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SURGICAL TISSUE ROUTINE SPECIMEN COLLECTION PROCEDURE

SPECIMEN COLLECTION*:* Surgical specimens should be placed as soon as possible in 10% neutral buffered formalin. Close container lids securely to eliminate leakage and possible loss of tissue.

NOTES:

1. **Breast cases need documentation of the date and time specimen is placed in formalin in the box at the lower right hand corner of the requisition.**
2. **Amputated limbs should be wrapped in several red biohazard bags secured and sent fresh (without 10% neutral buffered formalin).**
3. **Specimens intended for culture must be collected in a sterile container or in sterile saline (without fixative) and split from the main tissue specimen prior to transport with the proper requisition order for microbiology culture. Consult your clinical lab test catalog for specific specimen requirements.**

FIXATIVE: 10% neutral buffered formalin

LABELING*:* Surgical specimen containers are considered a primary specimen and must be labeled with at least two patient specific identifiers. These include the PATIENT **NAME AND SITE/SOURCE OF SPECIMEN and one other identifier such as date of birth, hospital number, social security number, requisition number or unique random number**. Laterality and specimen source are critical to analysis.

STORAGE: Ambient temperature

REQUISITION: Fill out all information completely including **Billing Information** in the appropriate boxes. Include a copy of all insurance cards (front and back) and a copy of the facility data sheet, if available.

NOTES: Fill out the "Additional Reports To" field with any additional referring physicians and/or facilities making sure to INCLUDE their corresponding fax number.

SURGICAL PATHOLOGY SPECIMEN SITES: Include any preop/postop diagnoses and clinical information in the appropriate boxes on the requisition. Include the anatomic site under "Specimen Origin" using 1 line for each specimen. **One requisition can be used for multiple specimens.**

TRANSPORT: Place specimen container in a **biohazard specimen transport bag** and seal closure.

FROZEN SECTIONS: For pathology services for frozen sections, please contact one of the Heartland Pathology Consultants pathologists at 405.715.4500.

SPECIMEN PICKUP and SUPPLIES: Contact HPC at 405.715.4500 for scheduling pickup and to order supplies.

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BONE MARROW/PERIPHERAL SMEAR COLLECTION PROCEDURE

CLINICAL HISTORY: Provide clear, complete clinical history including current CBC and relevant laboratory results.

LABELING*:* Bone marrow and peripheral blood smear specimens, particularly prepared slides, are considered a primary specimen and must be labeled with at least two patient specific identifiers. These include the PATIENT **NAME AND SITE/SOURCE OF SPECIMEN and one other identifier such as date of birth, hospital number, social security number, requisition number or unique random number**. Laterality and specimen source are critical to analysis.

BONE MARROW CORE BIOPSY PREPARATION:

1. Obtain biopsy core specimen from posterior iliac crest.
2. Make several touch preps of the bone marrow core by touching the core lightly to a clean glass slide.
3. Place bone marrow core biopsy in 10% buffered formalin.

BONE MARROW ASPIRATE PREPARATION:

1. Draw bone marrow aspirate in a syringe containing no anticoagulant.
2. Make four to eight slides by putting a single drop of marrow aspirate from the syringe or needle on each slide and a making a blood film as you would with a peripheral blood smear. Thoroughly air dry. Label these slides “ASP”. Proper labeling of this material is very important since it is not always possible to identify the source of smears, especially when marrow aspirates are markedly hypocellular.
3. Fill a 5 mL blood tube (containing approximately 0.5 mL of EDTA) with 1.5 mL of bone marrow aspirate material. Mix well to prevent clotting as soon as possible after the aspirate is drawn. Label this tube, “Aspirate”.
4. Place the remainder of the aspirate material in a tube with no anti-coagulant and allow the material to clot. The clot should then be placed in 10% buffered formalin.
5. Also collect a tube of EDTA peripheral blood. 3-4 smears labeled “peripheral” and a copy of the CBC printout should be submitted with the bone marrow specimens.

