

Specimen Collection

Accuracy of any laboratory test can be no better than the specimen on which the test is performed. Instructions for proper patient preparation (ie, fasting), specific specimen requirements, and specimen handling are provided in this service manual. Only when proper procedures are followed in the area of patient preparation, specimen collection, and specimen handling will the test results be valid and usable by the physician.

It is important that patient identification and specimen labeling policies be followed diligently. Please refer to these policies in Specimen Labeling in Specimen Identification in General Information.

Specimen Collection Tubes

Blue-Stoppered (Sodium Citrate) Tube: Sodium citrate prevents coagulation by binding calcium. Testing performed on this tube consists of mainly coagulation studies using plasma. Plasma is the whole fluid portion of the blood when cells are removed. As with all additive tubes, the tube should be inverted gently 4 to 6 times to ensure proper mixing. Tubes must be filled completely. Clot activator in plastic serum tubes are inappropriate for use before coagulation draws. (See Correct Order of Draw in Specimen Collection in General Information.) Examples of acceptable tests are:

- Prothrombin time (PT)
- Partial thromboplastin time (PTT)
- Fibrinogen
- D-Dimer

Gold-Stoppered or Red/Black Serum Gel (Clot Activator and Gel Separator) Tube: This tube yields serum. It cannot be used when serum is required for Blood Banking procedures, cold agglutinin testing, and a slight few tests sent out of our laboratory. The volume of whole blood needed to perform testing is approximately 2 1/2 times the required volume of serum. For example, a full 3.5-mL gold-top tube will yield approximately 1.4 mL of serum. Examples of acceptable tests are:

- Procalcitonin
- Vitamin D 25 Hydroxy
- HIV
- PSA (screening and diagnostic)
- RA
- LDH
- LH

Red-Stoppered (Glass, Non-Additive or if Plastic, Clot Activator) Tube: This tube is to be used when serum is needed. Serum is the fluid portion of the blood obtained after removal of the fibrin clot and blood cells. As a general rule, serum is roughly half of the blood content. The volume of whole blood needed to perform testing is approximately 2 1/2 times the required volume of serum. For example, a full 10-mL tube will yield approximately 4 mL of serum. Examples of acceptable tests are:

- Antibody screen (type and screen), when lavender-top only is insufficient
- Cold agglutinin
- Occasional specimen referral testing

Light Green or Dark Green-Stoppered (Lithium or Sodium Heparin) Tube: Heparin prevents coagulation by inhibiting the clotting components thrombin and thromboplastin. Heparinized blood specimens yield plasma for laboratory testing. Heparinized tubes can also include separator gels and can contain heparin in either liquid or powder form. Volume requirements are similar to serum specimen tubes and the tube should be inverted 4 to 6 times to ensure proper mixing.

Examples of acceptable tests are:

- Chemistry panels (Basic, lytes, metabolic, LFTs, Lipids)
- Ammonia, lactic acid (on ice)
- Troponin
- Chromosome analysis (sodium heparin only)

Lavender-Stoppered (EDTA) Tube or Pink EDTA: Ethylenediamine tetra- acetic acid (EDTA) prevents coagulation by binding calcium. Testing performed in this tube consists of mainly whole blood studies. Tube must be at least half full and should be inverted gently 4 to 6 times to ensure proper mixing. Examples of acceptable tests are:

- Complete blood count (CBC)
- Hemoglobin A1c
- BNP
- PTH
- Type and screen

Grey-Stoppered (Potassium Oxalate/Sodium Fluoride) Tube: Together, additives prevent coagulation by binding calcium and also act as an antiglycolitic agent that prohibits metabolism of glucose and destroys many enzymes. The 3-mL size tube needs a minimum of 1 mL of blood specimen. Examples of acceptable tests are:

- Ethylene glycol
- Legal blood alcohol

Special Tube: Call CentraCare Laboratory Services at 320-255-5999 or 320-255-5632 for special collection tubes.

Correct Order of Draw

According to the National Committee for Clinical Laboratory Standards (NCCLS) standards, revised in 1998, the following order is recommended to avoid contamination of non-additive tubes by additive tubes, as well as cross-contamination between different types of additive tubes. This order is applicable to both evacuated and syringe methods of collection.

