Directory of Services

PathGroup
Physician Centered. Patient Focused.
Welcome to PathGroup, a medical diagnostic laboratory that provides anatomic and clinical lab services to physicians' offices, multi-specialty groups, surgery centers and hospitals. In the tradition of PathGroup, we strive to maintain excellence in laboratory services and to provide you with the best professional assistance in the field of Laboratory Medicine.

Our laboratories employ more than 400 highly trained professionals, clinical laboratory scientists, technicians, and support staff. State-of-the-art instrumentation, techniques, and data processing advancements enable us to deliver sophisticated laboratory services backed by extensive quality assurance programs and accreditation by the College of American Pathologists.

This Directory of Services Manual represents the efforts of managers, supervisors, pathologists, technologists, and clerical staff to develop a comprehensive and practical guide to PathGroup’s services. We trust that our services will exceed your expectations and truly enhance the care you provide to your patients.

Respectfully,

Samuel A. Smith, M.D. /Terence T. Casey, M.D.
Pathologists / Medical Directors

Associated Pathologists • 5301 Virginia Way • Suite 320 • Brentwood TN • 37027 • 615-221-4500 • 800-366-5847
PathGroup Labs • 658 Grassmere Park • Suite 101 • Nashville TN • 37221 • 615-562-9200 • 888-474-5227
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Medical Directors

MEDICAL DIRECTORS/PATHOLOGISTS

Our pathologists are available for consultation on laboratory test results, clinical indications for test ordering, etc. Laboratory managers and technical staff are also available to answer questions. These professionals are available by calling our Client Services department at 615-562-9300 or toll-free at 888-474-5227.

TERENCE T. CASEY, M.D.
Medical Director, Anatomic Pathology Laboratory, PathGroup

Board Certification: Anatomic and Clinical Pathology
American Board of Pathology, 1985

Education: B.S., University of New Orleans, New Orleans, Louisiana, 1978
M.D., Tulane University School of Medicine, New Orleans, 1981

Post Doctoral Training: Resident Physician, Department of Pathology, Vanderbilt
University Medical Center, Nashville, Tennessee, 1981-1985
Fellow in Hematopathology, Department of Pathology,
Vanderbilt University Medical Center, Nashville (Robert D. Collins, M.D.), 1985-1987

Professional Organizations: American Society for Clinical Pathology, 1988
Arthur Purdy Stout Society of Surgical Pathologists, 1992
United States and Canadian Academy of Pathology, 1988

SAMUEL A. SMITH, M.D.
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Pathology Residency, Anatomic and Clinical Pathology,
University of Louisville School of Medicine, 1974-1978
Clinical Fellow, American Cancer Society, University of
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Professional Organizations: American Medical Association; Tennessee Medical Association;
Nashville Academy of Medicine; American Society for Clinical
Pathology; College of American Pathologists; William
Christopherson Society; Tennessee Society of Pathologists
ACCREDITATIONS OF ASSOCIATED PATHOLOGISTS AND PATHGROUP LABORATORIES

Licensure & Accreditation Certificates

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<th>College of American Pathologists (CAP)</th>
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<td>SKYRIDGE MEDICAL CENTER</td>
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BILLING OF SERVICES

Our commitment is to maintain and provide quality customer service and satisfaction. We are here to help answer any of your questions regarding our billing.

PathGroup’s Business Office handles the billing of our services. The client guides us to either bill the patient/patient’s insurance or bill the client directly.

The Business Office will bill all insurance companies on behalf of our clients/patients. It is, however, the responsibility of the client and/or the patient to check with the insurance company prior to using our laboratories to assure PathGroup is an in-network provider. If you have questions regarding our in-network provider status with an insurance company, please contact your Territory Sales Manager or Client Services.

Within the Business Office, there are two customer service areas:

**Patient Billing Customer Service** handles patient calls regarding patient statements.

- Hours of operation are:
  - Monday through Thursday - 6:30 a.m. to 5:00 p.m.
  - Friday – 7:30 a.m. to 1:00 p.m.

- You may call us at:
  - Associated Pathologists – 877-456-6706
  - PathGroup Labs – 800-773-0234

**Client Billing Customer Service** handles client calls regarding client statements.

- Hours of operation are:
  - Monday through Thursday – 7:00 a.m. to 4:30 p.m.
  - Friday – 7:00 a.m. to 1:00 p.m.

- You may call us at:
  - Local calls – 615-221-4463
  - Toll-free calls – 866-728-4435

**Client Consultation Services**

PathGroup’s pathologists are available for medical and technical consultations on laboratory test results and/or clinical conditions. Our managers and technical staff are also available whenever needed to resolve problems or answer questions, either by telephone or personal visit.

**Client Services**

PathGroup’s Client Services department is available Monday through Friday, 8:00 a.m. to 5:00 p.m. (central standard time) to provide you with information concerning:

- Turnaround times
- Test information
- Specimen and special handling requirements
- Additional test requests
- Test results
- Test prices
- Courier pickup requests
Our Client Services Representatives are highly trained to provide prompt answers to any questions or concerns. Calls may also be directed to technical or medical personnel if necessary. Our goals are to meet your needs and exceed your expectations.

Client Services toll-free - 888-474-5227
Client Services toll-free fax – 866-325-5890

Courier Services

Courier services are available for transporting specimens to PathGroup from your location. This service provides delivery of specimens (including frozen) under controlled conditions. Special courier services will be established if appropriate arrangements can be made. Our PathGroup Territory Sales Manager is available to discuss the criteria with you. Pick up frequency is determined by referral volume.

Our couriers provide regular Monday through Friday pickup and delivery service for laboratory specimens and patient reports. If you have laboratory specimens for PathGroup and are not on a regular courier schedule, please call Client Services to arrange for a special pickup.

Laboratory Tours

We are proud of our laboratory, our technical capabilities, and the people who work together to provide the highest quality laboratory services to the medical community. We welcome the opportunity to show our laboratory to current and prospective clients and their staff members. To arrange a visit with us, please call Client Services at 615-592-9300 or toll-free 888-474-5227.

Referral Testing

PathGroup is a full service laboratory. We perform most tests at our own facilities; however, a few highly complex procedures are referred to reliable reference laboratories, primarily ARUP Clinical Laboratories.

Sales and Marketing Services

PathGroup employs a team of sales and marketing professionals to serve our clients. They are trained to meet the unique needs of a diverse client base which includes physicians, multispecialty groups, surgery centers, and hospitals. Our representatives provide our customers with the most current industry information such as legislative updates, infection control, medical necessity, billing, new technologies, new tests managed care updates, and laboratory policies and procedures.

A representative is assigned to each of our customers based on needs and specialty. To speak to your representative today, please call 615-234-2532 or 800-366-5847 extension 4532.
Supply Requests

PathGroup provides all forms and supplies necessary for the collection and transport of laboratory testing. Please complete a supply order form and return by fax to 800-730-8892.
Specimen Collection and Preparation

Handling and Processing of Blood Specimens

There are multiple factors associated with the handling and processing of laboratory specimens that can introduce test result inaccuracy, both before the specimen has been obtained and after it has been collected.

These pre-analytical factors can produce pre-analytical changes that result in erroneous laboratory test results. Examples include:

- Failure to draw a patient at correct time (fasting, post prandial, post- or pre-medication)
- Failure to centrifuge specimens in a timely manner
- Hemolysis secondary to venipuncture technique or specimen mishandling
- Analyte concentration changes due to evaporation
- Incorrect storage temperature
- Using improper Vacutainer® tube with inappropriate additive
- Incorrect transport

Recognition and control of these pre-analytical variables should reduce error and contribute to the medical usefulness of patient test results.

Labeling Specimens

Each specimen container must be labeled with at least two patient identifiers to include patient name in combination with one of the following:

- Date of Birth
- Medical Record Number
- Social Security Number
- Requisition Number

Specimen Packaging

Specimens

OSHA requires that all shipments containing clinical specimens be marked with a “Biohazard Label.” Bags and labels for shipments sent to PathGroup will be provided.

