Cytology Specimen Collection Manual

SHARP GROSSMONT HOSPITAL

December 2017
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 Pathology Department at Sharp Grossmont Hospital laboratory at 619-740-4497.

*Sharp Grossmont Hospital*

*5555 Grossmont Center Drive*

*La Mesa, CA 91942*
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I. GENERAL INFORMATION

A. LAB HOURS

Processing of specimens:  
- Mon to Fri: 8:00 am to 5:00 pm  
- Saturday: 8:00 am to 12:00 pm  
- Sunday: CLOSED

Emergencies:  
- Pathologist always ON-CALL  
- 24 hours per day, 7 days per week

B. TELEPHONE NUMBERS

- Cytology results: 619-740-4497
- Pathologist ON-CALL: 619-740-4497 (ask for pathologist on-call)
- Supplies and Requisitions: 619-740-4497 (Fax: 619-740-4418)

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 the Main Laboratory at Sharp Grossmont Hospital.

3) Ideally, if a specimen is to be used for any lab test other than Cytology, the specimen should be submitted to the appropriate department (i.e., Microbiology) first and then to Cytology. Fixatives and refrigeration may render the specimen inadequate for other tests.

4) 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 within 2-3 business days.

2) Cytology specimens are not processed at the Sharp Grossmont Hospital Laboratory.

3) Gyn specimens are sent to Sharp Pathology Laboratory Att: Pacific Rim Pathology.  
- Non-Gyn specimens are sent to the Cytology Department at SPL.

4) 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 at SPL including:
- Spray Fixative
- FNA supplies (slides, Cytolyt solution, 95% alcohol)
- PreserveCyt solution
- Requisition Forms

Please call 858-262-8620

II. **GENERAL SPECIMEN REQUIREMENTS**

A. **GENERAL CRITERIA REQUIRED FOR EVALUATION OF SPECIMENS**

1) Patient’s name on all slides and two identifiers on fluid specimen containers.

2) Properly completed and matching requisition form including patient name, DOB, history, IDC 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 delayed until the issue is resolved.

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 or rejected.

    **Patient information:**

    - Patient’s full name
    - Date of birth
    - Sex
    - Patient’s address and phone number

    **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 codes

    **Specimen:**

    - Exact type or source of each specimen
    - Method of collection
    - Date of collection
III. SPECIMEN COLLECTION PROCEDURES

A. GENERAL DIRECTIONS FOR ALL DIRECT SMEAR SPECIMENS—
including, but not limited to, 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. It is desirable to prepare at least one fixed smear (#4) and one air-
dried smear (#5) from each specimen.

4) Immediately spray smears thoroughly with cytology spray fixative at a distance of
approximately 10 to 12 inches from the smear. Write “fixed” on slides. Allow slides
to dry and send to lab in folder or container. (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) Air dried slides can also be submitted. Prepare slides as in #3 above and let the
smear air dry. Write “air dried” on the slides.

6) Send a completed requisition form and insurance information with all specimens.

***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).

C. SPUTUMS

1) Delivered to lab within 30 minutes of collection, if possible.

2) If not possible, add equal amounts of CytoLyt fixative (up to a 100 ml aliquot) to the
specimen or refrigerate until delivery the same day.

3) Early morning sputum is preferable. The teeth should be brushed, the mouth
washed, and the dentures taken out. Nasal passages and sinuses should be clear.
An excellent way to produce sputum is to have the patient take ten good, deep
breaths, followed by a very deep, low cough. The sputum is then expectorated into
the container.

4) Label and send to laboratory.

5) Order AP Tissue Request on LIS (inpatients), state the specimen and any special
requests under comments.

6) Write special requests on the specimen label/requisition.

D. WASHINGS AND LAVAGES—BRONCHIAL OR ALIMENTARY TRACT SPECIMENS

1) Deliver to lab within 30 minutes of collection, if possible.

2) If not possible, add equal amounts of CytoLyt fixative (up to 100 ml aliquot) to the
specimen or refrigerate until delivery the same day.

3) Order AP Tissue Request, state under comments “bronchial washing,” which lung
and lobe, and any special requests.

