PATHOLOGY MEDICAL SERVICES, P.C.
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LINCOLN, NEBRASKA 68506

TELEPHONE: (402) 465-1900
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Specimen Collection Manual

Diagnostic excellence for better patient care

We strive to be the recognized leader in laboratory medicine, by providing timely, efficient and high quality anatomic and clinical pathology services to patients and health care providers. We offer state of the art technology tailored to meet the needs of our clients. We serve as a diagnostic and consultative resource to facilitate better patient care.

Accreditation

Pathology Medical Services, P.C. participates in proficiency evaluation programs in conformance with the Clinical Laboratory Improvement Act (CLIA) as well as the proficiency testing programs of the College of American Pathologists (CAP). Pathology Medical Services, P.C. has been accredited by the College of American Pathologists since 1973.

Quality Assurance Programs

Our Quality Improvement program includes proficiency testing / external audits and a documented operational plan covering all aspects of laboratory services provided at our various locations. To assess its effectiveness, the Quality Improvement program is regularly evaluated by the Laboratory Directors. In addition, our board certified pathologists and technical personnel serve as inspectors for other accredited laboratories.

An on-call pathologist is available 24 hours a day, 365 days a year at 402-465-1900 to assist with any patient care issues or questions.

For all pathology reports a microscopic examination was performed in order to arrive at the diagnostic conclusion reported. All specimens were formalin-fixed and submitted for paraffin embedded sections unless otherwise noted on the pathology report

Histology:

Key quality indicators (including pre-analytical and post-analytic variables) are monitored and compared to benchmarks where available and applicable. A designated pathologist is responsible for quality indicator monitoring and reporting; pathologists are also directly responsible for coordination with other medical and administrative staff members through various divisional, departmental and institutional committee meetings. Our quality control policies include clearly defined goals for monitoring analytic performance, tolerance limits, procedures and corrective action. Our quality variance reporting system is designed to identify and correct problems that may interfere with patient care services.

Cytology:

Pathology Medical Services, P.C.'s Quality Assurance Program is in accordance with CLIA '88 requirements, the American Society of Cytopathology requirements and the College of American Pathologists requirements. Each of our cytotechnologists is ASCP certified.

All slides are initially screened by a qualified cytotechnologist. Cases interpreted as "Unsatisfactory" are re-screened by a senior cytotechnologist that has three or

more years of experience. All patients that are considered to be high-risk for developing cervical cancer as indicated on the Pap test requisition are also re-screened by a senior cytotechnologist. At least ten percent of the cases interpreted as normal by the cytotechnologist are quality control selected for senior cytotechnologist re-screening. Re-screening is performed prior to issuing a final report.

All other cases with abnormal cells including reactive/typical repair are evaluated by a board certified pathologist for final diagnosis.

Dr. Ryan DeHaan, Dr. Patrick Keelan, Dr. Darrell Lester, Dr. Charles Reese

REVIEWED

By Drs. DeHaan, Keelan, Lester, and Reese at 5:13 pm, Jun 23, 2022

Pathology Medical Services

Specimen Submission Instructions

Test Requisition: All specimens must be submitted with a paper/printed test requisition. This may be a manual requisition or a requisition generated from an office/hospital EHR order system/interface. The requisition must include:

1. Patient demographics: complete patient name, date of birth, sex, Social Security number or medical record number.
2. Complete billing instructions and insurance information; include copies of insurance cards if this information is not provided on the requisition.
3. Ordering provider's complete name; note that Pathology Medical Services will accept specimens from licensed health care providers only.
4. Place of service/patient status: physician office, hospital in-patient, hospital out-patient.
5. Test order(s), ICD code(s) and clinical information/history.
6. Specimen source/body site/procedure used for specimen collection.
7. Date and time of specimen collection.
8. Time the specimen was placed into formalin.
9. Include Advanced Beneficiary Notice (ABN) as indicated.

Specimen Labeling: All specimens must be completely and adequately labeled to ensure accurate patient identification.

1. Each specimen container label must include **2**-unique patient identifiers (i.e. the complete patient name, DOB, medical record number, SSN or requisition number).
2. Slides must be labeled with **2**-unique patient identifiers (i.e. the complete patient name, DOB, medical record number, SSN, or requisition number). If slides are obtained from multiple body sites, the specific body site must be included on each slide label.
3. For multi-part specimens, label each container with the letter part (A, B, etc.) and body site that corresponds to the letter and body site listed on the requisition.
4. **Specimens are considered mislabeled when there is a mismatch between the patient identifiers on the specimen and the requisition submitted with the specimen. Mislabeled and completely unlabeled specimens or specimens with only one unique identifier will be returned to the client office.**

Specimen Submission: Specimen containers and requisitions should be submitted in biohazard specimen bags.

1. Submit specimen(s) from one patient per specimen bag.
2. Place specimen container(s) into specimen bag.
3. Place prepared slides into a cardboard or plastic slide holder and place slide holder into specimen bag.
4. Place requisition in the side pocket of the specimen bag.

Verbal Order Authorization/Test Request: Federal and state regulations require that a verbal request for laboratory testing be documented with a written verification of the request including

the signature of the ordering clinician. A Verbal Order Authorization form will be faxed to the client office when a verbal test request is placed. The test order will be processed upon receipt of a signed Verbal Order Authorization.

Dr. Ryan DeHaan, Dr. Patrick Keelan, Dr. Darrell Lester, Dr. Charles Reese

REVIEWED

By Drs. DeHaan, Keelan, Lester, and Reese at 5:24 pm, Jun 23, 2022

Bone Marrow Aspiration and Biopsy/Routine Peripheral Blood Smear/ Malaria

See **Specimen Submission Instructions** for test requisition, specimen labeling and specimen packaging requirements.

Bone Marrow Procedure

Ideally, bone marrows specimens should include:

- 8 aspirate slide smears
- 4 touch prep slides
- 2 peripheral blood smears

Clot Procedure:

1. Allow the aspirate to clot in the aspirating syringe for 10-15 minutes at room temperature.

2. Remove plunger from syringe. Using a wooden applicator stick, gently dislodge clot and place into container of ten percent formalin. Record the time the clot is placed on formalin. Volume of fixative must be ten times that of the specimen.

Core Biopsy Procedure:

1. Prepare four touch preps. Touch the core specimen to four glass slides and air dry.

2. Place core specimen into ten percent formalin. Volume of fixative must be ten times that of the specimen. Mark container with patient data and collection time. Fix core specimens at room temperature.