- Blood culture or other sterile tubes/bottles
- Non-additive tubes (plain, red-top tubes contain clot activators)
- Light blue-top (3.2% sodium citrate) tubes
- Red-top, red/black-top serum gel tubes, or gold-top serum gel tubes
- Light or dark green-top (heparin) tubes
- Lavender-top (EDTA) tubes or Pink EDTA
- Grey-top (potassium oxalate/sodium fluoride) tubes

Indwelling Catheters and Coagulation

Testing The laboratory discourages obtaining blood for coagulation tests from indwelling vascular catheters. Heparin affects coagulation tests and since heparin is often used to keep indwelling vascular catheter lines open, blood obtained from these lines can yield falsely elevated coagulation results.

If blood for coagulation tests must be collected through an indwelling catheter, sheath, or line, blood must be discarded or used for other laboratory tests before the specimen for coagulation testing is obtained. The amount of blood to discard is determined by taking 5 times the amount of dwell space in the catheter. The more common catheters are listed below:

Catheter	Dwell	Waste*
Arterial sheath	1.5 mL	10 mL
Arterial line	0.9 mL	5 mL
CVS arterial line	3 mL	15 mL
PA catheter-prox	1 mL	5 mL
Pace port	1.5 mL	10 mL
Introducer	2 mL	10 mL
*Waste or use for other laboratory work		
If results obtained with the coagulation specimen indicate that heparin is present, a new coagulation specimen should be obtained by venipuncture. If this is not possible, draw from the line, discarding 30 mL of blood.		

Capillary (Skin Puncture) Blood Collection

When collecting blood specimens by skin puncture, performing a good puncture is critical to the success of the procedure. Skin punctures are particularly useful for both adult (when specimen is unattainable by venipuncture) and pediatric patients (infants and small children) when small amounts of blood can be obtained and adequately tested.

Blood obtained through skin puncture is a mixture of arterial, venous, and capillary blood, along with interstitial and intracellular fluids from the surrounding tissues. It contains a higher proportion of arterial blood than venous blood because of the pressure with which the arterial blood enters the capillaries. This is especially true if the area has been warmed, as warming increases arterial flow into the area. Composition of skin puncture blood, therefore, more closely resembles arterial blood than venous blood obtained by venipuncture.

Capillary Collection by Finger Puncture

1. If capillary puncture is indicated, choose appropriate site.

- Recommended site for adults and older children is the end segment of third (middle) or fourth (ring) fingers
- Choose a site that is warm, pink, and free of scars, cuts, bruises, or rashes
- Puncture central, fleshy portion of finger, slightly to side of center



2. **Do not** puncture:

- Side or very tip of finger (these areas are half as fleshy as the desired site)
- Index finger (this dominant finger is more sensitive to pain and, through use, will notice pain longer)
- Fifth (little) finger (tissue between skin and bone is thinnest in this finger)
- Fingers of infants and very young children (bone injury is likely due to the small amount of tissue between skin and bone)
- Through previous puncture sites as it may spread infection

3. Warm site for 5 to 10 minutes, if indicated. Site warming can increase arterial blood flow to the area by 7-fold. Warming can be accomplished with/by:

- Commercial warmers
 - Wrapping site with a diaper or towel that has been run under warm water
4. Cleanse site with a 70% isopropyl alcohol pad.
- Do not use povidone iodine because it interferes with many tests including bilirubin, potassium, phosphorus, and uric acid.
 - Wipe site dry to remove residual alcohol or allow to air dry. Alcohol causes rapid hemolysis of RBCs and will sting if introduced into the puncture.
5. Grasp finger firmly and, using appropriate automated lancet, perform skin puncture.
6. Position the site downward and gently massage finger from base to tip to allow a bead of blood to form.

Excessive massaging can lead to contamination and hemolysis.

- Wipe away first drop of blood; contamination from residual alcohol or interstitial fluids is likely to alter laboratory results.
- Collect blood using the tube or device appropriate for the testing procedure, such as MICROTAINER blood collection tube.
- Micro collection tubes fill by capillary action, whereby blood flows freely into tube on contact. Do not touch device to the wound or “scrape” skin. This activates platelets and can cause hemolysis.

7. Gently invert micro collection tube 8 to 10 times to assure proper mixing. Lavender-top micro collection tube should be continually mixed by gentle tapping during draw.

Capillary Collection by Heel Puncture

Heel puncture is the preferred method of obtaining blood from newborns and infants no older than 1 year. Because this patient population has such a small blood volume, removing large quantities of blood can lead to anemia. Large quantities removed rapidly can also cause cardiac arrest.