4) Write special requests on the specimen label/requisition.

E. BRUSHINGS—BRONCHIAL OR ALIMENTARY TRACT SPECIMENS

1) Containers for this procedure are available from the Cytology Department in the
Laboratory.

2) The brush used in the procedure should be cut off from the long wire and
immediately placed in the CytoLyt solution.

3) If both cytology and culture are requested, submit two brushes, one in CytoLyt and
one in Sterile Saline. If only one brush is submitted, it must go to Microbiology first
without fixative on it. Clearly label that the specimen is for both departments.

4) Appropriately label the container.

5) Order AP Tissue Request on LIS.

6) State under comments “bronchial brushings,” which lung and lobe, and any special
requests.

F. TRACHEAL ASPIRATES

1) Deliver to lab within 30 minutes of collection, if possible.

2) If not possible, add equal amounts of CytoLyt fixative (up to 100 ml aliquot) to the
specimen or refrigerate until delivery.

3) Appropriately label and send to the laboratory.
4) Order **AP Tissue Request** on LIS. Put “tracheal aspirate” and any special request under comments and on specimen label.

G. **THORACENTESIS AND PARACENTESIS**

1) Deliver to lab within 30 minutes of collection, or add an equal volume of CytoLyt fixative to a 100-200 ml aliquot of the fluid. (Since there is often abundant fluid, do not add CytoLyt to the entire specimen).

2) If CytoLyt is not available, refrigerate specimen until delivery to the lab.

3) Order **AP Tissue Request** on LIS; state the specimen and any special request under comments.

H. **URINARY TRACT SPECIMENS**

1) Attempt to get the second urinary specimen in the morning. Labeled as specified below.

2) If the specimen is a catheterized/foley urine, or bladder wash, it must be labeled as such.

3) Label and send urine to the laboratory immediately as the acidic nature of urine causes cellular degeneration.

4) If unable to send to the laboratory immediately, refrigerate until transport (up to 2 hours only) or add equal amounts of CytoLyt (up to 100 ml aliquot to the specimen).

5) Label the specimen.

6) Order **AP Tissue Request** on LIS; state the type of specimen and any special requests under comments.

I. **CEREBROSPINAL FLUID**

1) The cells in CSF are very delicate and the specimen must be delivered ASAP. Fill out a CSF order form per Doctor’s orders, keeping the top copy for the patient’s chart.

2) Order **AP Tissue Request** on LIS; state the type of specimen and any special requests under comments.

J. **SEX CHROMATIN (BARR BODY) DETERMINATION**

1) Scrape the buccal mucosa with a sterile wooden or metal spatula.

2) Immediately apply the specimen to a labeled glass slide using a gently circular motion on the center portion of the slide. Make 2-3 slides if possible.

3) Each slide needs to be immediately dropped in alcohol or sprayed with fixative. Do not allow the slides to air dry.
4) Send to lab and order AP Tissue Request on LIS – put “Barr Body” exam under comments.

K. FINE NEEDLE ASPIRATION

1) Slide/Specimen Preparation:

a. After the needle is removed from the lesion, it is detached from the syringe; the syringe is filled with air and then reattached to the needle.

b. Placing the bevel of the needle against a properly labeled glass slide, express the aspirated material onto the slide.

   o If a very scant amount of material is present, repeat steps a and b several times to express any material in the needle hub.

   o If abundant blood or fluid is present, let the excess fluid drop into a labeled CytoLyt container, or wipe the edge of the slide with gauze to remove the excess blood, being very careful to leave all particulate matter on the slide.

   o If there is abundant cellular material on the slide, transfer some of the material to another slide by scraping the original slide with a clean slide, moving the material onto another labeled slide.

c. Prepare the smears:

i. One-step technique (see Addendum A): The smear is made by holding the stationary slide with its long axis away from the observer and the smearing slide perpendicular to the stationary slide. The smearing slide initially contacts the stationary slide along a line below the drop, so that when the smearing slide is gently rotated downward, the upper edge just overlays the biopsy material. The smearing slide is then drawn toward the observer.