Additional Information

- Chromosomal studies are performed by Regional Pathology Services (UNMC). Include a copy of the CBC results.
- Place formalin container(s) in one biohazard bag and the slides in another biohazard bag or slide tray. DO NOT place formalin container and slides in the same biohazard bag. Doing so will cause a staining artifact, rendering the smears unreadable.

Aspirate Smears

8-12 aspirate smears should be made the same day as the marrow is drawn using the aspirate. To make smears pre-label the slides using an ML black lab marker or No. 2 lead pencil. Mix the tube of aspirate, put a small amount in a weigh boat from the tube. Using a disposable pipette drop aspirate from the weigh boat onto a slide that is propped up at an angle so the droplet starts to run down the slide, take another slide and lay the bottom edge on the angled slide holding the blood and spicules. Collect a small amount of the blood onto the bottom edge of the slide by moving the slide over the blood and spicules. Take the edge of the slide

and place it on the upper ¼ of the pre labeled slide. Slide it up a little towards the top of the slide and pull down quickly at a slight angle. Do not lay the slides on top of one another. Continue this process until you have the required 8-12 slides. Place slides in a 20 slot cardboard slide folder, (do not close). Allow the slides to dry for 10-15 min. The slides are then ready to be taken to the special stains area.

Peripheral Smear Preparation

Peripheral blood or potassium EDTA anticoagulated blood (1 to 2 mg EDTA/1ml blood may be used). Smears of peripheral blood must be made immediately. Smears may be made from EDTA-anticoagulated blood as long as two to three hours after collection. All specimens must be free of clots.

Specimen Requirements

1. Anticoagulated specimens must be checked with two applicator sticks to be certain no clots are present. If clots are present, the specimen is unsatisfactory.

2. A small drop of blood is placed on the surface of a clean glass slide near the end. If blood is taken from the finger, care must be taken to avoid touching the slide to the skin.

3. The slide is held between two fingers and the thumb of the left hand with the drip of blood on the upper surface towards the right. (Reverse for left-handed individuals.)

4. An edge of the spreader slide is placed on the first slide to the left of the drop of blood and is pulled to the edge of the drop. The angle between the two slides will vary according to the size of the drop and the viscosity of the blood. The larger the drop and the lower the hematocrit of the blood, the greater the angle must be to avoid running off the slide when spreading. Blood with a high hematocrit must be spread with a lower angle or the smear will be short and too thick to allow differentiation of cells. The approximate angle for normal blood is 30 to 40 degrees.

5. The drop of blood should be allowed to bank evenly behind the spreader which is then pushed to the left in a smooth, quick motion. The more rapid the motion, the shorter and thicker the smear. The smear should cover approximately half the slide with a gradual transition from thick to thin. No ridges should be present and the end (feather edge) should be smooth and even. In the feather edge, the red blood cells should not be routinely overlapped.

6. Label the frosted edge of the slide with the patient's first and last name, accession numbers, and date.

Malaria Smear Preparation

Process Overview

- For stat requests from the Lincoln hospitals, the hospital lab will prepare one WG stained peripheral smear. The pathologist will review the peripheral smear at the hospital and call the result to the ordering physician. If the smear is negative, the ordering physician should be informed that the result is from a thin smear only and that a thick smear preparation will also be evaluated and reported the next business day. A final report will be issued after examination of the thick smears.

- For the purposes of preparing thick smears, a separate lavender top tube (EDTA) of blood will be sent to PMS. Three thick smears and three thin smears will be prepared at PMS according to procedure. One unfixed thick smear will then be stained. The other slides will serve as a backup in case of an error staining the first slide or to send the slides to Mayo for speciation. The thin smear will be stained according to the procedure utilized for routine peripheral blood smear evaluation. The stained slides will be turned out to a hematopathologist for review the next business day, at the discretion of the pathologist on call.

- For non-stat requests, an EDTA tube of blood will be sent to PMS for routine preparation of thin and thick smears and turned out to a hematopathologist as a routine peripheral smear consult. If the slides and tube of blood are received before 2:00pm, the smears will be turned out to a hematopathologist for review the same day. If they are received after 2:00pm, the smears will be turned out to a hematopathologist for review the next business day.

- If an EDTA tube of blood is not available and only peripheral smears are provided (this might be the case for cases originating at hospitals outside of Lincoln), the case will be handled like any peripheral smear.

Specimen Requirements

Submit three unstained peripheral blood smear slides. Stained slides are acceptable, but not preferable. Submit a tube of Peripheral blood or potassium EDTA anticoagulated blood (1 to 2 EDTA/1ml blood may be used)

Additional Information

Include a copy of the CBC results with the blood smear slides. Smears must be made within 24 hours of blood collection.

Bone Marrow Aspiration and Biopsy/Routine Peripheral Blood Smear/ Malaria

Peripheral Smear Review

Specimen Requirements

Submit two unstained peripheral blood smear slides. Stained slides are acceptable, but not preferable.

Additional Information

Include a copy of the CBC results with the blood smear slides. Smears must be made within 24 hours of blood collection.

Chromosome Analysis

Test Description

Includes 5-20 G-banded metaphase cells analyzed and 3 or more G- banded karyotypes prepared.

Specimen Requirements

1. Collect 1.5 mL, (.5mL minimum) bone marrow from aspirate; 20 mL peripheral blood
2. Immediately aliquot into a 3 mL sodium heparin vacutainer.
3. Deliver to Pathology Medical Services, P.C immediately at room temperature.

Flow Cytometry - Leukemia / Lymphoma

Test Description

Leukemia Cell Surface Markers:

Cytofluorometric quantitation of mononuclear cell surface antigens using monoclonal antibodies. Useful in distinguishing leukemic cell lineage and stage of differentiation. Cytoplasmic IgM will be included on all specimens from children under 15 years of age with pre-B ALL.

Lymphoma Cell Surface Markers:

Cytofluorometric quantitation of mononuclear cell surface antigens using monoclonal antibodies to distinguish T cell or B cell lineage. DNA ploidy analysis will be performed if required.

Specimen Requirements

1. Collect 1mL bone marrow; 20mL peripheral blood.
2. Immediately aliquot into a 3 mL EDTA vacutainer.
3. Deliver to Pathology Medical Services, P.C. immediately at room temperature.

Dr. Ryan DeHaan, Dr. Patrick Keelan, Dr. Darrell Lester, Dr. Charles Reese

REVIEWED

By Drs. DeHaan, Keelan, Lester, and Reese at 5:14 pm, Jun 23, 2022

Breast Tissue Specimens

See **Specimen Submission Instructions** for test requisition, specimen labeling and specimen packaging requirements.

