Title: Laboratory - Pre-Analytical Blood Culture Collection - Guideline

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I. Purpose Statement

a. The detection of microorganisms in a patient's blood has diagnostic and prognostic importance. Bacteria, Mycobacterium, yeast, and fungus enter the blood from extravascular sites such as the genitourinary tract, respiratory tract, abscesses, surgical wound infections and other miscellaneous sites either directly or via the lymphatic vessels. When infectious agents enter and/or multiply in the bloodstream at a rate that exceeds the capacity of the reticuloendothelial system to remove them, septicemia results.

b. Blood cultures are essential in the diagnosis and treatment of the etiologic agents of sepsis. Bacterial sepsis constitutes one of the most serious infectious diseases and, therefore, the expeditious detection and identification of blood borne bacterial pathogens is an important function of the diagnostic microbiology laboratory.

c. The presence of fungal and Mycobacterium septicemia is more frequent with the increasing population of immunosuppressed individuals, those with HIV infections, and those under treatment with immuno-suppressing chemotherapy. Collection bottles and tubes for these specimens have agents to lyse the RBC's to release intracellular organisms. In addition, the fungal collection system allows for centrifugation to enhance the recovery of the fungus.

d. The ***BACTEC*** instrument is designed for the rapid detection of microorganisms in clinical cultures of blood. The sample to be tested is inoculated into the vial that is entered into the ***BACTEC*** for incubation and periodic reading. Each vial contains a sensor that detects increases in CO_2 produced by the growth of microorganisms. The sensor is monitored by the instrument every ten minutes for an increase in its fluorescence, which is proportional to the amount of CO_2 present. A positive reading indicates the presumptive presence of viable microorganisms in the vial.

II. Policy

a. Timing and numbers of cultures collected

i. The following recommendations are intended to maximize the chances of recovering organisms from the blood, and to assure proper test utilization. In all instances the ordering physician should specify draw times.
    1. Acute Sepsis
        a. 2 to 3 sets from separate sites, all within 10 minutes.
    2. Acute Endocarditis
        a. 3 sets from 3 sites at least 1 hour apart.
    3. Sub-acute Endocarditis
        a. 3 sets from 3 sites taken > 15 minutes to 1 hour apart. If negative at 24 hours obtain 3 more sets.
    4. Fever of unknown origin
        a. 2 to 3 sets from separate sites 15 minutes to 1 hour apart depending on the urgency to start antimicrobial therapy. If negative at 24 hours, obtain 2 to 3 more sets.
    5. Suspected colonized central line
        a. 1 set peripherally drawn and 1 set drawn through central line.
    6. Suspected Mycobacterium (TB) septicemia
        a. Patient permitting, two-three separate blood cultures should be drawn at no less than hourly intervals over a 24-hour period. Culture should be ordered as Culture Blood AFB.
    7. Suspected Fungus septicemia-
        a. Patient permitting, three separate specimens should be drawn on three consecutive days when no other orders supersede. The ordering physician may order any frequency in an effort to enhance recovery of the suspected organisms. The cultures should be separated by at least fifteen minutes, preferably 30 minutes or more, between venipuncture events. Culture should be ordered as a Culture Blood Fungus.
    8. NOTE: If more than 3 blood cultures or blood culture orders are received on a patient within 24 hours, the chart should be reviewed or the ordering physician called to investigate the reason for this request. Studies have revealed that a fourth blood culture is of no additional clinical value.

b. Volume of Blood Needed for routine Blood Culture

i. Obtaining an adequate volume of blood for each blood culture is critical in detecting bacteremia. Every effort should be made to obtain the optimum amount of blood based on patient weight. See table below.

BLOOD CULTURE VOLUMES by Patient Weight	**Blood Volume Drawn**	**Blood Culture Bottle(s)**
<4 kg (<8.8 lbs)	1 ml	All in Peds Plus
4.0 – 13 kg (8.8-28.6 lbs)	3 ml	All in Peds Plus
13 – 25 kg (28.6-55 lbs)	**Optimum: 10 ml**	**Aerobic Plus (6 ml)** **Anaerobic (4 ml)**
	8-9ml	Aerobic Plus (5 ml) Anaerobic (3-4ml)
	4-7ml	Peds Plus (1-4 ml) Anaerobic (3 ml)
	1-3 ml	All in Peds Plus
>25 kg (>55lbs)	**Ideal: 20mL**	**Aerobic (blue) - 10mL** **Anaerobic (purple) - 10mL**
	16-19 mL	**Aerobic (blue) -9- 10mL** **Anaerobic (purple) – 7-9mL**
	11-15 mL	**Aerobic (blue) – 6-10mL** **Anaerobic (purple) - 5mL**
	6-10 mL	**Aerobic (blue) – 3-7mL** **Anaerobic (purple) - 3mL**
	1-3ml	**Peds (pink) – 1-5mL**

ii. Pediatric volume guide

1. One can usually draw 1 ml/yr of age. The more blood collected the better chance of detecting the presence of bacteria. However, for pediatrics, there must be a balance between volume of blood collected, the child's weight and the clinical condition of the patient.