PERIPHERAL SMEAR CONSULT:

1. Collect 4 mL blood in an EDTA (lavender-top) tube and prepare 3-4 peripheral smear slides and label “peripheral.”
2. Store peripheral smear slides at ambient temperature.
3. Submit peripheral blood smear slides along with copies of CBC and relevant laboratory results.

FLOW CYTOMETRY FOR BONE MARROW

1. For bone marrow, collect 2-3 mL of marrow in Na Heparin (green-top tube).
2. For peripheral blood, collect 7 mL whole blood in Na Heparin (green-top tube).
3. Store at ambient temperature.
4. Contact HPC at 405.715.4500 and inform of specimen collection. Mark requisition with appropriate patient history, source information and “flow cytometry for bone marrow.”

STORAGE: The slides should be separated from the rest of the specimen and kept at room temperature until pick up. The remainder of the specimen should be refrigerated until pick up

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TRANSPORT: All collected samples, including all slides, aspirate material, clotted material in formalin, and bone marrow core biopsy in formalin should be placed in a biohazard transport bag with a clinical information sheet, and any appropriate laboratory information.

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NONGYNECOLOGICAL CYTOLOGY COLLECTION PROCEDURE

GENERAL INFORMATION:

1. **Specimens processed for nongyn cytology specimens:** Add 70-95% alcohol as soon as possible in a volume equal to the specimen collected and label each container with the patient's name and site source. Submit the specimen along with the completed HPC requisition at room temperature.
2. **Specimens Processed for Microbiology or Clinical Analysis:** Specimens intended for culture must be collected in a sterile container or in sterile saline (without fixative) and split from the main Non-Gynecological specimen prior to transport with the proper requisition for microbiology culture or clinical testing. Consult your clinical lab test catalog for specific specimen requirements.

LABELING*:* Non gyn specimens particularly submitted slides are considered a primary specimen and must be labeled with at least two patient specific identifiers. These include the PATIENT **NAME AND SITE/SOURCE OF SPECIMEN and one other identifier such as date of birth, hospital number, social security number, requisition number or unique random number**. Laterality and specimen source are critical to analysis.

PULMONARY SPECIMENS

SPUTUM

1. **Specimen Required:** 5 mL (about one teaspoon) or more if possible, of sputum obtained from a deep cough specimen.
2. **Supplies:** 120 mL clean plastic specimen container; fixative (70% alcohol).
3. **Collection Procedure:**
   a. Patient Preparation: 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.
   b. Collect the specimen in the container, attempting to obtain at least one teaspoon of sputum. Specimen should be a deep cough specimen and not saliva. Saliva is of no diagnostic value. Greater diagnostic yield may be obtained if specimens are submitted on three to five successive mornings.
   c. Add 70%-95% alcohol as soon as possible in a volume equal to the specimen collected. Label each container with the patient name and a second unique identifier such as date of birth and site source and laterality.
   d. Submit the specimen at room temperature along with the completed HPC requisition.

   NOTE: If a good specimen is not obtainable by this method or if the patient is unable to comply, obtain an induced sputum or tracheal aspirate.

POST-BRONCHOSCOPY SPUTUM

1. **Patient Preparation:** Collect **<u>one</u>** good deep cough specimen at any time during the 24-hour period following bronchoscopy, as outlined in "Sputum" above.
2. Add 70%-95% alcohol as soon as possible in a volume equal to the specimen collected. Label each container with the patient name, site source and requisition peel-off number.
3. Submit the specimen at room temperature along with the completed HPC requisition.

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BRONCHIAL BRUSHINGS

1. **Specimen Required:** Bronchoscopically directed brushing of the identified lesion.
2. **Supplies:** Standard bronchoscopy equipment, one (or more if necessary) 5-10 mL vial and fixative of 70%-95% alcohol.