ii. Two-step technique (see Addendum B): If the cellular material is diluted by a small amount (1-2cc) of fluid, the smear is made by holding the stationary slide with its long axis away from the observer and the smearing slide parallel to the stationary slide. The smearing slide contacts the stationary slide along a line below the drop, then contacts the drop. The fluid is drawn towards the observer in the same way as a blood smear, but is stopped 2/3 of the way down. The stationary slide then rotates downward onto the particles. The slides are then pulled apart.

iii. The smears are usually allowed to air dry. If fixed slides are desired, the slides must immediately be dropped in alcohol fixative as soon as they are smeared or sprayed with a fixative. "Air dried" or "fixed" should be indicated on all slides, labels and requisition slips.

d. After preparing the smears (or if there is abundant material) rinse the syringe and needle in a labeled CytoLyt container.
o If cultures are desired, **do not** put the material in CytoLyt. Place material in a sterile tube to be sent to Microbiology.

o If only a cell block is desired, the needle and syringe can be rinsed in Formalin. This is only warranted if large cores of tissue are present. **NOTE:** Once the specimen is in Formalin, it **cannot** be used to make smears – it is only good for cores of tissue. Use the CytoLyt fixative whenever possible as it can be used with cores or scanty amounts of fluid.

IV. REFERENCES


V. APPROVALS

A. Dr. James Carry, M.D.; SGH Pathologist, Hector Herrera, Anatomic Pathology Manager; Maggie Van Aken, Pathology Supervisor: December 2017.
The purpose of this manual is to provide general instructions for surgical pathology specimen collection and handling where specimen quality may affect the 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 Pathology Department at Sharp Grossmont Hospital 619-740-4497

*Sharp Grossmont Hospital
5555 Grossmont Center Drive
La Mesa, CA 91942*
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I. GENERAL INFORMATION

A. LAB HOURS
   Processing of specimens:  
   Mon to Fri:  8:00 am – 5:00 pm  
   Saturday:   8:00 am – 12:00 pm  
   Sunday:     CLOSED

   Emergencies:          Pathologist ON-CALL  
                         24 hours per day, 7 days per week

B. TELEPHONE NUMBERS
   Surgical Pathology: 619-740-4497
   Pathologist ON-CALL: 619-740-4497 (ask for pathologist on-call)
   Supplies and Requisitions: 619-740-4497 (Fax: 619-740-4418)

C. SPECIMEN SUBMISSION TO LABORATORY
   1) Specimens may be submitted to the lab at any time, but generally will be processed within the schedule indicated above.
   2) Send specimens by appropriate courier to Main Laboratory at Sharp Grossmont Hospital.
   3) Ideally, if a specimen is to be used for any lab test other than Surgical Pathology/Histology, the specimen should be submitted to the appropriate department (i.e. Microbiology) first and then to Surgical Pathology. Histology fixatives and refrigeration may render the specimen inadequate for other tests.
   4) Do NOT refrigerate or fix any specimen that must be shared with Microbiology or any other department.

D. REPORTING TIME AND STAT SPECIMENS
   1) Routine surgical specimens will generally be reported within 2-3 working days.
   2) 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 at Sharp Grossmont Hospital Laboratory:
   - Formalin or other fixative (for tissue fixation)
   - Specimen bags/containers.
• Requisition Forms

Please call: 619-740-4497

II. GENERAL SPECIMEN REQUIREMENTS

A. GENERAL CRITERIA REQUIRED FOR EVALUATION OF SPECIMENS

1) Patient’s name and birthdate on all slides and two patient identifiers on specimen containers.

2) Properly completed and matching requisition form including patient name, DOB, history, ICD code(s), and specimen source. Do not put the requisition form in the bag with the specimen container (in case of leakage).

3) Specimen intact (i.e., container not leaking).
   *Needles must be removed from syringes before sending to lab.*

   **NOTE:** If the above conditions are not met, the specimen may either be rejected without being processed or processing delayed until the issue is resolved.

B. REQUISITION FORMS

1) Use a separate pathology 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 or rejected.