Ambient Temperature (room temperature)

Our standard specimen bags are designed to transport serum and urine specimens that do not require special temperatures or handling.

If you have any concerns regarding the effect of extreme weather conditions on routine or refrigerated specimens, please call Client Services (888-474-5227).
Exposure to Light

It is important to avoid exposing blood specimens for photosensitive analytes to artificial or sunlight for any length of time. Examples: Vitamin B6 and porphyrins. These specimens are to be protected with an aluminum wrap or equivalent.

Refrigerated (on coolant) Specimens

Place specimen in the refrigerator for storage before pickup by the courier. When packing for transport, place specimen tube or urine tube into zip-lock portion of bag and place the requisition into the outer sleeve. Place coolant in transport bag (box) along with any specimens in a way so that there is no direct contact of the specimens with the coolant.

Frozen Specimens

Label each tube with the patient’s name, date, and type of specimen (EDTA plasma, serum, etc.). Phone Client Services for special transport arrangements of all critical specimens.

Specimen Transport

Laboratory biohazard zip-lock bags are available and must be used for the transport of all specimens. These bags are designed to transport specimens that do not require special temperatures or handling.

Appropriate test requisition(s) must accompany labeled specimens and should be placed in the sleeve conveniently located on the front side of the transport bag.

Each bag should include the following:

- ONE patient ONLY per bag.
- Requisition legibly filled out with all patient demographics, billing information, ICD-9 codes and test information
- Labeled specimens

Rejection of Specimens

As part of our active quality assurance program and as part of the requirements of various certifying agencies, we have developed the following list of specimen rejection criteria. These criteria were developed with the intention of insuring accurate, meaningful patient results.

Unsatisfactory Information

All specimens must be properly identified by full name. All specimens for blood group and type testing must be labeled with the patient’s name, date of birth, Social Security Number, date and time, and initials of phlebotomist.

All specimens must be accompanied by a requisition which includes name, birth date, sex, date and time of collection, and name of requesting physician.
• The source of the specimen should be noted when appropriate
• A specimen not labeled properly may be discarded

**Inadequate Specimen Due to Collection and Transportation Problems**

• Contamination of the specimen (e.g., bacterial contamination, hemolysis, etc.)
• Insufficient specimen for test requested, such as quantities less than those stated in this Directory of Services manual
• Collection in improper container (e.g., incorrect anticoagulant, unsterile container for cultures, improper preservative, or holding medium)
• Failure to follow special instructions (e.g., draw and place in ice, protect from light, separate plasma immediately)
• Prolonged delay in transportation

**Inadequate Specimen Due to Patient Preparation**

• Non-fasting patient for test that requires fasting state
• Incorrect preparation of patient for test
• Specimen drawn at incorrect time (e.g., drug levels which should be drawn at peak or trough concentrations)

If a compromised specimen is accepted, a note will be made on the final report as to the nature of the problem and caution should be used when interpreting the results. If a specimen is rejected, the client/physician will be contacted to decide disposition. Specimens rejected due to collection problems are held in proper storage for 3-7 days depending on the specimen type.

All specimens are examined upon receipt by the laboratory to insure suitability for analysis. If the specimen volume is insufficient or if the specimen has been improperly handled, the reliability of test results could be compromised and the specimen will not be processed. The client will be contacted.

Laboratory test results are dependent on the quality of the specimen submitted. It is important that all specimens and request slips be properly labeled with the full name of the patient, collection date, and the origin (source) of the sample, when applicable.

If there is any doubt or question regarding the type of specimen that should be collected, it is imperative that our Client Services department be called at 888-474-5227 to clarify the order and sample requirements.

**Blood Collection – Performance of a Routine Venipuncture**

Most laboratory tests are performed on anticoagulated whole blood, plasma, or serum. Please see our individual Test Directory section for specific storage and transport requirements.

• **Plasma** – Draw a sufficient amount of blood with the indicated anticoagulant to yield the necessary plasma volume. Gently mix the blood collection tube by inverting 8-10 times immediately after collection. If required, separate plasma from cells by centrifugation within 30 minutes.
Serum – Draw a sufficient amount of blood to yield the necessary serum volume. Gently mix the blood 5 times if SST or plain red tube is used. Allow blood to clot at ambient temperature, approximately 30 minutes for SST tube and 60 minutes for plain red tube. Separate serum from clot by centrifugation within 2 hours at RPMs required to give a clean spin.

Whole Blood – Draw a sufficient amount of blood with the indicated anticoagulant. Gently mix the blood collection tube by inverting 8-10 times immediately after collection.

Blood samples used for laboratory testing are typically obtained by venipuncture. The proper procedures for routine venipuncture are outlined below. All these procedures should be conducted observing OSHA’s “Universal Precaution Procedures” regulations. When collecting, processing, or handling specimens, they should be considered a biohazard source with the potential of transmitting infectious diseases.

Venipuncture Procedure

Properly identify the patient by asking the patient to state his or her full name and confirming a second identifier such as the Social Security Number or date of birth. Prepare the tubes and other needed equipment:

- Gloves
- Tourniquet
- 70% alcohol prep pads
- Dry cotton balls
- Appropriate evacuated tubes for test ordered
- Holder or syringe and needle (21-22 gauge, 1-1/5" long) with appropriate safety device
- Adhesive pressure strip (micropore tape) or bandaids
- Biohazard waste container

Review the request form(s) or physician order to determine that you have the appropriate evacuated tubes (Table 1: PathGroup Specimen Tube Guide, page 18). Check for diet restrictions. If the test requires that the patient be fasting, make sure that these requirements have been followed.

Position the patient so that the arm is supported by a stationary object, such as a drawing chair, drawing table, or bed. Never draw blood from a standing patient. Do not draw blood from a compromised limb (i.e., due to mastectomy, stroke, surgery, etc.). Do not draw above an intravenous infusion.

Always wear gloves and work quickly so that the tourniquet does not remain on the patient’s arm longer than one minute. Apply the tourniquet approximately 2-4 inches above the elbow, snug – not tight. Ask the patient to make and hold a fist; avoid vigorous hand pumping.

Palpate (feel) for a vein. The most commonly used veins are the median cubital, cephalic, and basilic veins. The preferred selection of veins is the median cubital first, then the cephalic, and last, the basilic vein. A vein should have an elastic feel and “gives” under pressure.
Clean the chosen puncture site using the alcohol pad, starting at the center of the site, moving in an ever-widening concentric circle. Allow the skin to dry. Place the index finger on the vein above the puncture site, the thumb on the vein below the puncture site, and pull the skin tight to prevent the vein from “rolling.”

With the needle bevel facing upward, line up the needle with the vein at an upward angle of approximately 15-30°. Puncture the vein in a rapid smooth motion, without penetrating through the vein. Push the evacuated tube forward until the back of the needle punctures the rubber stopper.

Reassure the patient. Explain that there will be slight pain associated with the procedure. Never tell the patient, “This will not hurt.”

**Order of Draw**

When drawing for multiple specimen types, establish the correct order of draw to avoid contamination with additives. Draw the tubes in the following order:

1. Blood cultures
2. Light blue-top (citrate)
3. Gold top (SST gel)
4. Plain red-top (serum)
5. Green-top (heparin with or without gel)
6. Lavender-top (EDTA)

Fill the light blue-top tube until the vacuum is exhausted, 4.5 mLs. Partially filled citrate tubes are unacceptable. Never pour the contents of one tube into another.

When using a winged blood collection set for venipuncture, and a coagulation (citrate) tube is the first specimen tube to be drawn, a discard tube should be drawn first. The discard tube must be used to fill the blood collection set tubing’s “dead space” with blood, but the discard tube does not need to be completely filled. This important step will insure maintenance of the proper blood-to-additive ratio of the blood specimen. The discard tube should be a non-additive or coagulation tube.

Remove the tourniquet and ask the patient to relax his/her hand. Do not keep the tourniquet on a patient’s arm for more than 1 minute. After the tube has completed filling, remove and insert other tubes as required, into the tube holder. Immediately and gently invert all additive tubes after filling. Refer to Table 1: PathGroup Specimen Tube Guide, special instructions on page 18.

