Cambridge, U.K.

2. Corry, J. E. L., Post, D. E., Colin, P. *et al.* (1995) Culture media for the isolation of campylobacters. *Int. J. Food Microbiol.* 26. 43-76.

C. DIFFICILE TEST KIT

Code: DR1107

Introduction

Clostridium difficile is recognised as a major cause of infectious diarrhoea in hospital patients. Infection with the organism is commonly responsible for pseudomembranous colitis and antibiotic associated diarrhoea, as well as post-operative diarrhoea[1]. The isolation and identification of *Clostridium difficile* is important in establishing the aetiology of these pathologies.

Methods for the selective isolation of *Clostridium difficile* have been described[2] and the rate of isolation is increased by culturing on selective media[7]. However, subsequent identification of the organism has required the use of gas liquid chromatography since conventional biochemical tests are often of little value. Although detection of *Clostridium difficile* cytotoxin is widely used in diagnosis, the association between the presence of cytotoxin and the symptoms of infection is inconsistent[3,4].

The Oxoid *Clostridium difficile* Test Kit is a rapid and simple latex agglutination test for the early identification of *Clostridium difficile* and is ideally suited to screening selective and enrichment broth cultures. The test can also be used for the identification of *Clostridium difficile* colonies from selective solid media[2,6]. The recommended procedure for the identification of *Clostridium difficile* using this kit is compared with a typical procedure below:

A comparison of methods for Isolation and identification of *Clostridium difficile*

Time	Oxoid Suggested Procedure	Conventional Laboratory Procedure
Faeces Faeces		
24 hours	Selective enrichment broth (e.g. GCC) 37°C	Alcohol shock Procedure Enrichment broth 37°C, 18-24 hours
Test with Oxoid *Clostridium difficile* latex (2 mins)		Report
Negative Presumptive Report Positive		Report
48 hours		
Subculture onto selective Medium (*Clostridium difficile* agar base + selective supplements - SR0096 or SR0173) 37°C Anaerobically, 24-48 hours		
Subculture onto selective medium (CCFA)		
Test with Oxoid *Clostridium difficile* latex (2 mins)		Incubate at 37°C Anaerobically 48-72 hours
Negative Report		Confirmed Positive Report
72 hours		Examine morphologically for *Clostridium difficile*
Negative		Positive Report
Gram Stain	Long Wave UV light	G.L.C. (Approx 30 minutes) Set up biochemical Identification (24-48 Hours)
Read Results		

The advantages of using the Oxoid C. difficile Test Kit are:

1. Earlier identification of *Clostridium difficile* in primary selective cultures allowing presumptive positive identification overnight, and confirmed positive results within two days. Identification by anaerobic culture and confirmatory tests requires 3-6 days.
2. The high predictive value of a negative result allows early elimination of negative selective cultures.
3. Reduction in the required number of subcultures.
4. No requirement for extended anaerobic subcultures of 48-72 hours.
5. No requirement for expensive equipment.

Principle of the test

Latex particles are coated with IgG antibodes specific for *Clostridium difficile* cell wall antigens. When mixed on a reaction card with selective or enrichment broth containing the organism, or with a suspension of *Clostridium difficile* colonies from solid media, the latex particles agglutinate in large visible clumps within 2 minutes.

Components of the kit

Clostridium difficile Reagent: Latex particles coated with rabbit IgG against *Clostridium difficile*; preserved with 0.02% merthiolate (2.5 ml)

Positive Control: Inactivated *Clostridium difficile* (0.5 ml)

0.85% isotonic saline, preserved with 0. 1% sodium azide (5 ml)

Disposable reaction cards

Disposable mixing sticks

Materials required but not provided

Pasteur pipettes

Storage

The Oxoid *Clostridium difficile* Test Kit should be stored at 2- 8°C. Do not freeze. The kit should not be used after the expiry date printed on the outside of the carton.

Precautions

The Positive Control contains inactivated *Clostridium difficile* but should be treated as a potentially infectious agent.

The isotonic saline is preserved with sodium azide which can form potentially explosive compounds on contact with copper and lead plumbing. On disposal of reagent, flush with copious quantities of water to prevent azide build up.

References

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E. COLI O157 LATEX TEST KIT

Code: DR0620

A latex agglutination test for the identification of Escherichia coli serogroup O157.

Introduction

Certain strains of *Escherichia coli* have been implicated in cases of haemorrhagic colitis (HC) and haemolytic ureamic syndrome (HUS).

It has been shown that these strains produce a vero-cytotoxin (VT). The *Escherichia coli* serotype most frequently isolated from HC and HUS case is O157:H7. Isolation of this serotype from a diarrhoeal stool particularly with the presence of blood is indicative of a vero-cytotoxin producing strain.

The Oxoid *Escherichia coli* O157 Latex Test will demonstrate by slide agglutination *Escherichia coli* strains possessing the O157 serogroup antigen. The test is best used in conjunction with Sorbitol MacConkey Agar CM813. *Escherichia coli* O157:H7 strains do not ferment Sorbitol and therefore give colourless colonies on this medium. The majority of *Escherichia coli* isolates do ferment sorbitol and give characteristic pink colonies.

Sorbitol MacConkey Agar CM0813 should be used as the primary screen. Non-Sorbitol fermenting colonies can then be tested with the latex reagents, to determine whether the isolate belongs to the O157 serogroup and therefore a potential VT-producing strain.

Components of the kit

DR0621 Test Latex
Consists of blue latex particles sensitised with specific rabbit antibody reactive with the O157 somatic antigen. Each kit contains sufficient reagent for 100 tests.

DR0622 Control Latex
Consists of blue latex particles sensitised with pre-immune rabbit globulin. Each kit contains sufficient reagent for 100 tests.

DR0623 Positive Control Suspension
A suspension of inactivated *Escherichia coli* O157 cells in buffer. Sufficient for 25 tests.

DR0624 Negative Control Suspension
A suspension of *Escherichia coli* O116 cells in buffer. Sufficient for 25 tests.

DR0500 Reaction Cards
There are 35 disposable reaction cards provided in the kit.
Instruction leaflet.

Materials required but not provided
Microbiological loop and bunsen burner.
0.85% saline.
Suitable disinfectant e.g. sodium hypochlorite solution >1.3% W/W.

Storage
This kit should be stored at 2-8°C. Do not freeze. Do not use after the expiry date printed on the outside of the carton.

Precautions
Reagents contain 0.1% sodium azide as a preservative.

Sodium azide may react with lead or copper plumbing to produce metal azides which are explosive by contact detonation. To prevent azide accumulation in plumbing, flush with copious amounts of water immediately after waste disposal.

INFECTIOUS MONONUCLEOSIS KIT

Code: DR0680

A latex agglutination test for the detection of infectious mononucleosis heterophile antibody in serum and plasma.

Introduction
Infectious mononucleosis (glandular fever) is an acute infectious disease caused by the Epstein-Barr virus and primarily affects lymphoid tissue. It is characterised by the appearance of enlarged and often tender lymph nodes, enlarged spleen, and abnormal lymphocytes in blood. Patients usually, but not always, develop a transient heterophile antibody response.

The detection of heterophile antibodies to infectious mononucleosis by the agglutination of sheep red blood cells was first reported by Paul and Bunnell[1]. Subsequent work by Davidsohn[2,3], Lee[3] and Beer[4] showed the need for differential absorption of sera to remove non-infectious mononucleosis heterophile antibodies. Fletcher and Woolfolk[5] showed that antigens obtained from the membranes of bovine erythrocytes were more effective in combining with the infectious mononucleosis heterophile antibodies than those antigens obtained from either sheep or horse erythrocytes[6,7].

The Oxoid Infectious Mononucleosis Kit is a simple two minute latex agglutination test for the detection of the specific heterophile antibody associated with infectious mononucleosis in serum and plasma. The purified specific heterophile antigen from bovine red cell membranes is used to coat uniform latex particles. The purity and potency of the antigen used in the Oxoid Infectious Mononucleosis Kit leads to improved sensitivity and specificity, and eliminates the need to perform differential absorptions on test samples.

When a drop of serum or plasma containing the heterophile antibody associated with infectious mononucleosis is mixed with a drop of the latex, visible agglutination of the latex occurs within two minutes. When no such antibody is present, agglutination will not occur.

If required, the latex reagent may be used in a semi-quantitative assay for the antibody.

An accurate diagnosis of infectious mononucleosis however, should only be made when clinical and haematological findings as well as the results from the Oxoid Infectious Mononucleosis Test have been taken into consideration.

Components of the kit

DR0681M Test Latex: Consists of latex particles sensitised with purified bovine antigen. Each kit contains sufficient reagent for 50 tests.

DR0682M Positive Control Serum: Consists of rabbit antiserum containing specific antibody reactive with the test reagent. Sufficient for 15 tests.

DR0683M Negative Control Serum: Consists of rabbit antiserum tested for the absence of heterophile antibodies to infectious mononucleosis.

DR0500G Disposable Reaction Cards: There are 15 reaction cards provided in the pack. Each card may be used for testing six sera. If fewer sera are to be tested, the card may be cut prior to use with scissors and the unused rings saved for use later.

DR0699M Paddle Pastettes: A minimum of 55 paddle pastettes are provided. These may be used to apply a drop of the serum to the test card and to mix the serum and latex together.

Instruction Leaflet.

Materials required but not provided

Timer
Sodium hypochlorite solution (> 1.3% w/w).

Additional items required for the optional semi-quantitative assay

Test tube (12 x 75 mm)
Pipettes (for delivery of 0.5 ml)
0.85% Nacl (w/v) solution

Storage

The Oxoid Infectious Mononucleosis Test Kit should be stored at 2-8°C. Do not freeze. The kit should not be used after the expiry date printed on the outside of the carton.

Precautions

The reagents contain sodium azide as a preservative. Sodium azide may react with lead or copper plumbing to produce metal azides which are explosive by contact detonation. To prevent azide accumulation in plumbing, flush with copious amounts of water immediately after waste disposal.

The appropriate precautions should be taken when handling human serum or plasma. Pipettes, test cards, etc. should be disposed of into hypochlorite disinfectant (> 1.3% w/w).

References

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2. Davidsohn I. Serologic diagnosis of infectious mononucleosis. *JAMA* 1937: 108. 289-295.
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LEGIONELLA LATEX TEST

Code: DR0800

The Oxoid Legionella Latex Test is a latex agglutination test for the identification of predominantLegionella species grown on plate media from patients with suspected Legionellosis or from environmental sources. The Oxoid Legionella Latex Test allows separate identification of Legionella pneumophila serogroup 1 and serogroups 2-14 and detection of seven other Legionella species which have been implicated in human disease.

Introduction

Legionnaires' disease named after the outbreak in 1976 at the American Legion Convention in Philadelphia, is caused by *Legionella pneumophila* and other *Legionella* species. It is characterised as an acute febrile respiratory illness ranging in severity from mild illness to fatal pneumonia. Since that time, it has been recognised that the disease occurs in both epidemic and endemic form and that the sporadic cases are not readily differentiated from other respiratory infections by clinical symptons. It is estimated that worldwide about 25,000 cases of Legionella infections occur annually. Known risk factors include immunosuppression, cigarette smoking, alcohol consumption and concomitant pulmonary disease. The mortality rate, which can be as high as 25% in untreated immuno suppressed patients, can be lowered if the disease is diagnosed rapidly and appropriate antimicrobial therapy started earlier.

Legionella pneumophila has been shown to be a major cause of both pneumonia and an acute self limiting febrile disease called Pontiac Fever. *Legionella pneumophila* strains and other *Legionella* species are isolated from patients with pneumonia and from the environment (mainly water).

Rare isolations have also been made in cases other than pneumonia, such as wound abscesses. The major reservoir of *Legionella* species appears to be fresh water sites, air-conditioning units and various water plumbing fixtures.

Legionella pneumophila is the most common cause of Legionnaires' disease. At present, 14 different serotypes exist of which *Legionella pneumophila* serogroup 1 accounts for 90% of cases.

The Oxoid Legionella Latex Test uses antibody sensitised blue latex particles which will agglutinate in the presence of specific Legionella cell wall antigens to form visible clumps. This provides a fast and simple screening procedure for predominant pathogenic Legionella species and serotypes[1,2].

Components of the kit

DR0801 *Legionella pneumophila serogroup* 1 Test Reagent
Consists of blue latex particles sensitised with specific rabbit antibody reactive with Legionella pneumophila serogroup 1 antigen. Each kit contains sufficient reagent for 50 tests.

DR0802 *Legionella pneumophila* serogroup 2-14 Test Reagent
Consists of blue latex particles sensitised with specific rabbit antibody reactive with *Legionella pneumophila* serogroup 2-14 antigen. Each kit contains sufficient reagent for 50 tests.

DR0803 Legionella species Test Reagent
Consists of blue latex particles sensitised with specific rabbit antibody reactive with the following species and serotypes: *L. longbeachae* 1 & 2 *L. bozemanii* 1 & 2 *L. dumoffii*, *L. gormanii*, *L. jordanis*, *L. micdadei*, *L. anisa.*

Each kit contains sufficient reagent for 50 tests.

DR0804 Positive Control Suspension
A polyvalent suspension of Legionella cells in buffer, sufficient for 25 tests.

DR0805 Negative Control Suspension
A suspension of *L. spiritensis* cells in buffer non-reactive with the test reagents sufficient for 25 tests.

DR0806 Control Latex
Consists of blue latex particles sensitised with non-reactive rabbit globulin. Each kit contains sufficient reagent for 50 tests.

DR0807 Suspension Buffer
A phosphate buffered saline solution. pH 7.3.

DR0500 Reaction Cards
There are 50 disposable reaction cards provided in the kit.

Instruction leaflet

Materials required but not provided
Microbiological loop and bunsen burner.
0.85% saline (for optional tube method).
Suitable laboratory disinfectant e.g. Sodium hypochlorite solution >1.3% w/v.

References
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OXOID LISTERIA TEST KIT

Code: DR1126

The Oxoid Listeria Test Kit is a rapid latex agglutination test for the presumptive identification of Listeria spp. in selective and/or enrichment cultures. The Oxoid Listeria Test Kit will confirm the presence of Listeria spp. in culture and should be used in conjunction with biochemical analysis (e.g. MicobactTM 12L Kit-MB1128A) for full identification of Listeria spp.

Introduction
L. monocytogenes is the principal causative agent of human listeriosis, which is clinically characterized by Central Nervous System (CNS) involvement in non pregnant individuals and may be associated with infection of the foetus in pregnant women, leading to abortion, stillbirth and premature delivery[1]. Contaminated food including dairy and non dairy products are the primary sources of *L. monocytogenes* transmission in both sporadic and outbreak cases[2,3,4]. Because of the importance of *L. monocytogenes* in food borne infections and the widespread distribution of *Listeria* species in the environment, including the intestinal carriage of the organisms by many animal species[5], the rapid identification of *L. monocytogenes* is vital in both the food industry and diagnostic medicine. However, currently the presumptive identification of *L. monocytogenes* relies on the performance of biochemical tests which require prolonged incubation and are time consuming – up to 7 days[6]. The Oxoid Listeria Test Kit can provide presumptive identification of *Listeria* spp. from culture 24 to 48 hours earlier than conventional techniques.

Principle of the test
Polyvalent antisera are prepared against purified flagellin proteins from *L. monocytogenes* (antigens A, B and C) and *L. grayi* (antigen E) and are used to coat latex particles. When mixed with a suspension containing Listeria spp. the latex particles rapidly agglutinate to form visible clumps. The Oxoid Listeria Test Kit detects all motile strains of *Listeria* spp.

Components of the kit
Listeria Latex Reagent. Latex particles coated with rabbit antiserum against Listeria flagellin antigens, preserved with 0.02% Merthiolate. 1 x 5 ml
Listeria positive control antigen 0.5 ml
0.85% isotonic saline, preserved with 0. 09% sodium azide 5 ml
Disposable reaction cards
Disposable mixing sticks

Material required but not provided
Pasteur pipettes

Forfull proceedure please see product insert.