Specimen Requirements

1. The entire specimen must be submitted in 10% neutral buffered formalin fixative.
2. **The time the specimen was collected and the time placed in fixative must be recorded on the requisition.**
3. Excisional, incisional, core and mastectomies must fix in formalin for at least six (6) hours and no more than 72 hours for optimal results.
4. Formalin containers must have a formalin hazard label on the outside of the container.
5. The amount of formalin should be twenty (20) times the volume of the specimen.

Additional Information

Estrogen Receptor (ER), Progesterone Receptor (PR), Human epidermal growth factor receptor 2 (Her2-neu), and Ki67 may be performed on any tissue, but most common on breast specimens with carcinoma.

Dr. Ryan DeHaan, Dr. Patrick Keelan, Dr. Darrell Lester, Dr. Charles Reese

REVIEWED

By Drs. DeHaan, Keelan, Lester, and Reese at 5:15 pm, Jun 23, 2022

Bone (Decalcification)

See **Specimen Submission Instructions** for test requisition, specimen labeling and specimen packaging requirements.

Specimen Requirements

1. Bone or calcified soft tissue must be submitted in 10% neutral buffered formalin solution. Formalin containers must have a formalin hazard label on the outside of the container.

2. The time specimen is placed in formalin should be recorded on the tissue requisition. The amount of formalin should be ten times the volume of the specimen.

Dr. Ryan DeHaan, Dr. Patrick Keelan, Dr. Darrell Lester, Dr. Charles Reese

REVIEWED

By Drs. DeHaan, Keelan, Lester, and Reese at 5:15 pm, Jun 23, 2022

Gout/Crystals

See **Specimen Submission Instructions** for test requisition, specimen labeling and specimen packaging requirements.

Specimen Requirements

Tissue submitted for gout must be placed in 100% reagent alcohol or submitted fresh to the pathology lab. Since reagent alcohol is not readily available in most locations the tissue should be submitted fresh in a tissue container and transported to pathology, being kept cool with wet ice (ice pack).

Do not use 10% buffered formalin! Fixatives, such as formalin, containing water will destroy the crystals.

Dr. Ryan DeHaan, Dr. Patrick Keelan, Dr. Darrell Lester, Dr. Charles Reese

REVIEWED

By Drs. DeHaan, Keelan, Lester, and Reese at 5:15 pm, Jun 23, 2022

Lymph Node Biopsy-Lymphoma (Lincoln and Norfolk/Other)

See **Specimen Submission Instructions** for test requisition, specimen labeling and specimen packaging requirements.

Test Description

This procedure is meant for the evaluation of lymph nodes and lymphoid tissue when the possibility of lymphoma is in the differential. If lymphoma is not suspected routine specimen handling is adequate (submitted in formalin).

Pre-Surgical Instructions

Please call the PMS laboratory (402-465-1900) 24-48 hrs prior to the biopsy to help insure that all materials and media are available for all necessary steps and tests. RPMI media can be requested to be sent to your location for this procedure. Once the specimen is obtained, call the lab immediately for optimal pick-up.

Specimen Requirements

Lymph node samples should be submitted fresh so that portions may be obtained for microbiology studies, frozen section, Immunoperoxidase, and for molecular biologic studies (DNA gene rearrangement assay).

1. **If the biopsy is performed in Lincoln or Norfolk**:
   - Place fresh in a sterile container and deliver directly to Pathology Medical Services. If cultures are desired; write on tissue requisition "**for culture".**
   - Important: Notify someone in the laboratory that a fresh specimen has been delivered so that excessive drying artifact does not occur. The Pathologist will triage the specimen.

2. **If the biopsy is performed outside of Lincoln or Norfolk**:
   - If cultures are desired write on tissue requisition "**for culture".** under sterile conditions, obtain a small sample for microbiology (routine, fungus, and acid fast) and place the sample for culture into a sterile container wrapped in gauze moistened in sterile saline.
   - If sufficient tissue is present, a small (0.5 cm) sample should be placed into RPMI nutrient media (if available). If not available place into sterile, normal saline solution. Seal the container and keep refrigerated until pick up. DO NOT FREEZE. This portion of the specimen (used for possible special studies) should be transported to the laboratory in a refrigerated container. Please inform the courier of this.
   - Imprints of the sectioned surfaces of the node are desirable but not mandatory. Two or three slides should be air-dried after imprinting. DO NOT allow the air-dried imprints to be exposed to formalin or formalin fumes since this will render

them useless for further study. These imprints must be submitted in a container, which does not contain any specimens in formalin. Even a small amount of formalin fumes in a contained space such as a transportation container will affect the imprints.

- The remainder of the lymph node should be placed into ten percent buffered formalin fixative.

IMPORTANT: Please handle the fresh lymph nodes very carefully. They are generally rather soft and easily crushed or distorted which may severely impair the ability of the pathologist to render an accurate diagnosis, particularly on lymphomas.

3. Please contact a pathologist (at your location) if there are any additional questions or if any unusual procedures are required.

Dr. Ryan DeHaan, Dr. Patrick Keelan, Dr. Darrell Lester, Dr. Charles Reese

REVIEWED

By Drs. DeHaan, Keelan, Lester, and Reese at 5:16 pm, Jun 23, 2022

Muscle Biopsy

Instructions

Please call Customer Service at Pathology Medical Services, P.C. at 402-465-1900 24-48 hours prior to notify the laboratory of the time and date of the procedure and to request a STAT courier. ***Please schedule muscle biopsy procedures Monday through Thursday only. If this is impossible please notify the customer service at least 48 hours in advance to make special arrangements.***

If muscle biopsy clamps are unavailable, utilize tongue blades to stabilize the biopsy.

Please see requisition form instructions for complete information. Complete test requisition including last and first name of patient, patient's date of birth and social security number, body site and source of specimen collected. Label specimen container with patient's first and last name, and body site/source. Place container in a specimen bag with a biohazard label. Place the requisition in the side pocket of the specimen bag.

Include patient's history and physical, neurological reports, and results of any pertinent studies.

Specimen Requirements

1. Submit three separate muscle segments.
2. The biopsy site should be one that is clinically affected, but not severely atrophic or previously needled for electromyography. Avoid fascia and fat.
3. Select muscle bundles approximately 3 cm long x 0.5 cm in diameter.
4. Do not infiltrate muscle with local anesthetic. Avoid trauma or stretching during excision.
5. Tie a suture around one end of the muscle bundles to be removed using a small round curved needle. Tie a second suture at the other end of a 3 cm long and 0.5 cm in diameter bundle, cut across the bundle just beyond the suture. Continue retracting, undercutting the strip of muscle. Cut across the muscle attachment leaving both sutures attached to the specimen.
6. Attach the muscle bundle to muscle biopsy clamps.

