

# **CYTOLOGY SPECIMEN COLLECTION MANUAL**

**PACIFIC RIM PATHOLOGY**

**SHARP PATHOLOGY LABORATORY**

**September, 2006**

## **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 Cytology Department 619-295-0964 or Dr. Wayne Muller 858-939-3660.

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## I. GENERAL INFORMATION

### A. LAB HOURS:

Processing of specimens: Mon to Sat: 4:00 am - Noon  
Sunday: 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:

GYN Cytology results: 619-295-0964

NON-GYN Cytology results  
(And Biopsy results): 858-939-3660

Pathologist ON-CALL: 858-939-3650 (ask for pathologist on-call)

Supplies and Requisitions: 619-295-0964 (Fax: 619-295-0835)

### C. SPECIMEN SUBMISSION TO LABORATORY:

- 1) Specimens may be submitted to the lab at any time, but generally will not be processed until the following morning.
- 2) Send specimens by appropriate courier to Specimen Log-In Department in Main Laboratory at Sharp Memorial Hospital. (Sharp Rees-Stealy specimens should go to downtown SRS lab).
- 3) Ideally, if a specimen is to be used for any lab test other than cytology, the specimen should be split and sent separately with separate requisition forms. Cytology fixatives and refrigeration may render the specimen inadequate for other tests.
- 4) If the specimen can not be split at the point of origin, send the specimen directly to the 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.

D. REPORTING TIME AND STAT SPECIMENS:

- 1) Non-Gyn specimens will generally be reported out within 48 to 72 hours.
- 2) GYN specimens will generally be reported out within 5 business days.
- 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 phoned.

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

E. SUPPLIES:

Supplies are available from the Cytology Department including:

- Formalin (for tissue fixation)
- Pap Smear Collection Kits (Pap-Pak)
- Spray Fixative
- FNA supplies (slides, Saccomanno-green fluid, 95% alcohol)
- PreserveCyt solution for GYN and FNAs
- Requisition Forms
- Supplies Request Forms

Please call 619-295-0964

Or fax a "Supplies Request Form" to 619-295-0853.

II. **GENERAL SPECIMEN REQUIREMENTS**

A. GENERAL CRITERIA REQUIRED FOR EVALUATION OF SPECIMENS:

- 1) Patient's name on all slides and fluid specimen containers.
- 2) Properly completed and matching requisition form including patient name, DOB, history, IDC9 codes, and specimen source. (See next page for more information.)
- 3) 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 (strongly requested)
- Patient's address and phone number
- MRN (SRS patients)

Insurance information:

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

Patient history:

- Pertinent patient history and treatment
- Pathologic conditions
- Instrumentation or therapy
- ICD-9 codes

Specimen:

- Exact type or source of each specimen
- Method of collection
- Date of collection
- Name of submitting doctor with address and phone number

- 3) ADDITIONAL information required for GYN (pap smear) specimens:

Essential information:

- Exact source (i.e. Cervical and/or vaginal)
- First day of last menstrual period (LMP)
- Previous pap smear date and diagnosis
- ICD-9 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 (Pap Smears) and NON-GYN (including skin and oral lesions, buccal smears, and nipple secretions):

- 1) Identify all direct smear specimens with patient's last name and first initial 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. (Another option is to drop slides immediately into a Coplin jar containing 95% alcohol. Leave slides in alcohol for a minimum of 20 minutes.)
- 5) Allow slides to dry and send to lab in folder or container along with completed requisition form.

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

#### B. SKIN LESION SMEARS:

- 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 Smears):

- 1) The smear should not be taken during menstruation. Douching prior to the exam and lubricants should be avoided.
- 2) The cervical transformation zone should be thoroughly sampled in a patient with a cervix.

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.
- 3) If a hormonal evaluation is needed, a separate vaginal wall sampling is required.
  - 4) Use direct smear technique described on previous page (III.A.)

D. GYN SPECIMENS (Pap Smears)—ThinPrep® Method:

(from "ThinPrep® Pap Test™ Quick Reference Guide", 1997, Cytoc Corporation)

- 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 five times.
- 2) Rinse the broom in the PreservCyt® Solution vial by pushing the broom into the bottom 10 times, forcing the bristles apart. As a final step, 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, and send in a plastic bag with completed requisition.

- Endocervical Brush/Spatula Protocol:

- 1) Obtain an adequate sampling from the ectocervix using a plastic spatula.
- 2) Rinse the spatula 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 bottommost fibers are exposed. Slowly rotate  $\frac{1}{4}$  or  $\frac{1}{2}$  turn in on direction. DO NOT OVER-ROTATE.
- 4) Rinse the brush 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 and send in a plastic bag with completed requisition.

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. Refrigerate if delayed.

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

- 1) Send specimen directly to lab without fixative. Refrigerate if delayed.

G. BRUSHINGS—Bronchial or Alimentary Tract Specimens:

- 1) Insert brush into Saccomanno brush container and agitate.
- 2) Cut wire and send container to lab with brush enclosed.

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. 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) Send specimen directly to lab without fixative. 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 page 11 (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 insure that the cyst wall has been properly sampled.
- 9) Prepare slides / specimen as described on next page (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 Saccomanno fixative (or PreserveCyt 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 and sent with a completed requisition form.

## IV. REPORTING TERMINOLOGY

### A. NON-GYN SPECIMENS:

- 1) Statement of specimen adequacy
- 2) 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)
- 3) Descriptive diagnosis

### B. GYN SPECIMENS:

Reporting terminology follows The Bethesda System 2001.

- 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  $\geq$  40 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 SMEAR 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:

- 1) Koss, L., Diagnostic Cytology, 4<sup>th</sup> ed., 1992.
- 2) Bibbo, M., Comprehensive Cytopathology, 1991.
- 3) Keebler, C. M., Somrak, T. M., The Manual of Cytotechnology, 7<sup>th</sup> ed., 1993.
- 4) Cytoc Corporation, "ThinPrep® Pap Test™ Quick Reference Guide", 1997.
- 5) Solomon, D., Nayar, R., eds., The Bethesda System for Reporting Cervical Cytology, 2<sup>nd</sup> ed., 2004.