

CYTOLOGY

Non-Gynecological Specimens (refer to the Cytology Specimen Collection Guidelines for a quick reference).

A. General Considerations of Specimens

1. Specimens should be as fresh as possible.
2. Keep specimens refrigerated if a time lapse of one (1) hour or more between next delivery. Transport delivers specimens from the Central Lab to Cytology hourly between the hours of 8:00 a.m.-3:30 p.m. (STAT specimens should be taken directly to Cytology Lab)
3. The Cytology Lab at UCD-School of Medicine is open from 8:00 a.m. to 4:30 p.m., Monday through Friday. Closed weekends and holidays.
4. The Cytology Lab is located in AIP, Room 3.136, X84361
5. Contact Anatomic Pathology resident (call operator) for "BAL" after hours/weekend immediate processing and evaluation of critical specimens for the detection and identification of *Pneumocystis jirovecii* (carinii)

B. Cytology Request Forms

1. A supply of forms are available in all clinics and nursing stations, or the Cytology Lab (AIP Room 3.136).
2. Forms must be completely filled out, to include:
 - a. Patient's name
 - b. Hospital number
 - c. Date of birth
 - d. Patient's encounter #
 - e. Patient's insurance payor code
 - f. Ward/Clinic
 - g. Doctor's name (Attending Physician) and pager number who is requesting the lab work
 - h. Date specimen collected
 - i. Specimen source
 - j. Pertinent clinical history, including history of carcinoma, radiation or chemotherapy and dates
 - k. Referral Number - if required by patient's insurance.

*(Please use patient's card to stamp requisition forms.)

C. Bronchus, Esophagus or Gastric Specimens

1. Washings: Deliver unfixed specimen as soon as possible to Cytology Lab, or refrigerate until it can be delivered. If specimen is held over a weekend, add sputum fixative to container (Fixative available in Cytology Lab or Central Lab has a supply.) and indicate that the specimen was fixed.
2. Brushings: Label frosted end of slide with patient's name and hospital number. Roll the brush over slide and drop immediately (1-2 seconds) into 95% ethanol. Immediate fixation in alcohol is necessary to prevent drying and distortion of the cells. The cytology lab also has a new stain that can evaluate air dried specimens, however we must know before we stain the slides what fixative (if any) was used.

D. Cerebrospinal Fluid

1. The specimen should consist of at least three (3) milliliters.
2. CSF specimens are now processed by the Hematology section of the Clinical Laboratory and then sent to

Cytology for staining and reporting. Specimens MUST be taken ASAP to the Clinical Laboratory (Leprino Office Building Room 253).

E. Effusions

1. Specimens consist of:
 - a. Ascites or peritoneal fluid
 - b. Pleural or thoracentesis fluid
 - c. Pericardial fluid
 - d. Synovial fluid
2. Do not add anticoagulant or fixative. If specimen cannot be delivered fresh, it should be refrigerated. 150 cc of specimen is very adequate. SPECIMENS IN PLEUR-EVAC CONTAINERS, SPECIMENS IN EXCESS OF 500CC WILL NOT BE ACCEPTED. SPECIMENS WITH NEEDLES WILL NOT BE ACCEPTED. SPECIMENS IN GLASS CONTAINERS WILL NOT BE ACCEPTED.

F. Cyst Fluids

Specimen can be handled the same as effusions.

G. FNA (Fine Needle Aspiration)

1. 9th Avenue—For aspirates to be performed by a pathologist, call the Cytology Lab (X81793) with the following information: patient name, medical record number, specimen, room number and contact information. Pathologist on service will be contacted. Aspirates to be done by doctors outside of the Pathology Department (i.e., CT, EUS, Ultrasound), must be scheduled in advance (at least one hour) with the Cytology Lab (X81793). A cytotechnologist will attend the aspirate to prepare the slides, whenever possible.