Remove the needle and place clean gauze or a cotton ball on the puncture site and apply a slight pressure. Activate safe needle device immediately post draw. Dispose of the needle into an appropriate ‘sharps’ container. Request that the patient hold the gauze or cotton ball with pressure, usually 3-5 minutes. After labeling the tubes, inspect the puncture site. If bleeding has stopped, apply an adhesive strip over the gauze or cotton ball. Instruct the patient to leave the bandage in place for at least 15 minutes. Dispose of all contaminated items appropriately.

At the completion of a venipuncture, be sure that the bleeding has stopped. If blood flow has not stopped, apply pressure with a fresh gauze or cotton ball until it does. This is critical with patients receiving anticoagulants. If post puncture bleeding persists for longer than 5
minutes, alert the attending physician so he/she can be advised of any potential bleeding problems. Bandage the site.

There are many different types of bandages and tape available. Paper tape works well for elderly patients with fragile skin; however, it should not be used on latex-sensitive patients. Latex-sensitive patients should be bandaged with latex-free tape only. Coban elastic wrap can be used for patients on anticoagulant therapy.

**Skin Puncture Procedure**

Avoid a finger that is cold, cyanotic (blue), swollen, or inflamed.

1. A fresh pair of gloves must be worn.
2. With your left thumb and index finger, grasp either the patient’s long or ring finger about 3 inches from the tip of the finger. Moving your left hand toward the tip of the patient’s finger, apply a massaging motion to the fleshy portion of the finger.
3. Repeat this massaging process 5-6 times.
4. Cleanse the ball or pad of the finger with an alcohol swab. Do not use iodine solutions to cleanse the skin.
5. Thoroughly dry the ball or pad of the finger with a piece of dry cotton or gauze to avoid hemolysis due to residual alcohol.
6. Pick up a sterile lancet device and remove the lancet from its container.
7. With your right hand, firmly grasp the sterile lancet. With your left hand, firmly grasp the patient’s finger.
8. With a quick motion, depress the button on top of the lancet, making a deep cut on the side of the ball of the finger. The cut should be across the fingerprints.
9. If the blood flows freely, wipe away the first drop with a clean piece of cotton or gauze.
10. If the blood does not flow freely, increase the blood flow by holding the finger downward and gently massaging just above the puncture site. Avoid excessive massaging or rigorous pressure on the area since this may contaminate the blood sample with tissue fluid. If the blood does not flow easily after gentle massage, make another puncture at a different site.
11. **EDTA Microtainer**: Fill microtainer quickly, then stopper and mix thoroughly. Do not scrape the blood specimen from the finger as it may cause hemolysis. Blood specimen volume should be between 250-500ul (see lines on microtainer vial). Mix well by inverting 8-10 times.
12. Fill the appropriate microtainer tubes. **
13. Label the tube with the patient’s full name, date of birth, your initials, date and time drawn.

**Microtainers:**
1. PLAIN with red cap – no additive – used for blood bank or serum tests that cannot be collected in gel barrier tubes.
2. AMBER/gel barrier with gold cap – no additive – used for most chemistry tests.
3. PLAIN/gel barrier with green cap – lithium heparin additive – used for chemistry tests requiring plasma.
4. PLAIN with lavender cap – EDTA additive – used for all hematology tests (except Sed Rates).

**Glucose Tolerance Test**

Unless the physician tells the patient otherwise, for 3 days prior to testing, the patient should eat 3 balanced meals each day; include bread, starches or sweets (2 slices of bread at each meal is adequate). Beginning after dinner on the night before the test, the patient should not eat or drink anything except water until coming to the laboratory. Patients who smoke should abstain from smoking from the time they go to bed the night before until completion of the entire procedure.

**Specimen Preparation**

**Pre-Centrifugation Phase**

Strict adherence to all phases of collection and processing is essential for accurate test results.

Plasma specimens are obtained using a Vacutainer® tube, containing an anticoagulant. These specimens can be centrifuged within minutes after collection. Any vacuum tube containing an anticoagulant should be inverted gently 8-10 times immediately after blood collection to insure the intended action of the additive.

Serum specimens are obtained from tubes when the blood has been allowed to clot. Prior to centrifugation, the specimen must be thoroughly clotted.

**Clotting Instructions**

Clotting instructions with minimum clotting time recommendations:

- Non-additive tubes (red stoppers) – 60 minutes
- SST tubes – 30 minutes

Recommended times are based on an intact clotting process. Patients with abnormal clotting due to disease, or those receiving anticoagulant therapy require more time for complete clot formation. Separation of serum or plasma from cells should take place within 2 hours of collection to prevent erroneous test results.

When specimen requirements call for a chilled specimen (2-8°C), the specimen is to be immediately placed in a small plastic tube bag, tied and placed in crushed ice or a mixture of ice and water. Examples requiring a chilled specimen include ammonia, PT/APTT, and lactic acid.

**Centrifugation**

Blood specimens should **clot adequately prior to centrifugation**. Specimens should be centrifuged using the appropriate tube holders (black - 125mm, red - 100mm, green - 75mm). The recommended centrifuge time is 15 minutes at 3125 rpms for black, 3250 rpms for red, and 3300 rpms for green, all rpms (+/-) 100 rpms.
All specimens collected in tubes with gel barriers should be properly centrifuged prior to transport.

After centrifugation of the gel tubes, the serum/plasma is separated from the cells by the gel barrier. It is recommended that serum/plasma be physically separated from contact with cells as soon as possible, with a maximum time limit of 2 hours from the time of collection. After proper centrifugation, serum can be left in contact with the gel barrier of SST tubes for up to 7 days with proper storage.

The centrifuge must be properly balanced. This is to prevent excessive vibration and potential breakage of the specimen tube, and is also necessary to properly separate the serum/plasma from the cells.

**Centrifuge Safety**

With normal operation, the centrifuge does not present any safety hazards. It is important to follow the listed safety precautions while operating the centrifuge:

- **Lid**: Never open the lid while the rotor is moving. If the centrifuge comes with a safety interlock switch, do not tamper with this safety mechanism. If the switch is broken, do not operate the instrument until the switch is repaired.
- **Load Balance**: The centrifuge must be balanced before operating. When centrifuging single or multiple tubes, each tube has to be counterbalanced with a tube of blood or a tube filled with water to match the tube directly across from it in the centrifuge head. Never spin a single tube without installing a balance tube. Excessive noise or vibration is an indication that the centrifuge is not balanced.
- **Biohazard**: If a tube spills or breaks, there is a potential biological hazard and the instrument must be cleaned using an approved cleansing procedure.

**Specimen Storage**

Any specimen which must be stored for more than 1 hour prior to pickup should be refrigerated unless otherwise indicated under specimen requirements. Do not refrigerate unspun potassiums.

Any specimen which requires freezing should be frozen as soon as possible after collection. Always freeze specimens in a plastic vial, never glass.

Confirm that the specimens are properly spun, properly labeled and accompanied by a requisition. Place the corresponding specimen(s) and requisition into a specimen transport bag.

**Specimen Transport**

Transportation should occur at correct temperature so that the specimen integrity is always maintained.