Muscle Biopsy

7. Cover the biopsy with a saline moistened telfa. Do not immerse in saline solution. Submit specimen on wet ice immediately.

Dr. Ryan DeHaan, Dr. Patrick Keelan, Dr. Darrell Lester, Dr. Charles Reese

REVIEWED

By Drs. DeHaan, Keelan, Lester, and Reese at 5:16 pm, Jun 23, 2022

Nerve Biopsy

See **Specimen Submission Instructions** for test requisition, specimen labeling and specimen packaging requirements.

Pre-Surgical Instructions

Please call the laboratory at (402) 465-1900 24-48 hours prior to notify of the time and date of the procedure.

Submit with patient's history and physical as well as the neurological consult report.

Specimen Requirements

1. Isolate a 5-cm long fascicle of nerve.
2. Tie both ends of the dissected specimen. Free the specimen, and tie it isometrically to a tongue blade.
3. Immediately wrap the nerve biopsy in a saline moistened telfa pad or send fresh in an appropriate sized container. **DO NOT IMMERSE THE SPECIMEN IN SALINE OR FORMALIN**. Submit specimen refrigerated (ice pack) to pathology STAT.

Dr. Ryan DeHaan, Dr. Patrick Keelan, Dr. Darrell Lester, Dr. Charles Reese

REVIEWED

By Drs. DeHaan, Keelan, Lester, and Reese at 5:17 pm, Jun 23, 2022

Renal Biopsy

See **Specimen Submission Instructions** for test requisition, specimen labeling and specimen packaging requirements.

Specimen Collection:

Biopsy specimens may be obtained by radiology-guided percutaneous needle biopsy or open-wedge biopsy performed in surgery. Three needle biopsy pieces are preferable; however, instructions include handling of case with a single piece. Wedge biopsies from surgery are generally very adequate in size and pose no problem if glomeruli are present.

NECESSARY FIXATIVES:

- 10% Formalin - Light Microscopy
- Zeus or Michel's Fixative – Immunofluorescence
- Glutaraldehyde – Electron Microscopy (EM)

NOTE: All three tests can be done from Zeus/Michel's Fixative if necessary; however, the light microscopy isn't as distinctive. EM can be done from 10% formalin (and routinely is when case involves needle biopsy). Immunofluorescence can only be done from Zeus/Michel's fixative.

NEEDLE BIOPSY:

- If only one specimen can be obtained, it should be submitted in Zeus/Michel's fixative.
- If two specimens are obtained, they should be submitted (one each) in Zeus and formalin.

WEDGE:

- Slice the wedge biopsy into 1-2 mm pieces in 'bread-loaf' fashion, including a piece of the capsule in each.
- Take 1-2 pieces and cut into 2-mm squares and submit in Zeus/Michel's fixative.
- Take 1-2 pieces and cut into 1-mm squares and submit in Gluteraldehyde.
- Place remaining pieces in formalin.

Send all labeled containers down to pathology to be sent out to specified reference laboratory.

Dr. Ryan DeHaan, Dr. Patrick Keelan, Dr. Darrell Lester, Dr. Charles Reese

REVIEWED

By Drs. DeHaan, Keelan, Lester, and Reese at 5:17 pm, Jun 23, 2022

Sentinel Lymph Node Biopsy

See **Specimen Submission Instructions** for test requisition, specimen labeling and specimen packaging requirements.

Pre-Surgical Instructions

If a sentinel lymph node procedure using radioactive isotopes is performed, please contact histology at (402) 465-1933 at least 24 hours prior to the procedure to ensure proper handling procedures for radioactive specimens are followed.

Specimen Requirements

Specimens should be submitted in 10% neutral buffered formalin solution. Formalin containers must have a formalin hazard label on the outside of the container. The time the specimen is placed in formalin must be recorded on tissue requisition in the appropriate location. The amount of formalin should be ten times the volume of the specimen.

If the sentinel node is being sent for rapid intra-operative consultation at a hospital location then the specimen should be brought down to the pathology lab STAT by the Surgery staff. The specimen should be submitted fresh.

Dr. Ryan DeHaan, Dr. Patrick Keelan, Dr. Darrell Lester, Dr. Charles Reese

REVIEWED

By Drs. DeHaan, Keelan, Lester, and Reese at 5:17 pm, Jun 23, 2022

Tissue Biopsy

See **Specimen Submission Instructions** for test requisition, specimen labeling and specimen packaging requirements.

Specimen Requirements

Specimens for routine pathology must be submitted in 10% neutral buffered formalin. All containers with formalin must have a formalin hazard label on the outside of the container. The time specimen is placed in formalin must be recorded on tissue requisition in the appropriate location. The amount of formalin should be ten times the volume of the specimen. For large specimens the tissue should be free-floating or completely submerged by the formalin in the container (minimum 4:1 ratio).

Limitations

Tissues fixed in formalin cannot be submitted for microbiology testing. In order to culture tissue, submit one part of the tissue to your clinical laboratory prior to placing the specimen in formalin. Tissues submitted for microbiology testing must be collected utilizing sterile technique and must be placed in a sterile container. You must write on tissue requisition **"for culture"**. Submit the rest of the tissue to Pathology Medical Services, P.C. in formalin container.

Dr. Ryan DeHaan, Dr. Patrick Keelan, Dr. Darrell Lester, Dr. Charles Reese

REVIEWED

By Drs. DeHaan, Keelan, Lester, and Reese at 5:18 pm, Jun 23, 2022

Breast Secretions (Nipple Discharge)

See **Specimen Submission Instructions** for test requisition, specimen labeling and specimen packaging requirements.

Specimen Instructions

1. Express nipple secretion onto a glass slide.
2. Place a second slide on top of expressed fluid; allow fluid to spread, and using a horizontal motion, pull slides apart.
3. Immediately spray fix or allow to air dry. Label each slide “Fixed” for a fixed slide or “AD” for an air-dried slide. Note: slide must also be labeled with 2 unique patient identifiers.
4. Transport at ambient temperature.

