PROVIDENCE
Sacred Heart
Medical Center &
Children's Hospital

Blood Culture Procedure

Department of Microbiology

Effective date: 05/2007

Last Revision: 05/19/2015

Last reviewed: 05/19/2015

Table of Contents

1.0 Principle

The BacT/Alert Microbial Detection system uses a colorimetric sensor and reflected light to monitor the presence and production of CO_2 that is dissolved in the culture medium. If microorganisms are present in the test sample, CO_2 is produced as the organisms metabolize the substrates in the culture medium. When growth of the microorganisms produces CO_2, the color of the gas-permeable sensor in the bottom of each culture bottle changes from green to yellow.

2.0 Clinical Significance

Laboratory diagnosis of bacteremia and fungemia depends on blood cultures, which are probably the most important cultures performed in the microbiology laboratory. Rapid recognition of organisms and immediate implementation of appropriate treatment are essential. 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 byproducts of their metabolism can lead to septic shock, among the most serious complications of infectious diseases.

3.0 Scope

This procedure is classified under CLIA as Highly Complex. It should be carried out by technical personnel familiar with the operation of the BacT/Alert instrument and trained to identify bacterial and yeast isolates. They also need to understand and be able to perform antimicrobial susceptibility testing. Testing includes but is not limited to: troubleshooting, QC checks, and technical proficiency.

4.0 Safety - Personal Protective Equipment

Performance of this procedure will expose testing personnel to biohazardous material. All specimens must be handled as potentially infectious material, as outlined in the Providence Sacred Heart Microbiology Safety Guidelines.

The reagents and/or chemicals that are used in the identification of microorganisms may be hazardous to your health, if handled incorrectly. A brief listing of precautions for each chemical hazard is included in the reagent section of the respective procedures.

More extensive information concerning the safe handling of the reagents and/or chemicals used in this procedure, as well as other important safety information, may be obtained by consulting the Safety Data Sheet (SDS). Before performing any part of this procedure, the technologist must take any and all precautions and adhere to all prescribed policies.

This procedure may expose you to:

- Bloodborne pathogens
- Airborne pathogens
- Hazardous reagents

To perform this procedure, you must use:

- Gloves – must be worn when handling blood cultures.
- Laboratory Coat – must be worn when handling cultures and reagents.
- Biological Safety Cabinet – must be used when staining or subculturing instrument-positive bottles.

Disinfectant following procedure:

- Bleach dilution sprayers can be used for on demand disinfectant.

Reference for spill/decontamination:

- SDS
- Chemical hygiene plan

5.0 Specimen Collection, Handling and Storage

5.1 Volume

1. Neonates to 1 year: 0.5 to 1.5 mL/bottle, although at least 1.0 mL is preferred.
2. Children 1 to 6 years old: 1 mL per year of age, divided between two blood cultures. For example, for a 3-year-old, draw 1.5 mL from each of two sites, for a total of 3.0 mL of blood. Consult with the physician, who is responsible for ordering the amount of blood to be collected, especially if the child is below normal weight or has had previous venipuncture for other reasons.
3. Children weighing 30 to 80 lb: 10 to 20 mL, divided between two sets of blood cultures.
4. Adults and children weighing > 80 lb: 30 to 40 mL, divided between two sets of blood cultures.

5.2 Timing

Volume is more important than timing in the detection of agents of septicemia.

1. Collect at least two sets of blood cultures of maximum volume consecutively from different anatomic sites prior to initiating antimicrobial therapy.
2. When blood cultures are ordered on patients on antimicrobial therapy, they should be collected when antimicrobial agents are at their lowest concentration.
3. For fever of unknown origin, subacute bacterial endocarditis, or other cases of continuous bacteremia or fungemia, collect a maximum of three sets of blood cultures within a 24-hour period, with maximum volume from three different venipunctures.

5.3 Collection

Blood should be collected aseptically by venipuncture or from previously placed lines. Blood for cultures should not be drawn through an indwelling intravenous or indwelling intra-arterial catheter UNLESS it cannot be obtained by venipuncture. Proper disinfection is an essential requirement to reduce the incidence of contamination.