Some tests require that the specimen be shielded from light. These specimens, such as those being assayed for Vitamin A, B6, and porphyrins, should be protected from light by wrapping the specimen with foil or using an amber transfer container. Strict adherence to specimen requirements is essential.
### Table 1: PathGroup Specimen Tube Guide

<table>
<thead>
<tr>
<th>Tube Top Color (listed in order of draw)</th>
<th>Additive</th>
<th>Special Instructions and Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ADULT BLOOD CULTURES</strong> (detailed blood culture guidelines available upon request):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yellow/Black top-isolator Tube (Anaerobic/Aerobic)</td>
<td></td>
<td>Special prep for puncture site: 1. Cleanse with alcohol swab, outward with a circular motion. 2. Cleanse with betadine prep and allow site to dry. The tops of tube MUST be cleansed with betadine and allowed to dry before filling tube with blood.</td>
</tr>
<tr>
<td><strong>PEDIATRIC BLOOD CULTURES</strong> &lt; 7 years old (detailed blood culture guidelines available upon request):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yellow/Black top-isolator tube (Anaerobic/Aerobic)</td>
<td></td>
<td>Same as above.</td>
</tr>
<tr>
<td><strong>OTHER SPECIMEN CONTAINERS:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blue-top (citrate)</td>
<td>Sodium Citrate (3.2%)</td>
<td>Fill BLUE to capacity; gently invert 3-4 times. Used for coagulation tests.</td>
</tr>
<tr>
<td>Gold top (SST)</td>
<td>Inert barrier material/clot</td>
<td>Gently invert 5 times. Used for tests requiring serum.</td>
</tr>
<tr>
<td>Plain red-top (serum)</td>
<td>Clot Activator, silicone-coated</td>
<td>Gently invert 5 times. Used for tests requiring serum.</td>
</tr>
<tr>
<td>Green-top (heparin)</td>
<td>Sodium Heparin</td>
<td>Invert 8-10 times.</td>
</tr>
<tr>
<td>Light green-top (heparin/gel separator)</td>
<td>Lithium Heparin</td>
<td>Gently invert 8-10 times. Used for tests requiring Lithium Heparin or heparinized plasma.</td>
</tr>
<tr>
<td>Lavender (EDTA)</td>
<td>K2 EDTA</td>
<td>Gently invert 8-10 times. Used for most hematology tests.</td>
</tr>
<tr>
<td>Gray-top (K-Oxalate) plastic</td>
<td>Potassium oxalate/sodium fluoride</td>
<td>Gently invert 8-10 times. Used for some chemistry tests.</td>
</tr>
</tbody>
</table>

**NOTE:** Call PathGroup Client Services at 615-562-9300 or toll-free at 888-474-5227 to answer any questions regarding specimen requirements.

For proper additive performance, invert SST tubes 5 times. Invert all other filled additive tubes 8-10 times. Do not shake. Vigorous mixing may cause hemolysis. Insufficient mixing or delayed mixing in tubes with anticoagulant may result in clotting, platelet clumping, and incorrect test results.

### Urine Collection

**Clean Catch Urine Collection**
Male Collection Procedure

- Remove lid of the container. Take care to handle outside of container only.
- Wash hands with soap and water, rinse and dry on a disposable paper towel.
- Completely retract the foreskin and cleanse the glans penis with a towelette.
- Pass the first portion of the urine into the toilet bowl and without stopping, catch the remaining urine into the screw-capped, sterile container.
- Place the lid securely onto the container.

Female Collection Procedure

- Remove lid of the container. Take care to handle outside of container only.
- Wash hands with soap and water, rinse and dry on a disposable paper towel.
- With one hand, spread the labia and wash the area from front to back with a towelette.
- Hold labia apart.
- Pass the first portion of the urine into the toilet bowl and without stopping, catch the remaining urine into the screw-capped, sterile container.
- Place the lid securely onto the container.

12- or 24-Hour Urine Collection Procedure

For tests requiring a 12-hour or 24-hour urine collection, we provide the appropriate containers. For such tests, patients must be instructed to:

- Time the collection accurately during the 12- or 24-hour period.
- Collect all urine voided during the time period as described in the procedure below.

Make sure that patients are warned of the presence of potentially hazardous preservatives if they were added to the container.

College of American Pathologists (CAP) Recommended Procedure for Collection of Timed Urine Specimen

- Upon arising in the morning, urinate into the toilet, emptying the bladder completely. Do not collect this sample.
- Write down (on the container) the time; from this time forward, collect all urine voided for 12 or 24 hours in this container. Because direct contact with preservatives in the collection container may be hazardous, void into a clean container, then pour the urine into the collection container.
- Refrigerate (if indicated) the collected urine between all voidings.
- At exactly the same time the following morning (24-hour specimens), void completely again and add this sample to the collection container. Write down the time on the container.
- Keep the 12- or 24-hour urine specimen refrigerated (if indicated) and bring to the laboratory as soon as possible.

Microbiology Specimen Collection

General Collection Guidelines
1. Collect the specimen from the actual site of infection, avoiding contamination from adjacent tissues or secretions.
2. If appropriate, decontaminate the skin surface. Use 70-95% alcohol (ALC) and 1-2% tincture of iodine (TOI) to prepare the site. Allow a contact time of two minutes to maximize the antiseptic effect.
3. Collect the specimen at optimal times (for example, early morning sputum for AFB culture).
4. Collect a sufficient quantity of material. Use appropriate collection devices - sterile, leak-proof specimen containers. Use appropriate transport media (anaerobe transport vials, Amies or Stuart’s for bacterial culture, Cary-Blair for stool culture, M4 for viral, Chlamydia, and urea plasma cultures).
5. Whenever possible, collect specimens prior to administration of antimicrobials.
6. Properly label the specimen and complete the test request form. The source of specimen is required.
7. Minimize transport time. Maintain an appropriate environment between collection of specimens and delivery to the laboratory.
8. Package each specimen in a separate sealable transport bag.

**General Transport Guidelines**

1. Transport specimens to the laboratory as soon as possible. Prompt processing minimizes loss in viability of potential pathogens and insures an accurate appraisal of the different flora present.
2. If a delay in transport is anticipated, a transport medium must be used.
3. Wound specimens for anaerobic workup must be submitted in an anaerobic transport medium.
4. Most specimens can be refrigerated at 2-8°C with the following exceptions:
   - Blood culture tubes (ambient temperature)
   - Lavender-top tubes (ambient temperature)
   - Genital specimens for Neisseria gonorrhoeae (ambient temperature)
   - CSF and other sterile body fluids, except urine (ambient temperature or 35-37° for CSF)
   - Stool in preservatives and/or transport medium (ambient temperature)
   - Eyes and inner ear specimens (ambient temperature)
   - Specimens already inoculated onto primary culture media at the bedside or at a doctor’s office (ambient temperature)
5. Never transport syringes with needles to the laboratory; instead, transfer the contents to a sterile tube.

**Specific Procedures for Microbiologic Specimen Collection**

**Abscess**

1. Decontaminate the surface with 70-95% ALC and 1-2% TOI.
2. Collect purulent material aseptically from an undrained abscess using a sterile needle and syringe. Open miliary abscesses with a sterile scalpel and collect the expressed material with a sterile needle and syringe.
3. Expel air from the syringe, remove the needle, and cap the syringe. Alternatively, transfer 5-10 mL of the aspirated material to tightly sealed sterile container.

**Blood (bacterial only)**
1. Gather the collection blood bottles or tubes needed.
   NOTE: Blood culture bottles will be sent to a reference laboratory.
2. Clean the tops of each blood culture bottle and/or the stopper of an isolator® or ACD tube with alcohol. Do not allow alcohol to pool, as it could enter the system and kill organisms. Allow to dry while preparing the patient.
3. Cleanse the skin with 70-95% ALC.
4. Cleanse the skin with 1-2% TOI. Move in an ever-increasing circular pattern, starting at the point of projected needle insertion.
5. Apply a tourniquet proximal to the point of venous entry. The venipuncture site should not be palpated following disinfection unless sterile gloves are worn.
6. Use a sterile needle and syringe or closed system blood collection tubing. For fastidious microorganisms, use the Vacutainer® system for isolator® tubes.
7. Collect blood. Collecting the appropriate volume of blood is critical. Inoculate the bottles or tubes without changing needles.
   A. The adult isolator® tubes will accommodate 9.5-10 mL blood. Allow the vacuum to draw in the proper amount of blood. Do not force the blood into the tube.
   B. The ACD vacutainers hold 8 mL.
   C. For adult bacterial culture, inoculate aerobic bottle with 10 mL of blood or the anaerobic bottle with 7 mL of blood. If less than 17 mL is collected for 2 bottles, inoculate the aerobic bottle with 10 mL and inoculate the anaerobic bottle with the remainder.
   D. For pediatric specimens, inoculate 1-3 mL blood into a pediatric bottle or use the pediatric isolator tube. Allow the tube to draw 1.5 mL of blood.
   E. For fungal and AFB cultures, inoculate 5 mL blood into a Bactec™ Myco/F Lytic bottle or use an isolator® tube.
8. Invert tubes several times after specimen collection.
9. Remove the iodine from the skin after collection of the specimen.
10. Label and transport specimens as soon as possible. Do not refrigerate.
11. Hold at room temperature or at 35°C.