Dr. Ryan DeHaan, Dr. Patrick Keelan, Dr. Darrell Lester, Dr. Charles Reese

REVIEWED

By Drs. DeHaan, Keelan, Lester, and Reese at 5:18 pm, Jun 23, 2022

Bronchial Brushing

See **Specimen Submission Instructions** for test requisition, specimen labeling and specimen packaging requirements.

Specimen Instructions

1. If microbiology is ordered, submit brush in sterile saline. Specimen will be processed for cultures prior to cytology processing.
2. If microbiology is not ordered:
   a. Place brush in Cytolyt.
   b. If desired, direct smear/s may be prepared from the brush by rolling the brush onto a glass slide prior to placing into Cytolyt. Immediately spray fix or allow to air dry. Label each slide “Fixed” for a fixed slide or “AD” for an air-dried slide. Note: slide must also be labeled with 2 unique patient identifiers.
3. If submitted as a fresh specimen, refrigerate and transport on ice/cold pack.
4. If submitted in Cytolyt, transport at ambient temperature.

Dr. Ryan DeHaan, Dr. Patrick Keelan, Dr. Darrell Lester, Dr. Charles Reese

REVIEWED

By Drs. DeHaan, Keelan, Lester, and Reese at 5:19 pm, Jun 23, 2022

Bronchial Washings

See **Specimen Submission Instructions** for test requisition, specimen labeling and specimen packaging requirements.

Specimen Instructions

1. Submit as a fresh specimen.
2. If ordered, microbiology will be performed prior to cytology processing.
3. Refrigerate and transport on ice/cold pack.

Dr. Ryan DeHaan, Dr. Patrick Keelan, Dr. Darrell Lester, Dr. Charles Reese

REVIEWED

By Drs. DeHaan, Keelan, Lester, and Reese at 5:19 pm, Jun 23, 2022

Cerebrospinal Fluid

See **Specimen Submission Instructions** for test requisition, specimen labeling and specimen packaging requirements.

Specimen Instructions

1. Submit as a fresh specimen.
2. Refrigerate and transport ASAP on ice/cold pack.

Dr. Ryan DeHaan, Dr. Patrick Keelan, Dr. Darrell Lester, Dr. Charles Reese

REVIEWED

By Drs. DeHaan, Keelan, Lester, and Reese at 5:20 pm, Jun 23, 2022

Effusions: Pleural Fluid (Thoracentesis), Peritoneal Fluid (Paracentesis), Ascitic Fluid, Pericardial Fluid

See **Specimen Submission Instructions** for test requisition, specimen labeling and specimen packaging requirements.

Specimen Instructions

1. Submit as a fresh specimen.
2. Submit entire fluid, not an aliquot.
3. Refrigerate and transport on ice.

Dr. Ryan DeHaan, Dr. Patrick Keelan, Dr. Darrell Lester, Dr. Charles Reese

REVIEWED

By Drs. DeHaan, Keelan, Lester, and Reese at 5:20 pm, Jun 23, 2022

Gastrointestinal (Biliary, Esophageal, Pancreatic) Brushing

See **Specimen Submission Instructions** for test requisition, specimen labeling and specimen packaging requirements.

Specimen Instructions

1. Place brush into Cytolyt.
2. If desired, direct smear/s may be prepared from the brush by rolling the brush onto a glass slide prior to placing into Cytolyt. Immediately spray fix or allow to air dry. Label each slide “Fixed” for a fixed slide or “AD” for an air-dried slide. Note: slide must also be labeled with 2 unique patient identifiers.
3. If microbiology is ordered, submit brush in sterile saline. Specimen will be processed for cultures prior to cytology processing.
4. Transport at ambient temperature.

Dr. Ryan DeHaan, Dr. Patrick Keelan, Dr. Darrell Lester, Dr. Charles Reese

REVIEWED

By Drs. DeHaan, Keelan, Lester, and Reese at 5:20 pm, Jun 23, 2022

Gastrointestinal (Esophageal, Gastric) Washing

See **Specimen Submission Instructions** for test requisition, specimen labeling and specimen packaging requirements.

Specimen Instructions

1. Submit as a fresh specimen.
2. Refrigerate and transport on ice/cold pack.

Dr. Ryan DeHaan, Dr. Patrick Keelan, Dr. Darrell Lester, Dr. Charles Reese

REVIEWED

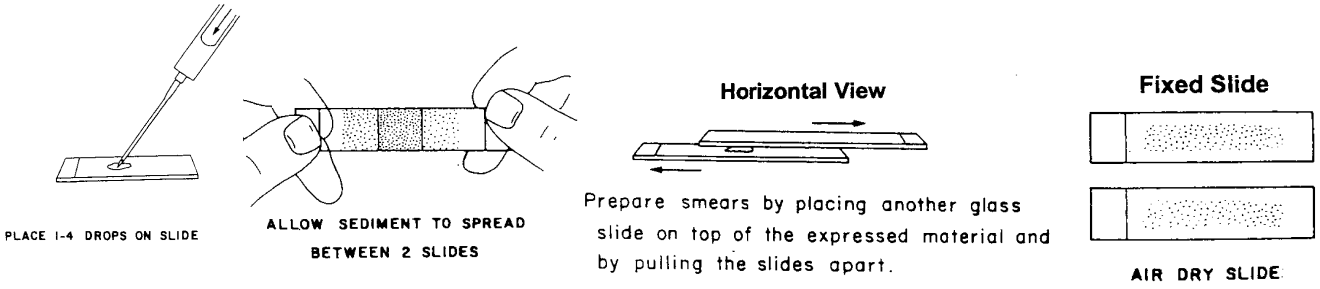
By Drs. DeHaan, Keelan, Lester, and Reese at 5:20 pm, Jun 23, 2022

Fine Needle Aspiration

See **Specimen Submission Instructions** for test requisition, specimen labeling and specimen packaging requirements.

Specimen Instructions

1. Prepare direct smears by placing 1-2 drops of aspirated material onto a glass slide. Place a second slide on top of the aspirated material; allow material to spread and using a horizontal motion pull the slides apart. See illustration below.
2. Immediately fix one slide with spray fixative. Allow the second slide to air dry. Label each slide “FIXED” for the fixed slide and “AD” for the aired dried slide.
3. Rinse remaining aspirate into Cytolyt by drawing Cytolyt fluid into the syringe and rapidly expelling through the needle into the container. Repeat as needed to rinse all aspirated material form the needle and syringe.
4. Prepare a maximum of 2 slides and one Cytolyt rinse per site aspirated, (*e.g.* right thyroid). Rinse subsequent passes from the same body site directly into the same Cytolyt container.
5. Label containers and slides with:
   a. Full patient name and one additional unique identifier
   b. Body site of specimen
   c. Fixation method for slide/s. See 2 above.
6. Transport at ambient temperature.



