CYTOLOGY SPECIMEN COLLECTION MANUAL

PACIFIC RIM PATHOLOGY
SHARP HEALTHCARE

November 2021

CT 045.0

PREFACE

The purpose of this manual is to provide general instructions for cytology specimen collection and handling where specimen quality may affect the cytologic diagnosis. It is not intended to encompass the whole process of specimen collection such as special techniques, patient preparation, or contraindications. Universal precautions should always be followed for any patient or specimen contact.

For further assistance with individual cases or general questions, please contact the Pacific Rim Cytology Department (858-261-7284), Sharp Copley Cytology Department (858-262-6973), or Dr. Wayne Muller (858-939-3660).

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San Diego, CA 92123
858-261-7284
858-939-3660
https://pacificrimpathology.com/

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I. GENERAL INFORMATION

A. LAB HOURS, CYTOLOGY DEPT. AT PACIFIC RIM PATHOLOGY:

Processing of specimens:	Mon to Fri:	6:00 am – 5:00 pm
	Sat & Sun:	CLOSED.
Screening of specimens:	Mon to Fri:	8:00 am – 4:00 pm
Emergencies:	Pathologist always ON-CALL 24 hours per day, 7 days per week	

B. TELEPHONE NUMBERS:

Cytology results, Pacific Rim:	858-261-7284
Cytology results, Copley Lab:	858-262-6973
Pathologist ON-CALL:	858-262-6810 (ask for pathologist on-call)
Supplies and Requisitions:	858-261-7284 (Fax: 858-939-1447)

C. SPECIMEN SUBMISSION TO LABORATORY:

1) Specimens may be submitted to the lab during business hours, but generally will not be processed until the following morning.

2) Specimens must be labeled properly and requisition forms completely filled out. Samples transported must be packed to assure that all personnel are protected from biologic hazards associated with handling of laboratory specimens.

3) Send all Gyn Cytology specimens and out-patient Non-Gyn specimens to Pacific Rim Pathology. Contact Pacific Rim laboratory for courier instructions.

   Pacific Rim Pathology
   9295 Farnham Street, Suite 100
   San Diego, CA 92123

   Send **in-patient** Non-Gyn Cytology specimens by hospital courier to Pathology Specimen Log-in Department at Sharp Copley Laboratory.

   Sharp Copley Laboratory
   5651 Copley Drive, Suite B
   San Diego, CA 92111

4) Ideally, if a specimen is to be used for any lab test other than cytology, split the specimen during collection and send separately with separate requisition forms. Cytology fixatives and refrigeration may render the specimen inadequate for other tests.

5) If the specimen can not be split at the point of origin, send the specimen to the hospital lab and clearly indicate on the requisition form which multiple tests are required. Do NOT refrigerate or fix any cytology specimen that must be shared with Microbiology or any other department.

6) Hospital lab: for large-volume body fluids, if an aliquot is prepared, please send the maximum volume possible for cytology processing, or at least 100 mL (e.g., two 50-mL centrifuge tubes). Evaluate the specimen prior to aliquoting, and write the description on the requisition, with initials and date.

7) Packaging instructions:
   - Assure all tubes are securely stoppered and lids are tight.
   - Assure that specimen temperature requirement is met.
   - Place labeled specimens in a Laboratory Specimen Transport zip-lock plastic bag and seal the bag.
   - Place the requisition in the outside pouch of the zip-lock bag.
   - Contaminated specimen containers or requisitions will be rejected.
   - Place specimen bags in a rigid, leak proof transport unit that maintains temperature requirements. The transport unit must be marked with a Biohazard Label. Deliver to lab immediately.

D. REPORTING TIME AND STAT SPECIMENS:

1) Non-Gyn specimens will generally be reported out within 3 business days from receipt date.

2) Gyn specimens will generally be reported out within 1 week.

3) If it is important for the results on any particular specimen to be reported to a physician sooner, please write "**STAT**" at the top of the requisition form with the phone number and name of doctor to whom the results should be telephoned.

NOTE: For critical specimens, please discuss the case with a pathologist.