Fitzsimons—Call the Pathology Front Desk (X84421) or Resident's Office (X84424) for a pathologist to perform a fine needle aspiration. For cytotechnologist assistance (i.e., CT, EUS, Ultrasound), please call X81792 or 303-266-1597 for a tech to assist and prepare slides.
2. If an aspirate must be done after normal hours, with no cytology assistance, the following steps must be adhered to:
 - a. The specimen must be labeled as to type of fixative used, if any.
 - b. For best results a series of air-dried slides can be prepared and shipped to the cytology laboratory. Slides prepared in this manner are safe for up to 30 days.
 - c. If Pro-Fix or other fixative is used it must be used immediately and liberally. DO NOT allow the slides to air-dry and then apply fixative. The slides will be unreadable.
 - d. Prepare the slides as you would a blood smear. Use the entire slide and spread the material as thinly as possible.
 - e. Prepare 3-5 slides per pass.
 - f. Complete a cytology requisition form and send the slides to cytology as soon as possible.
 - g. DO NOT ship cytology slides and formalin fixed specimens in the same container. If ANY formalin fumes contaminate the cytology material, it will be unreadable.

H. Sputum Collection

1. Early morning deep cough specimen is to be collected in a container with sputum fixative. Sputum cups with a fixative are available inside Central Lab in the basement of the critical care tower.
Sputum specimens received without fixative will be rejected.

Instruction to Patients for Sputum Specimen Collection

1. Label the sputum cup with patients name, hospital number and date of collection.
2. An early morning cough specimen gives the best yield of cells.
 - a. Rinse your mouth well (4-5 times) and brush your teeth before eating or taking any medication.
 - b. Take several deep breaths and then cough up material from your lungs.
 - c. Expel the fluid into an empty sputum cup and then pour cytology fixative over specimen. (Spitting saliva into the cup will result in an unsatisfactory specimen).
 - d. Cap the sputum cup securely so the fluid will not leak.
 - e. Specimen must be accompanied by a cytology request form with patient's name, hospital number, date of birth, patient's history and diagnosis, doctor's name, and care center location or nursing station, etc.
3. Outpatient Sputum Collection
 - a. Follow the directions above and collect an early morning cough specimen on three consecutive days.
 - b. Keep specimens in the refrigerator until brought to the hospital care center.

I. Urine Specimen

1. The second voided specimen in the morning is preferred, after the patient has been up and active. For males, a voided specimen is satisfactory. For females, a "clean catch" specimen is preferred.
2. If specimen cannot be refrigerated, add equal amount of cytology fixative to specimen to preserve until delivered. Fixative should only be added as a last resort. Adding fixative dilutes the specimen and may result in a less than satisfactory reading. Patients should be encouraged to come to the hospital to void a fresh specimen for best results.
3. 50-80 ml of urine is considered adequate
4. Adequate hydration is important to provide a sufficient volume of urine. Any liquid other than alcohol is considered satisfactory.

Gynecological Specimen Collection

A. Gynecological Smears (conventional)

Endocervical, Ectocervical and combined Smears

1. Each slide must be labeled with patient's name and hospital number and site of smear.
2. Adequate smears must have cells from the squamo-columnar junction. If a lesion is visible, a slide should be made of this area first.
3. Do not use lubricants when performing smears. If necessary use only saline to perform the examination and smear. Lubricants will obscure the cellular material and may result in a less than optimal specimen.
4. Spread cells evenly and quickly over the slide and fix IMMEDIATELY in 95% ethanol or by spraying with hair spray or Pro-fix. When spraying, the aerosol can should be held about twelve (12) inches from the slide to avoid freezing the cells. If a pump spray is used, pump 3-4 times at a distance of 6-8 inches.
5. Each specimen must be accompanied by a Cytology Requisition Form. These forms are available in all

clinics and nursing stations, by the Cytology Lab (57334). Information needed includes: *(Stamp Requisition Form with patient's card)

- a. Patient name
- b. Hospital number
- c. Date of birth
- d. Patient's encounter #
- e. Patient's insurance payer code
- f. Clinic
- g. Attending clinician
- h. Date collected
- i. LMP (Last Menstrual Period),
- j. Previous history concerning menstruation, abnormal paps or cancer history with dates of surgery, radiation treatment or chemo, etc.
- k. Birth control method.
- l. Reason for Cytology (Annual Exam, CM, etc)
- m. Source of Specimen
- n. Pap Collection Method
- o. Screening factors
- p. Number of slides
- q. Referral Number - if required by insurance.