iii. For Neonates

1. Approximately 1.5ml blood is drawn, with 0.5ml placed in the Peds bottle and 1.0ml placed in the Anaerobic bottle.
2. Alternately, the entire volume may be placed in the Pediatric bottle.

iv. Volume for Culture Blood AFB

1. BACTEC Myco/F-Lytic bottle for Mycobacterium (TB) detection inoculate with 1-5mls of blood aiming for the maximum 5 mls.

v. Volume for Culture Blood Fungus:
   1. Isolator Fungus blood culture tube is a vacutainer tube; inoculate with as much blood as will draw into tube. Pediatric=1.5ml, Adult=10ml

vi. Specimen Labeling
   1. Correctly label each vial at the patient's bedside following laboratory policy for specimen labeling. Label each vial with patient's full name, medical record number, date, time, site of venipuncture, and collector's initials.

c. Reagents and materials
   i. BACTEC PLUS Aerobic/F Culture Vial
   ii. BACTEC Standard Anaerobic/F Culture Vial
   iii. BACTEC PEDS PLUS/F Culture Vial
   iv. BACTEC Myco/F-Lytic Culture Vial
   v. Isolator Fungus Tubes.
   vi. Blood Collection Set ('butterfly') or 20 mL LUER-LOK syringe with a 21 gauge needle; or 3 mL LUER-LOK syringe with a 23 gauge needle.
   vii. ChloraPrep Swabstick or 1.5ml Frepp pack – 2% Chlorhexidine Gluconate and 70% Isopropyl alcohol. Store between 15-30C.
      1. WARNING: DO NOT use on children less than 2 months old.
   viii. 70% isopropyl alcohol (alcohol pad)
   ix. Tourniquet
   x. 2x2 Gauze sponges
   xi. Tape or Adhesive Bandages

d. General safety considerations
   i. Pathogenic microorganisms including Hepatitis B Virus and Human Immunodeficiency Virus may be present in specimens. "Universal Precautions" and institutional guidelines should be followed in handling all items contaminated with blood or other body fluids.
      1. Wear gloves while handling inoculated vials.
      2. Perform all blood culture processing in a biological safety cabinet.
      3. Properly dispose of all contaminated materials. Place syringes, needles, and other sharp contaminated materials in a puncture proof container.
      4. Never attempt to recap a needle.

III. Content

a. Apply the tourniquet.
    i. Palpate the area and select site of vein for venipuncture.
    ii. Venipuncture may be performed on any acceptable vein on the patient's arm or hand. Select a different site for each culture drawn.
b. Remove tourniquet.
c. Prepare venipucture site for blood cultures to be drawn as follows:
    i. IMPORTANT: If a blood alcohol level is also to be drawn, DO NOT use any product containing alcohol on the arm. Cleanse instead with Phisohex soap.
d. Procedure for adults and children > 2 months using Chloraprep Swabstick
    i. Remove swab from pouch. Do not touch foam applicator tip.
    ii. Place foam slat side down on the treatment area.
        1. Maximum treatment area for one applicator is approximately 2.5 in. x 2.5 in.
    iii. Use repeated back-and-forth strokes of the swabstick for 30 seconds. Completely wet the treatment area with the antiseptic.
    iv. Discard swab.
    v. Allow the area to air dry for 30 seconds.
        1. Do not blot or wipe away. Do not touch the venipuncture site again.
e. Procedure for adults and children >2 months using Chloraprep FREPP applicator
    i. Remove FREPP applicator from pouch.
    ii. Pinch the wings on the applicator to break the ampule and release the antiseptic.
        1. Do not touch sponge.
    iii. Wet the sponge by repeatedly pressing and releasing the sponge against treatment area until liquid is visible on the skin.
        1. Maximum treatment area for one applicator is approximately 2.5 in. x 2.5 in.
    iv. Use repeated back-and-forth strokes of the applicator for 30 seconds.
    v. Allow the area to air dry for 30 seconds.
        1. Do not blot or wipe away. Do not touch the venipuncture site again.
f. Procedure for infants <2 months old
    i. Wipe the venipuncture area for at least 30 seconds with an alcohol pad.
    ii. With a fresh alcohol pad, wipe the puncture site in a circular motion, moving concentrically outward for at least 30 seconds.

iii. Allow the area to dry completely 30-60 seconds.

g. Inspect and disinfect blood culture vials
   i. Inspect all vials and tubes for evidence of contamination, leakage, or damage. DO NOT use any vials or tubes that show evidence of contamination, leakage, or damage.
   ii. For Bactec bottles including Myco/F Lytic
      1. Remove flip-off caps from vials.
      2. Wipe the tops of the blood culture vials with a 70% isopropyl alcohol pad and discard the used pad.
      3. Do not use iodine to disinfect tops of vials.
   iii. For Isolator fungal tube
      1. Disinfect top of the isolator tube with PVP iodine, careful not to let the iodine pool and thereby be introduced into the tube (Iodine in the isolator media can significantly reduce growth of any organisms in the blood).
      2. Allow iodine to dry completely, about one minute.