**Body Fluids, Sterile (except Urine and CSF)**

1. Prepare the skin as for blood cultures.
2. Collect the fluid using a sterile needle and syringe.
3. Submit 10 mL of the specimen for analysis.
4. Transport the specimen in a capped syringe after expelling the air from the syringe and removing the needle. Transfer the aspirated material to a tightly sealed sterile container or collect with the appropriate transport vial as described below:
   A. For aerobic and anaerobic organisms, use an anaerobic transport vial to insure the survival of anaerobic organisms.
   B. 10 mL of peritoneal fluid may be added to a blood culture bottle. Peritoneal fluid is the only body fluid that may be cultured in blood culture media.
   C. For viral isolation, send 3 mL of less fluid in viral transport medium or a sterile vial.
   D. If tuberculosis or fungal infections are suspected, larger volumes are required. Collect in sterile container.
5. Immediately transport fungal specimens at 2-8°C, viral specimens at 2-8°C, and all other specimens at ambient temperature.

**Bone Marrow**

1. Physicians should wear gowns, masks, and gloves during specimen collection.
2. Prepare skin as for blood cultures.
3. Drape the surrounding skin with sterile linen.
4. Aspirate the marrow percutaneously using a sterile needle and syringe.
5. Transfer 3-5 mL to a sterile tube containing SPS for bacterial AFB and fungal cultures. EDTA is required for viral cultures and molecular tests.
6. Transport specimens immediately at ambient temperature.

**Bordetella pertussis Culture and PCR**

**Note:** Please contact Client Supplies for Regan-Lowe tubes.

1. Allow 2 tubes of the transport medium (Regan-Lowe) to equilibrate to ambient temperature.
2. Use 2 swabs on a flexible wire handle to collect the specimen. One swab is used to inoculate the transport medium.
3. Seat the patient comfortably. Tilt the head back.
4. If available, insert a nasal speculum. Press the swab through the nares until resistance is met due to contact with the nasopharynx.
5. Rotate the swab gently and allow the swab to maintain contact with the nasopharynx for 20-30 seconds or until coughing is induced.
6. Place the swab into the transport medium. Label the tube with the patient’s name and identification number. Leave the swab embedded in the tube during transport. Transport the specimens at ambient temperature.

**Note:** Specimen will be sent to Tennessee Department of Health Laboratory Services for testing.

**Bronchial Brush/Washing/Lavage**

1. This technique should only be performed by an experienced individual. Descriptions of the methodology are readily available in literature.
2. Transport in a tightly sealed sterile container at 2-8°C for cultures, or frozen for molecular tests.

**Bullae, Vesicles**

1. Cleanse the skin as for blood cultures.
2. Aspirate the fluid/purulent material using a sterile needle and syringe.
3. If an aspirate is obtained, place in appropriate viral or bacterial transport.
4. If no material is obtained, unroof vesicle or bullous lesion and use a swab to collect cells from the base of the lesion. Place in appropriate viral or bacterial transport media.

**Cellulitis**

1. Swabs and leading-edge aspirates with or without injection of saline fail to yield etiologic agents in the majority of cases. If an unusual organism is suspected, a leading-edge (advancing margin) punch biopsy is the recommended specimen of choice.

**Cerebrospinal Fluid**
1. Physicians should wear gowns, masks, and gloves to collect the specimen. Because an open tube is held to collect the fluid, other personnel should stand away or wear masks in order to avoid respiratory contamination.
2. Decontaminate the skin with 1-2% TOI, followed by 70-90% ALC using an increasingly outward circular movement.
3. Drape sterile linen over the skin surrounding the puncture site.
4. Insert the needle. Collect the fluid into 3 sterile leak-proof tubes. Collect an adequate volume of fluid as recommended below.
   A. bacterial culture > 1 mL
   B. fungal culture 8-10 mL
   C. molecular > 1 mL
   D. mycobacterial culture 8-10 mL
   E. viral culture > 2 mL
5. Cap the tubes tightly. Submit the third tube for culture to reduce the possibility of contamination due to skin flora. Transport immediately.
6. Transport other bacterial cultures at ambient temperature. If a delay in transport occurs, incubate at 37°C or leave the fluid at ambient temperature for transport.
7. Freeze specimens for molecular (PCR) analysis.
8. For viral culture, if volume is greater than 3 mL, refrigerate and transport at once. If volume is less than 3 mL, add fluid to M4 viral transport media and transport at 2-8°C.

**Chlamydia/Gonorrhea**

1. Chlamydia/Gonorrhea testing is available by several methods. A DNA amplification method that detects *Chlamydia trachomatis/Neisseria gonorrhoeae* nucleic acid in urogenital specimens is the preferred diagnostic method. The nonamplified direct DNA probe is also available; but in general, it is less sensitive than an amplified test. Culture for *Chlamydia trachomatis* or *Neisseria gonorrhoeae* is the method of choice in cases of treatment failure and sexual abuse and for nongenital sources. Test orders will be changed to match test-specific transport media submitted.
2. Specimens for all of the above can be collected following the procedures below. Test-specific collection and transport kits are required for DNA tests and are available from PGL.
3. Females (endocervical):
   A. Place patient in the lithotomy position.
   B. Insert speculum and visualize the cervical os.
   C. Remove excess mucus from cervical os and surrounding mucosa using the large swab provided in the kit. Discard this swab.
   D. Insert second swab from kit, 1-1.5 cm into endocervical canal.
   E. Rotate swab for 30 seconds in endocervical canal to insure adequate sampling.
   F. Withdraw swab carefully, avoiding any contact with vaginal mucosa.
Males (urethral):
   A. Do not allow patient to urinate for at least 1 hour prior to collection.
   B. If purulent discharge is present, collect discharge directly on swab.
   C. If no discharge is present, insert smaller swab 2-4 cm into urethra. Rotate gently to insure contact with all urethral surfaces. Leave inserted for 2-3 seconds. Rotate gently while withdrawing swab.
4. Place swab into the test-specific transport tube.
   A. Break swab shaft to fit tube, if required.
   B. Cap tube tightly.
   C. Transport at 2-8°C.
D. For culture, inoculate specimen as specified below.
5. For culture of *N. gonorrhoeae*, use calcium alginate or Dacron swabs for specimen collection. Cotton fibers contain fatty acids that are inhibitory to the gonococcus. Avoid swabs with wood sticks.
6. Rectal culture:
   A. Moisten a swab with sterile water and insert the swab into the anal canal just beyond the anal sphincter.
   B. Allow 10-30 seconds for absorption of the organisms onto the swab.
   C. Withdraw swab gently and inoculate plate as described above.
   D. Stool is not an acceptable specimen for gonorrheal culture.
7. If disseminated gonococcal infection is suspected, culture blood and suspicious sites such as petechiae or joint fluid.

**Cutaneous (fungus only)**

1. Hair:
   A. Scrape the scalp with a blunt scalpel.
   B. Place specimen in a dry sterile container.
   C. Transport at ambient temperature.
   D. The following specimens are also acceptable:
      - Hair stubs
      - Contents of plugged follicles
      - Skin scales
      - Hair plucked from the scalp with forceps
2. Note: Cut hair is NOT an acceptable specimen.
3. Nails:
   A. Cleanse the nail with 70-95% ALC.
   B. Remove the outermost layer by scraping with a scalpel.
   C. Place specimen in a dry, sterile container.
   D. Transport at ambient temperature.
   E. The following specimens are also acceptable:
      - Clippings from any discolored or brittle parts of nail
      - Deeper scrapings and debris under the edges of the nail
4. Skin:
   A. Cleanse the skin with 70-95% ALC.
   B. Collect epidermal scales with a scalpel, at the active border of the lesion.
   C. Place specimen in a dry, sterile container.
   D. Transport at ambient temperature.