E. SUPPLIES:

Supplies are available from the Cytology Department including:
- PreserveCyt solution for GYN
- Cytolyt solution for FNAs
- Requisition Forms

Please call 858-261-7284, or fax a "Supplies Request Form" to 858-939-1447.

II. GENERAL SPECIMEN REQUIREMENTS

A. GENERAL CRITERIA REQUIRED FOR EVALUATION OF SPECIMENS:

1) Patient's name AND second identifier (e.g., MRN or date of birth) on all slides and fluid specimen containers.

2) Source of specimen (if multiple sources were sampled) on all slides and fluid specimen containers.

3) Properly completed and matching requisition form including patient name, DOB, history, ICD10 codes, and specimen source. (See next page for more information.)

4) Specimen intact (*i.e.*, slides not broken, container not leaking).
***Please remove needles from all syringes before sending to lab.*

NOTE: If the above conditions are not met, the specimen may either be rejected without being processed, or an "Unsatisfactory" report will be issued.

B. REQUISITION FORMS:

1) Use a separate cytology requisition for each patient (but multiple specimens on one patient may be placed on the same form).

2) The form must contain the following information or the specimen may be delayed, rejected, or reported as "Unsatisfactory".

Patient information:
- Patient's full name
- Date of birth
- Sex
- SSN or MRN (strongly requested)
- Patient's address and phone number

Insurance information:
- Complete insurance information or copy of insurance card (both sides)

Patient history:
- Pertinent patient history and treatment
- Pathologic conditions
- Instrumentation or therapy
- ICD10 codes

Specimen:
- Exact type or source of each specimen
- Method of collection
- Date of collection
- Test(s) requested
- Name of ordering provider with address and phone number

3) ADDITIONAL information required for Gyn (Pap) specimens:

Essential information:
- Exact source (i.e. Cervical and/or vaginal)
- First day of last menstrual period (LMP)
- Previous pap smear date and diagnosis
- ICD10 Code(s)

Pertinent history includes:
- Hysterectomy (Total or Cervix remaining)
- Pregnant or Post-partum
- Post-menopausal
- Hormones or BCPs
- Recent biopsy and diagnosis
- Radiation or other treatments
- Abnormal bleeding or other symptoms
- IUD
- Other neoplasms

III. SPECIMEN COLLECTION PROCEDURES

A. GENERAL DIRECTIONS FOR ALL DIRECT SMEAR SPECIMENS— Gyn (Conventional Pap Smears) and Non-Gyn (including skin and oral lesions, buccal smears, and nipple secretions):

1) Identify all direct smear specimens with patient's full last name, first initial, AND second identifier (e.g., MRN or DOB) written in pencil on the frosted end of each glass slide ***before the smear is taken***.

2) Using spatula or brush, gently and smoothly spread the sample over the surface of the slide creating a uniform layer (labeled side up!) For nipple secretions, touch glass slide to drop of secretion and gently slide across areolar area.

3) Complete the smearing procedure within ***2 seconds*** in order to avoid cellular degeneration. If multiple slides are prepared, fix (step #4) each slide separately, as it is prepared.

4) ***Immediately*** spray smears thoroughly with cytology spray fixative at a distance of approximately 10 to 12 inches from the smear. Allow slides to dry and send to lab in folder or container along with completed requisition form.

5) Another option is to drop slides immediately into a Coplin jar containing 95% alcohol. Ensure jar is capped tightly before sending to the lab.

NOTE: Do NOT place smears in same bag as biopsies because formalin vapors will adversely affect smear quality.

B. SKIN LESION SMEARS (TZANCK):

1) Open vesicle or blister with sterile scalpel.

2) Scrape base and sides of vesicle with a sterile metal spatula or scalpel. Use direct smear technique described above (III.A.).

3) The slide may be air-dried if stat results are desired.

C. GYN SPECIMENS (PAP TEST):
(from "ThinPrep Pap Test Quick Reference Guide", Hologic, 2015.)

i. General Guidelines

1) Paps should not be taken during menstruation. Ideal is 2 weeks after the first day of her last menstrual period.

2) The patient should not use vaginal medication, vaginal contraceptives, or douches for 48 hours before the exam.

3) The patient should refrain from intercourse 48 hours prior to the exam.