Storage
The Oxoid Listeria Test Kit should be stored at 2-8°C. Do not freeze. The kit should not be used after the expiry date printed on the outside of the carton.

References
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6. McLaughlin J. The identification of Listeria species. *Public Health Laboratory Service. DMRQC Newsletter* (1988) 3, 13.

OXOID PYLORI TEST

Code: DR0130

Oxoid Pylori Test is a rapid latex agglutination test for the qualitative detection of Helicobacter pylori total antibodies in serum as an aid in the diagnosis of infection by Helicobacter pylori. The product is intended for use to test patients with symptoms of gastro-intestinal disorders.

Introduction

In 1983, Marshall and Warren cultured a new pathogen from patients with gastritis[1]. These findings stimulated research into the relationship between *Helicobacter pylori* and gastric disease. Studies have established that *Helicobacter pylori* (formerly *Campylobacter pylori*) can cause chronic gastritis[2], and an increasing amount of evidence indicates that there is an association between *Helicobacter pylori* infection and peptic ulcers[3,4,5]. Recently *Helicobacter pylori* has also been identified as a risk factor for gastric cancer[6,7]. Nearly 100% of patients with duodenal ulcers[8,9], 70% of those with gastric ulcer[2,10,11] and more than 80% of patients with gastric cancer[6,12] have *Helicobacter pylori* infection.

Once infection caused by *Helicobacter pylori* has been diagnosed, the patient can be treated with antimicrobial drugs. Successful eradication of *Helicobacter pylori* leads to disappearance of gastric inflammation[13]. Among duodenal ulcer patients, eradication of *Helicobacter pylori* has been followed by healing of the ulcer and by reduced rate of ulcer relapse[14,15]. Several techniques, both invasive and non-invasive, are now available for diagnosing *Helicobacter pylori* infection. The invasive methods include culture, histological examination and urease testing of biopsy specimens. They require the collection of multiple pinch biopsy samples taken during upper gastro-intestinal endoscopy. Although commonly used, the invasive methods are rather tedious and time consuming, and require a sampling procedure that may cause patient discomfort. In addition, *Helicobacter pylori* is a delicate organism, and is therefore easily destroyed if transported.

The non-invasive methods available include the urea breath test requiring patient ingestion of carbon isotope derivatives of urea[16,17,18] and serological detection of serum antibodies to *Helicobacter pylori*[19,20.21]. *Helicobacter pylori* elicits a specific serological response in the infected person and detection of antibodies to *Helicobacter pylori* in the patient's serum is a reliable indicator of *Helicobacter pylori* infection[22] . Serology has also proven to be a useful tool for monitoring efficacy of antimicrobial treatment[22] .

Principle of the test

Oxoid Pylori Test Latex Reagent containing latex particles sensitised with partially purified *Helicobacter pylori* antigens, is dried on the test card as dry spots. *Helicobacter pylori* antibodies present in serum specimens will react with the sensitised latex particles, resulting in visually detectable agglutination. Sera containing antibodies reactive to *Helicobacter pylori*, and sera free of antibodies to *Helicobacter pylori*, are included in the kit as positive and negative controls respectively.

Components of the kit

Test Cards DR0131

Four card packages, each containing two test cards with three reaction circles. Latex reagent sensitised with partially purified *Helicobacter pylori* antigens, and containing <1% sodium azide, is dried on the reaction circles.

Positive Control DR0133

One vial (0.5 ml) of diluted rabbit serum having antibodies reactive to *Helicobacter pylori*, and containing <0.1% sodium azide as a preservative. This reagent is supplied ready for use. Allow the reagent to warm to 18–25°C prior to conducting an assay.

Negative Control DR0134

One vial (0.5 ml) of diluted newborn calf serum, non-reactive to *Helicobacter pylori*, and containing <0.1% sodium azide as a preservative. This reagent is supplied ready for use. Allow the reagent to warm to 18–25°C prior to conducting an assay.

Dilution Buffer DR0132

One bottle (30 ml) of phosphate buffered saline, pH 7.2 ± 0.1 containing <0.1% sodium azide as a preservative. The buffer is supplied ready for use, but should be allowed to warm to 18–25°C prior to use.

Mixing sticks

Thirty double ended mixing sticks for mixing latex and diluted serum.

Plastic storage pouch
One plastic pouch for storage of the opened test card package containing unused test cards.

Materials required but not provided

Micropipette
Micropipettes capable of delivering volumes of 40 µl, 50 µl and 150 µl (see Procedure, Dilution Method A), or 10 µl and 30 µl (see Procedure, Dilution Method B) are needed.

Test Tubes
Plastic or glass tubes for diluting patient serum samples prior to testing, if using Dilution Method A.

Precautions
The dried latex reagent on the cards contain as preservative <1% sodium azide which is harmful when inhaled, swallowed, on in contact with skin. The dissolved latex reagent contains <0.1% sodium azide which is not considered to be a harmful concentration. The other reagents in the kit contain <0.1% sodium azide, as preservative. The copper and lead used in some plumbing systems can react with azides to form explosive salts. The quantities of azide used in this kit are small; nevertheless when disposing of azide-containing materials, they should be flushed away with a large volume of water.

Although the reagents do not contain *Helicobacter pylori* infectious agents, they are prepared from biological materials and should be handled and discarded as a potential biohazard.

References

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OXOID PENICILLIN BINDING PROTEIN (PBP2′) LATEX AGGLUTINATION TEST

Code DR0900

This test is a rapid latex agglutination assay, detecting PBP2′ (also called PBP2a)[7], in isolates of *Staphylococcus*, as an aid in identifying methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant coagulase-negative staphylococci.

Introduction
Staphylococci are a leading cause of nosocomial and community-acquired infections worldwide[2]. In many institutions, approximately 25% to 50% of *Staphylococcus aureus* strains and 75% of coagulase-negative staphylococci (CoNS) are resistant to methicillin[12]. MRSA are of particular concern because of the ease with which certain epidemic strains spread and colonise debilitated patients. Treatment of sensitive strains with penicillinase-resistant penicillins (PRP), is preferred as beta-lactam drugs are more easily absorbed into body fluids and tissues, cause fewer complications from treatment, and do not select for vancomycin-resistant organisms. Reliable identification of methicillin-resistance is therefore important.

Strains of *Staphylococcus aureus* with reduced susceptibility to PRP are categorized as follows:

(i) Methicillin-resistant *Staphylococcus aureus* (MRSA), which produce the low-affinity penicillin binding protein PBP2′, encoded by the mecA gene[3,6]

(ii) Borderline methicillin-resistant *Staphylococcus aureus* (BORSA), generally considered to be due to hyperproduction of type A – beta lactamase[10]

(iii) Strains with modified PBPs due to altered penicillin binding capacity or hyperproduction of PBPs (MODSA)[1,2]. MODSA have only rarely been isolated and their clinical response to beta-lactam therapy has not been well studied. Thus for clinical purposes, with rare exceptions, the presence of PBP2′ is responsible for methicillin-resistance in the treatment of infections with *Staphylococcus aureus* and CoNS[2,6].

The methicillin-resistant phenotype can be highly heterogeneous, making it difficult to detect by conventional antimicrobial susceptibility test methods, such as Minimum Inhibitory Concentration (MIC), disc and agar screen. The accuracy of these methods is affected by inoculum size, incubation time and temperature, medium, pH, salt concentration and other factors[8,9]. In addition, these culture methods require 24 h incubation for accurate results. CoNS often produce lower amounts of PBP2′ and require induction by exposure to one of the PRPs to produce sufficient product to be detected[2,3,12].

Detection of the mecA gene has been considered the gold standard in the determination of methicillin-resistance because of its accuracy, but this method is labour-intensive and expensive to perform[1,6]. The Oxoid PBP2′ Latex test has the advantage of direct detection of the PBP2′ protein performed in a rapid timeframe with minimal labour. It has the potential for being even more accurate than the detection of the mecA gene, as false-positive results will not occur with strains that possess mecA but are unable to produce the protein product of the gene. In addition, the assay does not detect strains that are hyperproducers of either beta-lactamase or PBPs.

The Oxoid PBP2′ test has previously been evaluated world-wide, demonstrating its high sensitivity and specificity[4,5,11].

Latex particles sensitized with a monoclonal antibody against PBP2′ will specifically react with methicillin-resistant staphylococci to cause agglutination visible to the unaided eye.

Components of the Kit

DR0901
Test Latex sensitised with a monoclonal antibody against PBP2′

DR0902
Control Latex sensitised with a monoclonal antibody of the same IgG subclass showing no reactivity with proteins of *Staphylococcus aureus*.

DR0903
Extraction Reagent 1

DR0904
Extraction Reagent 2
Test Cards
Mixing Sticks
Instruction Leaflet

Materials required but not provided
Micropipette and tips (50 ml)
Microbiological loops (5 ml/1 ml)
Boiling water bath or heating block
Centrifuge (1500 x g)
Microcentifuge tubes (safe lock)
Suitable laboratory disinfectant

Storage
Store the kit at 2-8° C. Do not freeze.the kit should not be used after the expiry date printed on the outside of the carton.

Precautions
The extraction procedure may not kill bacteria; therefore the extract must be handled with the same precautions.

Extraction Reagents 1 and 2 contain a mild irritant and a weak acid. Avoid direct contact by wearing suitable protective equipment. If the material comes into contact with the skin, mucous membranes or eyes immediately wash the area by rinsing with plenty of water.

Control procedures
For each new lot of the kit and weekly thereafter, the following control procedures must be performed.

Positive Control – Use a known MRSA strain such as ATCC® 43300 Oxoid Culti-Loops® C9022L. Follow the method given in the test procedure. Ensure that agglutination occurs within 3 minutes.

Negative Control – Use a known Methicillin-Sensitive Staphylococcus aureus (MSSA) strain such as ATCC® 25923 or ATCC® 29213, Oxoid Culti-Loops® C7010L or C7011L. Follow the method given in the test procedure. Ensure that no agglutination occurs within 3 minutes. Do not use the test if reactions with the control organisms are incorrect.

Do not use kits beyond their expiry date.

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OXOID SALMONELLA TEST KIT

Code: DR1108

Introduction
The genus *Salmonella* consists of over 2400 serotypes which may cause acute infectious gastroenteritis frequently associated with food poisoning. Since many *Salmonella* species infect domestic animals, either clinically or subclinically, cases of Salmonella food poisoning usually originate from animal sources. In contrast, *S. typhi* and *S. paratyphi* only infect humans and cause enteric fever. *Salmonella* are often present in a sample in small numbers, together with other Enterobacteriaceae. In order to promote the growth of *Salmonella*, enrichment and/or selective media are used, such as Rappaport Vassiliadis Medium broths for inducing motility and media containing selenite, deoxycholate or bismuth sulphite[1,2].

The Oxoid Salmonella Test Kit is a rapid latex agglutination test for the presumptive identification ofSalmonella in selective and/or enrichment cultures. The use of latex technology makes this test more sensitive than direct agglutination methods, so that use of the Oxoid Salmonella Test Kit permits identification of Salmonella at least 24 hours earlier than when using conventional techniques. The Oxoid Salmonella Test Kit is particularly suited to screening cultures due to the high predictive value of a negative result. When screening clinical specimens, these features enable decisions on appropriate patient management to be taken sooner. The kit is also ideal for screening samples in the food industry where prompt results minimise the delay associated with routine testing for *Salmonella* contamination.

Principle of the test
Polyvalent antisera are prepared against a wide range of Salmonella flagellar antigens and used to coat latex particles. When mixed with a suspension of *Salmonella* containing these antigens, the latex particles rapidly agglutinate to form visible clumps. The Oxoid Salmonella Test Kit detects the majority of common *Salmonella* species including *S. typhimurium* and *S. enteritidis*. In order to minimise cross reactions with other Enterobacteriaceae, antibodies to the principal somatic antigens are removed during preparation of the antisera. Consequently, this test reacts predominantly with flagellar antigens although the reagent will react with the non-motile species *S. pullorumand*, *S. gallinarum*.

Components of the kit
The reagents supplied are for *in vitro* diagnostic use only.
Salmonella Latex Reagent: Latex particles coated with rabbit antiserum against Salmonella antigens, preserved with 0.02% merthiolate. 2.5 ml
0.85% isotonic saline, preserved with 0.1% sodium azide. 5ml
Disposable reaction cards
Disposable mixing sticks
Materials required but not provided
Pasteur pipettes

References

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STAPHYTECT PLUS

Code: DR0850

Staphytect Plus is a latex slide agglutination test[1] for the differentiation of Staphylococcus aureus by detection of clumping factor, Protein A and certain polysaccharides found in methicillin resistant Staphylococcus aureus (MRSA) from those Staphylococci that do not possess these properties.

Introduction
Traditionally, differentiation between coagulase-positive and coagulase-negative staphylococci has been performed either with the tube coagulase test that detects extracellular staphylocoagulase or the slide coagulase test that detects the clumping factor (bound coagulase) present on the bacterial cell surface. Several other differentiation tests are also available including the passive haemagglutination test (Oxoid Staphylase DR0595) and the DNase test.

It has been reported that approximately 97% of human strains of *Staphylococcus aureus* possess both bound coagulase and extracelluar staphylocoagulase.

Protein A is found on the cell surface of about 95% of human strains of *Staphylococcus aureus* and has the ability to bind the Fc portion of immunoglobulin G (IgG)[2].

It has been observed that certain methicillin-resistant strains of *Staphylococcus aureus* (MRSA) may express undetectable levels of clumping factor and Protein A[3,4,5]. It has been shown however that these strains all possess capsular polysaccharide[6]. The capsule can mask both Protein A and the clumping factor thereby preventing agglutination.

Staphytect Plus uses blue latex particles coated with porcine fibrinogen and rabbit IgG including specific polyclonal antibodies raised against capsular polysaccharides of *Staphylococcus aureus*[7,8].

When the reagent is mixed on a card with colonies of *Staphylococcus aureus*, rapid agglutination occurs through the reaction between (i) fibrinogen and clumping factor, (ii) Fc portion of IgG and Protein A (iii) specific IgG and capsular polysaccharide. Agglutination may also occur with other species which possess clumping factor or Protein A such as *Staphylococcus hyicus* and *Staphylococcus intermedius*. If neither clumping factor, Protein A or specific capsular polysaccharides are present, agglutination will not occur and the result will be regarded as negative. The most frequent coagulase and Protein A negative isolates of staphylococci are *Staphylococcus epidermidis*.

Components of the Kit

DR0851 Staphytect Plus Test Reagent (5.6 ml)
Blue latex particles coated with both porcine, fibrinogen and rabbit IgG together with specific polyclonal antibodies raised against capsular polysaccharide of *Staphylococcus aureus*. Each bottle contains sufficient reagent for 100 tests.

DR0852 Staphytect Plus Control Reagent (5.6 ml)
Blue unsensitised latex particles. Each bottle contains sufficient reagents for 100 tests.

DR0500 Reaction Cards
There are 35 disposable reaction cards provided in the kit
Instruction Leaflet

Materials required but not provided
Timer
Microbiological Loop
A suitable laboratory disinfectant
Positive Control: *Staphylococcus aureus* strain such as ATCC®25923.
Negative Control: *S. epidermidis* strain such as ATCC®12228.

Storage
This kit must be stored at 2-8°C away from direct sunlight or heat sources. Do not Freeze.

The kit should not be used after the expiry date printed on the outside of the carton.

Precautions
Reagents contain 0.095% sodium azide as a preservative. Sodium azide is toxic and may react with lead or copper plumbing to produce metal azides which are explosive by contact detonation. To prevent azide accumulation in plumbing flush with copious amounts of water immediately after waste disposal.

Specimen materials may contain pathogenic organisms handle with appropriate precautions.

References

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STAPHYLASE TEST KIT

Code: DR0595

A rapid slide identification test for Staphylococcus aureus

Introduction
The generally accepted identifying characteristic of *Staphylococcus aureus* is the ability to produce free and bound coagulase (or clumping factor). The presence of clumping factor may be detected in a number of ways. The Oxoid Staphylase Test detects the presence of clumping factor through clumping of fibrinogen-sensitised sheep red blood cells[1,2]. The specificity of the reaction is ensured by a simultaneous test with a control reagent (unsensitised sheep red blood cells), when of course no clumping reaction should be observed.

