

CYTOLOGY LABORATORY

Hours of operation:

University of Florida Health Pathology Laboratories

Monday – Friday | 8 a.m. – 4:30 p.m.

Phone: 352.265.0172, ext. 72120

Fax: 352.265.6935

FNA at UF Health Shands Hospital (North Tower) 8:30 a.m. – 3:30 p.m. | Pager: 352.413.6834

After-hours, page the resident-on-call: 352.413.6266

Manager: Mai Ta
Cell: 352.219.3644

Medical director: Larry J. Fowler, M.D.
Pager: 352.413.1620

The function of the UF Health Pathology Laboratories Cytology Laboratory is to evaluate exfoliated or aspirated cells for abnormalities, including (but not limited to) the presence of cancer, pre-cancerous conditions, infection due to fungus, virus, parasites and bacteria. Our cytology services include processing, evaluating and performing diagnostic interpretation on submitted specimens. All specimens are examined and interpreted by cytotechnologists and given to a pathologist for review and final diagnosis. The Cytology Laboratory staff assists in fine-needle aspiration (FNA) procedures in various areas at UF Health Shands Hospital and only during regular hours (8:30 a.m. - 3:30 p.m.). Please be aware that staff availability for assisting with FNA procedures is limited. Patience with scheduling is appreciated. Should an FNA be anticipated after 3:30 p.m., contact the FNA service during operating hours for needed supplies. Residents are available for questions but do not assist with FNA slide preparation and assessment of adequacy.

STAT specimens are processed between 7 a.m. and 3 p.m. Call our Client Services team at 352.265.9900 for rush specimen pick-up and delivery to UF Health Pathology Laboratories. For after-hours STAT requests, contact resident on-call at 352.413.6266. STAT specimens MUST have a direct contact number for the requesting clinician written on the requisition.

Collection guidelines (other than FNA)

- 1) Contact laboratory before obtaining cerebrospinal fluid specimen on patient suspected to have Creutzfeldt-Jakob disease.
- 2) All specimens must be capped/sealed tightly.
- 3) When ordering cytologic evaluation and tests for other labs, whenever feasible, provide a separate specimen for the Cytology Laboratory. If one specimen is to be shared between two laboratories, please indicate so.
- 4) Refrigerate specimens that cannot be sent immediately.

Breast secretions: Secretion from the nipple can be obtained in up to 70 percent of women who have borne children. There are two procedures that are acceptable for handling breast cytology specimens:

1. **Six-slide technique:** Secretion from the nipple is expressed on six slides, labeled with the patient's last name and a second identifier. As the drop of secretion appears, the first slide is gently brought to the drop, and the secretion is smeared on the slide using a slide-push technique, as employed in hematology. In this technique, a second slide is simply brought up to the drop. The drop is permitted to spread across the joining edges of the slide, and at that point, the forwarding slide is pushed across the surface, smearing the sample. The sample is immediately placed into 95 percent alcohol or sprayed using an appropriate fixative, being certain the spraying nozzle is at least 12 inches away from the sample.
2. **Breast secretion employing the cytocentrifuge technique:** Collection of a sample employing these technologies requires that the sample be collected fresh in a clean, covered container and submitted directly to the Cytopathology Lab. In these cases, at least 2 mL of sample is generally required to perform cytologic evaluation. If the sample cannot be submitted directly to Cytopathology, the sample must be refrigerated until it can be delivered, along with the appropriate requisition form.
3. Fine needle aspiration may also be used for breast masses.

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Corneal (eye): Samples are collected by the ophthalmology clinicians and submitted dependent of the clinical differential.

Corneal (eye) (continued): If looking **ONLY** for fungi or acanthamoeba, the slide(s) should be submitted as air-dried smears accompanied by a request for GMS (silver) stains. The slide, which must have the patient's last name and 2nd identifier written on the frosted end, is given an accession number and submitted to Histology for a Gomori methanamine silver stain and evaluated for the presence of fungi or Acanthamoeba.

If herpes, Chlamydia, or pre-cancer is considered, then the clinician should IMMEDIATELY place slide in 95 percent ethanol and submit for Pap stain. Also, submit the scraping in PreservCyt for preparation and Pap stain.

Fluid samples from the eye should be transported immediately to the Cytopathology Lab for processing. The delivery to the Cytopathology Lab is delayed, the fluid samples must be refrigerated.

Effusions-Ascites, pleural OR pericardial: If a specimen can be transported promptly to the lab, we prefer fresh fluid. If it cannot be immediately delivered, add three units of heparin per mL of fluid to prevent clotting, and place in refrigerator until it can be delivered. We prefer at least 30 mL of sample or more if possible.

Female genital tract – Monolayer Paps: Obtain an adequate sample (See the ThinPrep Pap kit for detailed collection information.). Rinse the spatula/broom in preservative solution and discard the collection device. Tighten the cap on the vial and label it with the patient's name before placing in a transport bag. HPV, GC and CT testing is now available.