Dr. Ryan DeHaan, Dr. Patrick Keelan, Dr. Darrell Lester, Dr. Charles Reese

REVIEWED

By Drs. DeHaan, Keelan, Lester, and Reese at 5:21 pm, Jun 23, 2022

Sputum

See **Specimen Submission Instructions** for test requisition, specimen labeling and specimen packaging requirements.

Specimen Instructions

1. Submit fresh (if cultures are ordered) or in Cytolyt.
2. Specimen must be produced from a deep cough (mucus/saliva is not an adequate specimen).
3. Fresh specimens must be refrigerated and transported on ice/cold pack.
4. Cytolyt can be transported at ambient temperature.

Dr. Ryan DeHaan, Dr. Patrick Keelan, Dr. Darrell Lester, Dr. Charles Reese

REVIEWED

By Drs. DeHaan, Keelan, Lester, and Reese at 5:21 pm, Jun 23, 2022

Tzanck Smear for Viral Inclusions (Herpes)

See **Specimen Submission Instructions** for test requisition, specimen labeling and specimen packaging requirements.

Specimen Instructions

1. Rupture vesicle and obtain the specimen by scraping the base and margins of the lesion.
2. Spread specimen on glass slides and immediately spray fix or allow to air dry. Label each slide “Fixed” for a fixed slide or “AD” for an air-dried slide. Note: slide must also be labeled with 2 unique patient identifiers.
3. Provide body site of lesion on the requisition.
4. Transport at ambient temperature.

Dr. Ryan DeHaan, Dr. Patrick Keelan, Dr. Darrell Lester, Dr. Charles Reese

REVIEWED

By Drs. DeHaan, Keelan, Lester, and Reese at 5:22 pm, Jun 23, 2022

Urine: Voided or Catheterized, Bladder Washing, Cystoscopy, Ileal Conduit

See **Specimen Submission Instructions** for test requisition, specimen labeling and specimen packaging requirements.

Specimen Instructions

1. Submit random fresh voided urine, catheterized urine or bladder washing. Do not submit first morning urine.
2. Specify source of urine *e.g.* voided, catheterized, cystoscopy, bladder washing, ileal conduit etc.
3. Minimum volume is 10 ml.
4. Refrigerate and transport on ice/cold pack.

Dr. Ryan DeHaan, Dr. Patrick Keelan, Dr. Darrell Lester, Dr. Charles Reese

REVIEWED

By Drs. DeHaan, Keelan, Lester, and Reese at 5:22 pm, Jun 23, 2022

Urine for UroVysion™/FISH

See **Specimen Submission Instructions** for test requisition, specimen labeling and specimen packaging requirements.

Test Description **Urine for UroVysion™/FISH.**

Indications

UroVysion™ is a FISH assay for the detection chromosomal abnormalities associated with bladder carcinoma. Results are intended for use in conjunction with current standard diagnostic procedures as an aid for initial diagnosis of bladder carcinoma in patients with hematuria and subsequent monitoring for tumor recurrence in patients previously diagnosed with bladder carcinoma.

Specimen Requirements

1. Use **UroVysion™ Specimen Collection Kit**. Kit provides one specimen collection container and one 30 ml vial of PreservCyt (preservative solution).
2. Collect a minimum of 30 ml of urine. If urine volume exceeds 60 ml, pour off excess urine.
3. Immediately add PreservCyt in a 2:1 ratio (2 parts urine to 1 part PreservCyt).
4. Example:

Urine	+	PreservCyt	=	Total Volume
30 ml		15 ml		45 ml
60 ml		30 ml (entire vial)		90 ml

5. Total volume should not exceed 90 ml of urine + preservative solution in the specimen cup.
6. Place urine/PreservCyt mixture in original kit packaging and refrigerate (4°C) immediately. **Decomposition of urine begins within 30 minutes.**
7. Transport ASAP via courier.
8. Ship with ice pack. Do not allow direct contact of ice pack with specimen container.

Stability

Urine specimen must be combined with PreservCyt solution at the time of collection. Specimen submitted without PreservCyt will be rejected. Ship ASAP.

Reference Value An interpretative report is provided.

Dr. Ryan DeHaan, Dr. Patrick Keelan, Dr. Darrell Lester, Dr. Charles Reese

REVIEWED

By Drs. DeHaan, Keelan, Lester, and Reese at 5:22 pm, Jun 23, 2022

ThinPrep™ Pap Test: Collection Procedures

See **Specimen Submission Instructions** for test requisition, specimen labeling and specimen packaging requirements.

Patient Instructions

1. The ThinPrep ™ Pap test should ideally be taken two weeks after the first day of the last menstrual period. The patient should avoid scheduling her appointment during heavy menstrual bleeding; excessive amounts of blood may compromise the quality of the specimen and may lead to an unsatisfactory result.
2. The patient should avoid the use of vaginal medications, vaginal contraceptives, douches, lubricants, and sexual intercourse at least 48 hours prior to examination.

Use of Lubricant

1. NCCLS guidelines recommend that no lubricant be used during the pap test. [1] ACOG recommends that care be taken not to contaminate the specimen with lubricant [2]. Use of lubricant may lead to an unsatisfactory result and insufficient specimen remaining for ancillary testing.

[1] Papanicolaou Technique Approved Guidelines (NCCLS Document GP 15-A)
[2] ACOG Practice Bulletin, no. 45, August, 2003

Pertinent Clinical Information

It is essential to provide all pertinent clinical information. Please include:

1. Specimen source, *i.e.* cervicovaginal, vaginal
2. ICD code
3. Date of last menstrual period or menstrual status; pregnant, post-partum, postmenopausal, or has had a hysterectomy.
4. Clinical history to include contraceptive/hormone treatment, abnormal bleeding, +HPV, lesion, high risk factors, surgical/treatment history including biopsy, LEEP, radiation etc.

Collection Procedure

Specimen collection and cell transfer technique is critical. See ThinPrep™ Pap test collection procedure. Note: if a separate collection (swab) is planned for GC/CT or other testing, the **Pap specimen must be collected first**. Failure to collect Pap specimen first may cause a false negative Pap result.

ThinPrep™ Pap Test: Collection Procedures

Endocervical Brush / Spatula Protocol:

1. Specimen Collection

- Record the patient's first and last name and 1 additional unique patient identifier on the ThinPrep™ vial.