   **Patient information:**
   • Patient’s full name
   • Date of birth
   • Sex
   • Patient’s address and phone number

   **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 code(s).

   **Specimen:**
   • Exact type or source of each specimen
   • Method of collection
   • Date of collection
   • Name of submitting doctor with address and phone number
III. SPECIMEN COLLECTION PROCEDURES

A. COMMONLY USED TERMS

- Specimen: any product of a medical procedure. These can be soft tissues, bone tissue, fluids, foreign bodies, surgical appliances/hardware. The terms “sample”, “specimen”, and “tissue” are often used interchangeably.

- Fixative: a solution used to stabilize cellular components in preparation for histological examination. Proper fixation is essential for histology, but it kills cells and acts in other ways that limits many other tests (i.e., Cultures) and research applications. 10% neutral buffered zinc formalin is the most common routine fixative.

- Fresh: no fixative has been used. Fresh tissue samples must be frozen, placed in a transport media, such as RPMI, to keep the cells alive, or stabilized in some other way as soon as possible to prevent autolysis.

- Frozen Sections: specimens that are or have been frozen for rapid microscopic exam during an intraoperative consultation. Frozen section slides are cut on a cryostat for rapid microscopic analysis. The remaining tissue is placed in fixative to be made into permanent sections.

- Permanent sections: a specimen that is treated with fixative and processed to a paraffin matrix that permanently preserves them at ambient temperature. “Paraffin block” is an equivalent term. “Permanents”, or paraffin blocks, are specimens for which a microscopic histological exam can be performed. The histology of permanents is superior to that of frozens.

B. GENERAL HANDLING AND PRESERVATION OF TISSUE SPECIMENS

1) Fresh Tissues - submitted with no fixative:

   a. Frozen sections or gross exam - must be received fresh

   b. Lymphocyte markers - to rule out lymphoma. Send fresh immediately to surgical pathology or place in RPMI transport media and send to surgical pathology.

   c. Uric Acid crystals - note on requisition to rule out gout (see special requirements below).

   d. Microbiologic culture – specify cultures desired. Submit fresh and sterile.

   e. Muscle biopsies – contact surgical pathology 619-740-4497 for instructions before procedure. Submit Fresh.


2) Cytogenetic studies - Tissues for Cytogenetic analysis should be immediately placed in RPMI media to preserve viability and sent to the laboratory as soon as possible.
3) **10% Formalin:**
   a. Surgical pathology specimens should be placed in at least 10 times their volume of formalin immediately upon procurement for adequate fixation.
   b. The laboratory provides prefilled formalin containers. If the specimen is a large specimen the formalin can be added by histology staff to the specimen container during regular histology hours. The OR is provided with formalin which can be added by OR staff.

4) Any questions regarding handling of tissues should be directed to the pathologist in charge. There is a pathologist on duty or on-call at all times.

C. **SPECIAL HANDLING**

All specimens submitted to surgical pathology for examination will be submitted in 10% Formalin with a sufficient amount of fluid to achieve a 10:1 ratio of formalin to specimen. Specimens without sufficient formalin may require extra time of fixation, causing a delay in processing.

Exceptions to the 10% formalin are listed below.

1) **Amputated limbs:** Amputated limbs with a pathology requisition are delivered to surgical pathology by the OR personnel.

2) **Renal biopsies:** Renal biopsies will be collected in saline soaked gauze. The containers are labeled and sent immediately to surgical pathology with the accompanying tissue requisition, the patient’s insurance information and a comprehensive clinical history. The pathologist or pathologist assistant (PA) will then divide the specimen for appropriate studies that include light microscopy, Electron Microscopy, and Immunofluorescence. Immunofluorescence studies (Zeus transport media), electron microscopy (3% buffered glutaraldehyde) and light microscopy (10% Buffered Zinc Formalin). For specific instructions and supplies contact surgical pathology at 619-740-4497.

3) **Muscle biopsies:** Muscle biopsy specimens are immediately delivered to surgical pathology. Special handling is required. For detailed information contact surgical pathology ahead of time, preferably in the morning.

4) **Nerve biopsies:** Nerve biopsy specimens are immediately delivered to surgical pathology. Special handling is required. For detailed information contact surgical pathology ahead of time, preferably in the morning.

