Surgical Pathology Specimens

**Gross Examination Only**

**Days Performed:**
Monday to Friday, 7:30 am to 5:00 pm (Sinai), 8:00 am to 5:00 pm (Northwest).

**Turn-around-Time:**
Two (2) to three (3) days.

**Collection & Handling Requirements:**
- 10% Neutral Buffered Formalin (NBF)
- Sterile Container – if appropriate for size of specimen
- Must be placed in a “Biohazard Safety Bag.”
- Transport as soon as possible.

**Rejection Criteria:**
- Inadequate information / Missing Requisition
- Unlabeled / Mislabeled Specimen

**Retention Time of Specimen:**
Two (2) weeks after final report is issued (other than specifically requested medicolegal hold).

**NOTE:** The following may be submitted for gross exam only: Accessory digits, Calculi (all urinary calculi are sent to a reference laboratory for chemical analysis following gross description, other calculi may be similarly sent for chemical analysis at the request of the submitting physician), Extraocular muscles from corrective surgical procedures (e.g. strabismus repair), Foreign bodies, Ingrown fingernails and toenails, Ligament or tendon fragments (traumatic laceration), Nasal bone and cartilage from rhinoplasty or septoplasty, Optic lens, Prosthetic devices and Teeth (other than trauma).

**Bone Marrow**

**Days Performed:**
Monday to Friday, 7:30 am to 3:00 pm.

**Expected Turn-around-Time:**
Two (2) to three (3) days.

**Collection & Handling Requirements:**
- Peripheral Blood
- Bone Core in 10% Neutral Buffered Formalin
- Clot in syringe
- Room Temperature
- Send to laboratory STAT/RUSH

Rejection Criteria:
- Inadequate information / Missing Requisition
- Unlabeled / Mislabeled Specimen
- Specimens Submitted in preservative other than that required for testing.

Retention Time of Specimen: Two (2) weeks after final report is issued.

NOTE: Bone marrow kits are ordered by individual departments and no kits are stored in the Laboratory. All Medical Staff seeking kits will be referred to the appropriate department where the procedure will take place.

Specimens should be brought directly to Hematology during business hours (Monday through Friday, 7:00 a.m.-5:00 p.m.). If the specimen is received in Hematology before 2:00 p.m., it is processed the same day, and results will generally be available the following day. If received after 2:00 p.m., every effort will be made to process the specimen on the same day, but due to processing time constraints, the specimen may not be completely processed until the following day. If the specimen is received during the weekend or on a holiday, it will be stored and processed on the next business day.

The following specimens should be collected at the time of the procedure:
1. Core biopsy (1-2) in formalin.
2. Two Lavender top tubes; one with blood (for CBC) and one EDTA with bone marrow aspirate.
3. Two Green Top tubes with bone marrow aspirate.
4. Unstained smears (number of slides varies; may be unilateral or bilateral).
5. Clot in a syringe without the needle.

Specimens should be sent with an appropriately labeled Hematopathology Consultation Request Form including requests for special testing (e.g., cytogenetics, flow cytometry, iron stain) and a Surgical Requisition Form.

Nerve & Renal Biopsy Specimens

Days Performed: Monday to Friday, 7:30 am to 3:00 pm (Sinai), 8:00am to 3:00 pm (Northwest).

Expected Turn-around-Time: Up to ten (10) business days.

Collection & Handling Requirements:
- Fresh- no fixative
- Sterile Specimen Container

Written by: Siobhan Wroten
Effective Date: 12/30/2016
Send to laboratory STAT/RUSH
- Inadequate information / Missing Requisition
- Unlabeled / Mislabeled Specimen
- Specimens Submitted in preservative other than that required for testing.

Retention Time of Specimen: N/A; material is entirely submitted to reference laboratory.

NOTE: for Renal biopsies assessing non-neoplastic disease, performed under CT- or ultrasound guidance, contact the Pathology Department at x2-5963 or x2-3110 (Sinai)/x5-5910 or x5-5815 (Northwest) prior to obtaining the biopsy.

Pathology staff including a Pathologist will perform an on-site evaluation to assess the tissue for adequate glomeruli.

The Pathology Department will package the biopsy for submission to the Reference Laboratory. The reference laboratory requires current clinical information and laboratory results; if outside reports or laboratory results are available, please photocopy the reports for submission to the reference lab with the biopsy.

Core biopsies for full evaluation including routine histology and potential immunofluorescence and electron microscopy must be received fresh (without fixative).

**Surgical Specimens (most routine specimen types, unless specifically listed)**

Days Performed: Monday to Friday, 7:30 am to 5:00 pm

Expected Turn-around-Time: Two (2) to three (3) days

Collection & Handling Requirements:
- 10% Neutral Buffered Formalin
- Send specimen fresh if there is no formalin container large enough to hold specimen
- Send to laboratory as soon as possible
- Refrigerate if delayed delivery is anticipated and specimen is fresh

Rejection Criteria:
- Inadequate information / Missing Requisition
- Unlabeled / Mislabeled Specimen
- Specimens Submitted in preservative other than that required for testing.

