1. **Purpose**
   1.1. To provide accurate specimen collection information to the units as part of our on-line collection manual.

2. **Principles**
   2.1. Proper selection, collection, and handling of specimens for microbiology is necessary to ensure quality results that have the greatest impact on patient care.

3. **Procedure**
   3.1. The following page(s) have been posted on the on-line collection manual:
Blood Culture Collection

1. General Considerations:

A physician should perform any collection method requiring an invasive technique. Only a physician specialist with advanced training and skills should perform some specimen collection techniques.

<table>
<thead>
<tr>
<th>Proper recovery of blood pathogens require that all criteria below are met:</th>
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<tbody>
<tr>
<td>• Select the correct Blood Cultures Bottles/Media. (Example: resin bottles should be submitted for patients on antibiotic therapy)</td>
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<tr>
<td>• Pay attention to the Number and Timing of Blood Cultures. This may affect whether a pathogen will grow in the bottles and may help in the determination of contamination vs. sepsis. Submit full sets when possible. Both an aerobic and anaerobic bottle should be submitted as one set. Only peds plus and myco-f-lytic bottles were designed to be processed as single bottles</td>
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<tr>
<td>• Use Proper collection techniques. Improper collection techniques can give inaccurate results (Example: growth of contaminants).</td>
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DO NOT cover the barcodes please. Each bottle has a unique barcode, that is used by our instruments to identify the bottle to the patient.

a. Blood Culture Bottles/Media -- It is important to choose the correct blood culture bottles to ensure optimal recovery of blood pathogens.

1) **Standard Bottles** -- The most commonly used bottles: media contained in blood culture bottles designed for optimal recovery of blood pathogens. One aerobic (blue label) and one anaerobic bottle (yellow label) are processed as a set for each blood culture test.

   **Available through distribution**
   **Optimal volume: 8-10 ml blood**

2) **Plus (Resin) Bottles** – Resin beads in the media neutralize a wide variety of antibiotics, allowing growth of microorganisms that would not occur with conventional media. One aerobic (silver label) and one anaerobic (gold label) bottle are processed as a set for each blood culture test.

   **Available through distribution**
   **Optimal volume: 8-10 ml blood**
3) **Peds Plus** -- these bottles were designed to optimize detection of the most common pediatric pathogens in smaller volumes (<3mL) of blood. The medium also contains resins for antibiotic neutralization. Unless anaerobes are suspected, only the aerobic bottle should be submitted.

**Available through distribution**
**Optimal volume:** 1-3 ml blood

4) **Myco-F-Lytic** – the bottles are an adjunct to aerobic blood culture media for optimal recovery of mycobacteria (AFB), yeast, and fungi from blood samples.

**Available through the microbiology lab x24843**
**Optimal volume:** 1-5 ml blood

5) **Viral Culture** -- Heparin top blood tube (green) – typically, CMV, enteroviruses (coxackie virus, poliovirus, echovirus, and enterovirus), HSV, and VZV are isolated from blood. Note: Viral PCR may require an EDTA tube (purple top), depending on the laboratory that performs the test – call x24973 to confirm tube type.

**Adults:** 5 cc of blood
**Children:** 1 to 3 cc of blood

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b. **Number and timing of blood cultures** – Most cases of bacteremia are detected by using three sets of separately collected blood cultures. More than three sets of blood cultures yield little additional information. Conversely, a single blood culture may miss intermittently occurring bacteremia and make it difficult to interpret the clinical significance of certain isolated organisms.

**Acute sepsis**
Collect two or three cultures from separately prepared sites prior to starting therapy.

**Endocarditis**
**Acute:** Obtain three blood cultures with three separate venipunctures over 1 to 2 hours, and begin therapy.
**Subacute:** Obtain three blood cultures on day 1 (15 minutes or more apart). If all are negative 24 hrs later, obtain 3 more.
**Antimicrobial therapy 1 to 2 weeks before admission:** Obtain two separate blood cultures on each of three successive days.
c. **Order of Blood draw** – Clinical and Laboratory Standards Institute (CLSI), recommends the following order of blood draw when collecting multiple specimens for the clinical laboratory. Refer to the individual test information for additional details:

1) Blood culture tube
2) Coagulation tube
3) Serum tube with or without clot activator
4) Heparin tube with or without gel plasma separator
5) EDTA tube with or without gel separator
6) Glycolytic inhibitor.

d. Blood culture collection

1) **Step 1: Examine the blood culture bottles** -- Discard the bottles if any of the following is observed:
   a) Contamination, damage, or deterioration
   b) Cloudy or turbid Media
   c) More or significantly less than 2 inches of liquid media is inside the bottle
   d) Media is expired
   e) Sensor is the wrong color. The sensor is located on the bottom of the bottles and is used by the blood culture instrument to detect growth.