**Ear**

1. External ear cultures are processed as superficial wounds.
2. Middle ear fluid will be processed as a sterile body fluid. If the diagnosis is otitis media, the specimen of choice is middle ear fluid collected by tympanocentesis.

**Eye**

1. Cleanse the skin around the eye with a mild antiseptic.
2. Purulent conjunctivitis:
   A. Collect purulent material with a regular cotton swab.
   B. Place the swab into transport media and transport at ambient temperature or 2-8°C for viral cultures.
3. Corneal infections:
   A. Swab the conjunctiva as described above.
   B. Collect multiple corneal scrapings and inoculate directly onto bacterial agar media (chocolate agar, potato dextrose agar, and sheep blood agar) or viral transport media.
   C. Transport at ambient temperature or 2-8°C for viral cultures.

4. Intraocular fluid:
   A. Collect fluid by surgical needle aspiration.
   B. Transport bacterial cultures at ambient temperature, viral cultures at 2-8°C, or frozen for molecular tests.

**Nasopharyngeal Aspirates/Washings (virus only)**

1. For aspirate, attach mucus trap to suction pump and catheter, leaving wrapper on suction catheter. Turn on suction and adjust to suggested pressure.
2. Without applying suction, insert catheter into the nose, directed posteriorly and toward the opening of the external ear. Note: Depth of insertion necessary to reach posterior pharynx is equivalent to distance between anterior nares and external opening of the ear.
3. Apply suction. Using a rotating movement, slowly withdraw the catheter.
4. Transport at 2-8°C for viral cultures or frozen for molecular tests.
5. For washings, suction 3-5 mL of sterile saline into a new sterile bulb.
6. Insert bulb into one nostril until nostril is occluded.
7. Instill saline into one nostril with one squeeze of the bulb and immediately release bulb to collect recoverable nasal specimen.
8. Empty bulb into suitable dry, sterile specimen container or add 3 mL or less to viral transport media (M4).
9. Transport at 2-8°C.

**Nasopharyngeal Swab**

1. Seat the patient comfortably and tilt the head back.
2. Insert a nasal speculum.
3. Insert a nasopharyngeal swab (on a malleable wire) through the speculum into the nasopharyngeal area.
4. Rotate the swab gently and allow to remain for 20-30 seconds.
5. Remove the swab and place in a non-growth promoting transport medium (such as the culturette container from which the original swab has been removed). Place swab in M4 media for viral cultures.
6. Transport at ambient temperature or 2-8°C for viral cultures.

**Notes:**
- Transport media must be used because the swab tip is small and vulnerable to drying. The organisms likely to be present are fastidious.
- For infants, special bulb suction procedures are available.
- If unusual organisms such as *Bordetella pertussis* are suspected, special culture media is necessary for collection and transport. (Refer to *Bordetella pertussis* culture)

**Nose**
1. Note: This is an inappropriate specimen for anything other than assessment of staphylococcal colonization.
2. Collect anterior nares culture with a regular cotton swab. In small children, use a nasopharyngeal swab to facilitate collection.
3. Transport at ambient temperature.

Prostate

1. Cleanse the glans with soap and water.
2. Obtain prostate fluid by digital massage through the rectum.
3. Collect fluid using a sterile swab.
4. Transport at room temperature.
5. Alternatively, a urine specimen obtained immediately before and after massage may be submitted for culture.

Skin

Refer to Abscess, Bullae, Cellulitis, Vesicles, and Wounds.

Sputum

1. Assure patient cooperation to get an adequate specimen.
2. Instruct the patient as follows:
   A. Rinse mouth with tap water to remove food particles and debris.
   B. Have patient breathe deeply and cough several times to receive deep specimen.
   C. Patient should expectorate into dry, sterile container.
3. If patient is unable to produce sputum, induce using saline nebulization.
4. Transport immediately at ambient temperature. Refrigerate if a delay of more than one hour is anticipated; freeze for molecular tests.

Stool, Feces

1. Collect specimen in a clean bed pan or use plastic wrap placed between the toilet seat and the bowl. Do not submit feces contaminated with urine or toilet water.
2. Immediately transfer specimen into a clean, dry container or the appropriate preservative.
3. Transport unpreserved stool refrigerated.

Notes:
- Only loose or diarrheal stools are recommended for routine bacterial and C. difficile cultures.
- Place the specimen in an appropriate stool preservative or transport media, immediately after collection. For ova and parasite, use SAF vial or 10% formalin and modified PVA; for routine stool culture, use Cary-Blair transport media.
- If a stool specimen is not available, the following are suitable alternatives for culture:
  a. a swab of rectal mucus, or
  b. a rectal swab inserted 1 inch into the anal canal (not acceptable for Rotavirus/Adenovirus EIA).

Throat
1. Use a cotton or Dacron swab.
2. Use a tongue blade and an adequate light source to insure proper visualization.
3. Reach behind the uvula and swab:
   - both tonsillar fauces, and
   - the posterior pharynx, and
   - any ulceration, exudate, lesion or area of inflammation.
4. Place the swab into the transport media and transport at ambient temperature or 2-8°C for viral cultures.

**Tissues**

1. Tissue collection is an invasive procedure and requires surgery by a trained physician.
2. Collect tissue aseptically. Include material from both the center and the edge of the lesion. Submit actual tissue specimen; not swab of tissue surface.
3. Place the specimen in a sterile container on sterile gauze moistened with sterile nonbacteriostatic saline.
4. Transport immediately at ambient temperature, in a manner to insure recovery of anaerobic organisms. For virology cultures, do not allow the tissue to dry. Transport tissue suspended in viral transport media (M4) at 2-8°C, or frozen for molecular tests.
5. Do not submit tissue in formalin.

**Urethra**

Refer to Chlamydia/Gonorrhea.

**Urine**

1. Instructions for female patients to collect midstream urine for bacterial culture:
   A. Remove undergarments.
   B. Wash hands thoroughly with soap and water, rinse them, and dry them on a disposable paper towel or shake off excess water.
   C. Spread labia with one hand, and keep them continuously apart.
   D. Take the open sterile cup in the other hand, without touching the rim or inner surface of the cup or lid.
   E. Void 20-25 mL into the toilet and catch a portion of the rest of the urine in the container without stopping the stream. Do not touch the legs, vulva, or clothing with the cup.
   F. Place the lid on the cup.
2. Instructions for male patients to collect midstream urine for bacterial culture:
   A. Wash hands.
   B. Retract the foreskin completely.
   C. Void 20-25 mL into the toilet and catch a portion of the remaining urine in the cup without stopping the stream. Do not touch the cup with the penis.
   D. Place the lid on the cup.
   E. First-void urine for nucleic acid amplification tests (Chlamydia/Gonorrhea).
   F. Patient must not have urinated during the previous 2 hours.
   G. Collect the first 10-50 mL of the urine stream in a clean, empty plastic cup.
   H. Place the lid on the cup.
   I. Transport urine refrigerated in test-specific transport media.
3. Suprapubic aspiration:
   Notes:
• This is not a routine technique and is best performed by an experienced individual. Descriptions of the method are readily available in literature.
• These specimens are acceptable for anaerobic culture and should be submitted in an anaerobic environment if an anaerobic culture is requested.

4. Indwelling catheter urine:
   A. Do not collect urine from the drainage bag because growth of bacteria outside the catheter may have occurred at this site.
   B. Clean the catheter with an alcohol pad.
   C. Use a sterile needle and syringe to puncture the tubing. Aspirate the urine directly from the tubing.
   D. Transfer the urine to a sterile specimen container.

5. Urine catheter tip cultures are not acceptable.

6. Specimen handling:
   A. Label the container immediately and refrigerate at 2-8°C within 10 minutes of collection or transfer > 2 mL urine into a boric acid transport tube.