Retention Time of Specimen: Two (2) weeks after final report is issued.
NOTE: Most “routine” specimens can be placed in 10% buffered formalin containers (see exceptions above). Formalin containers can be retrieved from the Department of Pathology during business hours (Monday through Friday, 7:30 a.m.-5:00 p.m.).

All specimens must be properly labeled with two patient identifiers (including patient name, and/or date of birth, and/or medical record number). Patient hospital labels include all necessary information, and should be used when possible.

The specimen container should also be labeled with the specimen type (e.g., uterus and cervix), and the designation must match the requisition form. Mislabeled specimens (i.e., no label on container, specimen container designation does not match requisition, improperly completed requisition form, etc.) must be corrected by the OR or clinic representative, and an error report must be completed.

Tissue specimens for culture should be collected fresh in a sterile container and submitted directly to Microbiology, with the appropriate laboratory requisition indicating cultures of interest (e.g., aerobic, anaerobic, AFB, fungus).

For cases requiring dual evaluation by Microbiology and Anatomic Pathology, separate tissue (and/or swabs) should be sent to both areas with accompanying requisitions.

For cases requiring dual evaluation of one sample, the tissue should be sent fresh in a sterile container to Anatomic Pathology, with clear instructions for culture on the requisition.

For cases of suspected lymphoma requiring flow cytometric analysis, send the tissue fresh (without fixative) to the Pathology Department with a notation on the requisition (e.g., “R/O lymphoma” or “Flow cytometry”). Tissue in RPMI media is also acceptable. Tissue placed in formalin cannot be sent for flow cytometry.

For cases requiring chromosomal analysis (e.g., products of conception, some malignancies), send the tissue fresh (without fixative) to the Pathology Department with a notation on the requisition (e.g., “For chromosomal analysis” or “Karyotyping”).

Tissue in RPMI or FBS (fetal bovine serum) is also acceptable. Tissue placed in formalin cannot be sent for chromosomal analysis. If chromosomal analysis is desired on tissue submitted in formalin, additional methods are available on paraffin-embedded tissue using FISH.

For cases requiring submission of tissue to Precision Therapeutics or Caris laboratories for analysis, submit fresh tumor tissue to Pathology and include a notation on the requisition.

ChemoFx testing for Precision Therapeutics requires fresh tissue for tissue cultures; testing on formalin-fixed tissue cannot be performed.
BioSpeciFx testing at Precision Therapeutics and molecular testing at Caris is performed on formalin-fixed tissue, but both require limited fixation time.

Fresh tissue submitted to Pathology for Precision Therapeutics or Caris testing will be packaged and submitted directly to the appropriate site for processing and evaluation.

**Biopsy Specimens (most biopsy specimen types, unless specifically listed)**

**Days Performed:** Monday to Friday, 7:30 am to 5:00 pm

**Expected Turn-around-Time:** One (1) to two (2) days.

**Collection & Handling Requirements:**
- 10% Neutral Buffered Formalin
- Send to laboratory as soon as possible
- Refrigerate if delayed delivery is anticipated and specimen is fresh

**Rejection Criteria:**
- Inadequate information / Missing Requisition
- Unlabeled / Mislabeled Specimen
- Specimens Submitted in preservative other than that required for testing.

**Retention Time of Specimen:** Two (2) weeks after final report is issued.

**NOTE:** Most biopsy specimens can be placed in a 10% buffered formalin jar (which can be obtained from the Department of Pathology). This includes any specimen for routine histologic evaluation with or without immunostains. However, many exceptions exist (see below).

All specimens must be properly labeled with two patient identifiers (including patient name, and/or date of birth, and/or medical record number). Patient hospital labels include all necessary information, and should be used when possible.

The specimen container should also be labeled with the specimen type (e.g., uterus and cervix), and the designation must match the requisition form. Mislabeled specimens (i.e., no label on container, specimen container designation does not match requisition, improperly completed requisition form, etc.) must be corrected by the OR or clinic representative, and an error report must be completed.

Routine small biopsy specimens received before 4:00 p.m. on business days are usually reported the day following removal.

Specimens marked “RUSH” that are received prior to 5:00 p.m. on business days are reported first, usually before 1:00 p.m. on the following day. For RUSH specimens collected on a Friday requiring immediate diagnosis for rapid treatment, state on the requisition that a Saturday read is necessary. Please call the laboratory (x2-5963- Sinai/ x5-5910- Northwest) and ask for the
Pathologist on-call for the following Saturday to discuss the need for a Saturday Rush case. RUSH specimens should be immediately delivered to the Pathology Department to avoid delay in processing and evaluation.

Specimens received after 5:00 p.m. on business days are processed the following day, and a diagnosis will not be rendered until the second day following biopsy.

For emergency situations, it is possible to rapidly process small biopsies and report them the same day they are received (including specimens received after hours the prior evening), provided they are received before 9:00 a.m. on business days. Contact the laboratory (x2-5963-Sinai/ x5-5910- Northwest) in these situations.