5.3.1 Venipuncture

1. Select a different venipuncture site for each blood culture.
    a. If poor access requires that blood for culture be drawn through a port in an indwelling catheter, the second culture must be collected from a peripheral site, because cultures drawn through catheters can indicate catheter colonization but may not be indicative of sepsis.
    b. Do not collect blood from a vein into which an intravenous solution is running.
    c. Except for neonates, collect the two sets of blood cultures in succession. If the phlebotomy must be performed at the same site, perform a second venipuncture at that site.
2. Remove the plastic flip top from each bottle, and disinfect the septum with an alcohol pad.
3. Prepare the site.
    a. After palpation, cleanse the site with 70% isopropyl alcohol to remove surface dirt and oils. Allow to dry.
    b. Cleanse the site in a concentric pattern, starting at the center, and moving outward with an iodine pad (Betadine, 2% tincture of iodine, or 10% povidone-iodine) or chlorhexidine gluconate. Patients with known hypersensitivity to iodine can be prepared with a double application of 70% alcohol.
    c. For maximal effectiveness, the disinfectant must be allowed to dry 30-60 s for tincture of iodine or chlorhexidine gluconate and 1.5 to 2 min for povidone-iodine, before venipuncture is performed. If further palpation of the vein is necessary, the finger must be disinfected or a sterile glove may be worn.
4. While wearing gloves, insert the needle into the vein, and draw up to 20 mL of blood for a single set of cultures. Each bottle contains sufficient vacuum to draw 10 mL of blood. . **Do not overfill.** Use a new needle if the first attempt is not successful. Do not repalpate the skin after it is disinfected.

5. Apply the safety device to prevent needle exposure.
6. Transfer the blood to the bottles using aseptic technique. Remove the safety needle, and replace it with a Blunt Fill needle to transfer the blood to the bottles. **Do not touch the needle or hub**. If the optimal blood volume is obtained (i.e. 10 mL per vial), inoculate the blue top bottle first, and then inoculate 10 mL into the purple-top anaerobic bottle.
7. Thoroughly mix bottles to avoid clotting.
8. Label the bottles with the following patient information: name, date, time of collection, site of collection, and collector's identification.
9. After phlebotomy, dispose of needles in sharps container, and remove residual iodine from the patient's skin by cleansing with alcohol to avoid development of irritation.

5.3.2 Collection from Intravascular Catheters (performed by nursing personnel)

1. Remove the plastic flip top from each bottle, and disinfect the septum with 70% alcohol and allow complete drying.
2. Using two separate alcohol preps, scrub catheter hub connection for 15 s with 70% alcohol and air dry.
3. While wearing gloves, disconnect tubing or cap of the catheter and attach a syringe to collect discard blood, which is not used for culture (suggested amounts are 3 mL for adults and 0.2 mL for pediatric patients).
4. Using a new syringe, collect blood for culture through the hub. Quickly reconnect tubing.
10. Connect filled syringe to a Blunt Fill needle, or other safety transfer device, and transfer the blood into the bottles. If the optimal blood volume is obtained (i.e. 10 mL per vial), inoculate the blue top bottle vial first, and then inoculate 10 mL into the purple-top anaerobic bottle.
5. Thoroughly mix bottles to avoid clotting.
6. Label the bottles with the following patient information: name, date, time of collection, site of collection, and collector's identification.

5.4 Bottle Inoculation

The optimal volume for each set of bottles is 20 mL (10 mL per bottle). However, if it is not possible to obtain the 20 mL, the following guide should be used to distribute the blood between the aerobic and anaerobic bottles.

Table 1: Bottle Inoculation Guide

Total Volume Drawn	Aerobic (blue)	Anaerobic (purple)
20 mL	10 mL	10 mL
11-19 mL	10 mL	Balance
10 mL or less	All	0 mL

5.5 Specimen Transport

Do not refrigerate blood cultures. Hold and transport at room temperature.

