The Pathology Laboratory

Microbiology Policy

830 BAYOU PINES WEST · LAKE CHARLES, LA. 70601

BLOOD CULTURE COLLECTION/ WORK-UP OF POSITIVE BLOOD CULTURES

MIC. 22630
MC-108

Adopted Date: 7/13/16

Purpose:
To provide information to phlebotomists, medical technologists and other medical personnel regarding the proper collection of blood cultures. To provide instructions for work-up of positive blood cultures submitted from outside laboratories.

Principle:
When bacteria or fungi overcome the host's normal defense mechanisms and enter the bloodstream through the lymphatics or from extravascular sites, they can quickly disseminate throughout the body causing severe illness. In addition, the by-products of their metabolism can lead to septic shock, among the most serious complications of infectious diseases. Rapid recognition and immediate institution of appropriate treatment are essential. Laboratory diagnosis of bacteremia and fungemia depends on blood cultures. Because the culture methods are so sensitive, the procedure must be carefully controlled beginning at the pre-analytical stage (collection) to avoid the misinterpretation of procurement associated skin commensal microorganism as an agent of infection. Contamination could lead to a specimen being determined positive when a clinically relevant isolate is not actually present.

One blood culture consists of blood from a single venipuncture inoculated into either an aerobic and anaerobic bottle (used for adults) or one pediatric bottle (used for difficult draws or pediatric patients). Optimal blood-to-broth ratios are 1:5 to 1:10. Adequate volume is one of the most important factors in the laboratory detection of microorganisms in the bloodstream.

An understanding of the circumstances in which different types of bacteremia and fungemia are likely to occur is helpful in planning diagnostic studies and interpreting results of blood cultures. The common sources of blood stream infections are intravascular devices (19%), the genitourinary tract (17%), the respiratory tract (12%), the bowel and peritoneum (5%), skin (5%), the biliary tract (4%), intra-abdominal abscess (3%), other known sites (8%), and unknown sites (27%).

Specimen Collection:

I. Bottle Preparation
   1) Inspect each blood culture bottle before use to ensure the integrity of the bottle and that the sensor on the bottom is intact.
      - The sensor is normally a uniform grayish-green color and a yellow color indicates contamination of the broth.
      - The medium should not have excess gas pressure. This indicates contamination of

broth.
- Ensure that the bottles have been stored appropriately at room temperature (15-30°C) and protected from direct sunlight.
- An expiration date is printed on each bottle's label. Do not use the culture bottles beyond the last day of the month indicated.
- Check the bottles for damage, leakage, or deterioration.
- Discard any bottle found to be damaged, stored improperly, or with a yellow sensor. ***Note:*** *Report bottles found in poor condition to the Microbiology personnel or the Laboratory Manager.*

2) Remove the plastic flip-top over the cap.
3) Sterilize the exposed rubber stopper with a sterile 70% alcohol pad. Allow to air dry.

II. Site Preparation
   a. Clean the venipuncture area with a Chloraprep swab. Pinch the wings on the applicator to break the ampule and release the antiseptic. DO NOT TOUCH THE SPONGE. Wet the sponge by repeatedly pressing the sponge against the venipuncture site until liquid is visible on the skin. Use repeated back and forth strokes for approximately 30 seconds. Allow the area to completely air dry for approximately 30 seconds. DO NOT BLOT OR WIPE AWAY.
   b. Do not touch the venipuncture site with unsterile fingers. If you find it necessary to palpate the site before drawing the blood culture, cleanse the finger used to palpate with an alcohol prep and then Chloraprep swab.

III. Venipuncture
   1) Using a syringe or collection device, aseptically draw an appropriate amount of blood for the appropriate bottles being collected.
      - Aerobic and anaerobic bottles require 5-10 mls per bottle (10 mls being preferable).
      - Pediatric bottles require 2-4 mls per bottle (4 mls being preferable).
      - Recommended blood to broth ratio is 1:5 to 1:10. As the volume of blood drawn increases, the yield of a positive blood culture increases. Optimally, a total of 20 mls of blood should be drawn from adults (10 mls per bottle).
      - Fill the aerobic bottle first, then the anaerobic bottle so that any oxygen trapped in the tubing will not be transferred to the anaerobic bottle.
      - Do not overfill the bottles, as this may cause false positive readings.
      - Inoculate the blood culture bottles first to avoid contamination, then fill additional blood collection tubes.

IV. After drawing the appropriate bottles for the patient, carefully apply the patient's label to the bottle as to not cover the bottle's barcode or lot number. Blood culture bottles should be brought to the send out department as soon as possible and stored at room temperature.