Components of the kit
DR0596 Staphylase Test Reagent consists of rabbit fibrinogen-sensitised sheep red blood cells.
DR0597 Staphylase Control Reagent consists of unsensitised sheep red blood cells.
Disposable Reaction Cards
Each kit contains sufficient reagent for 100 tests.

Storage
The Staphylase Test Kit should be stored at 2-8°C. Do not freeze. The kit should not be used after the expiry date printed on the outside of the carton.

Precautions
Both reagents contain 0.1% sodium azide as preservative.

Reference
1. Flandrois J. P. and Carret G. (1981) *Zbl. Bakt. Hyg. Orig. A* 251, 171-176
2. Duthie E. S. (1955) *J. Gen. Microbiol.* 13, 383-393.

TPHA TEST KIT

Code: DR0530

Treponema pallidum haemagglutination test (TPHA) for the serodiagnosis of Syphilis.

Introduction
Syphilis is a sexually transmitted disease. The causative organism is *Treponema pallidum*, a spirochaete which cannot be grown on culture media or in tissue culture. Diagnosis of infection is normally by the detection of antibody specific for *Treponema pallidum* in the patient's blood or CSF.

Detection of the antibody becomes possible 3-4 weeks following infection. Detectable levels may remain for long periods after treatment.

Two groups of antibodies are formed in response to infection:

1. Antibodies reactive with non-treponemal antigens (reagin antibodies)

Reagin antibodies are normally found in the active disease. They are detected by the VDRL/Carbon Antigen and RPR tests (Oxoid VDRL Carbon Antigen Test DR0525). Reagin antibodies levels subside after successful treatment[1].

The non-treponemal antibodies may arise for reasons other than syphilitic infection. Positive tests for these should therefore be confirmed by a test for the specific antibodies.

2. Antibodies reactive with the specific antigens of *Treponema pallidum*:

Specific antibodies persist long after the infection has been successfully treated.

The TPHA test will detect these antibodies. It is a sensitive passive haemagglutination test specifically for the detection of antibodies to *Treponema pallidum*.

Principle of the test
Tanned fowl erythrocytes are coated with specific antigen and suspended in a diluent[2,3,4]. When diluted positive samples are mixed with the test suspension, antibody to the sensitising antigen causes agglutination of the cells. The cells form a characteristic pattern in the bottom of a microtitration plate well. In the absence of reacting antibody, the cells form a compact button in the well. Uncoated tanned fowl erythrocytes are used as the control cells.

Components of the Kit

DR0531 Test Cell Suspension
2 bottles each containing 8.5 ml of antigen coated formolised tanned fowl erythrocytes. The dropper bottle will dispense 75 ml drops. Each kit contains sufficient suspension for 200 tests.

DR0532 Control Cell Suspension
2 bottles each containing 8.5 ml of uncoated formolised tanned fowl erythrocytes. The dropper bottle will dispense 75 ml drops. Each kit contains sufficient suspension for 200 tests.

DR0533 Diluent Buffer
2 bottles each containing 20 ml of buffer.

DR0534 Positive Control Serum
1 bottle containing 2 ml of pre-diluted (1/20) serum, positive for antibodies to *Treponema pallidum*. The serum should cause agglutination in the screening test and remain positive to a serum dilution of 1/2560 plus or minus one doubling dilution in the quantitative test.

DR0535 Negative Control Serum
1 bottle containing 2 ml of pre-diluted (1/20) serum negative for antibodies to *Treponema pallidum*.

The human sera used in the manufacture of the controls have been shown to be negative for HBsAG (Hepatitis B surface antigen), Hepatitis C and HIV 1 and 2 antibodies by FDA approved tests.

Instruction Leaflet

Materials required but not provided
U-well microtitration plate.
Micropipettes and tips to deliver 25 and 100 ml volumes.
Suitable laboratory disinfectant.

Storage
This kit must be stored at 2-8° C. Do not freeze. The kit should not be used after the expiry date printed on the outside of the carton.

Precautions
Reagents contain 0.095% sodium azide as a preservative. Sodium azide is toxic and may react with lead or copper plumbing to produce metal azides which are explosive by contact detonation. To prevent azide accumulation in plumbing flush with copious amounts of water immediately after waste disposal (refer to local environmental reulations).

References
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VDRL CARBON ANTIGEN

Code: DR0520

Oxoid VDRL Carbon Antigen is used for the detection of reagin antibodies, which indicate a serological diagnosis of syphilis. It is a modified form of the VDRL antigen utilising micro-particulate carbon[1] to enhance the visual reading of results. The product is suitable for use on both single and multi-channel Auto-Analyzer equipment and for a manual slide test[2,3,4,5,6].

Components of the kit
VDRL Carbon Antigen Reagent

Materials required but not provided

1. Manual testing
White backed ringed slides
Pipettes to deliver 17 µl and 50 µl
Rotary shaking table

2. Automated testing
Single or multi-channel Auto-Analyzer equipment
A magnetic stirrer
For full proceedure please see product insert.

Storage
Store the antigen at 2-8° C. Ensure that the cap is fully tightened. The kit should not be used after the expiry date printed on the label.

References
1. Portnoy J., Brewer J. H. and Harris A. D. (1962) *US Public Health Report* 77. 645.
2. McGrew B. E., Stout G. W. and Falcone V. H. (1968) *Am.J. Med.Tech* 34. 634.
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6. Stout G. W., McGrew B. E. and Falcone, V. H. (1968) *J. Conf. Public Health Lab. Directors*, 26. 7.

VDRL TEST KIT

Code: DR0525

A test for the detection and quantitative assessment of reagin antibodies in syphilis screening.

Introduction

The Oxoid VDRL Carbon Antigen Test is a macroscopic non-treponemal flocculation test for use in the detection and quantification of reagin antibodies. A presumptive diagnosis of syphilis can be made when these antibodies are detected in serum. The Card Test uses a modified form of the VDRL antigen[1] containing micro-particulate carbon to improve the reading of results.

The test can be performed using unheated serum or plasma. The VDRL Carbon Antigen Suspension can also be used on both single- and multi-channel Auto-Analyzer equipment[2,3,4,5,6].

Components of the Kit DR0525/DR0526

DR0527 VDRL Carbon Antigen Suspension
DR0528 Positive Control Serum
DR0529 Negative Control Serum
Test Cards
Mixing Sticks
Instruction leaflet.

Precautions

The human sera used in the manufacture of the controls were shown to be negative for HbsAg (Hepatitis B surface antigen), Hepatitis C and HIV 1 and 2 antibodies by FDA approved tests. However, this cannot guarantee the absence of virus, and therefore the reagents should be handled as capable of transmitting hepatitis.

The positive and negative control sera contain sodium azide (0.1%). The VDRL Carbon Antigen Suspension contains thiomersalate (0.1%).

Storage

The VDRL Carbon Antigen Suspension must be stored at 2-8°C. Under these conditions it will retain its reactivity until the date shown on the label. Do not freeze the reagent.

The positive and negative control sera can be divided into 0.1 ml amounts and stored at -20°C.

Store test cards at ambient temperature; use each card once and discard. Take care not to finger mark the circled areas.

References

1. Portnoy J., Brewer J. H. and Harris A.D.(1962) *U.S. Public Health Report* 77. 645
2. McGrew B. E., Stout G. W. and Falcone V. H. (1968) *Am. J.Med. Tech* 34. 634
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TOXIN DETECTION KITS

These products detect a wide variety of bacterial toxins and enterotoxins in food, faecal or culture samples.

The kits are simple to operate but are reliable for toxin detection in the majority of laboratories where sophisticated equipment or special skills in chemistry are not available.

Both industrial clinical applications benefit from their use. Improved quality control of foods and raw materials, better detection of food poisoning outbreaks and rapid clinical diagnosis are examples of these benefits. The ability to carry out such tests in the laboratory, without sending them to external reference laboratories, is an additional benefit.

BCET-RPLA TOXIN DETECTION KIT

Code: TD0950

A kit for the detection of Bacillus cereus enterotoxin (diarrhoeal type) in foods and culture filtrates by reversed passive latex agglutination.

INTRODUCTION

In both its spore and vegetative forms, the organism *Bacillus cereus* is a common inhabitant of many different environments and can easily contaminate food. If contaminated foods are not cooled sufficiently after cooking and there is an extended time between preparation and consumption, then surviving heat-resistant spores can germinate, enabling the organism to multiply and produce toxins. Under these circumstances, *B. cereus* can cause food poisoning. Rice, pasta, meat, poultry, vegetable dishes, various soups, puddings and sauces have been implicated in *B. cereus* food poisoning[1,2,3].

Two distinct types of illness can be caused by this organism: the acute-onset 'emetic-syndrome' type which is mainly associated with cooked rice; and the longer-onset 'diarrhoeal-syndrome' type in which a wide range of foods have been implicated. Separate toxins are responsible for the characteristic symptoms of the two forms of illness: the emetic toxin and the diarrhoeal enterotoxin[4].

The BCET-RPLA Toxin Detection kit (TD0950) was developed for the purpose of detecting the diarrhoeal enterotoxin by reversed passive latex agglutination (RPLA). The technique of reversed passive latex agglutination (RPLA) enables soluble antigen such as bacterial toxins to be detected in an agglutination assay.

In a standard agglutination assay, soluble antibody reacts with particulate antigen such as bacterial cells. However, in a REVERSED agglutination assay the antibody, which is attached to particles, reacts with the soluble antigen. The particles (in this case, latex) do not themselves play a part in the reaction and they are therefore PASSIVE. The cross-linking of the latex particles by the specific antigen/antibody reaction results in the visible LATEX AGGLUTINATION reaction.

The BCET-RPLA test (TD0950) may be used to detect *B. cereus* enterotoxin in a variety of foods and to give a semi-quantitative result. The test may also be used to demonstrate enterotoxin production by isolates of *B. cereus* grown in culture.

PRINCIPLE OF ASSAY

Polystyrene latex particles are sensitised with purified antiserum taken from rabbits immunised with purified *B. cereus* diarrhoeal enterotoxin. These latex particles will agglutinate in the presence of *B. cereus* enterotoxin. A control reagent is provided which consists of latex particles sensitised with non-immune rabbit globulins. The test is performed in V-well microtitre plates. Dilutions of the food extract or culture filtrate are made in two rows of wells, a volume of the appropriate latex suspension is added to each well and the contents mixed. If *B. cereus* enterotoxin is present, agglutination occurs due to the formation of a lattice structure. Upon settling, this forms a diffuse layer on the base of the well. If *B. cereus* enterotoxin is absent or at a concentration below the assay detection level, no such lattice structure can be formed, and a tight button will, therefore, be observed.

PRECAUTIONS

This product is for *in vitro* diagnostic use only.

Do not freeze.

Reagents with different lot numbers should not be interchanged.

Reagents and diluent contain 0.1% sodium azide as a preservative. Sodium azide may react with lead or copper plumbing to produce metal azides which are explosive by contact detonation. To prevent azide accumulation in plumbing, flush with copious amounts of water immediately after waste disposal.

STORAGE

The BCET-PRLA kit (TD0950) must be stored at 2-8°C. Under these conditions the reagents will retain their reactivity until the date shown on the box. After reconstitution, the enterotoxin control should be stored at 2-8°C. Under these conditions, the reconstituted enterotoxin control will retain its reactivity for 3 months, or until the date shown on the box, whichever is the sooner.

SAMPLE PREPARATION

Food Matrices

A wide range of foods may be tested for enterotoxin. The extraction procedure may, however, require modifications for particular foods. The main requirement is to achieve a non-turbid, fat-free extract. A low dilution factor is desirable for optimum sensitivity, but if the nature of the food dictates a greater dilution during extraction, a reduced sensitivity will result.

To gain a representative sample of a batch, a series of 10 g portions are collected from different locations within the batch (see T.P.I., U.S.D.A. sampling plans or equivalent).

Culture Filtrates

B. cereus may be recovered from food or faecal samples and identified using suitable techniques described in standard textbooks. The use of *Bacillus Cereus* Selective Agar (Oxoid CM0617 and SR0099) will aid the isolation and presumptive identification of *B. cereus* prior to toxin detection[5].

METHOD OF USE

Materials required but not provided.

Blender or homogeniser (required for food matrices only).

Microtitre plates (V-well) and lids

Fixed or variable pipette and tips (25 µg)

Centrifuge capable of generating 900 g (typically 3000 rpm in a small bench top centrifuge) **or** membrane filtration unit using low protein-binding disposable filters with a porosity of 0.2 µm to 0.45 µm (such as Millipore SLGV)

Brain Heart Infusion (Oxoid CM0225)

Sodium chloride solution (0.85%)

Sodium hypochlorite solution (>1.3% w/w (disinfectant))

25 µl dropper (optional)

25 µl diluter (optional)

Moisture box (optional)

Components of the Kit

TD0951 Sensitised Latex. Latex sensitised with specific *B. cereus* anti-enterotoxin (rabbit IgG).

TD0952 Latex control. Latex suspension sensitised with non-immune rabbit globulins.

TD0953 Enterotoxin control (lyophilized). Lyophilized *B. cereus* enterotoxin.

TD0954 Diluent. Phosphate buffered saline containing bovine serum albumin.

Instruction leaflet

Toxin Extraction or Production

Extraction from Food Matrices

Blend 10 g of sample with 10 ml of sodium chloride solution (0.85%) in a blender or homogeniser.

Centrifuge the blended sample at 900 g at 4°C for 30 minutes. **NOTE:** If refrigerated centrifuge is not available, cool the sample to 4°C before centrifugation.

Filter the supernatant through a 0.2 µm-0.45 µm low protein-binding membrane filter. **Retain the filtrate for assay of toxin content**.

Production of Enterotoxin in Culture Fluids

Inoculate the isolated organism into Brain Heart Infusion (CM0225) and incubate at 32-37°C for 6-18 hours, preferably with shaking (250 cycles/min).

After growth, either centrifuge at 900 g for 20 minutes at 4°C **or** membrane filter using a 0.2 µm-0.45 µm low protein-binding filter.

Retain the filtrate for assay of toxin content. NOTE: It is advisable to check the particular cultural method of use with a standard enterotoxin-producing strain such as *B. cereus* NCTC 11145.

Control
The reconstituted toxin control (TD0953) will agglutinate the sensitised latex (TD0951). The use of the toxin control will provide a reference for the positive patterns illustrated below (see Interpretation of Test Results). The control should be used occasionally only to confirm the correct working of the test latex. The toxin control is not provided at a specified level and therefore must not be used as a means of quantifying the level of toxin detected in the test sample.

Assay Method
Working Reagents

The latex reagents (TD0951, TD0952) and diluent (TD0954) are ready for use. The latex reagents should be thoroughly shaken before use to ensure a homogenous suspension.

To reconstitute the enterotoxin control (TD0953), add 0.5 ml of diluent (TD0954) to each vial. Shake gently until the contents are dissolved.

Arrange the plate so that each row consists of 8 wells. Each sample needs the use of 2 such rows.

Using a pipette or dropper dispense 25 µl of diluent (TD0954) in each well of the 2 rows except the first well in each row.

Add 25 µl of test sample to the first and second well of both rows.

Using a pipette or diluter and starting at the second well of each row, pick up 25 µl and perform doubling dilutions along each of the rows. Stop at the 7th well to leave the last well containing diluent (TD0954 only).

To each well in the first row add 25 µl of sensitised latex (TD0951).

To each well in the second row add 25 µl of latex control (TD0952).

To mix the contents of each well, rotate the plate by micromixer or agitate by hand. Take care that no spillage occurs from the wells.

To avoid evaporation, either cover the plate with a lid, or place the plate in a moisture box. **Leave the plate undisturbed** on a vibration-free surface at room temperature for 20-24 hours. It will help subsequent reading of the test if the plate is placed on black paper for the duration of the incubation.

Examine each well in each row for agglutination against a black background.