5.6 Rejection Criteria

1. Reject blood cultures that are received unlabeled.
2. Do not process cracked or broken bottles.

5.7 Blood Culture for Fungus (CBF)

If dimorphic fungi (*Blastomyces, Coccidioides,* or *Histoplasma*) or *Cryptococcus* is suspected, blood should be collected in an ISOLATOR or SPS tube.

5.8 Blood Culture for AFB (CAFBNS)

Collect at least 10 mL of blood aseptically into an ISOLATOR or SPS tube, and transport to Microbiology. Direct AFB smears on blood are not productive and should not be made. Refer to the AFB procedure.

5.9 Blood for Detection of Unusual Microorganisms

- ***Bartonella* species**

  Collect 10 mL of blood for culture into an ISOLATOR tube using aseptic technique. Collection of more than one specimen may increase the yield of culture.

- ***Brucella* species**

  Collect 2 sets of blood cultures in BacT/Alert bottles. Process and incubate as for routine blood cultures. Extended incubation is not required.

6.0 Materials and Equipment

6.1 Equipment

- BacT/Alert instrument
- Incubators for subcultures
- Biosafety hood with HEPA filtration

6.2 Consumables

- 70% alcohol preps
- Transfer needles
- Glass slides

6.3 Media & Reagents

- BacT/Alert bottles: Standard Aerobic (SA - blue) and Standard Anaerobic (SN - purple)
- Agar plate media listed in Table 2
- Gram stain reagents

7.0 Procedure

7.1 Processing Blood Cultures

Upon receipt, bottles should be inspected for indications of microbial growth. This is especially important for specimens received from PAML or any bottles that have been delayed in transport. Microbial growth should be suspected if the pH indicator located on the bottom of the bottle has changed to yellow (see examples below). If microbial growth is suspected, prepare a Gram stain and proceed with the instructions for processing a positive bottle described below.

Inpatient blood cultures may require accessioning through function ORM, depending on who collected the specimen. All blood cultures received must be accessioned by adding or modifying the following information:

1. Collection time
2. Receipt time
3. Phlebotomist code or nurse collected
4. Site of blood draw (under SDES)
5. Bottle type(s) received (under SREQ)

7.2 Loading Bottles into the Instrument

1. Touch the “bottle icon” at the bottom of the screen.
2. Scan the bottle ID bar code.
3. Scan the accession bar code.
4. Open the drawer that is lit up, and place the bottle in an empty space.
5. Continue until all bottles are loaded.

7.3 Unloading Positive Bottles

1. An audible beep will alert you to a positive bottle in the 3D.
2. Push the (+) icon.
3. The drawer with the positive bottle will have a green light.
4. Unload the bottle.
5. Press the √ icon on the screen.

7.4 Processing Positive Blood Cultures

Prepare a gram stain from the blood culture bottle(s) under the biosafety hood. Gloves must be worn while manipulating blood bottles. All blood gram stains are stored in slide boxes.

7.4.1 Organisms Seen on Gram Stain

1. Perform FilmArray blood culture identification testing as indicated below. Refer to the FilmArray procedure for additional information.
2. Subculture according to the morphology and gram reaction of the organisms seen (see chart below). Use only ONE drop to inoculate each plate.
    a. Write the subculture date and time on the plates.
    b. Write which bottle the subs are from on each plate.
    c. Incubate plates at 35 ± 2°C in the appropriate atmosphere.

Table 2: Positive Blood Culture Testing & Set-up

Gram Stain	FilmArray	CHOC	BAP	BAP[1]	MAC	Other
GPC (staph)	If tube coag +	CO_2	CO_2			CHROM MRSA in O_2
GPC (strep)	Yes	CO_2	CO_2	ANA P disk		Bile Esculin in O_2
GPR (small/diphtheroid)	If motile	CO_2	CO_2	ANA		Bile Esculin in O_2
GPR (large/*Bacillus*/*Clost.*)	No		CO_2	ANA		
GNR[2]	Yes	CO_2	CO_2	ANA K disk	O_2	Direct Kirby Bauer[3] in O_2
GNDC	Yes	CO_2	CO_2	ANA	O_2	MTM in CO_2
Mixed Gram ±	Yes	CO_2	CO_2	ANA	O_2	CNA in CO_2 CHROM/TSA in O_2
Yeast	Yes					CHROM Candida & SAB in O_2

[1]Anaerobic subcultures are only necessary for isolates growing in anaerobic bottles or for strep growing in aerobic or anaerobic bottles.
[2]Add BAP microaerophilically if morphology resembles *Campylobacter.*
[3]Only one direct KB needs to be set up per patient in a 24 h period.