1. Choose appropriate site. It is important that the puncture be performed in an area of the heel where there is little risk of puncturing the bone. This could lead to:

- Osteomyelitis (bone infection)
- Osteochondritis (inflammation of the bone and cartilage)

2. **Do not** puncture:

- Deeper than 2.4 mm (automated devices eliminate this possibility)
- Through previous puncture sites, as it may spread infection
- Areas that are cold, bruised, or edematous
- Between boundaries
- Posterior curvature of the heel
- In area of the arch
- Areas of foot other than heel



3. Warm site for 5 to 10 minutes, if indicated. Site warming can increase arterial blood flow to the area by 7-fold. Warming can be accomplished with/by:

- Commercial warmers
- Wrapping site in a diaper or towel that has been run under warm water

4. Cleanse site with a 70% isopropyl alcohol pad.

- Do not use povidone iodine because it interferes with many tests including bilirubin, potassium, phosphorus, and uric acid.
- Wipe site dry to remove residual alcohol or allow to air dry. Alcohol causes rapid hemolysis of red blood cells and will sting if introduced into the puncture.

5. Grasp heel firmly but gently as to not bruise the leg, ankle, or heel. Rest appropriate automated heel incision device flat over intended site. Perform skin puncture.

- “Micro preemie” infants, as determined by nursing personnel, should warrant the use of the **Tenderfoot Preemie** (depth 0.85 mm, length 1.75 mm).
- All other heel punctures can be performed with the standard **Tenderfoot** device (depth 1 mm, length 2.5 mm).

6. Position site downward and gently massage heel intermittently, allowing beads of blood to form. Excessive massaging can lead to contamination and hemolysis.

- Wipe away first drop of blood (contamination from residual alcohol or interstitial fluids is likely to alter laboratory results).
- Collect blood using the tube or device appropriate for the testing procedure, such as MICROTAINER blood collection tubes or S/P Caraway Capillary Tubes for PKU or I-Stat testing.
- Micro collection tubes fill by capillary action, whereby blood flows freely into tube on contact. Do not touch device to the wound or “scrape” skin. This activates platelets and can cause hemolysis.

7. Gently invert micro collection tube 8 to 10 times to assure proper mixing. Lavender-top micro collection tube should be continually mixed by gentle tapping during draw.

Correct Order of Capillary Draw

Although today's technology allows many tests to be performed on very small quantities of blood, some tests cannot be performed on skin puncture specimens due to volume, additive requirements, or the nature of the test. Included are coagulation tests (where tissue thromboplastin interferes), chromosome studies, and blood cultures (where site sterility is a factor). These tests are therefore not included in the basic order of capillary draw, which is as follows:

1. i-STAT Testing (Caraway Tubes).
2. Lavender-top (EDTA) tube: Initial specimen is most free flowing and less likely to contain platelet clumping. Must be filled past 0.25 mL (first line), but not over 0.5 mL (second line). Mix well to avoid micro clotting or platelet clumping.
3. Green-top (lithium heparin and plasma gel) tube: Chemistry tube of choice for capillary specimens. Attention must be given, however, to those tests that cannot be drawn in a heparinized tube or require sodium heparin (see chart under Chemistry Specimen Requirements in Specimen Collection in General Information).
4. Gold-top serum gel tube: Micro equivalent to SST, but contains no clot activator.
5. Red-top (non-additive) tube: Equivalent to the plain, red tube. It can also be used for chemistry, or utilized when green-top tube is not appropriate.

Blood Culture for the Phlebotomist - Routine and Fungal Procedures

Background

Blood cultures are among the most important cultures performed by the Clinical Microbiology Department. Blood cultures are used by physicians to rule out or confirm septicemia and/or bacteremia.

Blood cultures indicate the severity of an infection and identify the causative organism. The procedure also determines the antimicrobial susceptibility of the causative organism.

Normally, there should be no organisms of any kind present in a person's blood stream. Therefore, normal values are negative for growth.

Proper collection techniques are essential to the accuracy of blood culture results. False-positive results due to incorrect collection techniques can have serious ramifications for the patient such as inappropriate and potentially risky treatments or prolonged hospitalization. False-negative results may delay a patient's diagnosis and treatment, which may cause their condition to worsen, resulting in needless suffering and prolonged hospitalization.