Centrifuge tubes, membrane filters, microtitre plates, lids and pipette tips should be sterilised by autoclaving at 121°C for 15 minutes or disinfected, before disposal, in hypochlorite solutions (>1.3%w/w).

Dispose of culture extracts, food extracts, samples and enterotoxin controls in hypochlorite solution (>1.3%w/w).

INTERPRETATION OF TEST RESULTS
The agglutination pattern should be judged by comparison with the following illustration:

Results classified as (+), (++) are considered to be positive.

Results in the row of wells containing latex control (TD0952) should be negative. In some cases, non-specific agglutination may be observed. In such cases, the results should be interpreted as positive, provided that the reaction with sensitised latex (TD0951) is positive to a higher dilution of test sample that that seen with the latex control (TD0952). The last well in all rows should be negative. If positive patterns are observed in some of these wells, the reaction should be regarded as invalid.

LIMITATIONS OF THE TEST
The sensitivity of this test in detecting the enterotoxin is 2 ng/ml in the test extract. When a food extract is made with a dilution ratio of 1:1 with diluent (TD0954), the sensitivity is, therefore, 4 ng/g of food matrix. The detection limit will vary according to any extra dilution conditions dictated by the type of food matrix. Concentration of the enterotoxin in the food extract can be effected by a variety of methods, such as ultrafiltration.

Production of enterotoxin in culture filtrate depends on the growth conditions. A positive result obtained in this way demonstrates the production of enterotoxin; it does not imply the *in vitro* production of toxins to those levels.

References
1. Kramer J. M. and Gilbert R. J. (1988). *In Foodborne Bacterial Pathogens* (ed. M.P. Doyle) pp. 21-70 Marcel Dekker Inc,. New York
2. Hauge S. (1955) *J. Appl. Bacterial* **18:** pp. 591-595.
3. Mortimer P. R. and McGann G. (1974). *Lancet* **1:** pp. 1043-1045
4. Turnbull P. C. B. (1936). *In Pharmacology of Bacterial Toxins* (ed. F. Dorner and J. Drews) pp. 397-448. Pergamon Press, Oxford.
5. Holbrook R. and Anderson J. M. (1980). *Can. J. Microbiol.* **26:** pp. 753-759.

PET-RPLA TOXIN DETECTION KIT

Code: TD0930

A kit for the detection of enterotoxin type A of Clostridium perfringens in faecal samples or culture filtrates by reversed passive latex agglutination.

INTRODUCTION

Food poisoning can result from eating foods contaminated with *Clostridium perfringens*. The ingested cells multiply in the patient's intestines and produce spores. The production of enterotoxin is associated with this spore-forming process. It is, therefore, important to detect the enterotoxin in faecal specimens obtained from the patient or in the culture fluid of the bacterial isolates.

The direct detection of *C. perfringens* enterotoxins in faeces is the most reliable method due to the much larger amount of toxin formed *in vivo*. The culture method is less reliable due to problems in encouraging *C. perfringens* to produce sufficient toxin in artificial media.

Reversed passive latex agglutination (RPLA) has been reported to be a reliable method for the detection of *C. perfringens* enterotoxin.[1] The technique of reversed passive latex agglutination (RPLA) enables soluble antigen such as bacterial toxins to be detected in an agglutination assay.

In a **standard** agglutination assay, soluble antibody reacts with particulate antigen such as bacterial cells. However, in a **reversed** agglutination assay the antibody, which is attached to particles, reacts with the soluble antigen. The particles (in this case, latex) do not themselves play a part in the reaction and they are therefore **passive**. The cross-linking of the latex particles by the specific antigen/antibody reaction results in the visible **latex agglutination** reaction.

PRINCIPLE OF ASSAY

Polystyrene latex particles are sensitised with purified antiserum taken from rabbits immunised[2] with purified *C. perfringens* enterotoxin. These latex particles will agglutinate in the presence of *Cl. perfringens* enterotoxin. A control reagent is provided which consists of latex particles sensitised with non-immune rabbit globulins.

The test is performed in V-well microtitre plates. Dilutions of the culture filtrate are made in two rows of wells, a volume of the latex suspension is added to each well and the contents mixed. If *C. perfringens* enterotoxin is present, agglutination occurs due to the formation of a lattice structure. Upon settling, this forms a diffuse layer on the base of the well. If *C. perfringens* enterotoxin is absent or at a concentration below the assay detection level, no such lattice structure can be formed. A tight button will therefore be observed.

PRECAUTIONS

This product is for *in vitro* diagnostic use only.

Do not freeze.

Reagents with different lot numbers should not be interchanged.

Reagents and diluent contain 0.1% sodium azide as a preservative. Sodium azide may react with lead or copper plumbing to produce metal azides which are explosive by contact detonation. To prevent azide accumulation in plumbing, flush with copious amounts of water immediately after waste disposal.

STORAGE

The PET-RPLA Kit (TD0930A)must be stored at 2-8°C. Under these conditions the reagents will retain their reactivity until the date shown on the box. After reconstitution, the enterotoxin control should be stored at 2-8°C. Under these conditions, the reconstituted enterotoxin control will retain its reactivity for 3 months, or until the date shown on the kit box, whichever is the sooner.

SAMPLE PREPARATION

The direct detection of *C. perfringens* enterotoxin in faeces is the most reliable method and it should be

followed unless insufficient faecal material is available. It is much quicker than the cultural method, taking approximately 24 hours to obtain a result from sampling the faeces.

If the culture method is to be used for diagnosis of *C. perfringens* food poisoning it should be noted that *C. perfringens* is a common inhabitant of the bowel. It is recommended that at least 6 colonies are separately tested to ensure a higher probability of detecting an enterotoxin-producing strain.

There is no single culture procedure which is suitable for all enterotoxin-producing strains of *C. perfringens*. Any culture method used may fail, due to insufficient toxin production. This may be due to inadequate growth, poor spore formation or low-level toxin production.

It should also be noted that some enterotoxin-positive strains may actually be killed by heat treatment and will not, therefore, produce enterotoxin in the second medium. It is recommended that each culture is checked for viability after heating.

It is advisable to test the particular cultural method used with a known enterotoxin-producing strain such as *C. perfringens* NCTC 8239 or *C. perfringens* ATCC® 12917.

METHOD OF USE

Materials required but not provided.

Microtitre plate (V-well) and lid.

Fixed or variable pipette and tips (25 µl)

Centrifuge capable of generating 900 g (typically 3000 rpm in a small bench top centrifuge) **or** membrane filtration unit, using low protein-binding disposable filters with a porosity of 0.2 µm-0.45 µm (such as Millipore SLGV)

Culture media for proportion of enterotoxin production of *C. perfringens* strains (a suitable medium is that developed by Duncan and Strong[3] modified by Harmon and Kautter[1] (see Appendix)).

Sodium hypochlorite solution (>1.3%w/w)

25 µl dropper (optional)

25 µl diluter (optional)

Micromixer (optional)

Moisture box (optional)

Components of the Kit

TD0931 Sensitised latex. Latex suspension sensitised with specific antibodies (rabbit lgG) against *C. perfringens* enterotoxin.

TD0932 Latex control. Latex suspension sensitised with non-immune rabbit globulins.

TD0933 Enterotoxin control. Dried *C. perfringens* enterotoxin.

TD0934 Diluent. Phosphate buffered saline containing bovine serum albumin.

Instruction Leaflet

Toxin Extraction or Production

Extraction from Faeces

To one volume of faeces, add a similar volume of phosphate buffered saline (Oxoid BR0014A).

Homogenise completely and centrifuge at 1300 g for 20 minutes at 4°C then membrane filter, using a 0.2 µm - 0.45 µm low protein binding filter. The filtrate is then used as the test sample.

Production of Enterotoxin Culture Fluid

Culture the isolated organism in Cooked Meat Medium (Oxoid CM0081) at 37°C for 18-20 hours, then inactivate by heating at 75°C for 20 minutes. Subculture to a medium designed for promotion of enterotoxin production[3 4].

Modified Duncan and Strong Medium[3,4]

Formula	***gm/litre***
Yeast Extract (Oxoid LP0021)	4.0
Proteose Peptone (Oxoid LP0085)	15.0
Soluble starch	4.0
Sodium thioglycollate	1.0
$NA_sHPO_47H_2O$	10.0

Autoclave at 121°C for 15 minutes.

Add a sufficient amount of filter-sterilised 0.66M sodium carbonate to increase the pH to 7.8 ± 0.1.

Inoculate 16-18 ml of the medium with 0.8 ml of the Cooked Mead Medium culture (taken from the base of the tube). **NOTE:** If the dilution rate is greater than 1:20, the Cooked Meat Medium constituents may be transferred in amounts large enough to inhibit spore formation.

Incubate at 37°C for 24 hours. After incubation **either** centrifuge at 900 g for 20 minutes at 4°C and use the supernatant as the test sample **or** membrane filter, using a 0.2 µm-0.45 µm low protein-binding filter and use the filtrate as the test sample.

Control

The reconstituted toxin control (TD0933) will agglutinate the sensitised latex (TD0931). The use of the toxin control will provide a reference for the positive patterns illustrated below (see Interpretation of Test Results). The control should be used occasionally only to confirm the correct working of the test latex. The toxin control is not provided at a specified level and therefore must not be used as a means of quantifying the level of toxin detected in the test sample.

Assay Method

Working Reagents

The latex reagents (TD0931, TD0932) and diluent (TD0934) are ready for use. The latex reagents should be thoroughly shaken before use to ensure a homogeneous suspension.

To reconstitute the enterotoxin control (TD0933) add 0.5 ml of diluent (TD0934) to each vial. Shake gently until the contents are dissolved.

Arrange the plate so that each row consists of 8 wells. Each sample needs the use of 2 such rows.

Using a pipette or dropper, dispense 25 µl of diluent (TD0934) in each well of the 2 rows **except** for the first well in each row.

Add 25 µl of test sample to the first and second well of each row.

Using a pipette or diluter and starting at the **second well** of each row, pick up 25 µl and perform doubling dilutions along each of the 2 rows. **Stop at the 7th well** to leave the last well containing diluent (TD0934) only.

Add 25 µl of sensitised latex (TD0931) to each well of the first row.

Add 25 µl of latex control (TD0932) to each well of the second row.

To mix the contents of each well, rotate the plate by micromixer or agitate by hand. Take care that no spillage occurs from the wells.

To avoid evaporation, either cover the plate with a lid, or place the plate in a moisture box. **Leave the plate undisturbed** on a vibration-free surface at room temperature for 20-24 hours. It will help subsequent reading of the test if the plate is placed on black paper for the duration of the incubation.

Examine each well in each row for agglutination against a black background.

Centrifuge tubes, membrane filters, microtitre plates, lids and pipette tips should be sterilised by autoclaving at 121°C for 15 minutes or disinfected before disposal in hypochlorite solutions (>1.3% w/w).

Dispose of toxin controls and culture extracts in hypochlorite solutions (>1.3%w/w).

INTERPRETATION OF RESULTS

The agglutination pattern should be judged by comparison with the following illustration:

Results classified as (+), (++) and (+++) are considered to be positive.

In some cases, non-specific agglutination may be observed. If the faecal specimen or culture filtrate reacts with the sensitised latex to a dilution greater than that seen with the latex control, the test result should be regarded as positive. The last well in all rows should be regarded as invalid.

LIMITATIONS OF THE TEST

The sensitivity of this test in detecting the enterotoxin is approximately 2 ng/ml.

Enterotoxin present at concentrations lower than this will, therefore, give negative results.

This kit is not intended for the detection of enterotoxins of other types of *Clostridium perfringens*.

References
1. Harmon S. M. and Kautter D. A. (1986). *J. Food Prot.* **49:** 523.
2. Sakaguchi G. Vemura T. and Riemann H. P. (1973). *J Appl. Microbiol*, **26:** 762.
3. Duncan C. L. and Strong D. H. (1968). *J. Appl. Microbiol*. **1:** 82
4. Harmon S. M. and Kautter D. A. (1986). *J. Food Protection* **49:** 706.

SET-RPLA KIT TOXIN DETECTION KIT

Code: TD0900

Staphylococcal Enterotoxin Test Kit (TD0900) for the detection of staphylococcal enterotoxins A, B, C and D in food samples or culture filtrates by reversed passive latex agglutination

INTRODUCTION

Staphylococcal food poisoning is caused by eating foods contaminated with enterotoxins produced during the growth of certain strains of *Staphylococcus aureus*. Reports on the assay of these toxins by reversed passive latex agglutination (RPLA) have been published.[1,2,3] The technique of reversed passive latex agglutination (RPLA) enables soluble antigen such as bacterial toxins to be detected in an agglutination assay.

In a standard agglutination assay, soluble antibody reacts with particulate antigen such as bacterial cells. However, in a **REVERSED** agglutination assay the antibody, which is attached to particles, reacts with the soluble antigen. The particles (in this case, latex) do not themselves play a part in the reaction and they are therefore **PASSIVE**. The cross-linking of the latex particles by the specific antigen/antibody reaction results in the visible **LATEX AGGLUTINATION** reaction.

The SET-RPLA test kit (TD0900) is based upon the reports by Shingaki *et al*.[1] and Oda *et al*.[4] It was developed under the guidance of the Tokyo Metropolitan Research Laboratory of Public Health.

The SET-RPLA test (TD0900) may be used to detect staphylococcal enterotoxins in a wide variety of foods and to give a semi-quantitative result. The test may also be used to demonstrate enterotoxin production in isolates of *S. aureus* grown in culture. It should be noted that coagulase-negative staphylococci have been isolated which also produce enterotoxin in staphylococcal food poisoning.[5]

PRINCIPLE OF ASSAY

Polystyrene latex particles are sensitised with purified antiserum taken from rabbits, immunised individually with purified staphylococcal enterotoxins A, B, C and D. These latex particles will agglutinate in the presence of the corresponding enterotoxin. A control reagent is provided which consists of latex particles sensitised with non-immune rabbit globulins. The test is performed in V-well microtitre plates. Dilutions of the food extract or culture filtrate are made in five rows of wells, a volume of the appropriate latex suspension is added to each well and the contents mixed. If staphylococcal enterotoxins A, B, C or D are present, agglutination occurs, which results in the formation of a lattice structure. Upon settling, this forms a diffuse layer on the base of the well. If staphylococcal enterotoxins are absent or at a concentration below the assay detection level, no such lattice structure can be formed and, therefore a tight button will be observed.

The diluent provided contains sodium hexametaphosphate, which has been shown to reduce the incidence of non-specific reactions with components of food matrices.[6]

STORAGE

The SET-RPLA Kit (TD0900) must be stored at 2-8°C. Under these conditions the reagents will retain their reactivity until the date shown on the box. After reconstitution, the enterotoxin controls should be stored at 2-8°C. Under these conditions, the reconstituted enterotoxin controls will retain the reactivity for 3 months, or until the date shown on the kit box, whichever is the sooner.

PRECAUTIONS

This product is for *in vitro* diagnostic use only.

Do not freeze.

Reagents with different lot numbers should not be interchanged.

Reagents and diluent contain 0.1% sodium azide as a preservative. Sodium azide may react with lead or copper plumbing to produce metal azides which are explosive by contact detonation. To prevent azide accumulation in plumbing, flush with copious amounts of water immediately after waste disposal.

SAMPLE PREPARATION

Food Matrices

A wide range of foods may be tested for staphylococcal enterotoxins; the extraction procedure may, however, require modification for particular foods. The main requirement is to achieve a non-turbid, fat-free extract. A low dilution factor is desirable for optimum sensitivity, but if the nature of the food dictates a greater dilution during extraction, a reduced sensitivity will result.

To gain a representative sample of a batch, a series of 10g portions are collected from different locations within the batch (see T.P.I., U.S.D.A. sampling plans or equivalent).

Culture Filtrates

Staphylococci from either clinical sources or food matrices may be recovered and identified using suitable techniques described in standard textbooks.