3. If the organisms seen are gram-negative rods/coccobacilli, **or are questionable in any way,** another smear must be prepared and methanol fixed prior to performing the Gram stain.
    a. Prepare a thin smear, and air dry.
    b. Flood the dry smear with methanol.
    c. Shake off the methanol, and air dry.
    d. Proceed with routine Gram stain procedure.
4. Perform direct antimicrobial susceptibility testing by Kirby-Bauer disk diffusion only for positive bottles with Gram-negative rods.
    a. Place 2 drops of blood culture broth on the center of a Mueller Hinton Agar (MHA) plate.

b. Use a sterile swab to streak the inoculum over the entire surface of the MHA plate. Repeat this procedure by streaking two more times, rotating the plate approximately 60° each time to ensure an even distribution of inoculum.
c. Dispense the Gram-negative antimicrobial disks onto the surface of the MHA.
d. Invert the plate, and incubate in an ambient (non-CO_2) incubator at 35 ± 2°C for 16 to 18 h.

7.4.2 No Organisms Seen

1. Prepare a second smear for methanol fixation as outlined above.
2. If no organisms are seen, scan the smear on low power to make sure there aren't any areas with clumps of organisms.
3. If no organisms are seen on the methanol-fixed smear, perform a blind subculture to BAP-ANA and CHOC-CO_2. The plates should be labeled with the date, time, and "Blind Sub."
4. Reload the bottle into the BacT/Alert incubator using the "LOAD BOTTLE" function as soon as possible.
5. If the BacT/Alert flags a reloaded bottle as positive a second time, repeat the methanol-fixed gram stain. If no organisms are seen, proceed with manual testing.

Day 1

- Place the bottle with the original blind sub plates at 35 ± 2°C in the Blood bench incubator.
- Enter preliminary result in LIS: **No growth to date. Results pending further incubation.**
- Document smear and subcultures in workup.

Day 2 and 3

- Examine the plates for growth.
- Examine the bottle for hemolysis or turbidity at the beginning and at the end of 1st shift. If there are signs of growth perform methanol-fixed Gram stain.
- If no growth, document observations in the workup each day, and return the bottle and plates to the incubator.

Day 4

- Examine the plates for growth.
- Examine the bottle for hemolysis or turbidity. If there are signs of growth, perform methanol-fixed Gram stain.
- If no growth, document observations in the workup each day, and return the bottle and plates to the incubator.

Day 5

- Examine the plates for growth.
- Perform methanol-fixed Gram stain. If no organisms are seen, and no culture growth is observed on the original blind sub plates, perform a final blind subculture to BAP-ANA and CHOC-CO_2. The plates should be labeled with the date, time, and "Blind Sub."
- Incubate and examine the plates each day for 2 days. Document observations in workup. If the plates do not grow any organisms after 2 days, enter a final report: **No growth**.

8.0 Reporting Gram Stain Results

8.1 Critical Value

Gram stain results for <u>each set</u> of positive blood cultures should be handled as critical values. Refer to the Microbiology Critical Values, Notifiable Conditions and Select Agents Procedure for calling and documenting the Gram stain results. Each new positive set should be called. However, the Gram stain results from a second positive bottle of a set that has already been called does not need to be called again unless different organism morphologies are observed.

8.2 Gram Stain Result Entry in LIS

1. Access the accession number in Micro Results Entry.
2. In the first observation, enter “Direct Gram Stain Result:” followed by the appropriate description of the organism morphology.
3. In the second observation, document to whom and when the critical value was called with Read Back.
4. Enter work-up information. Identify bottle type and which media were inoculated.