V. Bottle Transportation

The bottles will be sent to West Calcasieu-Cameron Hospital lab with a courier on same day of collection. If it is past the time for the last courier run, a yellow cab will be called for pick up.
Note: *Delays in placing blood culture bottles into a CMBCS may slow or impede bacterial growth detection. Therefore, storing blood culture bottles or tubes at room temperature for ≥2 hours is not recommended.*

VI. Timing of Blood Cultures
   1) ***Note:*** *Although drawing blood cultures before or during the fever spike is optimal for recovery, volume is more important than timing in the detection of agents of septicemia.*
      - When acute sepsis or another condition (osteomyelitis, meningitis, pneumonia or pyelonephritis) requires immediate institution of antimicrobial agent therapy, draw blood cultures before starting therapy.
      - For fever of unknown origin, subacute bacterial endocarditis, or continuous bacteremia or fungemia, a maximum of three blood cultures is recommended.

VII. Work-up of Positive Blood Cultures Received From Outside Sources:

1) Order 8450 and specify whether it is aerobic or anaerobic bottle (BLDAER/BLDANA).
2) Perform a direct gram stain from the bottle if not received with specimen. We receive blood cultures from outside hospitals and do not have access to their gram stain results.

    - Sterilize the exposed rubber stopper with a sterile 70% alcohol pad. Allow to air dry.
    - Use a tuberculin syringe to remove approximately 0.3 ml of blood from the blood culture bottle. Apply to dry slide and let dry.

3) Blood culture bottles are received inoculated on agar from outside sources. For aerobic bottle, inoculate blood, chocolate, and MacConkey agars, should be streaked for isolation. If only anaerobic bottle is positive, inoculate anaerobic blood, anaerobic PEA and LKV/BBE, streak for isolation and incubate in anaerobic environment. If both bottles from blood culture set are positive, inoculate blood, chocolate and MacConkey for both bottles. Anaerobic media is not needed.
4) Perform Maldi-tof identification on growth from each positive bottle. Susceptibility testing should be performed on all bottles.
5) For Beauregard Memorial Hospital, if a coagulase negative staphylococci is identified, do not perform a susceptibility. Result the identification with message #8450.
6) For other probable contaminants such as *Bacillus* spp., *Corynebacterium* spp., and *Propionibacterium* spp., use message #8451 and #CONTACT. Save the isolate in the refrigerator in case isolate should need to be sent out for susceptibility testing. If a Bacillus spp. is non-hemolytic the state laboratory should be consulted. See MC-105 Bio threat Agents.

Materials:

A "set" of blood cultures is usually described as one aerobic and one anaerobic blood culture bottle drawn together. In infants, young children, and patients who are difficult to draw, a pediatric blood culture bottle may be drawn and considered a "set" of blood cultures.

Adults = one aerobic bottle + one anaerobic bottle (5-10 mls of blood in <u>each</u> bottle)
Infants, Low Volume Draws = one pediatric bottle (2-4 mls)

<u>Bottles</u>
ORANGE = anaerobic bottle
GREEN = aerobic bottle
Note: *ORANGE + GREEN = ONE (1) ADULT BLOOD CULTURE SET*

YELLOW = pediatric bottle
Note: *YELLOW = ONE (1) PEDIATRIC/LOW VOLUME DRAW BLOOD CULTURE SET*

Storage and Stability:

- Inoculated blood culture bottles that are collected in house are sent directly to WCCH after collection.

Precautions and Warnings:

<u>Laboratory Diagnosis of Sepsis Caused by a Colonized Indwelling Vascular Catheter</u>
Indwelling vascular catheters are a cornerstone of modern medical care, enabling administration of fluids, parenteral nutrition and drugs, easy blood sampling, and monitoring of a patient's physiological parameters. However, even when careful antisepsis is followed during the catheter insertion and maintenance, these devices tend to become colonized by commensal indigenous and pathological

skin flora over time, and catheter-related infections (CRI) are now among the most common sources of nosocomial bacteremia. Microorganisms attach to the inner and outer surfaces of the intravenous device, and bacteria such as *Staphylococcus epidermidis* and other species of coagulase-negative staphylococci secrete an excess of extracellular matrix ("slime") and form a tenacious biofilm that protects the organism from the host immunological response. Flushing of the colonized catheter by intravenous fluid solutions provides nutritional support to residing organisms and contributes to dispersal of the infection through the bloodstream.

References:

1. Manual of Clinical Microbiology, 8th, BacT/Alert Procedure Manual, BacT/Alert Bottle Package inserts
2. BacT/Alert Blood Culture Collection Procedure – Biomerieux, Clinical Microbiology Procedures Handbook third edition, BacT/Alert blood culture package inserts
3. Q-Tracks Blood culture Contamination - College of American Pathologists
4. CLSI. *Principles and Procedures for Blood Cultures.* 2nd ed. CLSI guideline M47. 2022.
5. The Pathology Laboratory
6. CAP Standards