Wounds

1. For closed wounds, refer to Abscess and Bullae, Cellulitis, Vesicles.
2. For open wounds:
   A. Clean the sinus tract opening of the wound surface mechanically, without using a germicidal agent, to remove as much of the superficial flora as possible.
   B. Attempt to culture the base or edges of the wound to avoid collecting “normal flora” organisms.
   C. The following are preferred specimens for sinus tracts:
      • aspiration material obtained by needle or catheterization
      • curettings from the lining of the sinus tract
   D. Specimen swabbings of sinus tracts are acceptable only if the above cannot be obtained. Swabs of sinus tracts may not accurately reflect underlying disease process.
   E. Do not submit cultures of superficial lesions for anaerobic culture. Biopsy of advancing margin of wound is the preferred specimen for anaerobes, mycobacteria, and fungi.

Viral Transport Media (M4)

1. Some specimens can be submitted without utilizing a transport media, with a reasonable expectation of virus viability. Specimens in this category include: sterile fluids such as cerebrospinal fluid, pleural fluid, blood or bone marrow submitted in EDTA, urine, as well as some non-sterile specimens such as nasopharyngeal washings, sputum, bronchoalveolar lavage, and feces. Whenever there is a question of stability, the specimen should be placed in a suitable virus transport media. Refer to specific test in the Laboratory Test Directory for more information.
2. Tissue and biopsy material can be placed directly into the viral transport media. Each specimen need not be more than 1-2 cm in diameter.
3. Abscess material, bullae, pustules, vesicles, lesions, and skin scrapings can be collected o the swab and placed directly into viral transport media. If the material has been aspirated, place no more than 3 mL (equal to the amount of transport media) in the vial of M4.
4. CSF should be submitted in a sterile container.
5. Urine should be submitted in a sterile container.
6. Bronchoalveolar washings, nasopharyngeal washings, sputums, and other sterile body fluids can be submitted in sterile containers or no more than 3 mL placed in the M4 tube.
7. Stool should be submitted in a sterile container, or a small aliquot the size of a walnut can be placed in the M4 tube.
8. Blood and bone marrow should be submitted in an EDTA tube. Do not extract the buffy coat.
9. Viral transport media (M4) criteria is the same for other liquid viral transport media such as those available from Bartels, Syva, etc. labeled for viral/Chlamydia transport. Swabs that are made of calcium alginate and wood are known to interfere with the recovery of some viruses. These can also act as PCR inhibitors and are not appropriate for this type of testing.
10. Not all types of viral transport media have been validated for all testing; some may require a disclaimer, dependent on the assay.

Cytopathology/Gynecologic Collection

**General**

Our Cytopathology Laboratory is a full service department providing routine screening and diagnostic cytopathology services, including gynecologic and non-gynecologic specimens. Special studies, including flow cytometry and Urovysion FISH are also available. Consultations are available on all cytologic materials. Please refer to the Test Directory for information regarding the collection and handling requirements for individual tests, or call our Client Services department at 615-562-9300 or toll-free at 888-474-5227 for additional instructions.

**Cytopathology Specimen Submission**

1. Complete a cytopathology test requisition. Be sure to include the following:
   - date specimen collected
   - patient’s first and last name
   - sex of patient
   - patient’s date of birth
   - patient’s Social Security Number
   - patient’s complete mailing address
   - patient’s insurance information
   - patient’s pertinent clinical history and appropriate ICD-9 code(s)
   - check proper box for specimen(s) submitted and test(s) requested
   - source of specimen/specimen type
2. Label all specimen contains and slides submitted with patient’s first and last name.
3. Fix all slides and fluids immediately following specimen collection.
4. Close all specimen containers securely. Place each specimen into a specimen bag with the accompanying requisition.

**Gynecologic Collection and Submission**

**Patient Preparation**

- Ideal sampling date is 14 days after the first day of the last menstrual period
- Patient should not use douches, vaginal medication, lubricants, or vaginal contraception for 48 hours prior to sampling
• The speculum should be introduced with no lubricant

**ThinPrep Pap Smear**

**Endocervical Brush/Spatula Protocol:**

1. Record patient’s name and ID number on a ThinPrep vial and complete cytology requisition with all requested information.
2. Obtain sampling from the ectocervix using a plastic spatula.
3. Rinse spatula into the PreservCyt vial by swirling it vigorously in the vial 10 times. Discard spatula.
4. Obtain sampling from the endocervix using an endocervical brush. Insert brush into cervix until only bottommost fibers are exposed. Slowly rotate 1/4-1/2 turn in 1 direction. DO NOT OVER-ROTATE.
5. Rinse brush in PreservCyt vial by rotating in solution 10 times while pushing against the vial wall. Swirl brush vigorously to further release material. Discard brush.
6. Tighten vial cap.
7. Place vial and completed requisition into specimen bag for transport to lab.

**Broom Device Protocol:**

1. Record patient’s name and ID number on a ThinPrep vial and complete cytology requisition with all requested information.
2. Obtain sampling from the ectocervix using a broom. Insert the central bristles into the endocervical canal deep enough to allow the shorter bristles to fully contact the ectocervix. Push gently and rotate broom clockwise 5 times.
3. Rinse broom in PreservCyt vial by pushing it into the bottom of the vial 10 times, forcing the bristles apart. Swirl broom vigorously to further release material. Discard broom.
4. Tighten vial cap.
5. Please vial and completed broom into specimen bag for transport to lab.

**Conventional Pap Smear**

**Cervical/Endocervical:**

1. Using a lead pencil, label frosted end of slide with patient’s first and last name.
2. Complete requisition with all requested information.
3. Tear open fixative pouch or place spray fixative bottle beside slide.
4. Rotate cervical scraper around ectocervix and spread material evenly in the middle section of the glass slide.
5. Insert endocervical brush into endocervical canal until only the bottommost fibers are exposed. Rotate brush 90-180 degrees. Gently remove brush and spread material from brush evenly onto slide on the end farthest from the frosted end (endocervical brush should not be used on pregnant patients).
6. Fix smear immediately by flooding entire slide with pouch fixative or spraying evenly and completely with fixative.
7. When slide is dry, secure in Pap Pak and place Pap Pak in specimen bag with accompanying requisition.
Vaginal:

1. Using a #2 lead pencil, label frosted end of glass slide with patient’s first and last name.
2. Complete a requisition with all requested information.
3. Tear open fixative pouch, or place spray fixative bottle beside slide.
4. Take vaginal smear with spatula end of cervical scraper and spread evenly in center of the slide.
5. Fix smear immediately by flooding entire slide with pouch fixative or spraying evenly and completely with spray fixative.
6. When slide is dry, secure in Pap Pak and place Pap Pak in specimen bag with accompanying requisition.

**Human Papillomavirus (HPV)**

HPV testing utilizing the Hybrid Capture II System for HPV DNA detection is offered on the ThinPrep Pap specimen or cervical sampler brush. The test may be ordered by marking/requesting it on the cytology requisition. Refer to the Test Directory for more information regarding the collection and handling requirements for individual tests, or call our Client Services department at 615-562-9300 or toll-free at 888-474-5227 for additional instructions.

**Chlamydia, GC, HSV**

Chlamydia, GC and HSV by PCR testing is offered on the ThinPrep Pap specimen, urine, or Aptima swab. The tests may be ordered by marking/requesting them on the cytology requisition. Please refer to the Test Directory for more information regarding the collection and handling requirements for individual tests, or call our Client Services department at 615-562-9300 or toll-free at 888-474-5227 for additional instructions.

**Result Reporting**

Please note that the Pap test is a screening test for cervical cancer with an inherent false negative rate.

**Non-Gynecologic Collection and Submission**

PathGroup provides a full spectrum of cytology services on non-gynecological specimens, e.g., fine needle aspirations, pulmonary specimens, urines, body fluids, CSFs, etc. Please refer to the Test Directory for more information regarding the collection and handling requirements for individual tests, or call our Client Services department at 615-562-9300 or toll-free at 888-474-5227 for additional instructions.