METHOD OF USE

Materials required but not provided

Blender or homogeniser

Microtitre plates (V-well) and lids

Fixed or variable pipette and tips (25 µl)

Centrifuge capable of generating 900 g (typically 300 rpm in a small bench top centrifuge) **or** membrane filtration unit, using low protein-binding disposable filters, with a porosity of 0.2 µm-0.45 µm (such as Millipore SLGV)

Tryptone Soya Broth (CM0129)

Sodium chloride solution (0.85%)

Sodium hypochlorite solution (>1.3% w/w)

25 µl dropper (optional)

25 µl diluter (optional)

Micromixer (optional)

Moisture box (optional)

Components of Kit

TD0901 Latex sensitised with anti-enterotoxin A. Latex suspension sensitised with specific antibodies (rabbit IgG) against staphylococcal enterotoxin A.

TD0902 Latex sensitised with anti-enterotoxin B. Latex suspension sensitised with specific antibodies (rabbit IgG) against staphylococcal enterotoxin B.

TD0903 Latex sensitised with anti-enterotoxin C. Latex suspension sensitised with specific antibodies (rabbit IgG) against staphylococcal enterotoxin C.

TD0904 Latex sensitised with anti-enterotoxin D. Latex suspension sensitised with specific antibodies (rabbit IgG) against staphylococcal enterotoxin D.

TD0905 Latex control. Latex suspension sensitised with non-immune rabbit globulins.

TD00906 Staphylococcal enterotoxin A control.

TD0907 Staphylococcal enterotoxin B control.

TD0908 Staphylococcal enterotoxin C control.

TD0909 Staphylococcal enterotoxin D control.

TD0910 Diluent. Phosphate buffered saline containing bovine serum albumin and sodium hexametaphosphate.

Instruction leaflet

Toxin Extraction or Production

Extraction from Food Matrices

Blend 10 g of sample with 10 ml of sodium chloride solution (0.85%) in a blender or homogeniser.

Centrifuge the blended sample at 900 g at 4°C for 30 minutes. **NOTE:** If a refrigerated centrifuge is not available, cool the sample to 4°C before centrifugation.

Filter the supernatant through a 0.2 µm-0.45 µm low protein-binding membrane filter.

Retain the filtrate for assay of toxin content.

Production of Enterotoxins in Culture Fluids

Inoculate the isolated organism into Tryptone Soya Broth (CM0129) and incubate at 37° C for 18-24 hours, preferably with shaking.

After growth, either centrifuge at 900 g for 20 minutes at 4°C **or** membrane filter using a 0.2 µm low protein-binding filter. **Retain the filtrate for assay of toxin content**.

Control

Each reconstituted toxin control (TD0906-TD0909) will cause agglutination with its respective sensitised latex (TD0901-TD0904). The use of the toxin controls will provide references for the positive patterns illustrated below (see Interpretation of Test Results). The controls should be used occasionally only to confirm the correct working of the test latex. The toxin controls are not provided at a specified level and therefore must not be used as a means of quantifying the level of toxin detected in the test sample.

Assay Method

Working Reagents

The latex reagents (TD0901-TD0905) and diluent are ready for use. The latex reagents should be thoroughly shaken before use to ensure a homogeneous suspension. To reconstitute the control reagents TD0906-TD0909), add 0.5 ml of diluent (TD0910) to each vial. Shake gently until the contents are dissolved.

Arrange the plate so that each row consists of 8 wells. Each sample needs the use of 5 such rows.

Using a pipette or dropper, dispense 25 µl of diluent (TD0910) in each well of each of the 5 rows.

Using a pipette or dropper, dispense 25 µl of test sample to the **first well** of each of the 5 rows.

Using a pipette or diluter and starting at the **first well** of each row, pick up 25 µl and perform doubling dilutions along each of the 5 rows. **Stop at the 7th well** to leave the last well containing diluent (TD0910) only.

To each well in the first row, add 25 µl of latex sensitised with anti-enterotoxin A (TD0901).

To each well in the second row, add 25 µl of latex sensitised with anti-enterotoxin B (TD0902).

To each well in the third row, add 25 µl of latex sensitised with anti-enterotoxin C (TD0903).

To each well in the fourth row, add 25 µl of latex sensitised with anti-enterotoxin D (TD0904).

To each well in the fifth row, add 25 µl of latex control (TD0905).

To mix the contents of each well, rotate the plate by micromixer or agitate by hand. Take care that no spillage occurs from the wells.

To avoid evaporation, either cover the plate with a lid, or place the plate in a moisture box. **Leave the plate undisturbed** on a vibration-free surface at room temperature for 20-24 hours. It will help subsequent reading of the test if the plate is placed on black paper for the duration of the incubation.

Examine each well in each row for agglutination, against a black background.

Centrifuge tubes, membrane filters, microtitre plates, lids and pipette tips should be sterilised by autoclaving at 121°C for 15 minutes or disinfected, before disposal, in hypochlorite solutions (>1.3% w/w).

Dispose of culture extracts, food extracts, samples and toxin controls in hypochlorite solutions (>1.3% w/w).

INTERPRETATION OF TEST RESULTS

The agglutination pattern should be judged by comparison with the following illustration.

Results classified as (+), (++), and (+++) are considered to be positive.

Results in the row of wells containing latex control (TD0905) should be negative. In some cases, non-specific agglutination may be observed. In such cases, the results should be interpreted as positive, provided that the reaction with sensitised latex is positive to a higher dilution of test sample than that seen with the latex control. The last well in all rows should be negative. If positive patterns are observed in some of these wells, the reaction should be regarded as invalid.

NOTE: Certain *staphylococcal* strains are known to produce more than one enterotoxin.

LIMITATIONS OF THE TEST

The sensitivity of this test in detecting the enterotoxins has been reported to be 0.5 ng/ml in the test extract. When a food extract is made with a dilution ratio of 1 : 1 with diluent, the sensitivity is, therefore, 1 ng/g of

food matrix. The detection limit will vary according to any extra dilution conditions dictated by the type of food matrix. Concentration of the enterotoxin in the food extract can be effected by a variety of methods, such as ultrafiltration. Production in culture of SET's depends on the growth conditions. A positive result obtained by the culture demonstrates the production of one or more SET under those circumstances; it does not imply the *in vivo* production of toxins to those levels.

References

1. Shingaki M. *et al*. (1981). *Ann. Rep. Tokyo Metro. Lab. Public Health* **32:** 128.
2. Oda T. *et al.* (1979) *Ann. Rep. Fukuoka City Lab Hyg.* **4:** 33.
3. Park C. and Szabo R. (1986). *Can. J. Microbiol.* **32:** 723.
4. Oda T. (1978) *Jap. J. Bacteriol.* **33:** 743.
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TST-RPLA TOXIN DETECTION KIT
STAPHYLOCOCCAL TOXIC SHOCK SYNDROME

Code: TD0940

A kit for the detection of staphylococcal toxic shock syndrome toxin in culture filtrates by reversed passive latex agglutination.

INTRODUCTION

Todd *et al*.[1] were the first to describe the disease known as toxic shock syndrome. *Staphylococcus aureus* strains isolated from patients with this syndrome have been reported to produce a toxin, toxic shock syndrome toxin-one (TSST-1)[1,2,3]. The TST-RPLA (TD0940) test for the detection of TSST-1 in the culture fluid of *Staphylococcus aureus* was developed under the guidance of the Tokyo Metropolitan Research Laboratory of Public Health.

The technique of reversed passive latex agglutination (RPLA) enables soluble antigen such as bacterial toxins to be detected in an agglutination assay.

In a **standard** agglutination assay, soluble antibody reacts with particulate antigen such as bacterial cells. However, in a **reversed** agglutination assay the antibody, which is attached to particles, reacts with the soluble antigen. The particles (in this case, latex) do not themselves play a part in the reaction and they are therefore **passive**. The cross-linking of the latex particles by the specific antigen/antibody reaction results in the visible **latex agglutination** reaction.

PRINCIPLE OF ASSAY

Polystyrene latex particles are sensitised with purified antiserum taken from rabbits immunised[4] with purified TSST-1. These latex particles will agglutinate in the presence of TSST-1. A control reagent is provided which consists of latex sensitised with non-immune rabbit globulins.

The test is performed in V-well microtitre plates. Dilutions of the culture filtrate are made in two rows of wells. A volume of the latex suspension is added to each well and the contents mixed. If TSST-1 is present, agglutination occurs which results in the formation of a lattice structure. Upon settling, this forms a diffuse layer on the base of the well. If TSST-1 is absent or at a concentration below the assay detection level, no such lattice structure can be formed. Therefore, a tight button will be observed.

PRECAUTIONS

This product is for *in vitro* diagnostic use only.

Do not freeze.

Reagents with different lot numbers should not be interchanged.

Reagents and diluent contain 0.1% sodium azide as a preservative. Sodium azide may react with lead or copper plumbing to produce metal azides which are explosive by contact detonation. To prevent azide accumulation in plumbing, flush with copious amounts of water immediately after waste disposal.

STORAGE

The TSE-RPLA kit (TD0940A) must be stored at 2-8°C. Under these conditions the reagents will retain their reactivity until the date shown on the box. After reconstitution, the TST control should be stored at 2-8°C. Under these conditions, the reconstituted TST control will retain its reactivity for 3 months, or until the date shown on the kit box, whichever is the sooner.

SAMPLE PREPARATION

Staphylococcus aureus for testing may be recovered from swabs, etc. and identified by suitable techniques described in standard textbooks.

If Staphylococci are recovered from body sites, such as the vagina, which are normally colonised with staphylococci, it is recommended that several colonies are separately tested to ensure a higher probability of detecting TSST-1 producing strains.

METHOD OF USE

Materials required but not provided

Microtitre plates (V-well) and lids

Fixed or variable pipette and tips (25 µl)

Centrifuge capable of generating 900g (typically 3000 rpm in a small bench top centrifuge) **or** membrane filtration unit, using low protein-binding disposable filters with a porosity of 0.2 µm – 0.45 µm (such as Millipore SLGV).

Brain Heart Infusion Broth (Oxoid CM0225)

Sodium hypochlorite solution (>1.3% w/w)

25 µl dropper (optional)

25 µl diluter (optional)

Micromixer (optional)

Moisture box (optional)

Components of the kit

TD0941 Sensitised latex - Latex suspension sensitised with specific antibodies (rabbit IgG) against staphylococcal TSST-1.

TD0942 Latex Control - Latex suspension sensitised with non-immune rabbit globulins.

TD0943 TST Control - Dried toxic shock syndrome toxin.

TD0944 Diluent - Phosphate buffered saline containing bovine serum albumin.

Toxin Production in Culture Fluid

Inoculate the isolate into Brain Heart Infusion Broth (CM0225). Incubate at 37°C for 18-24 hours, preferably with shaking. After growth, either centrifuge at 900 g for 20 minutes at 4°C and use the supernatant as the test sample **or** membrane filter using a 0.2 µm – 0.45 µm low protein-binding filter and use the filtrate as the test sample.

Control

The reconstituted toxin control will agglutinate the sensitised latex. The use of the toxin control will provide a reference for the positive patterns illustrated below (see Interpretation of Test Results). The control should be used occasionally only to confirm the correct working of the test latex. The toxin control is not provided at a specified level and therefore must not be used as a means of quantifying the level of toxin detected in the test sample.

Assay Method

Working Reagents

The latex reagents (TD0941, TD0942) and diluent (TD0944) are ready for use. The latex reagents should be thoroughly shaken before use to ensure a homogeneous suspension.

To reconstitute the TST control (TD0943), add 0.5 ml of diluent (TD0944) to the vial. Shake gently until the contents are dissolved.

Arrange the plate so that each row consists of 8 wells. Each sample needs the use of 2 such rows.

Using a pipette or dropper, dispense 25 µl of diluent (TD0944) in each well of the 2 rows except for the first well in each row.

Add 25 µl of test sample to the first and second well of each row

Using a pipette or diluter and starting at the second well of each row, pick up 25 µl and perform doubling dilutions along each of the 2 rows. Stop at the 7th well to leave the last well containing diluent (TD0944) only.

Add 25 µl of sensitised latex (TD0941) to each well of the first row.

Add 25 µl of latex (TD0942) control to each well of the second row.

To mix the contents of each well, rotate the plate by micromixer or agitate by hand. Take care that no spillage occurs from the wells.

To avoid evaporation, either cover the plate with a lid, or place the plate in a moisture box. **Leave the plate undisturbed** on a vibration-free surface at room temperature for 20-24 hours. It will help subsequent reading of the test if the plate is placed on black paper for the duration of the incubation.

Examine each well in each row for agglutination, against a black background.

Centrifuge tubes, membrane filters, microtitre plates, lids and pipette tips should be sterilised by autoclaving at 121°C for 15 minutes or disinfected before disposal, in hypochlorite solution (>1.3%w.w).

Dispose of toxin controls and culture extracts in hypochlorite solution (>1.3%w/w)

INTERPRETATION OF TEST RESULTS

The agglutination pattern should be judged by comparison with the following illustration:

(–) (±) (+) (++) (+++)

Results classified as (+), (++) and (+++) are considered to be positive.

Results in the row of wells containing control latex should be negative. In some cases, non-specific agglutination may be observed. In such cases, the results should be interpreted as positive, provided that the reaction with sensitised latex is positive to a higher dilution of test sample than that seen with the latex control. The last well in all rows should be negative. If positive patterns are observed in some of these wells the reaction should be regarded as invalid.

LIMITATIONS OF THE TEST

The sensitivity of this test in detecting TSST-1 is approximately 2 ng/ml in the extract. TSST-1 present at concentrations lower than this will, therefore, give negative results.

The production of TSST-1 by a *Staphylococcus aureus* isolated from an individual does not constitute a diagnosis of toxic shock syndrome. Strains producing TSST-1 have been isolated from healthy individuals, although with a lower frequency than from patients with toxic shock syndrome.

References

1. Todd J., Fishaut M., Kapral F. and Welch T. (1978). *Lancet* **ii:** 116.
2. Cohen M. L. and Falkow S. (1981). *Science* **211:** 842
3. Bergdoll M. S. and Schievert P. M. (1984) *Lancet* **ii:** 691
4. Igarashi H., Fujkawa H., Usami H., Kawabata S. and Morita T. (1984). *Infection and Immunity* **44:** 175.

VET-RPLA TOXIN DETECTION KIT

Code: TD0920

A kit for the detection of Vibrio cholerae enterotoxin and Escherichia coli heat-labile enterotoxin in culture filtrates by reversed passive latex agglutination.

INTRODUCTION

Certain strains of *Escherichia coli* are known to produce enterotoxins. These enterotoxingenic *E. coli* (ETEC) strains are a common cause of diarrhoea in developing countries and of travellers' diarrhoea. ETEC strains produce one or both of two different enterotoxins, a head-labile enterotoxin (LT) and a head-stable enterotoxin (ST). The LT enterotoxin as antigenic structures similar to those found on *Vibrio cholerae* enterotoxin (CT). Antiserum taken from rabbits, immunised with CT, will therefore react with both CT and LT[1].

The VET-RPLA (TD0920) test is designed for the detection of LT or CT in culture fluid. A positive result given in the test indicates that the organism produces the relevant enterotoxin. The technique of reversed passive latex agglutination (RPLA) enables soluble antigen such as bacterial toxins to be detected in an agglutination assay.

In a standard agglutination assay, soluble antibody reacts with particulate antigen such as bacterial cells. However, in a **reversed** agglutination assay. the antibody, which is attached to particles, reacts with the soluble antigen. The particles (in this case, latex) do not, themselves, play a part in the reaction and they are therefore **passive**. The cross-linking of the latex particles by the specific antigen/antibody reaction results in the visible **latex agglutination** reaction.

PRINCIPLE OF ASSAY

Polystyrene latex particles are sensitised with purified antiserum taken from rabbits immunised with purified

Vibrio cholerae enterotoxin. These latex particles will agglutinate in the presence of *V. cholerae* enterotoxin (CT) or *E. coli* heat-labile enterotoxin (LT). A control reagent is provided which consists of latex particles sensitised with non-immune rabbit globulins.