9.0 Organism Identification & Reporting

9.1 FilmArray Blood Culture Identification Panel

Refer to the FilmArray procedure.

9.2 Culture & Antimicrobial Susceptibility Testing

Refer to the Isolate Work-up Charts for appropriate identification and antimicrobial susceptibility testing methods. If an organism was detected by FilmArray PCR, the culture plates should be examined for growth characteristics that are consistent with the molecular result. If growth characteristics are consistent with the FilmArray result, further identification by phenotypic methods (e.g., spot tests, latex tests, Phoenix panels, etc.) is not necessary. If the growth characteristics are not consistent, or if there are additional morphotypes present that were not detected by FilmArray, perform identification tests as outlined in the Isolate Work-up Charts. Billing for organism identification (ORGB1) should only be performed when additional testing is necessary to confirm the identification of an isolate.

Staphylococcus Isolates Tested by PCR (Tube Coagulase-Positive at 4 h)

1. Examine the CHROMagar™ MRSA II and BAP plates after 18-26 h incubation.
2. If *S. aureus* was identified by PCR, verify that the *mecA* result correlates with the CHROMagar™ result. Discrepancies may occur if the culture is mixed with both *S. aureus* (MSSA) and *mecA*-positive coagulase-negative staph. Since the *S. aureus* target and the *mecA* gene are detected independently, the PCR result will appear to be MRSA. The *mecA*-positive coagulase-negative staph may be found growing on the CHROMagar™ plate as white or pale pink colonies while the *S. aureus* (MSSA) will only be growing on the blood agar plate.
    a. Verify that the growth on the CHROMagar™ plate is coagulase-negative.
    b. Enter the culture result as *Staph aureus* NOT MRSA and Coagulase-negative staph.
    c. Enter the following comment: **The FilmArray PCR antimicrobial resistance assays do not specifically link a resistance gene to the associated organism. In cultures with multiple *Staphylococcus* spp., *mecA*-mediated methicillin resistance may be associated with either *S. aureus* or another *Staphylococcus* spp. Additional testing determines the final methicillin susceptibility for each organism. [FLMSTC]**
    d. Call client with the updated results.
    e. Perform susceptibility testing and report results for both isolates. Add appropriate comments for the coagulase-negative staph based on the number of sets positive.

Staphylococcus Isolates not Tested by PCR (Tube Coagulase-Negative at 4 h)

1. Examine the CHROMagar™ MRSA II and BAP plates after 18-26 h incubation.
2. Determine coagulase results from BAP.
3. If *S. aureus* is identified, determine if isolate is MRSA or MSSA based on CHROMagar™ result (refer to the MRSA Screen Culture Procedure for instructions on interpreting CHROMagar™ MRSA II).
4. Enter identification in LIS as either MRSA or *Staph aureus,* not MRSA.
5. Call client with MRSA/MSSA results.
6. Complete antimicrobial susceptibility testing by testing with a Phoenix PMIC panel.
7. For coagulase-negative staph, discard CHROMagar and proceed with antimicrobial susceptibility testing, if multiple sets of blood cultures are positive with staph.

Enterobacteriaceae Isolates

For Gram-negative rods, only report the direct susceptibility results for isolates that are clearly lactose fermenters (coliforms). Enter **Preliminary susceptibility** [PSEN] as an observation in the report when releasing direct susceptibility results. This comment should be removed when the standardized antimicrobial susceptibility results are reported.

9.3 Positive Culture Result Entry in LIS

1. In Micro Results Entry, enter the specimen accession number.
2. In the next available observation line, enter "Culture Result:" followed by the preliminary or final identification of the isolate.
3. Enter all testing performed under the corresponding work-up number.