2) **Step 2: Mark bottles for appropriate specimen collection amount** -- The bottles are marked in 5 mL increments along the label. The only way to ensure that the appropriate amount of sample is collected is to mark off the broth level and indicate the fill level. 8-10 mL of blood per bottle is recommended for adults.

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**Fever of unknown origin**

Obtain two separate blood cultures at least 1 hour apart. If these are negative, then 24-36 hours later, obtain two more blood cultures 1 hour apart. The yield of information beyond four cultures is usually minimal.
3) **Step 3: Disinfect the bottle tops** -- Remove the protective caps from the Bactec blood culture bottles and disinfect the top of each bottle septum with 70% alcohol and allow to air dry. **Use 1 alcohol wipe for each bottle septum. Do Not Use Providine-Iodine on the Bactec blood culture bottles.** After disinfecting the bottle septums, do not retouch the bottle tops.

4) **Step 4: Identify the Patient** -- Appropriate positive identification of patient and explanation procedure to patient is required before proceeding.

5) **Step 5: Apply the Tourniquet** -- Just prior to blood collection, apply the tourniquet 4-6 inches from insertion site. The tourniquet should restrict venous flow only, can be easily released, and never tight enough to restrict the arterial blood flow. Never allow the tourniquet to be left on longer than two minutes. NEVER probe blindly in a patient’s arm. Make only two attempts per person taking the blood.

6) **Step 6: Skin Preparation** -- Select the site of venipuncture. The practice of drawing blood for culture from catheters of the groin should never be performed when a peripheral (i.e., non-catheterized) site is available. Blood should be obtained from peripheral venous or arterial sites. Obtaining blood cultures from central venous catheters, arterial lines, and inguinal vessels increases the likelihood of obtaining a false positive blood culture. Palpate for the site from which to draw the sample. Obtain 1 Chloroprep-Frepp. Place the sponge down onto the arm in the area of the intended puncture site and **prime the sponge** by pressing the sponge up and down on the arm until the alcohol can be seen on the skin. Scrub the arm **vigorously** with the Chloroprep sponge for **30 seconds**. Allow to **air dry** completely for **30 seconds**. **DO NOT BLOW, FAN, OR WIPE THE AREA WITH GAUZE. DO NOT TOUCH THE AREA!**
7) **Step 7: BD Butterfly Method**— To be performed after inspecting bottles, disinfecting the tops, applying tourniquet, and skin preparation:

a) Peel apart the package and remove blood collection set. Thread the Luer end of tubing set into Vacutainer holder. Remove sheath-covering needle at the wings.

b) Perform venipuncture.

c) Push and hold the Vacutainer holder over the top of the vial to puncture the septum. Collect blood in the aerobic bottle to the desired fill level on the vial. Inoculate the amount recommended on the bottle (8-10 cc for adults). The volume of the inoculum must never be less than 3.0 for the standard aerobic/F, standard anaerobic/F, aerobic plus, and anaerobic plus bottles. Pediatrics Plus bottles will except 1-3 ml.

d) Monitor to ensure proper blood flow and fill level. Always keep the culture bottles in the upright position while obtaining specimens.

e) Remove the holder from the vial. Immediately push and hold the holder onto the anaerobic bottle.

f) Collect blood to the desired fill level on the second vial. Remove the holder from the vial. Note: If more samples are required, additional tubes may be drawn at this time using the Vacutainer holder. Follow the appropriate order of draw if further blood tests are required.

g) Removal— When the final vial or tube is filled, activate the safety on the butterfly. Apply pressure to the insertion site. Only after bleeding has completely ceased apply a 2x2 gauze and tape or Band-Aid.

h) Attach the patient label vertically (being careful not to completely cover the bottle barcode) to both blood culture bottles. These labels must include the patient's name, medical record number, room number, procedure ordered, collection date and time. The staff member who
performed the venipuncture must also handwritten his/her initials, collection
date and time, area of puncture site or if drawn from a central line, on the
patient's specimen label. All blood cultures are to be sent to the Laboratory
within one hour.

4. References

4.1. Murray, P. R., E. J. Baron, J. H. Jorgensen, M. A. Pfaller, and R. H. Yolken,
Press, Washington, DC. (see ASM Press)

4.2. Clinical and Laboratory Standards Institute (CLSI). Laboratory Documents:
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