4) The cervical transformation zone should be thoroughly sampled in a patient with a cervix.

5) Use lukewarm water to lubricate the speculum. If necessary, sparingly apply *carbomer-free* lubricant (preferably Surgilube or PapTest Gel) on the exterior of the speculum blades avoiding the tip. Any questions, please call the laboratory.

6) Remove excess mucus or other discharge present before taking the sample. This should be gently removed with ring forceps holding a folded gauze pad.

7) Remove inflammatory exudate from the cervical canal by placing a dry 2x2" piece of gauze over the cervix and peeling away.

8) The cervix should not be cleaned by washing with saline.

9) The sample should be taken *before* application of acetic acid.

10) Please do not break off the device in the ThinPrep vial. Collection devices should be immediately rinsed in the vial and discarded.

11) If a hormonal evaluation is needed, a separate vaginal wall sampling is required.

ii. ThinPrep Pap: Broom-like Device Protocol

1) Insert the central bristles of the broom into the endocervical canal deep enough to allow the shorter bristles to fully contact the ectocervix. Push gently, and rotate the broom in a clockwise direction for five complete, 360 degree turns.

2) Rinse the broom as quickly as possible in the PreservCyt Solution vial by pushing the broom into the bottom 10 times, forcing the bristles apart. Swirl the broom **vigorously** to further release material. Discard the collection device.

3) Tighten the cap so that the torque line on the cap passes the torque line on the vial. Label with patient's name and second identifier, and send in a plastic bag with completed requisition.

iii. ThinPrep Pap: Endocervical Brush/Spatula Protocol

1) Obtain an adequate sampling from the ectocervix using a plastic spatula. Rotate the contoured end 360 degrees around the entire ectocervix while maintaining tight contact with the ectocervical surface.

2) Rinse the spatula at quickly as possible in the PreservCyt Solution vial by swirling the spatula **vigorously** 10 times. Discard the spatula.

3) Obtain an adequate sampling from the endocervix using an endocervical brush device. Insert the brush into the cervix until only the bottom-most fibers are exposed. Slowly rotate ¼ or ½ turn in one direction. DO NOT OVER-ROTATE.

4) Rinse the brush as soon as possible in the PreservCyt Solution by rotating the device 10 times while pushing against the PreservCyt vial wall. Swirl **vigorously**. Discard brush.

5) Tighten cap so that the torque line on the cap passes the torque line on the vial. Label with patient's name and second identifier, and send in a plastic bag with completed requisition.

iv. Conventional Pap Smears

1) Three suggested techniques:
   - Ectocervical followed by endocervical smear.
   - Smear slide with both sampling devices at once.
   - A single pass with one device sampling the entire t-zone.

2) Use direct smear technique described on previous page (III.A.)

v. Ancillary Tests on Gyn Specimens

Ancillary tests that may be ordered on the ThinPrep Pap specimen (up to 30 days from date of collection) or Hologic Aptima collection vial include:

- High Risk HPV test (or reflex if ASCUS)
- HPV 16 and 18/45 genotyping (or reflex if HPV positive)
- Chlamydia
- Gonorrhea
- Trichomoniasis
- BV/ CV (Vaginosis Panel)

D. ANAL SPECIMENS (ANAL PAP TEST):

1) Insert a water-moistened Dacron swab (or Cytobrush) 5-6 cm into the anus to ensure sampling of the anorectal transformation zone.

2) Slowly rotate the swab or brush in a cone-shaped arc while maintaining firm pressure against the mucosa.

3) Rinse swab/brush as quickly as possible in the PreservCyt solution by rotating the device in the solution 10 times while pushing against the PreservCyt vial wall. Swirl the device vigorously to further release material. Discard the brush.

4) Tighten the cap so that the cap torque line passes the torque line on the vial.

5) Record the patient's name and DOB on the vial and send in a specimen bag with completed requisition form, including date of collection and source of specimen. Also check-mark HPV molecular testing, if needed.

E. SPUTUMS:

1) Instruct patient to expectorate a deep cough specimen directly into container—saliva is NOT adequate for diagnostic purposes. The specimen must include material coughed up from the lungs. An induced specimen is preferred.