If special testing is required and the submitting physician is unclear on the submission requirements, please contact the Department of Pathology at x2-5963 (410-601-5963) to discuss specimen collection requirements and delivery. Most molecular testing can be performed on formalin-fixed, paraffin-embedded tissue.

For fine needle aspiration biopsies requiring on-site evaluation to determine adequacy, contact Cytology at Sinai (x2-5104) or APLT at Northwest (x5-5910) to set up the evaluation.

**Skin Biopsy Specimens (Punch or Shave)**

Days Performed: Monday to Friday, 7:30 am to 5:00 pm

Expected Turn-around-Time: One (1) to two (2) days.

Collection & Handling Requirements:
- 10% Neutral Buffered Formalin
- Send to laboratory as soon as possible
- Refrigerate if delayed delivery is anticipated and specimen is fresh

Rejection Criteria:
- Inadequate information / Missing Requisition
- Unlabeled / Mislabeled Specimen
- Specimens Submitted in preservative other than that required for testing.

Retention Time of Specimen: Two (2) weeks after final report is issued.

**NOTE:** If special testing is required and the submitting physician is unclear on the submission requirements, please contact the Department of Pathology at x2-5963 (410-601-5963) to discuss specimen collection requirements and delivery. Most molecular testing can be performed on formalin-fixed, paraffin-embedded tissue.
For skin biopsies requiring immunofluorescence studies (e.g., bullous disorders such as bullous pemphigoid; any suspected antibody- or complement-mediated disorder), submit the tissue to Pathology either fresh (without fixative) or in Michel’s medium.

**Muscle Biopsy Specimens (For Send Out)**

**Days Performed:** Monday to Friday, 7:30 am to 3:00 pm (Sinai), 8:00 am to 3:00 pm (Northwest).

**Expected Turn-around-Time:** Up to ten (10) business days.

**Collection & Handling Requirements:**
- Fresh - no fixative
- Sterile Specimen Container
- Send to laboratory STAT/RUSH

**Rejection Criteria:**
- Inadequate information / Missing Requisition
- Unlabeled / Mislabeled Specimen
- Specimens Submitted in preservative other than that required for testing.

**Retention Time of Specimen:** N/A; material is entirely submitted to reference laboratory

**NOTE:** For a muscle biopsy in the setting of neuromuscular disease, please notify the Pathology Department (Sinai x2-5963/ Northwest x5-5910) in advance of the pending biopsy. Muscle biopsies should be received by the Pathology Department prior to 2:00 p.m. to allow packaging and shipping to the University of Maryland Medical Center Department of Pathology. The specimen should consist of at least two cylindrical fragments of muscle measuring 2 x 0.5 cm. The specimen should be placed on saline-moistened Telfa pads in a properly labeled container, placed on ice and transported immediately to the Pathology Department. The UMMC laboratory receives specimens Monday through Friday, and the specimen must be received by 4:00 p.m. Muscle biopsies require immediate processing for proper histochemical studies, and collection of specimens after 2 pm on weekdays or on weekends may compromise the complete evaluation.
Cytopathology Specimens

**ThinPrep® Pap Preparations**

Indications: Cervical screening for the detection of premalignant or malignant lesions of the cervix which can be eradicated thus reducing the occurrence of invasive carcinoma of the cervix.

The use of the thin-layer/liquid-based PAP at LifeBridge Health has replaced the conventional Pap smear.

Specimens Required: Cervical sample from the junction of the ectocervix and the endocervix (transformation zone).

Expected Turn-around-Time: Three (3) to seven (7) days.

Supplies: Cytyc ThinPrep® PreservCyt Vial and Broom-type sampling device or Endocervical Brush/Spatula.

Collection Method:

- Properly fill out a LifeBridge Health Cytopathology Requisition with appropriate Patient demographics, pertinent clinical information and relevant history.
- Label ThinPrep PreservCyt collection vial with correct patient name and medical record number and/or DOB.
- Collect a sample with a broom-type sampling device or endocervical brush/spatula using one of the following procedures:

  **Broom-like Device Collection:**

  **Recommendation:** Lubricant is not recommended as it interferes with the processing of the specimen and may result in an inadequate slide. If lubricant has to be used in collection of specimen, suitable lubricants include KY Jelly (non sterile or sterile), Surgilube, or Astroglide. Other lubricants interfere with specimen and should not be used.

  - Label ThinPrep® PreservCyt vial with patient name and MRN and/or DOB.
  - Obtain sample using broom-like device.
  - Insert central bristles into the endocervical canal deep enough to allow the shorter bristles to contact the ectocervix.
  - Push gently and rotate CLOCKWISE five times.
  - Rinse the broom immediately in the PreservCyt vial by pushing the broom into the bottom of the vial 10 times forcing bristles apart.
  - Swirl brush vigorously in solution.
  - Discard brush- **DO NOT** submit brush in vial.
- Tighten cap on vial until torque line on cap passes torque line on vial.
- Transport to laboratory for processing.