The test is performed in V-well microtitre plates. Dilutions of the culture filtrate are made in two rows of wells. A volume of the latex suspension is added to each well and the contents mixed. If either toxin is present, agglutination occurs which results in the formation of a lattice structure. Upon settling, this forms a diffuse layer on the base of the well. If the enterotoxins are absent or at a concentration below the assay detection level, no such lattice structure can be formed and, therefore, a tight button will be observed.

PRECAUTIONS

This product is for *in vitro* diagnostic use only.

Do not freeze.

Reagents with different lot numbers should not be interchanged.

Reagents and diluent contain 0.1% sodium azide as a preservative. Sodium azide may react with lead or copper plumbing to produce metal azides which are explosive by contact detonation. To prevent azide accumulation in plumbing, flush with copious amounts of water immediately after waste disposal.

STORAGE

The VET-RPLA Kit (TD0920) must be stored at 2-8°C. Under these conditions the reagents will retain their reactivity until the date shown on the kit box. After reconstitution the enterotoxin control should be stored at 2-8°C. Under these conditions, the reconstituted enterotoxin control will retain its reactivity for 3 months, or until the date shown on the box, whichever is the sooner.

SAMPLE PREPARATION

V. cholerae and *E. coli* for testing may be recovered from clinical samples and identified by suitable techniques described in standard textbooks.

It should be noted that *E. coli* is a normal inhabitant of the bowel and there may be more than one serotype present in the sample. It is therefore recommended that at least 6 colonies are separately tested to ensure a higher probability of detecting an enterotoxin-producing strain. Some ETEC strains produce LT in culture at a low level. Treatment of the broth culture with polymixin B should release sufficient LT for detection.

V. cholerae normally produces a large quantity of enterotoxin in culture and no special medium or extraction technique is required.

METHOD OF USE

Materials required but not provided

Microtitre plate (V-well) and lid

Fixed or variable pipette and tips (25 µl)

Centrifuge capable of generating 900 g (typically 3000 rpm in a small bench top centrifuge) **or** membrane filtration, unit using low protein-binding disposable filters, with a porosity of 0.2 µm - 0.45 µm (such as Millipore SLGV)

Orbital Shaker

Culture media for promotion of enterotoxin production (suitable media are Peptone Water (Oxoid (CM0009)) adjusted to pH 8.4 for *V. cholerae* strains and Mundell's Medium[2].

Mundell's Medium[2] Formulation

Formula	***gm/litre***
Casein Peptone (Oxoid LP0042)	20.0
Sodium chloride	2.5
Yeast Extract (Oxoid LP0021)	6.0
Di-potassium hydrogen phosphate	8.7
Dextrose	2.5
Ferric chloride	0.005
Manganese chloride	0.005
Magnesium sulphate	0.05
pH 8.5 ± 0.2	

Polymixin B

Sodium hypochlorite solution (>1.3% w/w)

25 µl dropper (optional)

25 µl diluter (optional)

Micromixer (optional)

Moisture box (optional)

Components of the Kit
Instruction Leaflet

TD0921 Sensitised Latex - Latex suspension sensitised with specific antibodies (rabbit IgG) against *V. cholerae* enterotoxin.

TD0922 Latex control - Latex suspension sensitised with non-immune rabbit globulins.

TD0923 Enterotoxin control - Dried *V. cholerae* enterotoxin.

TD0924 Diluent - Phosphate buffered saline containing bovine serum albumin.

Toxin Production in Culture Fluid
***Vibrio cholerae* enterotoxin**

A suitable medium is Peptone Water (Oxoid CM0009) adjusted to pH 8.4.

Inoculate the strain to be tested into the culture medium and incubate, preferably with shaking, at more than 110 rpm on an orbital shaker at 30°C for 24 hours. After incubation, **either** centrifuge at 900 g for 20 minutes at 4°C and use the supernatant as the test sample **or** membrane filter using 0.2 µm - 0.45 µm low protein-binding filter and use the filtrate as the test sample.

***Escherichia coli* enterotoxin**

A suitable medium is Mundell's medium[2].

Inoculate the strain to be tested into the culture medium (suitable volumes of medium would be 2-20 ml) and incubate, preferably with shaking, at 37°C for 18-24 hours.

To the overnight broth culture (or a portion of it) add[3,4] polymixin B to a concentration of 10,000 units/ml. Incubate at 37°C for 4 hours. After incubation, either centrifuge at 900 g for 20 minutes at 4°C and use the supernatant as the sample **or** membrane filter using a 0.2 µm - 0.45 µm low protein-binding filter and use the filtrate as the test sample.

Note: It is advisable to check the particular cultural method of use with standard toxin-producing strains of *V. cholerae* or *E. coli* such as *E. coli* NCTC 11601.

Control
The reconstituted toxin control (TD0923) will agglutinate the sensitised latex (TD0921). The use of the toxin control will provide a reference for the positive patterns illustrated below (see Interpretation of Test Results). The control should be used occasionally only to confirm the correct working of the test latex. The toxin control is not provided at a specified level and therefore must not be used as a means of quantifying the level of toxin detected in the test sample.

Assay Method
Working Reagents

The latex reagents (TD0921, TD0922) and diluent (TD0924) are ready for use. The latex reagents should be thoroughly shaken before use to ensure a homogeneous suspension.

To reconstitute the enterotoxin control (TD0923), add 0.5 ml of diluent (TD0924) to each vial. Shake gently until the contents are dissolved.

Arrange the plate so that each row consists of a wells. Each sample needs the use of 2 such rows.

Using a pipette or dropper, dispense 25 µl of diluent (TD0924) in each well of the 2 rows **except** for the first well in each row.

Add 25 µl of test sample to the first and second well of each row.

Using a pipette or diluter and starting at the **second well** of each row, pick up 25 µl and perform doubling dilutions along each of the 2 rows. **Stop at the 7th well** to leave the last well containing diluent only.

Add 25 µl of sensitised latex (TD0921) to each well of the first row.

Add 25 µl of latex (TD0922) control to each well of the second row.

To mix the contents of each well, rotate the plate by micromixer or agitate by hand. Take care that no spillage occurs from the wells.

To avoid evaporation, either cover the plate with a lid, or place the plate in a moisture box. **Leave the plate undisturbed** on a vibration-free surface at room temperature for 20–24 hours. It will help subsequent reading of the test if the plate is placed on black paper for the duration of the incubation.

Examine each well in each row for agglutination, against a black background.

Centrifuge tubes, membrane filters, microtitre plates, lids and pipette tips should be sterilised by autoclaving at 121°C for 15 minutes or disinfected, before disposal in hypochlorite solutions (>1.3% w/w)

Dispose of toxin controls and culture extracts in hypochlorite solutions (>1.3% w/w)

INTERPRETATION OF TEST RESULTS

The agglutination pattern should be judged by comparison with the following illustration:

(–) (±) (+) (++) (+++)

Results classified as (+), (++), and (+++) are considered to be positive.

Results in the row of wells containing latex control (TD0922) should be negative. In some cases, non-specific agglutination may be observed. In such cases, the results should be interpreted as positive, provided that the reaction with sensitised latex (TD0921) is positive to a higher dilution of test sample than that seen with the latex control. The last well in all rows should be negative. If positive patterns are observed in some of these wells , the reaction should be regarded as invalid.

Note: With excess amount of CT, a prozone effect may be observed, i.e. negative pattern is obtained in wells containing test sample and sensitised latex. However, as a result of the doubling dilutions, the concentration of CT in each well along the row is progressively reduced and therefore the prozone effect due to excess amounts of CT is negated. A positive pattern of agglutination may, therefore, be seen after negative patterns in the first few wells of the row. With such results, the test sample should be classified as positive.

LIMITATIONS OF THE TEST

The sensitivity of this test kit in detecting CT is 1-2 ng/ml. Enterotoxin present at concentrations lower than this will, therefore, give negative results.

References

1. Ristaino P. A., Levine M., and Young C. R. (1983). *J. Clin. Microbiol.* **18:** 808-815.
2. Mundell D. H., Anselmo C. R. and Wishnow R. M. (1976), *Infection and Immunity* **14:** 383-388.
3. Evans D. J. Evans D. G and Gorbach S. L. (1974), *Infection and Immunity* **8:** 725.
4. Chapman P. (1987). Private communication.

FURTHER BACKGROUND INFORMATION

Almeida R. J., Hickman-Brenner F. W., Sowers, Evangeline G., Puhr, Nance D., Farmer III J. J. and Wachsmuth I. K. (1990) *J Clin. Microbiol.* **28:** 128-130.

E. COLI ST EIA

Code: TD0700

A test kit for the detection of heat-stable E. coli enterotoxin (ST) in culture filtrates or supernatants by competitive enzyme immunoassay (EIA).

INTRODUCTION

Certain strains of *Escherichia coli* are known to produce enterotoxins. These enterotoxigenic *E. coli* (ETEC) strains are a common cause of infantile diarrhoea in developing countries and of travellers' diarrhoea[1,3]. ETEC strains may produce a heat-liable enterotoxin (LT)* a heat-stable enterotoxin (ST) or broth. The ST enterotoxin which has traditionally been detected by means of the infant mouse assay has been termed ST_A ST_a or STI. This particular test has not been applicable to routine laboratory use due to its high cost, requirement for animal facilities and time-consuming nature. The other ST enterotoxin, which is not detected by the mouse assay, is termed ST_B, St_b and STII and has rarely been implicated in human disease.

The **Oxoid *E. coli* ST EIA kit** (TD0700), however, is suitable for use in any laboratory which requires a simple test for the detection of ST_a-producing *E. coli*. The test is rapid, sensitive and reliable and gives results consistent with the infant mouse assay (see Performance Characteristics)[2]. Based on the Competitive EIA format, the test uses a synthetic peptide toxin analogue and a monoclonal antibody to ensure specificity. The

assay is designed for the detection of ST_A in culture fluid. A positive result given in the test indicates that the organism produces the heat-stable enterotoxin.

*For the detection of E. coli LT enterotoxin use the Oxoid VET-RPLA kit (TD0920).

PRINCIPLE OF ASSAY

The test is performed in microtitre wells in the form of a competitive EIA technique. The microtitre wells are coated with pure synthetic *E. coli* heat-stable toxin. This solid-phase toxin is derived by the technique of peptide synthesis. The antibody-enzyme conjugate is composed of monoclonal antibody, chemically linked to the enzyme horseradish peroxidase (HRP). This conjugate binds specifically to the solid-phase toxin or to the toxin in the culture filtrate. The test is simple to perform. Culture fluid is added to the synthetic-toxin-coated wells followed by the antibody-enzyme conjugate.

After incubation, then washing, a substrate (hydrogen peroxide and *o*-phenylenediamine) is added. When heat-stable toxin is **not** present in the culture fluid, the conjugate will bind to the solid-phase toxin exclusively. After washing to remove the unbound conjugate, the substrate is added and a colour reaction will occur. This indicates a negative result. When heat-stable toxin is present in the culture fluid, it will compete with the solid-phase ST for conjugate, with the resultant reduction in binding of the conjugate to the solid-phase toxin. This will, therefore, cause a reduced colour intensity. At a sufficiently high concentration of heat-stable toxin, a very pale or even colourless reaction is seen. This indicates a positive result.

STORAGE

The Oxoid *E. coli* ST EIA kit (TD0700) must be stored at 2-8°C. **Note: Do not freeze.** Under these conditions the reagents as supplied will retain their activity until the date shown on the box. After dilution, the conjugate will retain its activity for 3 months and the wash buffer for 1 month when stored at 2-8°C. The substrate will only remain active for up to one hour after its preparation and should be discarded after use.

PRECAUTIONS

This product is for *in vivo* diagnostive use only. Phenylenediamine is toxic by inhalation, in contact with skin and if swallowed. May cause sensitisation by skin contact and is very toxic to aquatic organisms. May cause long term effects in the aquatic environment. After contact with skin, wash immediately with plenty of soap and water. Wear suitable protective clothing and gloves. In case of accident or if you feel unwell, seek medical advice immediately. *This material and/or container must be disposed of as hazardous waste*. Avoid release to the environment. Refer to Safety Data Sheet. Care should be exercised in the handling of cultures. Culture filtrates and the toxin controls. For correct disposal procedures, see the section given later in this instruction booklet.

METHOD OF USE

Materials required but not provided

Micropipettes, capable of delivering 10 µl, 100 µl, 200 µl and 1 ml.

Microtitre plate reader, capable of measuring optical density (OD) at 490 nm.

Spectrophotometer capable of reading absorbance at 650nm.

Culture medium suitable for enterotoxin production. (A suitable medium is 'CA-YE' broth[4] (see Appendix).)
Centrifuge, capable of generating 900 g (typically 3000 rpm in a small bench-top centrifuge) **or** membrane filtration unit using a low protein-binding disposable filter, with a porosity of 0.2 to 0.45 µm (such as Millipore SLGV).

Microtitre plate cover

Distilled water

Pasteur pipettes

Sodium hypochlorite solution (>1.3%w/v)

Micromixer (optional)

Paper towel

Components of the Kit

TD0701 ST-coated microtitre well-strips. 6 strips, each consisting of 16 wells held in a transit frame. Each well is coated with synthetic enterotoxin and contains a stabiliser.

TD0702 Positive control. 1.5 ml culture fluid of ST positive strain.

TD0703 Negative control. 1.5 ml culture fluid of ST-negative strain.

TD0704 Antibody-enzyme conjugate. 0.1 ml anti-ST monoclonal antibody labelled with horseradish peroxidase (supplied as a concentrate).

TD0705 Diluent for antibody-enzyme conjugate. 1.5 ml tris-HCl buffer containing bovine serum albumin.

TD0706 Substrate A. 6 vials of dried *o*-phenylenediamine (each for the preparation of 4 ml of substrate).

TD0707 Substrate B. 30 ml hydrogen peroxide solution. Substrate B (Hydrogen Peroxide Solution) and the Stopping Reagent (1.5 N Sulphuric Acid) are irritants. **Avoid contact with eyes and skin**.

TD0708 Wash buffer concentrate. 60 ml phosphate buffered saline containing Tween 20.

TD0709 Stopping reagent. 12 ml 1.5 N sulphuric acid.

TD0710 Holding frame.

Instruction leaflet.

Sample preparation

Isolation of *E. coli*

E. coli for testing may be recovered from clinical samples and identified using suitable techniques described in standard text books. It should be noted that *E. coli* is a normal inhabitant of the bowel and there may be more than one strain present in the sample. A higher probability of detecting an enterotoxin-producing strain will be ensured by the preparation of several cultures, each inoculated with a single colony.

Toxin Production in Culture Fluid

Inoculate 2 ml of sterile CA-YE broth with the *E. coli* isolate to be tested. Incubate the culture, with continuous and vigorous shaking, at 37°C for 18-24 hours. Ensure that sufficient growth has occurred. A minimum OD650 (1cm pat length) of 0.5 for a 1 in 10 dilution for the culture must be achieved. After incubation **either** centrifuge at 900g for 30 minutes at 4°C and use the supernatant as the test sample **or** membrane filter, using a 0.2 to 0.45 µm low protein-binding filter and use the filtrate as the test sample.

Notes

The medium volume and head space ratio is critical for achieving sufficient growth and therefore toxin production. A volume of 2 ml and a head space of at least 25 ml is required.

The heat-stable enterotoxin produced in culture will lose activity over a period of time. It is, therefore, necessary to test the culture as soon as possible after growth. Ensure that the supernatant or filtrate is non-turbid and of low viscosity. Do not heat the culture. Although the heat-stable enterotoxin will survive a temperature of 100°C, high temperatures may adversely affect the test.

Substances such as sodium azide and formalin, which affect the reactivity of peroxidase, should not be used in this test.

Failure to observe any of the above points may give false results.

Assay Method

Preparation of Working Reagents

Antibody-enzyme conjugate

Add 1 ml of diluent (TD 0705) to the antibody-enzyme conjugate (TD0704) and shake well. This will provide sufficient working reagent for 100 wells.

Store at 2-8°C. Use within 3 months.

Wash buffer

For each well-strip (16 wells), approximately 40 ml of wash buffer should be prepared. Dilute the wash buffer concentrate (TD0708) 1 in 10 i.e., 4 ml of concentrate with 36 ml of distilled water.