2) Send specimen directly to lab without fixative, with proper labeling and completed requisition. Refrigerate if delayed.

F. WASHINGS AND LAVAGES—Bronchial or Alimentary Tract Specimens:

1) Send specimen directly to lab without fixative, with proper labeling and completed requisition. Refrigerate if delayed.

G. BRUSHINGS—Bronchial or Alimentary Tract Specimens:

1) Insert brush into Cytolyt container and agitate.

2) Cut wire and send container to lab with brush enclosed, with proper labeling and completed requisition.

H. BODY CAVITY FLUIDS:

1) Please be sure to specify the exact specimen type (i.e. Paracentesis vs. abdominal washing).

2) Use appropriate size container for specimen volume. Small volume specimens (CSF or fluid less than 12 cc) should be sent in a small centrifugation tube.

3) Send specimen directly to lab without fixative, with proper labeling and completed requisition. Refrigerate specimens that are only for cytology if there is a significant delay.

I. URINARY TRACT SPECIMENS:

1) *****Please specify specimen collection method** (*i.e.*, voided urine, catheterized urine, urinary bladder washing, etc.)

2) Minimum volume of 30 ml is preferred.

3) Send specimen directly to lab without fixative, with proper labeling and completed requisition. Refrigerate if delayed.

J. FINE NEEDLE ASPIRATION OF NON-PALPABLE LESIONS (by CT scan, etc):

1) For rapid assessment of specimen adequacy, call for pathologist assistance (x3660) or send prepared slide(s) to pathology lab attention pathologist.

2) Prepare slides / specimen as described on next page (III.L.).

K. FINE NEEDLE ASPIRATION OF PALPABLE LESIONS:

1) The syringe gun, syringe, and needle are assembled. All slides and specimen containers are labeled with patient name and a requisition form is completed.

2) The lesion is palpated and its distance from the skin estimated.

3) Skin surface is sterilized with alcohol or other iodine-like solution.

4) The target is fixed with one hand, usually between index finger and thumb.

5) The needle is placed into the mass. Usually a distinct change in consistency of tissue is felt when a subcutaneous lesion is entered.

6) The plunger on the syringe is withdrawn to create a vacuum. The needle tip is moved within the mass with short movements, withdrawing approximately 2 to

5 mm and reinserting with redirection of the needle tip. At least three needle passes should be performed within the target lesion.

7) Negative pressure within the syringe is released when aspiration is complete or when any hemorrhagic material or fluid is noted within the needle hub or syringe.

8) If a cystic lesion is aspirated, the cyst fluid should be placed directly into a separate vial of Saccomanno solution. It is recommended that the cyst wall of the lesion then be re-aspirated to ensure that the cyst wall has been sampled.

9) Prepare slides / specimen as described below (III.L.).

L. FINE NEEDLE ASPIRATION—Specimen / Slide Preparation:

1) Have labeled slides and open alcohol ready.

2) A small drop of the aspirated material is carefully expressed onto the glass slide (labeled side up!). Several slides are usually prepared at one time in this fashion.

NOTE: If a drop can not be expressed, the syringe needle may be detached and the plunger withdrawn to fill the syringe with a small amount of air and the needle reattached to the syringe (Careful! Use single hand technique only). The specimen should then be more easily expressed.

3) Smears are prepared by placing one glass slide perpendicular to the other, over the expressed material. The expressed material is GENTLY smeared over the original glass slide surface by sliding the second perpendicular slide over its surface. (No resistance should be felt. It is not necessary to cover the entire slide with material.)

4) Once the smear is achieved, the original glass slide should be immediately placed in the alcohol fixative (have Coplin jar containing 95% alcohol ready). The remaining slides are smeared one at a time and placed in the alcohol fixative.

NOTE: If air-dried smears are desired, no fixative is required. These are sometimes helpful in lesions involving the thyroid gland, salivary gland, and lymph nodes. **The slides should also be labeled "air-dried"**.

5) The needle should then be rinsed with PreserveCyt or CytoLyt solution for cytospin or cell block preparations. This is done by drawing some fluid into the syringe and then expressing the entire contents into the vial.