**Endocervical Brush/Spatula Collection:**

**Recommendation:** Lubricant is not recommended as it interferes with the processing of the specimen and may result in an inadequate slide. If lubricant has to be used in collection of specimen, suitable lubricants include KY Jelly (non sterile or sterile), Surgilube, or Astroglide. Other lubricants interfere with specimen and should not be used. Label ThinPrep® PreservCyt vial with patient name and MRN and/or DOB.

- Obtain a sample from the ectocervix using a plastic spatula.
- Rinse the spatula into the PreservCyt vial by swirling vigorously in the vial 10 times.
- Discard the spatula.
- Obtain a sample from the endocervix using an endocervical brush.
- Insert brush in to the cervix until only the bottom fibers are exposed.
- Slowly rotate 1/4 to 1/2 turn in one direction. DO NOT OVER ROTATE.
- Rinse the brush quickly in the PreservCyt vial by rotating the device in the solution 10 times while pushing against the vial wall.
- Swirl vigorously to further release material.
- Discard brush-DO NOT submit in vial.
- Tighten cap on vial until torque line on cap passes torque line on vial.
- Transport to laboratory for processing.

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**Reflexive HPV-DNA Testing on ThinPREP® Specimens with ASCUS**

Indications: Human Papilloma Virus DNA (HPV-DNA) testing is available for those patients with the diagnosis of Atypical Squamous Cells of Undetermined Significance (ASCUS). Those patients in whom Atypical Squamous Cells of Undetermined Significance (ASCUS) are detected should have a HPV-DNA test performed. This test is most cost-effective when performed on the original liquid-based thin-layer specimen in which the ASCUS was identified. Patient management can then be based on an algorithm using the results of these combined tests.

Specimens Required: Liquid-Based Thin-Layer specimen.

Expected Turn-around-Time: Three (3) to seven (7) days.
Supplies: Cytyc ThinPrep® PreservCyt Vial & Broom-type sampling device OR Endocervical Brush/Spatula.

Collection Method:
- Collect Thin-layer specimen for gynecologic cytology following Thin-layer (ThinPrep®) Preparation collection method (see above).
- Select or provide handwritten instructions on the Cytology requisition for: "Thin-layer With Reflex HPV if ASCUS" (Recommended Standard of Care). HPV-DNA Testing results will appear as a Procedure/Addendum to the original Gynecologic Cytology Report when Reflexive HPV Testing is completed.

**Anal Cytology**
The “Anal Pap” is a screening used in high risk population to identify pre-malignant and malignant cytologic changes of the anal epithelium.

Indications: Detection and characterization of pre-malignant and malignant changes.

Specimens Required: Liquid-Based Thin-Layer specimen.

Expected Turn-around-Time: One (1) to two (2) days.

Supplies: ThinPrep vial, break off spatula and cytobrush.

Collection Procedure:
- Label ThinPrep vial with appropriate patient name and MRN that corresponds to the information provided on the requisition.
- Insert the cytobrush 1.5 cms into the anal canal.
- Slowly retract the cytobrush with a twisting motion.
- Break off the cytobrush tip and drop in vial. Use the spatula to perform a sweep of the anus.
- Break off the spatula tip and drop in vial.
- Send labeled vial with collection device tips submitted in vial and Cytology Requisition to lab for processing.

**Note:** HPV testing on anal specimens is a referred test. A separate specimen must be submitted. Specimens for HPV testing on anal samples must be collected using a Digene Cervical Sampler/Specimen Transport.

**Body Cavity Fluid Specimens**
Body cavity fluids are commonly evaluated for the presence of malignant cells from metastatic disease. Body cavity fluids in general are relatively easy to obtain and are relatively difficult to
compromise. However, in some instances, due to a large number of inflammatory cells, specimens may degenerate rapidly. In addition, if large amounts of protein are present, the specimen may clot, trapping diagnostic cells within the clot.

Indications: Detection and characterization of malignant cells in body cavity fluids.

Specimen required: Minimum 10 mL (< 10 mL preferred, 100 mL optimal) of fluid obtained from an appropriately performed paracentesis or thoracentesis.

Expected Turn-around-Time: One (1) to two (2) days.

Supplies: Standard paracentesis or thoracentesis equipment and clean collection container of appropriate size.

Collection Procedure:
- Using standard paracentesis or thoracentesis technique, obtain a fluid specimen from the desired body cavity. If necessary, move the patient into multiple positions to suspend cellular material in the fluid. A minimum of 10 mL (> 10 mL preferred, 100 mL optimal) of specimen is desirable for optimal cytologic evaluation.
- If other studies are required, withdraw a fraction of the specimen and submit it to the appropriate laboratory separately, following their guidelines for specimen collection. Heparin may be added to the specimen to reduce clotting. Place 3 units of heparin per mL capacity of the collection container. Gently agitate to thoroughly mix the specimen and heparin.
- Submit the specimen to the Cytopathology Laboratory along with the completed cytology request form. The specimen should be refrigerated or kept on wet ice until transport to the lab.