h. Perform venipuncture using the following guidelines
   i. Re-apply the tourniquet. To avoid contamination do not touch/palpate the cleansed site. If further palpation is required venipuncture site must be disinfected again.
   ii. Aseptically draw directly the appropriate volume (see section "Specimen Requirements-volume of blood needed" for specific volume guidelines) following the method of collection guidelines below for the method used.
   iii. Butterfly Guidelines
      1. When using the Blood Collection Set ("butterfly"), the phlebotomist must carefully monitor the volume collected by means of the 5 ml graduation marks on the vial label.
      2. If the volume is not monitored, the stated maximum amount collected may be exceeded.
      3. This condition may adversely create a "false" positive result due to high blood background.
      4. Mark the bottles to insure the following amount of blood is drawn.
      5. Aerobic vial
         a. Mark at the level the liquid will be after 10ml are drawn into it.
      6. Anaerobic vial
         a. Mark at the level the liquid will be after 7ml are drawn into it.
      7. Myco/F-Lytic vial
         a. Mark at the level the liquid will be after 5 ml are drawn into it.

8. Important: Keep the bottles upright during the time they are filling with blood. Fungal isolator tubes are vacutainer tubes and will draw up the correct amount of blood. Using a butterfly for the isolator tube is not recommended; the blood can prematurely clot and require a redraw as clotted specimens are rejected.
9. Place the butterfly protected needle into a barrel adapter. Perform the venipuncture by inserting the exposed needle into vein at the disinfected site.
10. While holding the needle steady place the barrel adapter over the aerobic bottle and allow blood to flow in up to the pre-marked line.
11. Remove the barrel from the aerobic bottle and place over the anaerobic bottle, again allowing the blood to flow in up to the pre-marked line.
12. Mix the bottles immediately after drawing by inverting several times to prevent clotting.
13. If blood needs to be obtained for other tests the blood culture should be drawn first before other vacutainers.
14. Important: Never invert an Aerobic, Anaerobic, Peds, or AFB Bactec bottle in an adapter with a regular straight needle on it. This could cause fluid and/or particles from the bottle to back flush into the venipuncture site. When using an adapter ALWAYS use a butterfly apparatus

iv. Syringe guidelines

1. If using a needle and syringe, typically a 20 ml syringe is used for adults. Draw 17 ml of blood for one blood culture set (aerobic and anaerobic). Add blood to bottles as directed in section 2.3.4.
2. For pediatric patients, a 3 ml syringe is frequently used. Draw 1-3 ml of blood and transfer the entire amount into Bactec Peds Plus/F vial.
3. For AFB add 5ml of blood – (1-5ml are acceptable).
4. Mix the bottles immediately after drawing by inverting several times to prevent clotting.

v. Fungal Isolator guidelines

1. For Fungal isolator 10 ml of blood will be drawn into the vacutainer tube. Holding the tube lower than the needle insertion site, allow the blood to flow into the tube until it stops.
2. Invert the tube immediately four or five times, gently, to insure the mixing of the contents of the tube and the blood. This prevents clots from forming. Clotted specimens are rejected for culture, so mixing the tube is essential.
3. Note: Using a butterfly to draw fungal blood cultures is not recommended because the specimen can easily clot and be

rejected. The fungal tube is best used with a vacutainer needle and hub.

vi. Indwelling catheter guidelines
    1. Blood may only be collected through an indwelling catheter line by qualified personnel (determined by scope of practice). MUHC phlebotomy staff are not permitted to collect specimens via indwelling catheter lines.
    2. Blood may be drawn through an indwelling catheter line if so requested by the physician or when venipuncture is unsuccessful.
    3. Inspect and cleanse the blood culture bottle tops according to above procedure.
    4. Wipe the port of entry with alcohol and allow to dry. (If the port is protected, this step may not be necessary.) Any blood or fluid resident in the line must be removed and discarded.
    5. Use a syringe and withdraw enough blood to be certain you have removed all of the fluid in the line and brought blood from peripheral circulation to the syringe. Discard this syringe.
    6. Attach a second syringe and withdraw the needed sample of blood – optimally 17ml for adults and 1-3ml for infants.
    7. Aseptically attach a needle to the syringe and inject the blood into the appropriate bottles following the guidelines.

vii. After venipuncture, apply pressure as needed to the site.

viii. Deliver inoculated bottles to the lab as soon as possible
    1. Do not incubate or refrigerate vials or tubes

i. Specimen handling
    i. Mycobacterium-Myco/F bottles
        1. Sent to microbiology department
        2. Tubes are to be kept at room temperature
    ii. Fungal isolator tubes
        1. Sent to microbiology department within 16 hours.
        2. Tubes are to be kept at room temperature
        3. There can be a delay of up to sixteen hours before the tubes are processed, but quick processing is optimal
    iii. Routine blood cultures
        1. Sent to microbiology department with minimal delay
        2. Bottles are kept at room temperature
    iv. Extra blood culture vials
        1. Sent to microbiology department with next courier
        2. Bottles are kept at room temperature

IV. Attachments

a. See attached PDFs via “links and attachments” tab at top right corner

V. References, Regulatory References, Related Documents, and Links

a. Not applicable