6) All slides and specimen containers must be labeled with the patient name, second identifier, and source, and sent with a completed requisition form.

7) For Afirma reflex-testing, use two passes for FNA CytoLyt solution and two passes for FNAprotect collection tube. Send together in same package. See instructional video at: https://www.afirma.com/physicians/practice-resources/.

IV. REPORTING TERMINOLOGY

A. THYROID FNA:

1) Statement of specimen adequacy

2) General diagnosis category (Bethesda System category):
   - BC I. Non-diagnostic or Unsatisfactory
   - BC II. Benign
   - BC III. Atypia of Undetermined Significance or Follicular Lesion of Undetermined Significance
   - BC IV. Follicular Neoplasm or Suspicious for a Follicular Neoplasm
   - BC V. Suspicious for Malignancy
   - BC VI. Malignant

3) Descriptive diagnosis

B. OTHER NON-GYN SPECIMENS:

4) Statement of specimen adequacy

5) The diagnosis includes a general diagnostic category:
   - Negative for malignant cells
   - Atypical or abnormal cell population
   - Suspicious for malignant cells
   - Positive for malignant cells
   - Unsatisfactory specimen (no diagnostic interpretation)

6) Descriptive diagnosis

C. GYN SPECIMENS:

Reporting terminology follows The Bethesda System 2014.

1) Statement of specimen adequacy

2) General categorization:
   - Negative for Intraepithelial Lesion or Malignancy
   - Epithelial Cell Abnormality (See Interpretation/Result)
   - Other (See Interpretation/Result)

3) Interpretation/Result:
   - Negative for Intraepithelial Lesion or Malignancy
     - No Cellular Evidence of Neoplasia
     - Organisms
     - Other Non-Neoplastic Findings

- Other
  - Endometrial Cells (In a Woman ≥ 45 Years of Age)

- Epithelial Cell Abnormality
  SQUAMOUS CELL
  - Atypical squamous cells
    - of undetermined significance (ASC-US)
    - cannot exclude HSIL (ASC-H)
  - Low grade squamous intraepithelial lesion (LSIL), encompassing: HPV/mild dysplasia/CIN 1
  - High grade squamous intraepithelial lesion (HSIL), encompassing: moderate and severe dysplasia, CIN2, CIN3, and CIS
    - With features suspicious for invasion *(if invasion is suspected)*
  - Squamous cell carcinoma

  GLANDULAR CELL
  - Atypical
    - endocervical cells
    - endometrial cells
    - glandular cells, not otherwise specified (NOS)
  - Atypical
    - endocervical cells, favor neoplastic
    - glandular cells, favor neoplastic
  - Endocervical adenocarcinoma *in situ*
  - Adenocarcinoma
    - endocervical
    - endometrial
    - extrauterine
    - not otherwise specified (NOS)

- Other Malignant Neoplasms *(specify)*

*****NOTE: THE PAP TEST IS A SCREENING PROCEDURE TO AID IN THE DETECTION OF CERVICAL CANCER AND ITS PRECURSORS. BOTH FALSE POSITIVE AND FALSE NEGATIVE RESULTS ARE KNOWN TO OCCUR.**

V. REFERENCES:

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2) Bibbo, M. and D. C. Wilbur. 2015. Comprehensive Cytopathology, 4th ed. Elsevier, London.

3) Hologic. 2015. ThinPrep Pap Test Quick Reference Guides. www.thinprep.com.

4) Koss, L. 2005. Koss' Diagnostic Cytology and Its Histopathologic Bases, 5th ed. Lippincott Williams & Wilkins, Philadelphia.

5) Michael, C. W. and B. Davidson. 2016. Pre-analytical issues in effusion cytology. Pleura Peritoneum 1(1): 45-56.

6) Nayar, R. and D. C. Wilbur, eds. 2015. The Bethesda System for Reporting Cervical Cytology, 3rd ed. Springer, Switzerland.

7) Veracyte 2019. Afirma Thyroid FNA Analysis Ultrasound-Guided Fine Needle Aspiration of the Thyroid: Tips & Techniques. Veracyte, Inc. 6000 Shoreline Court, Suite 300, South San Francisco, CA, 94080, www.veracyte.com.