B. Vaginal, Vulvar, Labial Smears

1. A good scrape should be made of the specified area, smeared as in 1-3 above and labeled as to site of smear.

C. Thin Prep/SurePath Collection of Gynecological Specimens

Cervical Sample Collection

1. Vials must be labeled with patient's name, hospital number and site of smear.
2. Adequate sample must have cells from the squamo-columnar junction. If visible, this area should be sampled first.
3. Rinse collection device thoroughly in thin prep/surpath vial.
4. Submit to laboratory with appropriate labeled requisition form (see #5 above).

HPV Testing

1. Collect cervical sample as described above.
2. Order HPV testing in one of two ways.
 - a. Order HPV reflex test. With the reflex test, HPV testing will only be done on samples diagnosed as "Atypical Squamous Cells of Undetermined Significance" (ASCUS).
 - b. Order HPV typing. HPV testing will be done on all samples regardless of the diagnosis.

Breast (Nipple Smear and Cyst Fluid)

Nipple Smear

Patient Preparation:

Do not massage breasts. If the nipple is ulcerated, the nipple area should be soaked with sterile gauze pads and saline for a few minutes prior to making the smear.

Equipment:

1. Fully frosted slides (Dakin)
2. Open bottle containing 95% ethanol fixative or Pro-Fix Spray Fixative
3. Sterile gauze pads
4. Sterile saline

Procedure:

1. Have the patient hold the open bottle of fixative beneath the breast.
2. Gently compress the areola, vertically and then in a clock-wise direction to include the entire area.
3. Support the areola with one hand while placing a slide across the nipple with the other hand. Draw the slide across the nipple and immediately drop into the jar of fixative or spray with Pro-Fix fixative. Repeat until all nipple secretion is used.

Note: If the nipple is ulcerated, a few drips of sterile saline may be applied to the nipple after soaking as above. A touch prep is then made by gently touching a slide to the lesion and then immediately dropping it into the fixative. Alternatively, a cotton swab may also be used to gently abrade the lesion. The material thus collected is then quickly spread in the center of the slide and dropped immediately into the fixative.

Breast - Cyst Aspiration

Equipment:

1. Lidocaine for local anesthesia and Tuberculin needle and syringe for application.
2. Betadine or alcohol prep pads or swabs
3. 22 or 23 gauge needle
4. 20 cc syringe
5. Small Collection cup or centrifuge tube with lid

Procedure:

1. After administering satisfactory local anesthesia, the tissue is supported and stabilized and the cyst punctured with the 22 gauge needle attached to the 20 cc syringe. The cyst contents are evacuated. Cytologic examination is recommended if the cyst fluid is bloody, the cyst is recurrent, or if the patient is post menopausal. To submit for cytologic examination, express the fluid from the syringe into a tube or small collection cup with a lid. Label the specimen and submit with accompanying requisition.
2. Following evacuation of the cyst, the area is palpated. If a residual mass is noted at the cyst site, it is aspirated utilizing the technique described for fine needle aspiration cytology. Slides are then prepared immediately and fixed in a container of 95% ethanol and Carnoy's to be transported to the Cytology Lab. Pressure is then applied to the puncture site with a sterile gauze pad.

Sex Chromatin Examination

Buccal Smears for Barr Bodies

Equipment:

1. 1 Coplin jar of 95% ethanol or Pro-Fix Spray Fixative
2. 2 blunt knives or tongue depressor blades
3. 8 slides

Procedure:

1. Have the patient rinse mouth well with water 3-4 times and brush teeth.
2. Scrape cheek very hard with blunt knife or tongue depressor blade.
3. Smear material on 2 slides to make a thin smear and fix immediately in 95% ethanol. Scrape opposite cheek also if material is scarce to make 2 slides. Fix slides for 15 minutes.
4. Control smears should be made from a child-bearing female.
5. Stain the 2 patient slides and 2 control slides together.