5) **Breast needle localization specimens:** These specimens are sent from the OR to surgical pathology. Fixative may or may not be added. If fixative has been added, the time the fixative was added has to be documented. A frozen section may be requested.

6) **Stones for analysis:** Renal calculi are collected and sent to pathology fresh, without fixative. The specimen(s) should be placed in a collection vial dry and transported to surgical pathology. The specimen container must not contain any fixative, saline or urine.

7) **Uric acid analysis (Gout):** Specimens for histologic evaluation for uric acid crystals should be collected and sent to surgical pathology in 100% ethanol or, if sent fresh, must be immediately transported to surgical pathology.
8) **Placenta/POC for chromosome analysis:** These specimens are collected fresh and sent immediately to surgical pathology. A tissue requisition along with a cytogenetics request (specifying chromosome testing) must accompany the specimen.

9) **Testicular biopsies:** The preferred fixative for testicular biopsies is 10% Buffered Zinc Formalin and can be obtained calling surgical pathology at extension 619-740-4497.

IV. REFERENCES - N/A

V. APPROVALS

A. Dr. James Carry M.D. SGH Pathologist; Hector Herrera, Anatomic Pathology Manager; Maggie Van Aken, Pathology Supervisor; December 2017
Cytology Specimen Collection Manual

SHARP CORONADO HOSPITAL,
SHARP CHULA VISTA MEDICAL CENTER,
SHARP MEMORIAL HOSPITAL,
AND
SHARP PATHOLOGY LABORATORY

January 2018
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. Any process or contact information that is unique to Sharp Chula Vista will be indicated by (SCV).

For further assistance with individual cases or general questions, please contact the Cytology Department 858-262-8621 or Dr. Wayne Muller 858-939-3660.

Sharp Metropolitan Medical Campus
7901 Frost Street
San Diego, CA 92123

Sharp Pathology Laboratory
5325 Metro Street
San Diego, CA 92110

Sharp Chula Vista Medical Center
751 Medical Center Ct.
Chula Vista, CA 91911

Sharp Coronado Hospital
250 Prospect Place
Coronado, CA 92118
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I. GENERAL INFORMATION

A. LAB HOURS:
   Processing of specimens: Mon to Sat: 8:00 am – 5:00 pm
   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: 858-262-8625
   NON-GYN Cytology results
   (and Biopsy results): 858-939-3660
   Pathologist ON-CALL: 858-939-3650 (ask for pathologist on-call)
   Supplies and Requisitions: 858-262-8626 (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 or Sharp Pathology Laboratory.
   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 cannot 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 within 48 to 72 hours.
   2) GYN specimens will generally be reported 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 telephoned.

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 Thin Prep
- Spray Fixative
- FNA supplies (slides, Cytolyt solution, 95% alcohol)
- PreserveCyt solution for GYN specimens.
- Requisition Forms
- Supplies Request Forms

Please call 858-262-8626
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 two identifiers on fluid specimen containers.

2) Properly completed and matching requisition form including patient name, DOB, history, IDC 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)
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:**

   **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 ¼ or ½ turn in one 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 Cytolyt or 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 in section 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 Cytolyt 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) The Licensed Care Provider must label all slides and specimen containers with two patient identifiers.

   **NOTE:** The Cytology laboratory will label all slides and containers with an additional cytology tracking number (xylene resistant label).

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 cannot 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 Cytolyt solution for Thin Prep 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 ≥ 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


VI. APPROVALS

A. Dr. Wayne Muller, SMMC Pathologist, Hector Herrera, Anatomic Pathology Manager; Maggie Van Aken, Pathology Supervisor; December 2017

B. Dr. Art Mendoza, SMMC Pathologist, Dr. Art Huffaker, SCOR Pathologist, Dr. Octavio Armas, SCV Pathologist; January 2018
The purpose of this manual is to provide general instructions for surgical pathology specimen collection and handling where specimen quality may affect the 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. Any process or contact information that is unique to Sharp Chula Vista will be indicated by (SCV).

For further assistance with individual cases or general questions, please contact the Pathology Department at Sharp Metropolitan Medical Campus 858-939-3660 or the Pathology Department at Sharp Chula Vista Medical Campus 619-502-3622.