Supplies

- Gloves
- Alcohol pads
- Chlorhexidine gluconate swab (2% CHG and 70% IPA) (recommended)
- Sterile syringe and needles
- Order containing patient's identification and special instructions
- For routine blood cultures:
 - BACTEC Plus Aerobic/F and BACTEC Standard Anaerobic/F bottles for 1 full set/adults
 - BACTEC Peds Plus/F bottle for pediatrics/difficult draws
- For fungus blood cultures:
 - BBL Septi-Chek bottle with Tryptic Soy Broth (TSB) Media

Procedure

1. Identify patient correctly using the order to verify medical record number with patient's wristband.
2. Explain procedure to patient, specifically that the venipuncture procedure will be performed multiple times over a given time span (when applicable).
3. **Skin antisepsis** is a very important part of any blood culture collection procedure. Failure to follow sterile technique can introduce skin surface bacteria into the blood culture bottle and interfere with interpretation of results. After selecting venipuncture site:

- Scrub with chlorhexidine swab for 30 seconds, using repeated back-and-forth strokes, covering an area of 3 to 4 square inches in diameter.
- Allow to air dry for at least 30 seconds. The scrubbing process as well as the drying process allows time for the antiseptic agent to be effective against skin surface bacteria.

If chlorhexidine swab is unavailable, prepare site using the following procedure:

- Scrub with 70% isopropyl alcohol pad for 1 minute, covering an area of 3 to 4 square inches in diameter to initially cleanse site.
- Scrub with povidone iodine swab for 1 minute covering chosen area.
- Scrub with a second povidone iodine swab for 1 minute, taking care to begin from the center of site and move outward in concentric circles.
- Allow to air dry for at least 1 minute. The scrubbing process as well as the drying process allows time for the antiseptic agent to be effective against skin surface bacteria.

4. While the area is drying:

- Tops of blood culture bottles are cleansed with alcohol pads, leaving pads on the bottle tops until just before inoculation of blood into the bottles occurs.
- Assemble syringe and needle.

5. Venipuncture procedure is performed by laboratory standards with prepared syringe and needle apparatus, taking care that nothing touches the prepped area in the process. Palpating the site once it has been prepared is not recommended. However, if the necessity to repalpate is anticipated, the phlebotomist's gloved finger should be cleansed in the same manner as the venipuncture site.

6. Remove alcohol pads from blood culture bottles and inoculate as follows, with aerobic bottles first:

- Routine:
 - 0.5 to 3 mL - BACTEC Peds Plus bottle
 - >3 to 10 mL - BACTEC Plus Aerobic bottle (8-10 mL optimum range)
 - >10 to 17 mL - 6 to 10 mL into BACTEC Plus Aerobic bottle (8-10 mL optimum range) or 4 to 7 mL into BACTEC Standard Anaerobic bottle (5-7 mL optimum range)
- Fungal:
 - 1 to 10 mL - 1 BBL Septi-Chek bottle (8-10 mL optimum range).

7. Label bottles with patient's name (first and last), medical record number, date and actual time of draw, and tech initials or code. Sunquest labels may be used provided the time and initials/code are added and the labels are placed vertically, not horizontally. **Do not cover** any part of the barcode on the blood culture bottles when labeling.

8. Blood culture bottles are carried to the laboratory or sent individually through the pneumatic tube system. They are brought into the Microbiology Department for entry into the appropriate instrument.

Additional Information

- **Greater volume of draw leads to better recovery and sensitivity of the test.** Please attempt to draw the largest volume appropriate for optimum specimens.
- The bottles, although evacuated, are not a controlled draw, so volume added must be carefully monitored. Adding too much blood may result in a false-positive result.

- Routine blood cultures should be drawn before antibiotic therapy is started. The BACTEC Plus bottles contain resins that remove antimicrobials from the blood so that growth of microorganisms will not be inhibited if patient has begun antibiotic therapy.
- Commonly, the first set of blood cultures is ordered and drawn in the hour prior to a fever spike, with subsequent sets to follow. Bacteria are most likely to be present in the bloodstream in the time frame prior to and just following these fever spikes.
- The first set of routine blood cultures is typically ordered as a STAT test. Subsequent orders are then placed into the computer system in the requested time intervals (usually 15 or 30 minutes later). If there are any questions about timing or site selection (ie, drawing from indwelling lines vs. a peripheral stick), please consult patient care staff to clarify physician requests.
- Good standard of care recommends a minimum of 2 routine blood cultures per 24 hours. Numbers should not exceed 4 sets without consultation from the CCLS Pathology representative.
- Fungus blood cultures are incubated and monitored for 30 days.