3. **Collection Procedure:**
   a. Using standard bronchoscopy technique identify the lesion in question and obtain a brushing sample of the lesion. Upon withdrawing the brush, agitate the brush vigorously in a 5-10 ML vial of sterile saline or fixative. If possible, detach the brush and leave it in the vial.
   b. Add 70%-95% alcohol as soon as possible in a volume equal to the specimen collected. Label each container with the patient name, site source.
   c. Submit the specimen at room temperature along with the completed HPC requisition. Label each slide with the patient name and a second unique identifier such as date of birth and site source and laterality.

BRONCHIAL WASHINGS

1. **Specimen Require:** Bronchoscopically obtained washing (preferably at least 10 mL) of the bronchi in the region of the suspected lesion.
2. **Supplies:** Standard bronchoscopy equipment, 120 mL clean plastic specimen container(s) and fixative (70% -95% alcohol).
3. **Collection Procedure:**
   a. Using standard bronchoscopy technique, lavage the distribution of the bronchus to be sampled. Collect the wash in a clean container.
   b. Add 70%- 95% alcohol as soon as possible in a volume equal to the specimen collected. Label each container with the patient name, site source and requisition peel-off number.
   c. Submit the specimen at room temperature along with the completed HPC requisition and copies of insurance card(s).

E. BRONCHOALVEOLAR LAVAGE

1. **Specimen Required:** Bronchoscopically obtained lavage (preferably at least 20 mL) of the distal airways and alveoli in the distribution of the suspected lesion.
2. **Supplies:** Standard bronchoscopy equipment and 120 mL clean plastic specimen containers, 70% - 95% alcohol.
3. **Collection Procedure**
   a. Using standard bronchoscopy BAL (bronchoalveolar lavage) technique, lavage the lung distribution in question with normal saline (or other physiologic solution). Collect the lavage specimen in a clean specimen container.
   b. Add 70%-95% alcohol as soon as possible in a volume equal to the specimen collected. Label each container with the patient name, site source and requisition peel-off number.
   c. Submit the specimen at room temperature along with the completed HPC requisition.

GASTROINTESTINAL SPECIMENS

BRUSHINGS (Esophageal, GE Junction, Gastric, Duodenal, Bile Duct, Other)

1. **Specimen Required:** Endoscopically directed brushing sample of the identified lesion.
2. **Supplies:** Standard endoscopy equipment, one (or more if necessary) 5-10 mL vials of sterile saline, fixative (70%-95% alcohol), frosted-end glass slides and spray fixative.

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3. **Collection Procedure**
   a. Using standard endoscopy technique identify the lesion in question and obtain a brushing sample of the lesion.
      NOTE: It is important to brush the edges of an ulcer, as well as the floor, in order to obtain diagnostic material.
   b. Upon withdrawing the brush, roll the brush onto clean slide labeled with patient's name. Spray fix immediately. Then, agitate the brush vigorously in a 5-10 mL vial of saline or fixative. If possible, detach the brush and leave it in the vial.
   c. Add 70%-95% alcohol as soon as possible in a volume equal to the specimen collected. Label each container with the patient name, site source.
   d. Submit the specimen at room temperature along with the completed HPC requisition.

WASHINGS (Esophageal, Gastric, Other)

1. **Specimen Required:** Endoscopically obtained washing (preferably at least 10 mL) of the region of the suspected lesion.
2. **Supplies:** Standard endoscopy equipment, 120 mL clean plastic specimen container(s) and fixative (70%-95% alcohol)
3. **Collection Procedure**
   a. Using standard endoscopy technique, lavage the area of interest using a physiologic solution. Aspirate the solution and place in a clean specimen container.
   b. Add 70%-95% alcohol as soon as possible in a volume equal to the specimen collected. Label each container with the patient name, site source and requisition peel-off number.
   c. Submit the specimen at room temperature along with the completed HPC requisition.

BILE DRAINAGE

1. **Specimen Required:** 10 mL or more of collected bile drainage.