Specimen Rejection

Procedure

1. Specimens received from unknown clinics or facilities are rejected. Only legally authorized physicians and facilities may submit specimens for processing.
2. Specimens received without patient identification on the specimen container are not acceptable. See # 7.
3. Specimens and request sheets having different patient names but sent together are not acceptable. See #7.
4. Specimens received in leaking or unsealed containers are not acceptable. See #7 below.
5. Specimens received with attached needles, in a glass bottle, in a Pleurevac container, or in excess of 500 ml in volume, are not acceptable. See # 7 below.
6. Sputums not fixed in cytology fixative are not acceptable. If a sputum culture is required, 2 specimens must be obtained. See #7 below.
7. The clinic or nursing station will be called, up to two times to report the problem so the specimen can be repeated or properly submitted. Due to the fragile nature of the cellular material we will not attempt to recover a specimen that is over 7 days old.
8. We record all specimen rejections in the computer system. Information concerning the reason for rejection and the ward or clinic contacted is available for review by appropriate personnel.
9. Mislabeled specimens require an incident report to be completed. This must be completed within 24 hours of discovery of the problem All discrepancies are submitted via "Patient Safety Net" (PSN).

Cytology Specimen Collection Guidelines—Quick Reference

Specimen	Fixative	Quantity	Storage	Deliver To
CSF	None	3-5 ml	Refrigerate	Clinical Laboratory—Attn: Hematology (within 30 min)
Ascitic (peritoneal) Fluid	None	25-150 ml	Refrigerate	Cytology, M-F, 8:00 am – 4:30 pm (closed holidays) Clinical Laboratory
Bronchial Brushing	None	Brush and 5-10 ml Fluid	Refrigerate	Cytology, M-F, 8:00 am – 4:30 pm (closed holidays) Clinical Laboratory
Bronchial Washing	None	5-20 ml	Refrigerate	Cytology, M-F, 8:00 am – 4:30 pm (closed holidays) Clinical Laboratory
Sputum ¹	Cytolyte	5-10 ml	Refrigerate	Cytology, M-F, 8:00 am – 4:30 pm (closed holidays) Clinical Laboratory
Cyst Aspiration ²	None	1-5 ml	Refrigerate	Cytology, M-F, 8:00 am – 4:30 pm (closed holidays) Clinical Laboratory
Brushing Slides	SprayCyte or Air Dry ³	1-5 Slides per Site	Refrigerate	Cytology, M-F, 8:00 am – 4:30 pm (closed holidays) Clinical Laboratory
Misc. Body Fluids (Pericardial, Pelvic, etc.)	None	25-150 ml	Refrigerate	Cytology, M-F, 8:00 am – 4:30 pm (closed holidays) Clinical Laboratory
Urine / Bladder Washings	None	50-80 ml	Refrigerate	Cytology, M-F, 8:00 am – 4:30 pm (closed holidays) Clinical Laboratory
Pleural Fluid	None	25-150 ml	Refrigerate	Cytology, M-F, 8:00 am – 4:30 pm (closed holidays) Clinical Laboratory

NOTE: Fixatives are always added in equal volume to the volume of specimen (i.e., 5 ml of washing add 5 ml of fixative).

¹ Sputums received without fixative will NOT be processed. Contact the Cytology Laboratory if you need Cytolyte fixative.

² Specimens received with NEEDLES attached will NOT be processed.

³ The requisition MUST be marked to indicate the type of fixative (if any) that was used. If the slide is improperly stained based on incorrect information, it may be rendered unreadable.

WE WILL NOT PROCESS
ANY SPECIMEN RECEIVED IN A GLASS BOTTLE, SPECIMENS WITH NEEDLES,
SPECIMENS IN A PLEUREVAC CONTAINER, OR IN EXCESS OF 500 ML IN
VOLUME PLEASE USE PROPER CONTAINERS, SEALED AND LABELED FOR
UNIVERSAL PRECAUTIONS.