Breast Nipple Secretions
Indications: Detection of malignant cells or characterization of components in nipple discharge specimens.

Specimens Required: Direct smear of nipple discharge.

Expected Turn-around-Time: One (1) to two (2) days.

Supplies: Two clean glass slides with label area, fixative (spray fixative or 95% ethanol), requisition form.
Collection Procedure:
- Label the two slides in pencil with the patient name and DOB.
- Collect a small amount of nipple secretion directly onto one of the slides.
- Oppose a second glass slide onto the first, allowing the collected material to provide surface tension between the two slides, and then gently and quickly pull the two slides apart in a horizontal motion to distribute the material in a thin film over both slides.
- The smears should be immediately fixed in either spray fixative or 95% ethyl alcohol to prevent air drying.
- Submit the specimen and the completed request to the Cytopathology Laboratory.

**Conjunctival Scrapings**
Indications: Detection and characterization of inflammatory/infectious processes of the conjunctiva.

Specimens Required: Direct smear of material collected from the conjunctival surface.

Expected Turn-around-Time: One (1) to two (2) days.

Collection Procedure:
- Label the slides with the patient name and DOB.
- Gently scrape the area of abnormality.
- Evenly smear the collected material on one of the glass slides.
- Repeat the process with a second slide if necessary for better diagnostic yield.
- Submit the specimen and the completed request to the Cytopathology Laboratory.

**Cerebrospinal Fluid Specimens (CSF)**
In cytology, cerebrospinal fluids are most commonly evaluated to detect and characterize malignancy which may have gained access to the central nervous system. While in most individuals CSF specimens are relatively easy to obtain, in some individuals collection may require radiographic guidance. In addition, due to lack of nutrients in most of these fluids, cells may rapidly degenerate rendering morphologic evaluation less than optimal if adequate care is not taken.

Indications: Detection and characterization of malignant cells in the central nervous system.

Specimen Required: Minimum of 0.5 mL cerebrospinal fluid.
Expected Turn-around-Time: One (1) to two (2) days.

Supplies: Standard cerebrospinal fluid collection equipment and clean, clear 10 mL collection container for CSF.

Collection Procedure:
- Using standard CSF collection procedures, collect a minimum 0.5 mL or more of CSF.
- In general, morphology of cells within the CSF fluid can be
- Adequately maintained with prompt refrigeration for 24 hours.
- Submit the specimen to the Cytopathology Laboratory along with the completed cytology request form.

**Fine Needle Aspirations (FNA) Specimens**

Fine needle aspiration of mass lesions is commonly utilized in the detection and characterization of a variety of malignant diseases. Obtaining an adequate specimen requires attention to good aspiration technique as well as to processing of material obtained.

Indications: to determine benignity or malignancy of mass lesions and to characterize the type of malignancy or benign disease that is present.

Specimen Required: Adequate cellular material for cytologic evaluation obtained from an appropriately performed fine needle aspiration. This will depend on the specimen site and character of the lesion being aspirated. In general, this requires that there be enough material for the examiner to at least determine that the aspirating needle sampled a mass lesion.

Expected Turn-around-Time: One (1) to two (2) days.

Supplies: 3, 5, 10 or 20 mL syringe. Syringe pistol (optional).
- 22 to 25 gauge needle of appropriate length.
- Single end label clear glass slides (for preparation of direct smears).
- Fixative to preserve fixed slides (either Cytology spray fixative, Saccomanno fixative or 95% ethyl alcohol in coplin jar). RPMI 1640 medium should be used to submit remaining aspirated material for additional studies.).

Collection Procedure: Please note that the following collection procedure is a suggested guideline. Aspiration techniques vary widely based on personal preference, and specific clinical
circumstances must be taken into account when deciding on the method of aspiration utilized. NOTE: If collecting FNA for flow cytometry studies, please refer to the T&B cell markers collection guidelines for storage and transport information.

- Identification and Localization of a Mass Lesion: Mass lesions usually come to attention either by simple identification of the development of a mass (usually superficially) or by the development of symptoms directly or indirectly caused by the mass. In order to be able to sample the identified lesions, some means of accurate localization must be available.

- Patient Preparation: For superficial aspirates, clean technique suffices for cleansing of the skin surface. Local anesthetic may or may not be used. If more than two or three attempts are anticipated, this is recommended. However, be certain not to contaminate the lesion with a large volume of anesthetic. Also, make attempts not to directly interfere with the ability to palpate and localize the lesion.

- For deep aspirates, sterile technique is required for cleansing of the skin and local anesthetic is usually required.

- Assemble the aspirating equipment. If direct smears are to be made, label the slides prior to the aspiration. With the target of aspiration fixed with the non-dominant hand between the thumb and index finger, and the syringe pistol in the dominant hand, the needle is placed against the skin. If the lesion is very superficial, the needle should approach the skin at approximately a 30 degree angle. If the mass is deep, it should approach the skin perpendicularly.