Store at 2-8°C. Use the diluted wash buffer within 1 month.

Substrate solution

Important: Prepare this reagent immediately prior to use. Ensure that it is used within 1 hour of preparation.

Add 4 ml of substrate B (TD0707) to 1 vial of substrate A (TD0706) and shake well.

This will provide sufficient working reagent for 40 tests.

Test Procedure

Identify the required number of strips. Each strip of 16 wells will accommodate 13 specimens, in addition to positive, negative and culture medium controls. If more than 13 tests are required during the test session, use further strips.

Note: At any one test session **only 3 wells** need be reserved for control purposes.

Remove individual strips from the transit frame by cutting the plastic sealer along the groove between strips. Remove the plastic sealer, then place the strip(s) in the holding frame (TD0710) and, just before use, discard the well contents. Add wash buffer (TD0708) to each of the wells using a Pasteur pipette and again discard the contents.

Note: After rinsing, avoid drying and use the wells within the same day.

Using a pipette and tip, add 200 µl of the negative control (TD0703) to the first well.

Using a fresh tip, add 200 µl of the positive control (TD0702) to the second well.

Using a fresh tip, add 200 µl of sterile culture medium, as used for the stains under test, to the third well.

Using fresh tips each time, add 200 µl of each test sample (culture supernatant) to the remaining wells as required. Carefully note the position in the microtitre well-strip of each control and sample.

Using a pipette and tip, add 10 µl of the diluted antibody-enzyme conjugate (see Preparation of Working Reagents) to every well. Care should be taken to avoid contamination of the pipette tip with the well contents when delivering this reagent.

To mix the contents of each well, rotate the plate using a micromixer or agitate by hand. Take care that no spillage occurs from the wells.

Cover and incubate the microtitre plate undisturbed at room temperature for 90 minutes.

After the incubation period, aspirate the contents from the wells using a Pasteur pipette and discard the liquid into a suitable disinfectant.

Add 200 µl of diluted wash buffer (see Preparation of Working Reagents) to each well.

Note: It is important that **all** blank wells are included at each wash stage.

Mix for 30 seconds and discard the liquid into disinfectant using a Pasteur pipette. Repeat this washing procedure a further 4 times, finally inverting the plate on a paper towel and gently remove thoroughly the residual wash buffer.

Add 100 µl of freshly prepared substrate solution (see Preparation of Working Reagents) to every well used, **including the three control wells**. Mix for a few seconds using a micromixer or agitate by hand.

Leave the plate at room temperature, avoiding strong sunlight, for 30 minutes to allow the colour to develop.

Carefully add 100 µl of the stopping reagent (TD0709) to each well.

READING AND INTERPRETATION OF RESULTS

The test has been performed correctly if

(a) the positive control well is colourless

(b) the negative control well shows a yellow colour

(c) the culture medium control shows a yellow colour and has a similar optical density as that of the negative control.

Compare (by eye) the colour intensity produced by each specimen with the three controls. A specimen with a colour intensity corresponding to that of the positive control should be regarded as a definite positive. Similarly, a specimen with a colour intensity corresponding to that of the negative control should be regarded as a definite negative. Specimens which show an ambiguous or uninterpretable result should be read by a microtitre plate reader at a wavelength of 490 mm, using the following procedure:-

Zero the machine on a blank well, (if a blank well is not available, temporarily aspirate the positive control into a pipette and zero on the empty well, then return the liquid to the well). Measure the optical density (OD) of all the wells including positive, negative and culture medium controls. Determine the adjusted OD of each of the test samples using the following formula:

Adjusted OD = Specimen OD divided by Negative Control OD minus Positive Control OD.

A positive result for ST_A is given by an Adjusted OD value of **0.5 or lower (<0.5)**

A negative result for ST is given by an Adjusted OD of **greater than 0.5 (>0.5)**

For a specimen to be regarded as ST_A – positive, it must have an OD which is lower than that of the culture medium control. If the culture medium control shows a lower OD than the negative control, it may be necessary to alter the medium constituents and repeat the test.

DISPOSAL

The microtitre strips should be removed from the frame after use and, together with centrifuge tubes, membrane filters, microtitre plate and lid, pipette tips and paper towels, sterilised by autoclaving at 121°C or disinfected before disposal.

Dispose of control toxins and culture extracts in hypochlorite solution (>1.3% w/v).

LIMITATIONS
This test must only be performed on culture supernatants or filtrates. The sensitivity of this test kit in detecting heat-stable toxin is 10 ng/ml. Enterotoxin present at concentrations lower than this will, therefore, give negative results.

PERFORMANCE CHARACTERISTICS
In clinical comparisons of the performance of the Oxoid *E. coli* ST EIA (TD0700) test and the infant mouse assay, results demonstrated 100% agreement (200 strains, 100 positive, 100 negative) between the two methods.[2]

APPENDIX

CA-YE Broth Formulation[4]

Formula	***gm/litre***
Casein hydrolysate (Oxoid LP0041)	20.00
Yeast extract (Oxoid LP0021)	6.00
Sodium chloride	2.50
Dipotassium hydrogen phosphate	8.71
Salt solution*	1 ml

*To prepare the salt solution, dissolve the following in distilled water:

Magnesium sulphate ($MgSO_4$ $7H_20$) – 5.0%

Manganese chloride ($MnCL_2$ $4H_20$) – 0.5%

Ferric chloride ($FeCL_3$ $6H_20$) – 0.5%

Adjust the pH to 8.0-8.2, dispense into 2 ml volumes in a Universal bottle or a stoppered test tube of at least 25 ml capacity. Autoclave at 121°C for 15 minutes.

Note: The medium head space to volume ratio is critical for achieving sufficient growth and toxin production.

References
1. Gross R. J. and Rowe B., (1985) *J. Hyg., Camb*., **95.** 531–550.
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Further background information
Takao T., Hitouji T., Aimoto S., *et al.* (1983). *FEBS Letters*, **152.** No 1., 1-5
Ikemura H., Watanabe H., Aimoto S., *et al.* (1984) . *Bull Chem. Soc*. Jpn., **57.** 2550-2556.

VTEC-RPLA TOXIN DETECTION KIT

Code: TD0960

A reverse passive latex agglutination test for the detection of verocytotoxins VT1 and VT2 produced by Escherichia coli from cultured from food and faecal samples.

INTRODUCTION
Verocytotoxin producing *E. coli* (VTEC) are transmitted through food, water and person-to-person contact, and are known to cause a range of illnesses from self limiting watery diarrhoea and haemorrhagic colitis to haemolytic uraemic syndrome (HUS) and thrombotic thrombocytopenic purpura.[1,2,3,4,5]

These illnesses can be fatal, making the increasing incidence of VTEC contamination a cause for widespread concern.

Unlike other tests which detect the presence of strains such as *E. coli* O157 (of which some, but not all, produce verocytotoxins), the Oxoid VTEC-RPLA (TD0960) test detects the toxins themselves, providing a clear and specific indication of VT1 or VT2 production. This overcomes the problem of positive results from other latex and culture assays (i.e. those which detect the organism rather than the toxin) where non-toxin-producing *E. coli* O157 strains are present. Similarly, it also overcomes the problem of negative results in cases where non-O157 strains are responsible for toxin production.

The test can be used with isolates cultured from both food and faecal samples.

PRINCIPLE OF THE ASSAY

Polymer latex particles are sensitised with purified rabbit anti-serum which is reactive either with *E. coli* verocytotoxin VT1 or VT2. The latex particles will agglutinate in the presence of one or both of the *E. coli* verocytotoxins. Agglutination results in the formation of a lattice structure. On settling, this forms a diffuse layer on the base of a V-bottom microtitre well. If *E. coli* verocytotoxin is absent, or at a concentration below the assay detection level, no such lattice structure can be formed, and a tight button will therefore be observed. The use of polymyxin B solution facilitates the release of verocytotoxins.[6,7]

SAMPLE PREPARATION

E. coli isolates are recovered and identified from food or clinical samples using standard procedures.

Serogroup O157 organisms may be identified using Sorbitol MacConkey Agar (Oxoid CM0813), since they are unable to ferment sorbitol. Other serogroups of *E. coli* do, however, produce verocytotoxin.

METHOD OF USE

1. Materials required but not provided:

Microtitre plates (V-well) and lids.

Fixed or variable pipette and tips 25 µl.

Centrifuge capable of generating 900 g (typically 4,000 rpm in a small bench-top centrifuge).

Membrane filtration unit using low protein-binding disposable filters with a porosity of 0.2 µm - 0.45 µm (such as Millipore SLGV).

CA-YE (see Appendix) Broth or Brain Heart Infusion Agar (Oxoid CM0375).

Polymyxin B (see 3.2.2).

Sodium chloride solution (0.85%).

Sodium hypochlorite solution (1.3% w/w).

25 µl dropper (optional).

25 µl diluter (optional).

Micromixer (optional).

Moisture box (optional).

2. Components of the kit:

TD0961 Sensitised latex VT1 Latex suspension sensitised with specific antibodies (rabbit IgG) against *E. coli* verocytotoxin type 1.

TD0962 Sensitised latex VT2 Latex suspension sensitised with specific antibodies (rabbit IgG) against *E. coli* verocytotoxin type 2.

TD0963 Latex Control Latex suspension sensitised with non-immune rabbit globulins.

TD0964 Verotoxin Control (VT1) Dried *E. coli* verocytotoxin type 1.

TD 965 Verotoxin Control (VT2) Dried *E. coli* verocytotoxin type 2.

TD0966 Diluent Phosphate buffered saline.

Instruction leaflet

3. Toxin Production and Extraction

E. coli may be tested for verocytotoxin production by growth in culture media. Growth of the organism may be performed in a liquid medium (CA-YE Broth (see Appendix)), or on a Brain Heart Infusion Agar (Oxoid CM0375). Growth on agar is followed by extraction in a polymyxin B solution.

3.1 Broth culture method

3.1.1 Isolated organism is inoculated into CA-YE Broth (see Appendix) and incubated at 37°C for 18-20 hours with vigorous shaking (120-150 oscillations per minute).

3.1.2 After growth, the culture is either centrifuged at 4,000 rpm for 20 minutes at 4°C or filtered using a 0.2 µm -0.45 µm low protein binding filter (such as Millipore SLGV). The filtrate is retained for the verocytotoxin assay.

3.2 Solid culture method

3.2.1 Isolated organism is inoculated onto Brain Heart Infusion Agar (Oxoid CM0375) slopes (10ml volumes) and incubated at 37°C for 18-20 hours.

3.2.2 After incubation, the growth is removed using a microbiological loop and suspended in 1 ml of a 0.85% sodium chloride solution containing polymyxin B at a concentration of 5,000 units per ml.

3.2.3 Extraction is continued for 30 minutes at 37°C, shaking occasionally.

3.2.4 After extraction the culture is either centrifuged at 4,000 rpm for 20 minutes at 4°C or filtered using a 0.2 µm - 0.45 µm low protein binding filter. The filtrate is retained for the verocytotoxin assay.

4. Control

Each reconstituted toxin control (TD0964, TD0965) will cause agglutination with its homologous test latex. The use of the toxin controls will provide reference for the positive patterns illustrated below (see Interpretation of Test Results). The controls need only be used occasionally in order to confirm that the test latex reagents (TD0961, TD0962) are working correctly. The toxin control is not provided at a specified level and must not therefore be used as a means of quantifying the level of toxin detected in the test sample.

5. Assay method

5.1 The latex reagents (TD0961, TD0962) and diluent (TD0966) are ready for use. The latex reagents should be thoroughly shaken before use to ensure an homogeneous suspension.

To reconstitute the control toxins (TD0964, TD0965), 0.5 ml of diluent is added to each vial. The contents are shaken gently until dissolved.

5.2 The plate is arranged so that there are 3 columns, each consisting of 8 wells.

5.3 Using a pipette or dropper, 25 µl of diluent (TD0966) is dispensed into each well.

5.4 25 µl of test sample supernatant is added to the first well of each column.

5.5 Starting at the first well of each column, a pipette or diluter is used to pick up 25 µl and perform doubling dilutions down each column, up to and including row 7. (NB: 25 µl of sample and Buffer mix must be removed from the 7th well and discarded). The last row of wells is left containing diluent (TD0966) only.

5.6 25 µl of the test latex VT1 (TD0961) is added to each well in the first column.

5.7 25 µl of the test latex VT2 (TD0962) is added to each well in the second column.

5.8 25 µl of latex control (TD0963) is added to each well in the third column for the purpose of detecting false agglutination reactions.

5.9 Toxin controls (TD0964, TD0965) are assayed in the same manner as test samples.

5.10 The contents of each well are mixed either by rotating the plate using a micromixer or agitating by hand. Care must be taken to avoid spillage.

5.11 To avoid evaporation, either cover the plate with a lid, or place the plate in a moisture box. Leave the plate undisturbed on a vibration-free surface at room temperature for 20-24 hours. It will help subsequent reading of the test if the plate is placed on black paper for the duration of the incubation.

5.12 Each well in each column is examined for agglutination against a black background.

5.13 Centrifuge tubes, membrane filters, microtitre plates, lids and pipette tips should be sterilised by autoclaving at 121°C for 15 minutes or disinfected, before disposal, in hypochlorite solutions (>1.3% w/w).

5.14 Culture extracts, food extracts, samples and toxin controls should be disposed of in hypochlorite solutions (>1.3% w/w).

INTERPRETATION OF TEST RESULTS

The agglutination pattern should be judged by comparison with the following illustration:

Results classified as (+), (++) and (+++) are considered to be positive. Results classified as (±) and (-) are considered to be negative. Results in the 3rd column of wells containing latex control (TD0963) should be negative as should the 8th row of columns 1 and 2 which contain no sample. If positive patterns are observed in these wells, the test should be regarded as invalid.

In some cases, non-specific agglutination may be observed. In such cases, the results should be interpreted as positive, provided that the reaction with the test latex (TD0961, TD0962) is positive to a dilution of test sample four times higher than that seen with the latex control (TD0963).

In samples from high-level verocytotoxin producing strains, negative patterns of agglutination in the lower dilution wells may be observed due to antigen excess (i.e. the prozone effect); since, however, the amount of toxin in each test well is reduced 2-fold at each dilution, inhibition of agglutination is overcome and true positives can be read.

LIMITATIONS OF THE TEST

The sensitivity of the test is 1 to 2 ng/ml of *E. coli* verocytotoxin. Verocytotoxin present at concentrations lower than this will therefore give negative results. The production of VT1 and VT2 by *E. coli* isolated from clinical or food sample does not constitute a diagnosis of disease. Strains producing verocytotoxin have been isolated from healthy individuals and farm animals. New unused V-well plates should be used, as scratched wells may cause inconsistent results. Reagents with different lot numbers should not be interchanged or mixed.

APPENDIX

CA-YE Broth Formulation[8]

	Grams per Litre
Casein hydrolysate (Oxoid LP0041)	20.00
Yeast extract (Oxoid LP0021)	6.00
Sodium chloride (NaCl)	2.50
Di-potassium hydrogen phosphate (K_2HPO_4)	8.71
Salts solution	1 ml
To prepare the salt solution, dissolve the following in distilled water:	
Magnesium sulphate ($MgSO_4.7H_2O$)	5.0%
Manganese chloride ($MnCl_2.4H_2O$)	0.5%
Ferric chloride ($FeCl_3.6H_2O$)	0.5%

Adjust the pH to 8.0 - 8.2 with 0.1N NaOH, dispense into suitable volumes (2-10 ml), then autoclave at 121°C for 15 minutes.