2. **Supplies:** Standard transcutaneous or endoscopic biliary drainage equipment and clean plastic specimen container of appropriate size.
3. **Collection Procedure:**
   a. Using appropriate sterile technique, collect as much bile drainage through the drainage apparatus as possible, into a clean plastic container.
   b. Add 70%-95% alcohol as soon as possible in a volume equal to the specimen collected. Label each container with the patient name, site source.
   c. Submit the specimen at room temperature along with the completed HPC requisition.

NOTE: Bile specimens will degenerate very rapidly due to enzymatic activity and bile salts. Therefore, a 24-hour bile collection is not suitable for cytologic evaluation.

UROLOGIC SPECIMENS

VOIDED/CATHERIZED URINE

1. **Specimen Required:** 50 mL of an appropriately collected voided or catheterized urine specimen.
2. **Supplies:** Clean collection container of appropriate size, standard catheterization equipment (for catheterized urine) and fixative (70%-95% alcohol).
3. **Collection Procedure**
   a. Patient Preparation: 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

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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.

b. Add 70%-95% alcohol as soon as possible in a volume equal to the specimen collected. Label each container with the patient name, site source and requisition peel-off number.
c. Submit the specimen at room temperature along with the completed HPC requisition.

OTHER UROLOGIC SPECIMENS (Bladder Washing/Ureteral Washing)

1. **Specimen Required:** 10 mL (or more) of an appropriately collected cystoscopically derived specimen.
2. **Supplies:** Standard cystoscopy equipment, clean collection container of appropriate size and fixative (70% -95% alcohol).
3. **Collection Procedure**
   a. Using standard cystoscopy technique, obtain washing specimens, carefully denoting specific specimen sites for each specimen on the requisition.
   b. Add 70%- 95% alcohol as soon as possible in a volume equal to the specimen collected. Label each container with the patient name, site source.
   c. Submit the specimen at room temperature along with the completed HPC requisition.

BODY CAVITY FLUID SPECIMENS

COLLECTION OF BODY CAVITY FLUIDS

1. **Specimen Required:** 10 mL (or more) of fluid obtained from an appropriately performed paracentesis.
2. **Supplies:** Standard paracentesis equipment, clean collection container of appropriate size and fixative (70% - 95% alcohol).
3. **Collection Procedure**
   a. Using standard paracentesis 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 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 clinical laboratory separately, following their guidelines for specimen collection. Heparin may be added to the specimen to reduce clotting. Place three (3) units of heparin per mL capacity of the collection container. Agitate the container to coat the sides with heparin. Rinse the paracentesis instrument with a small amount of heparin to prevent clotting of specimen before it is put into the collection container. Add specimen to the heparinized container. Gently agitate to thoroughly mix the specimen and heparin.
   b. Add 70%- 95% alcohol as soon as possible in a volume equal to the specimen collected. Label each container with the patient name and site source.
   c. Submit the specimen at room temperature along with the completed HPC requisition.

COLLECTION OF JOINT FLUID FOR CRYSTAL ANALYSIS

1. **Specimen Required:** 5 mL of joint fluid fresh (no preservative). Tissue may be submitted in 10% neutral buffered formalin if microbiology culture is not indicated. Submit the specimen at room temperature.
   NOTE: If a microbiology culture is indicated, please split the specimen into two (2) sterile containers. Consult your clinical lab testing catalog for specific specimen requirements.

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2. **Supplies:** Appropriate gauge needle and syringe, collection containers (i.e., sterile red top Vacutainer or 10% neutral buffered formalin)

FINE NEEDLE ASPIRATION SPECIMENS

Supplies
Plastic syringe of appropriate ml size.
Syringe pistol or tube extension (optional).
22, 25 or 27-gauge needle of appropriate length.
70% - 95% ethyl alcohol

Provided by HPC in FNA kit: (Call 405 705 1770 for additional kits)
Single end frosted glass slides
Fixative (70%- 95% ethyl alcohol) in container for fixed smears
Conical tube for cyst fluid or needle rinse.
Plastic slide holder