Sharp Metropolitan Medical Campus
7901 Frost Street
San Diego, CA 92123

Sharp Pathology Laboratory
5325 Metro Street
San Diego, CA 92110

Sharp Chula Vista Medical Center
751 Medical Center Ct.
Chula Vista, CA 91911

Sharp Coronado Hospital
250 Prospect Place
Coronado, CA 92118
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I. GENERAL INFORMATION

A. LAB HOURS:

Processing of specimens:
- Mon to Fri: 8:00 am – 6:30 pm
- Saturday: 8:00 am – 2:30 pm
- Sunday: CLOSED.

Sharp Chula Vista:
- Mon to Fri: 7:30 am – 5:30 pm
- Saturday & Sunday: CLOSED

Emergencies:
- Pathologist ON-CALL
  24 hours per day, 7 days per week

B. TELEPHONE NUMBERS:

Surgical Pathology:
- SCV 858-939-3660 and 858-939-3663
- 619-502-3622

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

Supplies and Requisitions:
- SCV 858-262-8626 (Fax: 619-295-0835)
- 619-502-3622

C. SPECIMEN SUBMISSION TO LABORATORY:

1) Specimens may be submitted to the lab at any time, but generally will be processed within the schedule indicated above.

2) Send specimens by appropriate courier to Specimen Log-In Department in Main Laboratory at Sharp Memorial Hospital, Sharp Pathology Laboratory or the Main Laboratory at Sharp Chula Vista.

3) Ideally, if a specimen is to be used for any lab test other than Surgical Pathology/Histology, the specimen should be split and sent separately with separate requisition forms. Histology fixatives and refrigeration may render the specimen inadequate for other tests.

4) If the specimen cannot 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 specimen that must be shared with Microbiology or any other department.

D. REPORTING TIME AND STAT SPECIMENS:

1) Routine surgical specimens will generally be reported within 2 to 3 working days.

2) 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 at Sharp Pathology Laboratory including:
- Formalin (for tissue fixation)
- Specimen bags
- Requisition Forms
- Supplies Request Forms

Please call 858-262-8626
Or fax a “Supplies Request Form” to 619-295-0853.
For supplies specific to Sharp Chula Vista, please call 619-502-3622.

II. **GENERAL SPECIMEN REQUIREMENTS**

A. **GENERAL CRITERIA REQUIRED FOR EVALUATION OF SPECIMENS:**

1) Patient’s name on all slides and two identifiers on fluid specimen containers.

2) Properly completed and matching requisition form including patient name, DOB, history, ICD codes, and specimen source.

3) Specimen intact (i.e., container not leaking).

*Please remove needles from syringes before sending to lab.*

**NOTE:** If the above conditions are not met, the specimen may either be rejected without being processed or processing delayed until the issue is resolved.

B. **REQUISITION FORMS:**

1) Use a separate pathology 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 or rejected.

**Patient information:**
- Patient’s full name
- Date of birth
- Sex
- SSN (strongly suggested)
- 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 codes
III. SPECIMEN COLLECTION PROCEDURES

A. COMMONLY USED TERMS

1) Specimen: any product of a medical procedure. These can be soft tissues, bone tissue, fluids, foreign bodies, surgical appliances/hardware. The terms “sample”, “specimen”, and “tissue” are often used interchangeably.

2) Fixative: a solution used to stabilize cellular components in preparation for histological examination. Proper fixation is essential for histology, but it kills cells and acts in other ways that limits many research applications. 10% neutral or Zinc buffered formalin is the most common routine fixative.

3) Fresh: no fixative has been used. Fresh tissue samples must be frozen, placed in a transport media to keep the cells alive, or stabilized in some other way as soon as possible to prevent autolysis.

4) Frozens Sections: specimens that are or have been frozen for rapid microscopic exam during an intraoperative consultation. Frozen section slides are cut on a cryostat for rapid microscopic analysis. The remaining tissue is placed in fixative to be made into permanent sections.

5) Permanent Sections: a specimen that is treated with fixative and processed to a paraffin matrix that permanently preserves them at ambient temperature. “Paraffin block” is an equivalent term. “Permanents”, or paraffin blocks, are specimens for which a microscopic histological exam can be performed. The histology of permanents is superior to that of frozens.