- Ectocervix Sample

  Insert contoured end of PLASTIC spatula into the cervical os and while maintaining tight contact with the ectocervical surface rotate one full turn (360 degrees).

  Rinse the spatula as quickly as possible into the PreservCyt Solution vial by swirling the spatula vigorously in the vial 10 times. Discard the spatula.

- Endocervix Sample

  Insert cytobrush into the cervix until only the bottom-most fibers are exposed. Slowly rotate ¼ turn to ½ turn in one direction. Do not over-rotate.

  Rinse the brush as quickly as possible in the PreservCyt Solution by rotating the device in the solution 10 times while pushing against the PreservCyt vial wall. Swirl the brush vigorously to further release material. Discard the brush.

- Tighten the cap so that the torque line on the cap passes the torque line on the vial.

ThinPrep™ Pap Test: Collection Procedures

Broom-Like Device Protocol:

1. Specimen Collection

- Record the patient's first and last name and 1 additional unique patient identifier on the ThinPrep™ vial.

- Ectocervix and Endocervix Sample

  Insert the central bristles of the broom into the endocervical canal deep enough to allow the shorter bristles to fully contact the ectocervix. Push gently, and rotate the broom in a clockwise direction five times.

  Rinse the broom as quickly as possible into the PreservCyt solution vial by pushing the broom into the bottom of the vial 10 times, forcing the bristles apart. As a final step, swirl the broom vigorously to further release material. Discard the collection device.

- Tighten the cap so that the torque line on the cap passes the torque line on the vial.

CPT Code 88175
88141 (Pathologist Review)

Dr. Ryan DeHaan, Dr. Patrick Keelan, Dr. Darrell Lester, Dr. Charles Reese

REVIEWED
By Drs. DeHaan, Keelan, Lester, and Reese at 5:23 pm, Jun 23, 2022

Conventional Pap Smear Collection Procedure

See **Specimen Submission Instructions** for test requisition, specimen labeling and specimen packaging requirements.

Patient Instructions

1. The Pap test should ideally be taken two weeks after the first day of the last menstrual period. The patient should avoid scheduling her appointment during heavy menstrual bleeding; excessive amounts of blood may compromise the quality of the specimen and may lead to an unsatisfactory result.
2. The patient should avoid the use of vaginal medications, vaginal contraceptives, douches, lubricants, and sexual intercourse at least 48 hours prior to examination.

Use of Lubricant

1. NCCLS guidelines recommend that no lubricant be used during the pap test. [1] ACOG recommends that care be taken not to contaminate the specimen with lubricant [2]. Use of lubricant may lead to an unsatisfactory result and insufficient specimen remaining for ancillary testing.

[1] Papanicolaou Technique Approved Guidelines (NCCLS Document GP 15-A)
[2] ACOG Practice Bulletin, no. 45, August, 2003

Pertinent Clinical Information

It is essential to provide all pertinent clinical information. Please include:

1. Specimen source, *i.e.* cervicovaginal, vaginal
2. ICD code
3. Date of last menstrual period or menstrual status; pregnant, post-partum, postmenopausal, or has had a hysterectomy.
4. Clinical history to include contraceptive/hormone treatment, abnormal bleeding, +HPV, lesion, high risk factors, surgical/treatment history including biopsy, LEEP, radiation etc.

Endocervical Brush / Spatula Protocol:

1. Specimen Collection

- With a pencil, label slide with patient's first and last name and one additional unique patient identifier.
- Ectocervix Sample

  Insert contoured end of spatula into the cervical os and while maintaining tight contact with the ectocervical surface rotate one full turn (360 degrees). Smear thinly on ½ of the slide and immediately spray fix.

Conventional Pap Smear Collection Procedure

- Endocervix Sample

  Insert cytobrush into the cervix until only the bottom-most fibers are exposed. Slowly rotate ¼ turn to ½ turn in one direction. Do not over-rotate. Using moderate pressure, roll brush on remaining ½ of the slide (make certain to complete 360 degree turn) and immediately spray fix.

- Spray fixative: hold fixative 3 inches from the slide and spray 1-3 times. Failure to fix immediately could cause Pap smear to be unsatisfactory for evaluation.

- Allow spray fixative to dry then place slide into cardboard or plastic slide holder.

Broom-Like Device Protocol:

1. <u>Specimen Collection</u>

   - With a pencil, label slide with the patient's first and last name and 1 additional unique patient identifier.

   - Ectocervix and Endocervix Sample

     Insert the central bristles of the broom into the endocervical canal deep enough to allow the shorter bristles to fully contact the ectocervix. Push gently, and rotate the broom in a clockwise direction five times.

     Smear each side of the broom onto a glass slide and immediately spray fix.

   - Spray fixative: hold fixative 3 inches from the slide and spray 1-3 times. Failure to fix immediately could cause Pap smear to be unsatisfactory for evaluation.

   - Allow spray fixative to dry then place slide into cardboard or plastic slide holder.

CPT code 88164
88141 (Pathologist Review)

Dr. Ryan DeHaan, Dr. Patrick Keelan, Dr. Darrell Lester, Dr. Charles Reese

REVIEWED

By Drs. DeHaan, Keelan, Lester, and Reese at 5:23 pm, Jun 23, 2022

HPV (Human Papillomavirus) HR (High Risk) Assay

See **Specimen Submission Instructions** for test requisition, specimen labeling and specimen packaging requirements.

Test Description

HPV (Human Papillomavirus) HR (High Risk) – The Cervista™ HPV HR test is an *in vitro* diagnostic test for the qualitative detection of DNA from 14 high-risk Human Papillomavirus (HPV) types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) in cervical specimens. The Cervista™ HPV HR test uses Invader® chemistry, a signal amplification method for the detection of specific nucleic acid sequences.

Indications

1. To screen patients with atypical squamous cells of undetermined significance (ASCUS) cervical cytology to determine the need for referral to colposcopy.
2. In women 30 years or older the Cervista™ HPV HR test can be used with cervical cytology to adjunctively screen to access the presence or absence of high-risk HPV types.

Specimen Requirements

Cervical specimen collected using routine pap specimen collection technique.
Note: when collected in conjunction with a colposcopy for biopsy, **HPV specimen must be collected prior to application of acetic acid/biopsy collection.** Failure to do so may yield an HPV specimen that is insufficient for testing or a false negative HPV result.