Collection Procedure
1. **Aspiration**
   a. Assemble the aspirating equipment. **If using ultrasound gel wipe the skin of the gel before aspirating as the gel obscures cellular detail and mimics colloid particularly in thyroid aspirates.**
   b. 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.
   c. With the needle in the mass, the needle tip should be moved in short motions initially to loosen cells within 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 lesion, and then reinserted at a slightly different angle. This maneuver should be repeated several times before complete withdrawal. 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.
2. **Preparation of Direct Smears**
   a. For preparation of smears, single-end frosted slides should be utilized. Slides should be labeled with patient name and site source. Gently express a drop of aspirated fluid onto a slide. If the aspirated material is abundant and fluid, a small drop may be easily expressed without force. If the material is more scant or more viscous or solid, the material must often be forcefully expelled.
   b. Once the specimen drop is on the slide it should by **quickly** smeared. The simplest way to accomplish this is to oppose a second glass slide onto the first, allowing the aspirated 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.
   c. Some or all of the smears should be air dried and left without fixation. Thyroid aspirates and lymph nodes are best suited for air drying as colloid, lymphocytes and cellular material is best seen by this technique. An additional slide may be prepared and must be quickly placed in 70%-95% ethyl alcohol container to preserve cells. Fixed specimens are particularly useful in lesions suspected of producing keratin such as squamous cell carcinomas. **If the specimen cannot be quickly fixed it is better to just let it air dry as post fixing an air dried specimen compromises cytological detail.**

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d. If material remains in the hub of the needle or a cyst is encountered, this material may be flushed into a conical tube containing 70%-95% ethyl alcohol. An alternative method is to take a hemostat and remove the needle and place the plastic hub into the alcohol. From this a cell pellet can be processed as a cell block preparation. Do not send needle to laboratory.
e. Cyst fluid that is considered for microbiology can be expressed into a sterile red top tube and sent for microbiology studies.
f. If a lymphoma is suspected aspiration material can also be placed in RPMI (tissue culture media) or viral transport media for possible flow cytometry study.
g. Submit the specimen at **room temperature** along with the completed HPC requisition.

BREAST NIPPLE SECRETION OR DISCHARGE SMEAR

1. **Specimen Required:** A "pea" size droplet for a direct smear
2. **Supplies:** Frosted end glass slide (Write the patient's name on the frosted end with a lead pencil). The slide can be air dried or fixed depending on how quickly fixation can occur. If the specimen begins to dry on the slide just let it continue to air dry and do not fix with spray or alcohol as it will disrupt cytologic features. 70-95% alcohol or container of spray fixative.
3. **Patient Preparation:** Smears of nipple secretions may be utilized in the detection of breast cancer that involve ducts. **DO NOT MASSAGE OR SQUEEZE THE BREAST.** The following method will express secretion without trauma.
4. **Collection Procedure**
   a. Open the bottle of fixative and have the patient hold the bottle near the breast.
   b. Gently express only the nipple and subareolar area of any secretions that may be lying in the collecting ducts. If NO SECRETION APPEARS AT THE NIPPLE WITH THIS GENTLE COMPRESSION, DO NOT MANIPULATE FURTHER.
   c. Allow a "pea size" drop of fluid to collect upon the nipple tip.
   d. Immobilize the breast and using the nipple, smear the material across a glass slide.
   e. IMMEDIATELY drop the slide into the fixative. Time is of the essence here. The smearing of the material across the slide and the dropping of the slide into the fixative should be accomplished in one motion. If this is not possible let the specimen air dry and send without fixative.
   f. Make as many smears as the amount of material allows.
   h. These should be collected by applying the slide directly to the nipple, followed by immediate fixation. Label each slide with the patient name and a second unique identifier such as date of birth and site source and laterality.
   i. Submit the specimen at room temperature along with the completed HPC requisition.