- A quick motion should be used in passing the needle through the skin. The needle is then advanced through the subcutaneous tissue into the mass. If the mass is small, the needle should be aimed toward the center; if it is large, the needle should be aimed toward the periphery as the center of larger masses may be necrotic. A noticeable difference in the consistency of the tissue should be noted when the needle penetrates the mass.

- With the needle in the mass, the needle tip should be moved in short motions initially to loosen cells with in the mass. Negative pressure is then applied by pulling back on the plunger of the syringe. Without releasing
pressure, the needle within the target is withdrawn slightly but not out of the lesions, and then reinserted at a slightly different angle. This maneuver should be repeated several times before complete withdrawal. While redirecting the needle, a corkscrew action may be used. If blood or material appears in the hub of the needle, the aspiration should be stopped.

- Prior to withdrawal of the needle, negative pressure must be released to prevent suction of the material into the barrel of the syringe when the needle exits the skin.

**Aspiration (Deep Lesions):**
- While the basic aspiration procedure is similar for deep lesions, specialized equipment for imaging, specialized needles, and setups for aspiration, and emergency equipment for handling major complications are required. Specific techniques are highly variable, according to personal preferences. While this guide will not attempt to provide methodological guidelines for aspiration of deep masses, the principles of not applying negative pressure until in the mass, stopping aspiration when blood or material appears in the hub of the needle, and not maintaining negative pressure when withdrawing the needle should be kept in mind.

**Preparation of Direct Smears:**
- For preparation of smears, single-end label clear glass slides should be utilized. Slides should be labeled prior to aspiration. Some author investigators recommend gently expressing a drop of aspirated fluid onto a slide, while others recommend forcefully expelling the material onto the slide. The actual method will be determined in part by the nature of the material present. If the aspirated material is abundant and fluid, a drop may be easily expressed without force. If the material is scant or more viscous or solid, the material must often be forcefully expelled. The latter method can result in splattering of material off of the slide and will utilize most of the specimen in the preparation of a minimal number of smears, necessitating more passes if additional material is required for additional studies. The former process allows for better controlling of the smear process.

- Once the specimen is on the slide, it must be smeared. The simplest way to accomplish this is to oppose a second glass slide onto the first, allowing the aspirated
material to provide better surface tension between the two slides, and then gently and quickly pull the two slides apart in a horizontal motion to distribute the material in a thin film over both slides.

- One smear from each pass should be immediately spray-fixed or immediately dropped into a Coplin jar containing enough 95% ethyl alcohol to cover the specimen area of the slide.

- The second slide from each pass should be allowed to air dry. The air dried slide can be stained with Diff Quick and used to provide an immediate assessment of cellularity if appropriate training has been provided (contact Cytology Laboratory).

- If the material remains in the hub of the needle, additional smears may be prepared or the material may be flushed into a tube containing CytoLyt.

- It is recommended that the additional aspirated material be flushed into CytoLyt for further processing by the Cytopreparatory Lab.

- Submit the specimen (smears and/or material expelled into solution) to the Cytopathology Laboratory along with the completed cytology request form. Each slide should be labeled with patient identifiers (Name & MRN or Name & DOB). If location of fine needle aspiration is changed then source should be noted on slide as well.

**NOTE:** Recommended collection procedure is to obtain three passes from each mass/lesion. One fixed slide and one air dried slide from each pass, remaining material to be submitted in CytoLyt. Perform a fourth pass to submit in CytoLyt for additional studies to be performed in Cytology Laboratory. If passes are from same mass/lesion, may be submitted in single tube of CytoLyt. A new tube CytoLyt should be used for each additional site sampled.

**Gastrointestinal Specimens**

The adequacy of a gastrointestinal specimen is determined primarily by the presence of well-preserved epithelial cells indicative of the type of epithelium present at the gastrointestinal site sampled. All GI specimens will tend to deteriorate rapidly due to enzymatic activity which is present throughout much of the GI tract. In addition, these specimens are easily contaminated by epithelia from sites proximal to that being sampled.

General information for all Gastrointestinal Specimens: As all GI specimens will rapidly deteriorate in the fresh state, collection of the specimen in CytoLyt fixative is requested. The specimen should be refrigerated or placed on wet ice until transport even if for a shorter period of time.
**Brushings (Esophageal, GE Junction, Gastric, Duodenal, Bile Duct, Other)**

Indications: For detection and characterization of endoscopically visible gastrointestinal lesions; for the identification of some microbiologic pathogens (primarily Herpes, CMV, and Candida).

Specimen Required: Endoscopically obtained brushing of region of the suspected lesion.

Expected Turn-around-Time: One (1) to two (2) days.

Supplies: - Standard endoscopy equipment, spray fixative and slide.
- Fixative (CytoLyt container).