**Surgical Pathology**
Introduction

A full spectrum of surgical pathology and consultative services are available through PathGroup. All pathologists affiliated with PathGroup are Board certified by the American Board of Pathology. PathGroup offers a full range of subspecialty pathology expertise including surgical, clinical, cytopathology, dermatopathology, hematopathology, neuropathology, oculopathology, pediatric/neonatal, gastrointestinal, hepatic, immunopathology, musculoskeletal, immunohematopathology, blood banking, autopsy studies, and forensic medicine. With this diverse selection of specialization available, either on-site or through intra-group consultation, we are able to equal or better most academic style practices including large university hospitals.

All requests for histopathologic examination and diagnosis are viewed as a request for consultation by another physician and, as such, you are welcome to contact our pathologists concerning their findings.

Submission Requirements - Routine

- A completed request form supplied by PathGroup must accompany the specimen(s). Please complete this form as indicated on the requisition.
- Submit each specimen separately in a plastic screw-capped container filled with 10% neutral buffered formalin supplied by PathGroup.
- The container must be labeled with the patient’s name. For large specimens, use large containers with enough 10% neutral buffered formalin to achieve a ratio of 5 parts fixative to 1 part tissue.
- Small specimens are to be placed in the sealable plastic bag supplied by PathGroup. The folded request form is placed in the sleeve on the back of the plastic bag, separated from the specimen container to avoid possible contamination from leakages of the specimen container.
- A PathGroup courier will transport the specimen together with the completed request form to the laboratory where the specimen routinely will be processed overnight for slide preparation and diagnosis the next day.
- For procedures and requirements of non-routine specimens, e.g., skin biopsies for immunofluorescence studies, please consult the specific section of the Test Directory.

Oncology/Hematopathology Specialty Testing

Associated Pathologists and PathGroup are leading oncology and hematopathology laboratories focused on quality, innovation, and client services. Our passion for quality and attention to detail is evident in the time and care our pathology staff takes with each case. Our pathologists have a broad range of experience to approach diagnostic challenges. Associated Pathologists and PathGroup deliver a personal touch in removing any barrier between client and pathologist, as our doctors are available throughout the day for consultation on difficult cases.

Employing state-of-the-art diagnostic technologies, Associated Pathologists and PathGroup are dedicated to comprehensive, high quality, rapid response cancer testing and the integration of these technologies into patient care. We provide valuable information that is critical for the categorization, assessment, and treatment of cancer. This leads to confident
treatment decisions by oncologists and a more efficient way to determine and employ drug therapies that make improved patient care possible.

Associated Pathologists and PathGroup also provide consultative services and second opinions to assist physicians and hospitals. For these clients, Associated Pathologists and PathGroup can provide the necessary technical components or ancillary anatomic pathology testing to complement flow cytometry, cytogenetics, FISH, and/or molecular studies performed in its laboratories.

**Hematopathology Consultations**

Hematopathology offers a variety of comprehensive laboratory and pathology tests for benign and malignant disorders of the peripheral blood and bone marrow. Our goal is to provide the referring physicians with an accurate hematologic evaluation in the most efficient and cost-effective manner. All requests will be processed as a consultation first. Special studies will be performed only if diagnostically indicated.

**PathWay Comprehensive Assessment**

Diagnostic hematopathology has become an increasingly complex subspecialty, particularly with neoplastic disorders of blood and bone marrow. The clinical, therapeutic, and prognostic features of these disorders are often distinctive; while the pathologic features are quite subtle, requiring the application of ancillary studies (e.g., cytochemistry, immunohistochemistry, flow cytometric immunophenotyping, cytogenetics, and molecular genetics) to establish a diagnosis. Furthermore, these ancillary studies are expensive, labor-intensive, and are most efficiently utilized and interpreted in the context of the morphologic features.

- PathWay Morphologic Interpretation, Flow Cytometry, Cytogenetics, FISH, Molecular Genetics (PCR) – Testing performed as necessary

**Flow Cytometry**

Flow cytometry is a rapid way to measure the characteristics of individual cells. Hematopoietic cells (blood, bone marrow, core biopsies, lymph nodes) are labeled with selective fluorescent antibodies and quantified according to their surface antigens. These fluorescent antibodies bind to specific abnormal cells in a pattern of antigen expression that is used to identify particular malignant cell types. Panels of antibodies are often used to help define which malignant cell types are present. Panels include acute and chronic leukemias, lymphomas, and MDS.

- Leukemia/Lymphoma Flow Analysis
- Stem Cell Analysis (CD34)
- DNA Analysis
- ZAP-70 Evaluation
- PNH Evaluation

**Cytogenetics**

Cytogenetics is the study of the structure of chromosome material. It includes routine analysis of G-banded chromosomes, other cytogenetic banding techniques. Acute leukemias, lymphomas, chronic myeloid and lymphoid disorders are examined cytogenetically in order
to establish the exact nature of the acquired genetic change. Rearrangements, also known as translocations, inversions, deletions, etc., can usually be detected under a light microscope. In most leukemias and lymphomas, changes in chromosome number (ploidy) or chromosome structure (rearrangements) are often observed.

- Cancer Cytogenetics
- FISH reflex if cytogenetics is normal with abnormal flow, culture failure, or possible clonal abnormality

**Constitutional Cytogenetics**

Cytogenetic analysis, to determine if constitutional abnormalities are present, is performed for a variety of indications including multiple congenital abnormalities, mental retardation of unknown etiology, abnormalities of growth, features of a recognized genetic syndrome, recurrent pregnancy loss, prenatal diagnosis via amniocentesis, mosaicism, stillbirth, fetal loss, or molar pregnancy. Adjunct studies such as fluorescence in situ hybridization (FISH) or other molecular and biochemical testing can be performed in addition to chromosomal analysis.

**FISH**

FISH (Fluorescent in situ hybridization) is a cytogenetic technique that can be used to detect and localize the presence or absence of specific DNA sequences on chromosomes. It uses fluorescent probes that bind to only those parts of the chromosome with which they show a high degree of sequence similarity. Fluorescent microscopy can be used to find out where the fluorescent probe is bound to the chromosome. FISH is often used for finding specific features in DNA.

- A complete listing of FISH probes and FISH profiles is available in the Test Directory.

**Molecular Genetics**

Cancer is a genetic disease. The progression of a neoplastic clone from a benign to a malignant state is the result of several successive genetic mutations. Molecular diagnostics seek to detect genetic errors that cause the disease at the molecular level. These highly sensitive and specific assays are inclusive of restriction digestion, PCR, RT-PCR, sequencing, and point mutation analyses using state-of-the-art technologies to provide the pathologist and oncologist with genetic information about the disease state. A variety of tests are currently available to characterize leukemias, lymphomas, and carcinomas.

- Jak-2 Quantitative
- BCR-ABL Quantitative
- FLT-3
- T-Cell Gene Rearrangement
Requests/Reporting

Fax Reporting

For those clients who prefer to have certain reports transmitted to their office facsimile (fax) machines, PathGroup will transmit the test results directly from its computer system via the fax modem. Please provide us with your fax number and other relevant instructions.

PathConnect Ordering System and LIS Interfaces

PathConnect has been designed to provide your practice the capability to view and print the results of your patients that have been seen in your office, hospital, or surgery center. With PathConnect, your practice will benefit by receiving results quicker, as well as the ability to retrieve your patients’ results wherever there is internet access. PathGroup also offers a laboratory information exchange that provides an easy, secure and flexible method for ordering tests.

Associated Pathologists and PathGroup Labs employ advanced technology in data transmission and encryption to provide a secure, HIPAA-compliant resource where physicians or staff can call up the patients’ test results while insuring patient data is protected from unauthorized access.

PathGroup utilizes the latest health care industry standards, such as Health Level Seven (HL7), Virtual Private Networks (VPN), and interface engine technology to develop and maintain interfaces to laboratory information systems and practice management systems. Interface clients who utilize PathGroup’s PathConnect system via the internet using VPN technology have their laboratory results delivered to their information system within seconds after result verification at PathGroup. This direct exchange of patient data provides clients with full control of storage, access, and retrieval of patient data, and allows clients to use their own LIS to order and report referral testing.

For more information or a demonstration, please contact our Sales and Marketing department at 615-234-2532 or 800-366-5847 extension 4532.