CONJUNCTIVAL SCRAPINGS – DIRECT SMEAR

1. **Specimen Required:** Direct smear of material collected from the conjunctival surface.
   NOTE: For Specimens Processed for Microbiology or Clinical Analysis: Specimens intended for culture must be collected in a sterile container or in sterile saline (without fixative) and split from the main Non-Gynecological specimen prior to transport with the proper requisition for microbiology culture or clinical testing. Consult your clinical lab test catalog for specific specimen requirements.
2. **Supplies:** Two clean glass slides (single end frosted), fixative (spray fixative or 70% - 95% alcohol), conjunctival scraping spatula and test requisition form.
3. **Collection Procedure**
   a. Label the slides with the patient's name and second unique identifier such as date of birth and place in a container filled with 70%- 95% alcohol so that the slides are completely covered.

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b. Gently scrape the area of abnormality. Remove one of the slides from the fixative. Quickly and evenly smear the collected material on one of the glass slides. Immediately reimmerse the slide in fixative. Repeat the process with the second slide, if necessary, for better diagnostic yield.
c. Label each slide with the patient name and a second unique identifier such as date of birth and site source and laterality.
d. Submit the specimen at room temperature along with the completed HPC requisition.

SKIN SCRAPINGS/TZANCK SMEARS – DIRECT SMEAR

1. **Specimen Required:** Direct smear of material collected from a skin lesion, usually a vesicle.
2. **Supplies:** Two (or more) clean glass slides (frosted end), fixative (70%-95% alcohol), skin scraping spatula and test requisition form.
3. **Collection Procedure**
   a. Label the slides with the patient's name and place in a container filled with 70%-95% alcohol so that the slides are completely covered.
   b. 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. Remove one of the slides from the fixative. Quickly and evenly smear the collected material on one of the glass slides. Immediately reimmerse 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.
   c. Label each slide with the patient name and a second unique identifier such as date of birth and site source and laterality.
   d. Submit the specimen at room temperature along with the completed HPC requisition.

MISCELLANEOUS SPECIMEN COLLECTIONS

PLACENTA

Collect placenta at the time of delivery and place in mammoth size container. Add 10% neutral buffered formalin to cover the specimen. Frozen placentas may also be submitted for surgical pathology and formalin should be added before sending to the lab. Store at room temperature and transport routinely to HPC laboratory.

FETAL DEMISE

Stillborns up to 500 grams weight or less than 20 weeks gestation are considered a surgical pathology specimen and can be submitted as such to HPC. Later gestation and increased weight specimens require an autopsy or funeral home disposal in the State of Oklahoma.

CHROMOSOME ANALYSIS

Small sections of tissue from the fetal skin or products of conception sent fresh in sterile saline can be used for chromosome analysis. Please call a HPC pathologist to arrange transport in the most expedient way as the cells are fragile and bacterial overgrowth causes cell death.

FLOW CYTOMETERY

Lymph node tissue, fine needle aspirates and bone marrow can be submitted for flow cytometry studies at the time of collections. If the surgeon can split a 2mm slice of the specimen under sterile conditions, cell preservation is best. This sample is then placed in RPMI tissue transport media and sent directly to flow laboratory or to HPC laboratory.
If this is not possible, the fresh specimen can be sent to HPC laboratory after calling and alerting the HPC pathologist that a fresh specimen is to arrive.

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LIVER BIOPSY FOR QUANTITATIVE IRON

A minimum of 2 mg of liver tissue is required. This should be submitted in special metal free container provided. Please call HPC to make arrangements for collection and transport.

MUSCLE BIOPSY/NERVE BIOPSY

These specimens are sent to a reference laboratory. We will NOT accept them on Friday as the reference laboratory is closed on Saturday. Please call HPC to make arrangements for the collection and transport of the specimens.

RENAL BIOPSY FOR MEDICAL DISEASE

These specimens are sent to a reference laboratory. Please contact HPC to make arrangements for collection and transport.

KIDNEY STONES FOR CONTENT ANALYSIS

Collect fresh **without** fixative in sterile urine cup. Send to HPC at room temperature.