Gastrointestinal tract: Brushing or aspirated material may be smeared directly on slides, which should have the patient's last name and a second identifier printed in pencil on one end. If prepared, the smears should be fixed IMMEDIATELY in 95 percent ethanol and the slides sent to the Cytopathology Lab. If the brush or needle is used in multiple passes in the same site, the brush or needle should be rinsed in a sterile container with 5mL of sterile Plasma-lyte® or sterile balanced electrolyte solution (BSS; e.g. Hanks' Balanced Salt solution). Immediately, after the procedure is finished, this rinse fluid should be added to the Cytolyt® (ThinPrep preservative) vial or Cytolyt® should be added to the rinsed fluid to fixed/preserve the material. The minimum Cytolyt® to sample ratio should be 1:3. The brush used in the procedure may be submitted in Cytolyt® (ThinPrep preservative) preservative fluid (provided by the Lab) for processing with prepared slides.

Respiratory tract (sputum): Instruct the patient to cough deeply (from the diaphragm) to expectorate a deep cough specimen and not saliva. We prefer a fresh unfixed samples. Specimens may be refrigerated until they can be delivered to the Cytopathology Lab. For best results, a series of early morning specimens should be submitted each morning for three consecutive days.

Bronchial: Send fresh bronchial secretions or washings to the Cytopathology Lab immediately, or refrigerate specimens until they can be delivered to the Lab for processing.

If slides are made, print the patient's last name and a second identifier with pencil on one end of all frosted slides. Smear the brushing or aspirated material and fix the slide immediately in 95 percent ethanol. **DO NOT ALLOW THE SMEAR TO AIR-DRY.** The brush may also be placed in saline and submitted. Do not forget to send three post-bronchoscopy sputum specimens to the Lab, obtained over the next three days. These are rich in exfoliated cells from the bronchial epithelium and are of great diagnostic value.

NOTES: 1. Bronchoalveolar lavage is preferred for detection of *Pneumocystis carinii*.
2. In cases where a sample is to be shared between the Cytology and Microbiology Labs, a cytology request form should accompany the specimen to the lab.

Cerebrospinal fluid (CSF): Bring the fresh specimen **PROMPTLY** to the Lab for processing. A sample size of at least 1 - 3 mL is needed. If a lymphoproliferative disorder is suspected, submit the CSF to the Hematopathology Lab for analysis.

Urinary tract (for cancer detection): Send fresh urine or a bladder washing immediately to the Cytopathology Lab. Please indicate whether the specimen is voided or catheterized urine, or a bladder washing. If the specimen cannot be brought immediately to the lab, it **MUST** be refrigerated.

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For cytomegalic inclusion disease (CMV) or polyoma (BK virus) detection: Fresh urine should be sent **IMMEDIATELY** to the Cytopathology Lab after collection. At least 5 mL of urine is required.

Virology Testing Note: Polymerase chain reaction (PCR) testing in virology is the preferred method of detection for BKV and CMV. BKV and CMV are performed on plasma specimens, and BKV is also performed on urine specimens.

Wound or lesion scrapes: Print the patient's last name and a second identifier with pencil on one end of all frosted slides. Scrape the wound with a moistened tongue blade and place the material directly on the slides. **IMMEDIATELY** place the slides in 95 percent ethanol, or spray them with an appropriate fixative, keeping the spray nozzle 12 inches away from the slide surface. If the wound is hard and crusted, it should be soaked with warm saline before obtaining the scrape. These slides will be Pap-stained.

Testing note: Old-style *Tzanck smears* for viral changes are of low quality, and we no longer will interpret them.

GUIDELINES FOR FINE-NEEDLE ASPIRATION (FNA)

Purpose: The purpose of an FNA is to obtain diagnostic cells from a designated site without using open biopsy techniques.

Principle: Tissue is obtained from a specific anatomic site with or without the aid of radiological assistance.

Specimen: FNA of masses for cytological examination

Safety note: All specimens **MUST** be submitted in closed, properly labeled containers and transported in biohazard bags.

When immediate assistance is not available: It is recommended for aspirate be placed into CellGro (RPMI or DMEM). Cell-transport fluid may be obtained from the Cytopathology Lab (Refer to the contact numbers above.).

Smears: Smears must be labeled with the patient's last name and a second identifier. They are prepared from a small drop of the semisolid aspirate placed on a glass slide. This is done by detaching the needle from the syringe and filling the syringe with air. Re-attach the needle and advance the plunger of the syringe to express a small drop of the aspirated material on the center of the glass side. Place the bevel of the needle against the slide while expressing this drop, so there is no intervening airspace that will cause drying artifacts in the alcohol-fixed smears.

Make the smear by laying another slide directly on top of the drop. After the drop spreads from the weight of the slide, rotate the slides apart by turning the upper-slide away from the lower-slide, like turning the page of a book. Immediately after separating the slides, place one into reagent alcohol and allow the other to air-dry for rapid staining.

Cyst fluid or predominately bloody specimens that fill the syringe can be submitted in a properly labeled sterile container or placed in transport solution and submitted to CLSC for transport to the Cytology Lab.