B. GENERAL HANDLING AND PRESERVATION OF TISSUE SPECIMENS

1) Fresh Tissues submitted with no fixative:
   a. Specimens for Frozen Sections or gross exam must be received fresh
   b. Lymphocyte markers to rule out lymphoma. Send fresh to surgical pathology or place in RPMI transport media and send to surgical pathology.
   c. Uric Acid crystals note on requisition to rule out gout (see special requirements below).
   d. Microbiologic culture – specify cultures desired – fresh specimen required.
   e. Muscle biopsies – contact surgical pathology 858-939-3663 (SCV: 619-502-3622) for instructions before procedure
2) Cytogenetic studies: Tissues for Cytogenetic analysis should be immediately placed in RPMI media to preserve viability and sent to the laboratory as soon as possible.

3) Routine specimens submitted in 10% Zinc Formalin:
   a. Surgical pathology specimens should be placed in at least 10 times their volume of formalin immediately upon procurement for adequate fixation.
   b. The laboratory provides prefilled formalin containers. If the specimen is a large specimen the formalin can be added by histology staff to the specimen container during regular histology hours. At other times the OR is provided with formalin which can be added by OR staff.

4) Any questions regarding handling of tissues should be directed to the pathologist in charge. There is a pathologist on duty or on-call at all times.

C. **SPECIAL HANDLING**

All specimens submitted to surgical pathology for examination will be submitted in 10% Zinc Formalin with a sufficient amount of fluid to achieve a 10:1 ratio of formalin to specimen. Specimens without sufficient formalin may require extra time of fixation, causing a delay in processing.

Exceptions to the 10% Zinc formalin are listed below.

1) **Amputated limbs**: Amputated limbs and pathology requisition are delivered to surgical pathology by the OR personnel.

2) **Renal biopsies**: Renal biopsies may be collected in containers with 0.9% normal physiological saline added. The containers are labeled and sent immediately to surgical pathology with the accompanying tissue requisition, the patient’s insurance information and a comprehensive clinical history. The pathologist or pathologist assistant (PA) will then divide the specimen for appropriate studies that include light microscopy, Electron Microscopy, and Immunofluorescence. Immunofluorescence studies (Zeus transport media), electron microscopy (3% buffered glutaraldehyde) and light microscopy (Bouins fixative with Glacial Acetic Acid). For a collection kit and specific instructions contact surgical pathology x3663.

3) **Muscle biopsies**: Muscle biopsy specimens are immediately delivered **Fresh** to surgical pathology. Special handling is required. For detailed information contact surgical pathology.

4) **Nerve biopsies**: Nerve biopsy specimens are immediately delivered **Fresh** to surgical pathology. Special handling is required. For detailed information contact surgical pathology.

5) **Breast needle localization specimens**: These specimens are sent from the OR or Radiology to surgical pathology immediately after removal from the patient. A frozen section may be requested.
6) **Stones for analysis:** Renal calculi are collected and sent to pathology fresh, without fixative. The specimen(s) should be placed in a collection vial dry and transported to surgical pathology. The specimen container must not contain any fixative, saline or urine.

7) **Uric acid analysis (Gout):** Specimens for histologic evaluation for uric acid crystals should be collected and sent to surgical pathology in 100% ethanol or, if sent fresh, must be immediately transported to surgical pathology.

8) **Placenta/POC for chromosome analysis:** These specimens are collected sent *fresh* immediately to surgical pathology. A tissue requisition along with a cytogenetics request (specifying chromosome testing) must accompany the specimen.

9) **Testicular biopsies:** The preferred fixative for testicular biopsies is Bouin’s fixative and can be obtained by calling surgical pathology at extension 3663.

IV. REFERENCES – N/A

V. APPROVALS

A. Dr. Art Mendoza, M.D., SMMC Pathologist, Hector Herrera, Anatomic Pathology Manager; December 2017

B. Dr. Art Huffaker, SCOR Pathologist, Dr. Octavio Armas, SCV Pathologist; January 2018