Collection Procedure: - Instruct the patient to fast overnight or for a minimum of six hours prior to the procedure.
- Using standard endoscopy technique, identify the lesion in question and obtain a brushing sample of the lesion.
- It is important to brush the edges of an ulcer, as well as the floor, in order to obtain diagnostic material.
- Upon withdrawing the brush, roll across a glass slide (label side up), depositing as much specimen as possible on the slide.
- Spray fix the slide(s) with Cytology fixative.
- Submit the brush into the CytoLyt container.
- Slide(s) must be labeled in pencil with patient name and MRN or DOB.
- Submit slides in slide mailer and CytoLyt container along with completed Cytology requisition to the Cytology Lab.

**Washings (Esophageal, GE Junction, Gastric, Duodenal, Bile Duct, Other)**

Indications: for detection and characterization of endoscopically ill-defined or invisible gastrointestinal lesion; for the identification of some microbiologic pathogens (primarily Herpes, CMV, and Candida).

Specimens Required: Endoscopically obtained washing (preferably at least 10 mL) of the region of the suspected lesion.

Expected Turn-around-Time: One (1) to two (2) days.

Supplies: - Standard endoscopy equipment and clean plastic specimen container(s).
- Fixative (CytoLyt fixative).

Collection Procedure: - Instruct the patient to fast overnight or for a minimum of six hours prior to the procedure.
Using standard endoscopy technique, lavage the area of interest using a physiologic solution. Aspirate the solution and place in a clean specimen container.

Label the container with the correct patient information and submit the specimen and the completed cytology request form to the Cytopathology Lab.

If transport of the specimen will be delayed more than 4 hours, add 50 mL of CytoLyt fixative or 50% ethyl alcohol.

If transport time will be less than 4 hours, or fixative is not available, the specimen should be refrigerated or kept on wet ice until transport to the lab.

**Oral Scrapings**

**Indications:** Detection of and characterization of malignancy and infectious process in the oral cavity.

**Specimen Required:** Direct smear of material collected from the oral mucosa.

**Expected Turn-around-Time:** One (1) to two (2) days.

**Collection Procedure:**

- Label the slides with the patient's name and place in a container filled with 95% ethyl alcohol so that the slides are completely covered.
- Gently scrape the area of abnormality.
- Remove one of the slides from the fixative and quickly and evenly smear the collected material on one of the glass slides.
- Immediately re-immers the slide in fixative.
- Repeat the process with the second slide if necessary for better diagnostic yield.
- Repeat the process for additional areas if necessary.
- Submit the specimen and the completed request form to the Cytopathology Laboratory.

**Pulmonary Specimens**

The adequacy of a sputum specimen is determined primarily by the presence of alveolar macrophages indicating that the specimen obtained is a deep cough specimen producing material from the lower airways. In addition, the specimen should not be obscured by oral or upper airway contaminants.

Adequate bronchial brushing and washing specimens should contain large numbers of well-preserved bronchial lining cells with as little contaminating oral and upper airway material as
possible. Bronchoalveolar lavage specimens should contain abundant, well-preserved, alveolar macrophages with as little contaminating upper airway material as possible.

### Sputum

**Indications:** For the detection and characterization of premalignant/malignant pulmonary lesions.

**Specimen Required:** Minimum 5 mL (> 5mL if possible), of sputum obtained from a deep cough specimen.

**Expected Turn-around-Time:** One (1) to two (2) days.

**Supplies:**
- Clean plastic specimen container;
- Fixative (CytoLyt fixative)

**Collection Procedure:** When clinically feasible, sputum specimens should be obtained as follows.

- The optimum time for specimen collection is within 15 to 30 minutes after waking and before eating breakfast. Brushing of teeth or rinsing of the mouth thoroughly with water will reduce contamination by saliva.
- Instruct the patient to inhale and exhale deeply, forcing air from the lungs using the diaphragm. Repeat until the patient coughs and is able to produce a sputum specimen.
- Collect the specimen in the container, attempting to obtain at least one teaspoon of sputum, add 50 mL of CytoLyt fixative.
- If transport time will be less than 24 hours, or fixative is not available, the specimen should be refrigerated or kept on wet ice until transport to the lab. Greater diagnostic yield may be obtained if specimens are submitted on three to five successive mornings.
- Label the container with correct patient information and submit the specimen along with the completed cytology request form to the Cytopathology Laboratory.

**Post-Bronchoscopy Sputum:**
- One good deep cough specimen at any time during the 24 hour period following bronchoscopy, as outlined above.
- Submit the specimen to the Cytopathology Laboratory, along with the completed cytology request form.

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### Bronchial Brushings

**Indications:** For the detection and characterization of bronchoscopically visible premalignant/malignant pulmonary lesions; for the identification of some microbiologic pathogens (primarily viral and fungal).

**Specimen Required:** Bronchoscopically-directed brushing of the identified lesion.

**Expected Turn-around-Time:** One (1) to two (2) days.

**Supplies:**
- Standard bronchoscopy equipment. Clean glass slides, labeled with 2 forms of patient identification on appropriate end of slide.
- Cytology spray fixative.

**Collection Procedure:**
- Using standard bronchoscopy technique, identify the lesion in question and obtain a brushing sample of the lesion.
- Roll/smear brush on slides; fix with appropriate spray fixative or brush tip may be submitted to Cytology Lab in CytoLyt for further processing.