Smears should be fixed in alcohol or spray-fixed while wet for Pap stain. Air-dried smears are helpful for special stains and immediate evaluation of adequacy using rapid stains.

It is preferable to make only two slides per aspiration attempt (one air-dried and one fixed). The rest of the material can then be placed in the transport fluid.

As much of the aspirate as possible should be placed in CellGro (DMEM or RPMI) for possible flow cytometry in suspected cases of lymphoma. If cultures for bacteria or virus are needed, a separate sample should be submitted directly to the Microbiology or Virology Labs for appropriate studies. If submitting smears, it is important to indicate which are spray-fixed and which are air-dried, as these are handled differently.

EACH SPECIMEN MUST BE SUBMITTED WITH A COMPLETED ANATOMIC PATHOLOGY/NON-GYN CYTOLOGY REQUISITION (BELOW).

Patient information*

Name (last, first, middle initial): _____ Sex: ☐ Male ☐ Female

Date of birth (MM/DD/YYYY): _____

Medical record/Patient ID#: _____

Place of service:

- ☐ Hospital inpatient ☐ Ambulatory surgical center
☐ Hospital outpatient ☐ Office/Non-hospital

Billing information

Along with this requisition, you **MUST** include copies of:

- The patient's demographics sheet;
- Both sides of the patient's insurance card(s); and
- Any secondary insurance information (if applicable).

Provider information

Ordering physician: _____

Phone: _____ Fax: _____

Ordering physician NPI #: _____

Duplicate report sent to: _____

Duplicate report fax: _____

Clinical history narrative/Clinical question*:

**An Advance Beneficiary Notice of Noncoverage form must be completed and attached for all Medicare patients.*

Collection date: _____ Time: _____ : _____ A.M./P.M.

Specimen information: Non-GYN cytology

Body fluid: ☐ CSF ☐ Pleural ☐ Peritoneal ☐ Other (*specify below*)

FNA (specify site): _____

Lung BAL/Brushing (specify lobe): _____

Flow cytometry: ☐ Flow cytometry for lymphoma (if indicated)

Urine: ☐ Voided ☐ Bladder washing ☐ Other: _____

☐ Reflex urine to UroVysion™ (if atypical/suspicious/positive)

*Other (specify): _____

Specimen information: Anatomic pathology

Tissue biopsy (designate sites):

A: _____ E: _____

B: _____ F: _____

C: _____ G: _____

D: _____ H: _____

Paraffin block testing

Accession #: _____ Block: _____

Breast carcinoma prognostic markers (image analysis)

Cold ischemia time < 1 hour: ☐ Yes ☐ No

Fixative (neutral-buffered formalin): ☐ Yes ☐ No

Fixation time 6 - 72 Hours: ☐ Yes ☐ No

☐ Comprehensive breast evaluation (ER/PR/Ki-67/HER2 IHC with reflex to FISH)

☐ Breast cancer evaluation (ER/PR/HER2 IHC with reflex to FISH)

☐ Estrogen Receptor (ER) ☐ Progesterone receptor (PR)

☐ HER2 IHC (HercepTest) ☐ Ki-67 (MIB1)

☐ HER2 (ERBB2) (FISH only) ☐ pHH3 IHC (mitotic figures)

Gastric/Gastroesophageal carcinoma

☐ HER2 immunohistochemistry with reflex to HER2 (ERBB2) FISH

☐ Gastric HER2 (ERBB2) (FISH only)

Colorectal carcinoma **Call for a complete list of genes tested.*

☐ Solid tumor next-generation sequencing panel (≥ 27 genes)*

☐ KRAS mutation ☐ Extended KRAS and NRAS

☐ Microsatellite instability (MSI by PCR) ☐ BRAF mutation

☐ MMR IHC (MLH1, MSH2, MSH6, PMS2) ☐ EGFR mutation

Lung carcinoma **Call for a complete list of genes tested.*

☐ Solid tumor next-generation sequencing panel (≥ 27 genes)*

☐ BRAF mutation ☐ KRAS mutation

☐ ALK FISH (FDA-approved) ☐ EGFR mutation

If EGFR/ALK are negative: ☐ Reflex ROS1 FISH ☐ ROS1 FISH

Melanoma/Thyroid

☐ BRAF mutation ☐ C-Kit mutation

Prostate

☐ PTEN/EGR FISH

Gestational Trophoblastic Disease/Molar Pregnancy Evaluation

☐ P57 and DNA ploidy

UroVysion™/Urine FISH

☐ UroVysion™ with cytology ☐ UroVysion™ only

FISH (formalin-fixed paraffin blocks)

☐ ALK FISH ☐ del 1p/19q FISH ☐ PTEN/EGR FISH

☐ DDIT3 (12q13.3) ☐ SS18 (SYT) (18q11.2) ☐ FUS (16p11.2)

☐ MDM2 (12q15) ☐ FOXO1 (13q14.11) ☐ EWSR (22q11)

☐ ROS1 FISH

Other (specify): _____