Acceptable specimen for in-house HR HPV assay

1. ThinPrep® Pap Test PreservCyt Solution***** vial collected with a Broom type device (Rovers Cervex® Brush, Wallach Papette®), or Endocervical Brush/Spatula.
   *****Cervista™ methodology includes an internal control for the presence of human DNA in each patient sample. Specimens with low cellularity or contaminating substances* may have insufficient DNA present for valid testing, yielding an “Insufficient or Indeterminate” result. *Contamination of the cervix with lubricant, contraceptive jellies or anti-fungal creams. (Clotrimizole or miconazole) may result in an insufficient specimen for HPV testing.

Note: Specimens submitted for HPV testing collected in SurePath Preservative ® vials or DNA Collection Devices will be forwarded to a reference laboratory for testing. Contact the cytology department for specimen requirements.

HPV (Human Papillomavirus) HR (High Risk) Assay

Stability

ThinPrep® collection vials are stable for 30 days at ambient temperatures for HPV HR

Reference Range

Test is reported as:
1. Negative (Normal reference value)
2. Positive
3. Insufficient human DNA detected – recommend recollection

CPT Code

87621

Dr. Ryan DeHaan, Dr. Patrick Keelan, Dr. Darrell Lester, Dr. Charles Reese

REVIEWED

By Drs. DeHaan, Keelan, Lester, and Reese at 5:24 pm, Jun 23, 2022

HPV (Human Papillomavirus) 16/18 Genotype Assay

See **Specimen Submission Instructions** for test requisition, specimen labeling and specimen packaging requirements.

Test Description

HPV (Human Papillomavirus) 16/18 Genotype – The Cervista™ HPV 16/18 test is an *in vitro* diagnostic test for the qualitative detection of DNA from Human Papillomavirus (HPV) Type 16 and Type 18 in cervical specimens. The Cervista™ HPV 16/18 test uses Invader® chemistry, a signal amplification method for the detection of specific nucleic acid sequences.

Indications

1. In women 30 years and older the Cervista™ HPV 16/18 test can be used adjunctively with the Cervista™ HPV HR test in combination with cervical cytology to assess the presence or absence of high-risk HPV types 16 and 18. This information, together with the physician's assessment of cytology history, other risk factors, and professional guidelines, may be used to guide patient management.
2. To be used adjunctively with the Cervista™ HPV HR test in patients with atypical squamous cells of undetermined significance (ASC-US) cervical cytology results, to assess the presence or absence of high-risk HPV types 16 and 18. This information, together with the physician's assessment of cytology history, other risk factors, and professional guidelines, may be used to guide patient management. The results of this test are not intended to prevent women for proceeding to colposcopy.

Specimen Requirements

Cervical specimen collected using routine pap specimen collection technique.
Note: when collected in conjunction with a colposcopy for biopsy, **HPV specimen must be collected prior to application of acetic acid/biopsy collection**. Failure to do so may yield an HPV specimen that is insufficient for testing or a false negative HPV result.

Acceptable specimen for in-house 16/18 HPV assay:

1. ThinPrep® Pap Test PreservCyt Solution***** vial collected with a Broom type device (Rovers Cervex® Brush, Wallach Papette®), or Endocervical Brush/Spatula

***** Cervista™ methodology includes an internal control for the presence of human DNA in each patient sample.

HPV (Human Papillomavirus) 16/18 Genotype Assay

Specimens with low cellularity or contaminating substances* may have insufficient DNA present for valid testing, yielding an “Insufficient or Indeterminate” result. *Contamination of the cervix with lubricant, contraceptive jellies or anti-fungal creams. (Clotrimizole or miconazole) may result in an insufficient specimen for HPV testing.

Note: Specimens submitted for HPV testing that are collected in SurePath Preservative® vials or DNA Collection Devices will be forwarded to a reference laboratory for testing. Contact the cytology department for specimen requirements

Stability

ThinPrep® collection vials are stable for 30 days at ambient temperatures for HPV 16/18

Reference Range

Test is reported as:

1. Negative (Normal reference value): HPV Type 16 and Type 18 not detected
2. Positive: HPV Type 16 and/or Type18 detected
3. Insufficient human DNA detected – recommend recollection

CPT Code

87621

Dr. Ryan DeHaan, Dr. Patrick Keelan, Dr. Darrell Lester, Dr. Charles Reese

REVIEWED

By Drs. DeHaan, Keelan, Lester, and Reese at 5:24 pm, Jun 23, 2022

Anal-Rectal Cytology: Collection Procedure

See **Specimen Submission Instructions** for test requisition, specimen labeling and specimen packaging requirements.

Indications

Anal-rectal cytology is indicated to screen select at risk patients to detect anal cancer and its precursor lesions.

Collection Procedure

1. Obtain specimen prior to use of lubricant gel.
2. Insert a moistened Dacron swab 5-6 cm into the anal canal.
3. Maintaining firm pressure against the mucosa, slowly rotate the swab10-20 revolutions while slowly withdrawing the swab from the anal canal.
4. Rinse the collection device as quickly as possible in the PreservCyt Solution vial by rotating the swab in the solution 10 times while pushing against the vial wall. Swirl the swab vigorously to further release material. Discard the swab.
5. Tighten the cap on the vial so that the torque line on the cap passes the torque line on the vial.
6. Label the vial with patient's full name and 1 additional unique identifier (DOB, medical record number).

CPT Code

88112

Dr. Ryan DeHaan, Dr. Patrick Keelan, Dr. Darrell Lester, Dr. Charles Reese

REVIEWED

By Drs. DeHaan, Keelan, Lester, and Reese at 5:24 pm, Jun 23, 2022

Bronchoalveolar Lavage (BAL)

See **Specimen Submission Instructions** for test requisition, specimen labeling and specimen packaging requirements.

Specimen Instructions

1. Submit as a fresh specimen.
2. If ordered, microbiology testing will be performed prior to cytology processing.
3. Refrigerate and transport on ice/cold pack.

Dr. Ryan DeHaan, Dr. Patrick Keelan, Dr. Darrell Lester, Dr. Charles Reese

REVIEWED

By Drs. DeHaan, Keelan, Lester, and Reese at 5:25 pm, Jun 23, 2022

Synovial Fluid

See **Specimen Submission Instructions** for test requisition, specimen labeling and specimen packaging requirements.

Specimen Instructions

1. Submit as a fresh specimen.
2. If ordered, microbiology will be performed prior to cytology processing.
3. Refrigerate and transport on ice.

Dr. Ryan DeHaan, Dr. Patrick Keelan, Dr. Darrell Lester, Dr. Charles Reese

REVIEWED

By Drs. DeHaan, Keelan, Lester, and Reese at 5:25 pm, Jun 23, 2022