### Bronchial Washings and Bronchoalveolar Lavage (BAL)

**Indications:** For the detection and characterization of bronchoscopically ill-defined or invisible premalignant/malignant pulmonary lesions; for the identification of some microbiologic pathogens (primarily viral and fungal).

**Specimen Required:** Bronchoscopically-obtained washing (preferably at least 10 mL) of the bronchi in the region of the suspected lesion.

**Expected Turn-around-Time:** One (1) to two (2) days.

**Supplies:**
- Standard bronchoscopy equipment. 120 mL clean plastic specimen container(s).
- Fixative (CytoLyt fixative).

**Collection Procedure:**
- Using standard bronchoscopy BAL technique, lavage the lung distribution in question with normal saline.
- Collect the lavage specimen in a clean specimen container.
- Label the container with the correct patient information and submit the specimen, along with the completed cytology request, to the Cytopathology Laboratory.
- If transport will be delayed, refrigerate the specimen. Do NOT add fixative to the specimen.
- Lipid-Laden Macrophages (Oil Red O): Do NOT add fixative to the BAL.
- Lipid ladens receive no fixative. MUST be received FRESH. Note on requisition that specimen is for lipid-laden macrophages.
- Hemosiderin-Laden Macrophages (Iron Stain): BAL should be received FRESH. Do NOT add fixative. Note on requisition specimen is for hemosiderin-laden macrophages.

### Tzanck Smears

**Indications:** Detection and characterization of inflammatory/infectious processes of the skin, especially herpetic infection (Tzanck smear).

**Specimen Required:** Direct smear of material collected from a skin lesion, usually a vesicle.

**Expected Turn-around-Time:** One (1) to two (2) days.

**Supplies:**
- Two (or more) clean glass slides with label end
- Skin scraping spatula
- Specimen requisition.

**Collection Procedure:**
- Label the slides in pencil with the patient name/DOB.
- Gently scrape the area of abnormality. If the abnormality is a vesicle, remove the covering and scrape both at the base of the vesicle and around the rim.
- Quickly and evenly smear the collected material on one of the glass slides.
- Repeat the process with the second slide if necessary for better diagnostic yield.
- Repeat the process for additional areas if necessary.
- Allow the slides to air dry (do not fix).
- Submit the specimen and the completed request form to the Cytopathology Laboratory

### Urologic Specimens

Urine is commonly evaluated cytologically for the presence of malignant cells in the detection of urologic malignancies. Method of specimen collection as well as time of collection will affect the cytologic evaluation in many instances. See below for specifics.
Voided/Catheterized Urine

Indications: Detection and characterization of malignant cells and other urologic abnormalities in symptomatic (usually hematuria) patients; screening for malignancy in selected individuals at high risk for the development of urologic malignancy.

Specimen Required: 50 mL of an appropriately collected voided, catheterized, or cystoscopically obtained urine specimen.

Expected Turn-around-Time: One (1) to two (2) days.

Supplies: - Clean collection container of appropriate size.
- Fixative (CytoLyt Fixative).

Collection Procedure: - For purposes of obtaining the greatest yield of diagnostic material, a second morning voided urine specimen should be obtained, if possible. A midstream, clean catch specimen is recommended to avoid vaginal contamination in female patients. A midstream specimen, not necessarily clean catch, is recommended for male patients.
- If the patient must be catheterized to obtain the specimen, this should be noted on the specimen requisition as catheterization can lead to artifacts that may be misinterpreted without the knowledge that the specimen was catheterized.
- Submit the specimen to the Cytopathology Laboratory along with the completed cytology request form.
- Add an equal volume of CytoLyt fixative (if sample size is too large to accommodate this volume, a well-mixed aliquot of the specimen with an equal volume of fixative may be utilized.
- With CytoLyt fixative added, specimen may be transported at room temperature.
- If fixative is not available, the specimen should be refrigerated or kept on wet ice until transported to the lab.

Other Urologic Specimens

Indications: Detection of suspected malignancy utilizing lavage specimens obtained cystoscopically (bladder washing, ureteral washing); staging of urologic malignancies. Specimen Required: 50 mL of an appropriately collected voided, catheterized, or cystoscopically obtained urine specimen.
Specimens Required: 10 mL (or more) of an appropriately collected cystoscopically derived specimen.

Expected Turn-around-Time: One (1) to two (2) days.

Supplies:
- Standard cystoscopy equipment.
- Clean collection container of appropriate size.
- Fixative (CytoLyt Fixative).

Collection Procedure:
- Using standard cystoscopy technique, obtain washing specimens, carefully denoting specific specimen sites for each specimen on the requisition.
- Add an equal volume of CytoLyt fixative to the specimen.
- Submit the specimen to the Cytopathology Laboratory along with the completed cytology request form.
- With CytoLyt fixative added, specimen may be transported at room temperature.
- If fixative is not available, the specimen should be refrigerated or kept on wet ice until transported to the lab.