References

1. Centers for Disease Control – United States, (1982) *Morb. Mortal. Wkly. Rep*., **31:** 580-585.
2. Konowalchuk J., Speirs J. I. and Stavric S. (1977) *Inf. Immun*., **18:** 775-779
3. Karmali M. A., Steele B. T., Petric M. and Lim, C. (1983) *Lancet* **1:** 619-620
4. Pal C. H., Gordon, Sims H. V. and Briant L. E., (1984) *Ann. Intern. Med*., **101:** 738-742
5. Waters J. R., (1985) *Con. Dis. Wkly. Rep*., **11:** 123-124
6. Karmali M. A., Petric M., Lim C., Fleming R. C., Arbus G .S. and Lior H. (1985) *J. InL Dis*., **151:** 775-782
7. Cerny G. and Tenber, M. (1971) *Arch.Microbiol.* **78:** 166-179
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C. DIFFICILE TOXIN A TEST

Code: TD0970

C. difficile Toxin A Test is a rapid immunoassay for the direct qualitative detection of Clostridium difficile toxin A in faecal samples.

INTRODUCTION

Clostridium difficile is a Gram-positive anaerobic bacillus which has been identified as a common nosocomial pathogen that causes diarrhoea and pseudomembranous colitis associated with antibiotic therapy. In the 1960's and 70's, antibiotic associated pseudomembranous colitis became a major clinical problem. This was particularly due to the use of broad spectrum antibiotics such as ampicillin and cephalosporins. Larson *et al.* (1977) reported that stool samples from affected patients contained a toxin that produced cytopathic changes in tissue culture cells[1]. Subsequently, *C. difficile* was identified as the organism responsible for toxin production[2,3]

C. difficile is known to produce at least two toxins, designated A and B.

Toxin A (mwt. 308 Kda) causes fluid secretion, mucosal damage and internal inflammation.

Toxin B (mwt. 250 Kda) is approximately 1000 fold more potent as a cytotoxin than toxin A, but is not enterotoxic[4]. It is therefore accepted that toxin A plays a more important role in the pathogenesis of *C. difficile*[5].

Both toxins A and B are produced at the same time. The symptoms are primarily due to toxin A, but toxin B is thought to play a role in the disease by acting synergistically with toxin A[6].

When established in the colon, *C. difficile* produces the toxins which cause diarrhoea and

pseudomembranous colitis. *C. difficile* infection is almost exclusively a nosocomial infection. Outbreaks once established, are difficult to control and re-infection of patients is common.

Laboratory diagnosis depends on demonstrating the presence of *C. difficile* toxins in the stool sample. The most widely used test for detection of *C. difficile* toxin is cell cytotoxicity with neutralisation (using, for example, vero cells) which primarily detects toxin B[7]. This test takes up to 48 hours to obtain a result.

The Test Unit provides a rapid 30 minute method for the detection of *C. difficile* toxin A from patient samples, which is suitable for single or batch testing.

The Test Unit utilises a unique technology. The test is performed by addition of the sample to the absorbent pad in the Sample Window.

The absorbent pad incorporates a latex-labelled monoclonal antibody to *Clostridium difficile* toxin A. The pad is in contact with a test strip which contains regions of immobilised antibody.

To perform the test, the user delivers 125 µ ml of sample to the Sample Window, thus saturating the absorbent pad. The sample moves from the pad to the test strip, mobilising anti *C. difficile* toxin A mouse monoclonal antibody attached to blue latex beads present in the pad.

The sample, antibody-latex and *C. difficile* toxin A antibody-latex continues to move up the test strip to an immobilised region of anti *C. difficile* toxin A antibody.

The *C. difficile* toxin A attaches to the immobilised antibody and a blue line becomes visible in the Result Window.

As the sample continues to move up the test strip, it comes into contact with an immobilised strip of rabbit anti-mouse antibody, forming a blue line in the Control Window.

TEST PRINCIPLE

Clostridium difficile toxin A is extracted from faecal samples as described in 'Sample Preparation'.

The Test Unit contains monoclonal antibodies to *C. difficile* toxin A.

The extracted antigen is added to the pad in the Sample Window. The pad contains blue latex labelled with antibody. The extract rehydrates the complex and, if present, the specific antigen reacts with the antibody.

The antibody-antigen-latex complex travels through the test strip by capillary action to an area midway along the Result Window. This area contains an immobilised line of monoclonal antibody to *C. difficile* toxin A.

A further reaction between the antigen-antibody-latex complex and the fixed antibody is shown by the formation of a blue line in the Result Window.

If no *C. difficile* toxin A is present no line will appear in the Result Window.

OXOID C. DIFFICLE TOXIN A STOOL FILTRATION SYSTEM

Code: TD0975A

INTENDED USE

The Oxoid *C. difficile* Toxin A stool filtration system (TD0975A) is an alternative preparation technique for the isolation of *C. difficile* toxin A from stool samples prior to testing with the Oxoid *C. difficile* Toxin A Test (TD0970A). The system is intended exclusively for for use with the Oxoid *C. difficile* Toxin A Test and does not require the use of a centrifuge.

SAMPLE PREPARATION

1. Note: All components and reagents should be allowed to reach room temperature (18-30°C) for 30 minutes before use.
2. **(a) Liquid Stools** – Pippette 1 ml of sample diluent to the sample cup of the stool filtration system. Using a pipette, add 100 µ of stool to the sample diluent in the the sample cup.

   (b) Solid or Semi-Solid Stools – Pippette 1 ml of sample diluent to the sample cup. Using a loop, add a 5mm diameter bead-like portion of stool into the sample cup.
3. Mix the sample thoroughly using a pipette or loop, as appropriate, to form an even suspension.
4. Place the filter body (with cap in place) into the sample cup, and push downward on the cap so that the filter body stops approximately 2 mm above the level of the liquid.
5. Allow the particulate matter to settle by standing the stool filter system on a level surface for 1 minute.
6. Apply further pressure to the cap of the filter body, and continue to push downwards so that the sample passes through the filter into the filter body.

TEST PROCEDURE

Remove the toxin test unit from the foil pouch and place on a level surface. Remove the cap from the body of the filter unit. Invert the stool filtration system and apply 6 drops of the stool filtrate to the sample window of the *C. difficile* Toxin A Test unit, by squeezing the sides of the nozzle.

INTERPRETATION OF RESULTS

Following application of sample from the stool filtration system to the test device results should be interpreted as directed in the *C. difficile* Toxin A Test product insert.

11
CULTI-LOOPS AND QUANTI-CULT

2006

QC CULTURES

Culti-Loops®
Culti-Loops® are ready to use, disposable inoculating loops containing stabilised, preserved, viable microorganisms. The loop may be dissolved in liquid medium, Culti-Loop® Anaerobic Set Rehydration fluid or streaked directly onto an appropriate media.

Culti-Loops® are recommended for use in performance testing of media, stains, reagents, and identification kits, for maintenance of stock cultures and for the evaluation of bacteriological procedures. The loops are sealed individually in foil pouches and packaged in 10 loops per pack. Over 400 reference strains are available in this format.

Quanti-Cult Plus®
Quanti-Cult Plus® consists of a film of preserved organisms on the inside of a cap on a plastic vial, ready for rehydration and use in quality control procedures. No serial dilutions are required. When reconstituted as stated in the Product Insert, Quanti-cult Plus® delivers <100 colony forming units (CFUs) per 0.1 ml inoculum. Each vial will give 10 tests. The kits are packaged as 10 vials, giving 100 tests per kit.

OXOID CULTI-LOOPS®

These enable standardised cultures for quality control testing to be prepared quickly, easily and safely.

Culti-Loops are ideal for:

Performance testing of culture media, stains and diagnostic reagents

Evaluation of bacteriological procedures

Maintenance of stock cultures

Culti-Loops are ready to use, disposable bacteriological loops containing stabilised viable micro-organisms.

Culti-Loops are recommended for use in the performance testing of culture media, stains, diagnostic kits and reagents, for the maintenance of stock cultures and in the evaluation of bacteriological procedures. Each loop is individually packaged in a foil pouch and each can contains ten such loops.

Precautions
Culti-Loops contain viable micro-organisms and should be used only by individuals with bacteriological training. Refer to national Guidelines for Microbiological Containment Category information. After use, all loops and packaging should be placed into an appropriate container and sterilised by autoclaving before their final disposal. Do not place the loops into bunsen burners.

Storage
Store Culti-Loops at 2-8°C (or frozen for *Campylobacter* spp.). Remove only the quantity of loops required for immediate use. Under these conditions, Culti-Loops will retain their viability until the date shown on the foil pouch.

To open
Cut open the end of the foil packet as indicated on the label.

Evidence of deterioration
Each loop should contain an intact dried film. Do not use the loop if there is any evidence of hydration.

Procedure
The film in each loop is made from a gelatin formulation and then dried by special processing. To rehydrate the film, the loops must come into contact with both warmth and moisture.

Direct inoculation of Culti-Loops onto selective media may result in slow or absent growth. It is therefore recommended that where this is observed, inoculation onto non-selective media (such as blood agar) should precede sub-culture onto selective media.

The following two methods may be used for inoculation. Utilise the appropriate method for the selected micro-organism.

Direct Streak Method
This procedure is recommended for all non-fastidious micro-organisms.

1. Warm the appropriate plate medium to 37°C.

2. Remove the sheath from the loop.
3. Stab the loop into the medium or lay it flat on the warm, moist surface. Hold it in this manner for 10-15 seconds to allow for absorption of moisture.
4. Streak the plate in the usual manner. As many as five plates may be streaked with the same loop.
5. Incubate the plates in an appropriate atmosphere and temperature for the optimal growth of the organism.

Indirect (Broth) Method
This procedure is recommended for all fastidious micro-organisms.

1. Remove the sheath from the loop.
2. Cut off the loop shaft from the handle using sterilised scissors into a tube containing 0.5 to 1.0 ml of liquid medium. Use: (a) Tryptone Soya Broth (Oxoid CM129) or freshly prepared Thioglycollate USP (CM391) for bacteria specimens. (b) Sterile saline for mycology specimens.
3. Place tube in a 37°C incubator just long enough for the film to dissolve completely out of the loop. Shake the tube gently to suspend the organism.
4. Using the Pasteur pipette, inoculate the appropriate media with several drops and streak in the usual manner.
5. Incubate the plates in an appropriate atmosphere and temperature for the optimal growth of the organism.

Most organisms grow in 24-48 hours under the proper conditions. However, some exhibit a considerable lag phase and should be incubated for an additional 24 hours.

Disclaimer
Those who receive Culti-Loops are responsible for their safe storage, handling and use. Oxoid is not liable for damages or injuries resulting from the receipt and/or use of Culti-Loops. Oxoid is not liable for damages arising from the misidentification or misrepresentation of strains.

Reference
Prier J., Bartola E. and Friedman H. (1973) *Quality Control in Microbiology*. University Park Press, Baltimore.

OXOID QUANTI-CULTPLUS™

This range features convenient ready-to-use preserved micro-organisms for use in quality control procedures. Each delivers a specific range of colony forming units (CFUs) and may be used for:

Growth promotion

Bacteriostasis and fungistasis

Microbial limit tests

Media quality control etc.

Use
This product contains a specified number of preserved micro-organisms for use in quality control procedures[1,2,3]. Colony forming units (CFUs) are specified under defined procedures and growing conditions and cannot be guaranteed under other conditions. Results may vary with more inhibitory or selective media or with the same medium if of inferior quality.

Description
Each set consists of two vials packaged in a plastic bag. One vial, sealed within a silvery mylar envelope, contains a film of micro-organisms attached to the inside of the cap of the vial. The microbial film can be seen through the red cap as a black area inside the black 'O' ring of the cap. Rehydrating fluid is provided in the second vial.

Precautions
QUANTI-CULT$^{PLUS(TM)}$ contains live micro-organisms and should be used only by individuals with microbiological training. Properly disinfect any spills and sterilise used containers by autoclaving before final disposal.

Storage
Both rehydrating fluid and micro-organisms should be stored at refrigerator temperature (2-8°C) until time of analysis.

Expiration date
The reagents are stable through the expiration date on the label when stored as directed.

Evidence of deterioration
Each RED CAP should contain an intact dried film. Do not use if the film has come out of the cap or shows evidence of hydration.

To open
The foil envelope can be cut open with scissors.

To rehydrate
CAUTION

THE REHYDRATED SUSPENSION MUST BE USED WITHIN 30 MINUTES AFTER THE 15 MINUTE REHYDRATION PERIOD. DO NOT PLAN TO USE A REHYDRATED SAMPLE THROUGHOUT THE WORK DAY OR THE FOLLOWING DAY. PROLONGED HOLDING WILL ADVERSELY EFFECT THE NUMBERS OF VIABLE ORGANISMS.

1. Remove vials from the refrigerator. Place the rehydrating fluid vial (blue cap) in a 35-37°C incubator to warm.
2. Allow the vial (red cap) containing the microbial film (silver envelope) to warm to room temperature.
3. Remove the vial (red cap) containing the microbial film from the envelope.
4. Remove and discard the blue cap from the rehydrating fluid.
5. Remove the red cap containing the micro-organisms and transfer to the rehydrating fluid vial. Tighten cap.
6. Invert this vial, tap to be sure liquid is in contact with the inside of the cap, and place in 35-37°C incubator in an INVERTED position for 15 minutes* to dissolve the preserved micro-organisms. Autoclavable polyester racks may be used to hold the vials in an inverted position. Please note that the foam is an efficient insulating material and will retard warming or cooling of liquids.
7. Grasp bottom of vial, hold firmly in an inverted position, tap cap gently to mix suspension and aid dissolution. Excessive vigorous shaking will produce foam.

***CAUTION**
Look at the cap to make certain that all of the micro-organisms are in solution. Undissolved intact black particles can be seen through the plastic cap and/or vial. If this happens, reinvert the vial and place back in the incubator and observe closely every 1 to 2 minutes for complete dissolution. Undissolved microbial film will cause reduced counts but prolonged heating may also result in incorrect counts.

Inoculation and sample analysis
QUANTI-CULT$^{PLUS(TM)}$ is designed to deliver ten 100 µl inocula each containing between 10 and 100 CFUs from a single source.

QUANTI-CULTPLUS(TM) is suitable for evaluation of culture media used for the growth promotion and/or bacteriostasis and fungistasis procedures[1], other media[3], or quality control of any quantitative microbiological procedure[2].

CAUTION
THE REHYDRATED SUSPENSION MUST BE USED WITHIN 30 MINUTES AFTER THE 15 MINUTE REHYDRATION PERIOD. DO NOT PLAN TO USE A REHYDRATED SAMPLE THROUGHOUT THE WORK DAY OR THE FOLLOWING DAY. PROLONGED HOLDING WILL ADVERSELY EFFECT THE NUMBERS OF VIABLE ORGANISMS.

Hold the rehydrated suspension at room temperature throughout the 30 minute use period. Re-mix sample between withdrawals. When inoculating or transferring to a liquid matrix, it is advisable to have the receiving liquid pre-warmed to 35-37°C. Dispensing into a cold liquid may interfere with even distribution of the micro-organisms.

References
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2. Clesceri L. S., Greenberg A.E. & Trussel R.R. eds. 1989. *Standard Methods for the Examination of Water and Wastewater*. 17th ed. A.P.H.A., A.W.W. A. & W.P.C.F.
3. Mehlman I. J. 1984. *Appendix I. Culture media. In Bacteriological Analytical Manual*. A.O.A.C. Arlington, VA.

QUANTI-CULTPLUS(™)
Each tube contains 10 packs (10 tests per pack).
Each kit is designed to deliver between 10-100 cfu/0.1 ml.
Total diluent volumes per vial is 1.2 ml.

Note
The organisms used in QUANTI-CULT$^{PLUS(TM)}$ are derived from original ATCC stock cultures according to the number shown.

QUANTI-CULTPLUS(TM) are manufactured for Oxoid Ltd by Chrisope Technologies Inc., an FDA approved company.

FDA Registration No: 1625984. QUANTI-CULT$^{PLUS(TM)}$ is a registered trademark of Chrisope Technologies Inc, a division of REMEL.

12
INDEXES

2006

PRODUCT INDEX

A

B

C

D

E

F

G

H

I

K

L

M

N

O

P

R

S

T

U

V

W

X

Y

NUMERICAL PRODUCT LISTING

(CM) CULTURE MEDIA

(SR) STERILE REAGENTS

(LP) LABORATORY PREPARATIONS

CM

SR

LP