Skin Contaminant vs. Opportunistic Pathogen

Organisms that inhabit the skin may be recovered in blood cultures. The following organisms are frequently blood culture contaminants:

- Coagulase-negative staphylococci
- *Corynebacterium* spp.
- Viridans streptococci
- *Aerococcus* spp.
- *Gemella* spp.
- *Peptostreptococcus* spp.
- *Propionibacterium* spp.
- *Bacillus* spp. not *anthracis*
- *Micrococcus*

When these organisms are recovered from a single set, it suggests that the isolate may represent contamination. In cases where only one set was drawn, the significance of the isolate is uncertain. Susceptibility testing is not routinely performed on the organisms listed above when they are isolated from a single set. However, these organisms may represent opportunistic infections, especially when the same organism is recovered from blood cultures drawn from separate anatomical sites. ID & AST should be performed on isolates from pediatric oncology/NICU patients, regardless of the number of sets positive. When one of the organisms listed above is identified, attach the following comment:

__ of __ total sets of blood cultures are positive to date with this organism. [BCSETS]

The BacT/Alert 3D computer must be used to determine the number of sets of blood cultures collected on a patient, not the Sunquest system. Include any sets collected within 48 h of the current set that is positive.

The following interpretive comments should be used to provide guidance to the clinician.

1. Single Set Positive
   - **This suggests the possibility that this organism represents a skin contaminant. [BC12]** is used if only 1 set out of 2 or more sets is positive with an organism generally considered to be a skin contaminant, as listed above. No susceptibility testing is necessary. For pediatric oncology/NICU patients, AST results are reported, but the comment is still used.
   - **The significance of this isolate is uncertain since only one blood culture set has been submitted to date. [BC11]** is used if 1 set of only 1 set received is positive with an organism generally considered to be a skin contaminant, as listed above. No susceptibility testing is necessary. For pediatric oncology/NICU patients, AST results are reported, but the comment is still used.
2. Multiple Sets Positive

   A set represents the bottles collected from a single venipuncture. It is NOT based on the number of positive bottles in the set. To determine if the same organism is present in

multiple sets, the colony morphology and rapid test results should be compared. For coagulase-negative staph and viridans strep, AST patterns should be compared to help determine if the isolates are the same. There may be minor differences in AST (S-I or I-R) with the same organism. However, major differences (S-R) would indicate two distinct organisms. If each set of blood cultures has a unique coagulase-negative staph or viridans strep, report the isolates with the skin contaminant comment listed above when a single set is positive (BC12). Report the AST results for the isolate recovered from each set. Consult Rounds if the test results are ambiguous.

If test results confirm that the same organism is present in multiple sets, the following comment should be added to the report. AST results should be reported for organisms that have CLSI standards for interpretation.

- **This organism may represent a significant finding since it is present in multiple blood culture sets. [BCMSET]**
  Note: When this comment is appended to a culture, modify any previously reported positive blood culture as follows:
    - Delete the comment regarding the isolate being a contaminant.
    - Insert the comment regarding the organism representing a significant finding.
    - Add appropriate susceptibility results or canned susceptibility comments on each set.

9.4 Negative Culture Result Entry in LIS

"No Growth" (final at 5 d) and "No Growth to Date" (preliminary at 2 d) reports are sent using a batch reporting function in Sunquest. No-growth reports should be sent once on 1st shift at about noon and again at midnight. Prior to sending the batch report, unload all negative bottles from the BacT/Alert instrument.

1. Touch the negative bottle icon on the instrument screen. The drawers with negative bottles are indicated with a green light.
2. Pull each drawer open, and remove the negative bottles. Cells with negative bottles should light up.
3. Discard bottles in a biohazard bin. Do not fill bins more than ¼ full with bottles, as they become too heavy to lift.
4. When finished removing all of the negative bottles, touch the checkmark button on the instrument screen.

LIS Batch No-Growth Resulting

1. Access Microbiology Automatic No-Growth Result Entry
2. Enter SHBCG for the Worksheet, and click the Add button.
3. Click a second Add button below to select the worksheets displayed.
4. Click the Start Update button.

10.0 Instrument Maintenance & Quality Control

1. Daily Temperature Checks
   The temperature in each BacT/Alert cabinet should be checked daily on first shift. The temperature should be 36 ± 1°C. Document the temperature reading from each cabinet in LIS. If one of the cabinets is out of range, notify the supervisor or lead/charge tech.
2. Check for Loading Errors
   If the bottle ID and accession number are not scanned properly before the bottle is loaded into the instrument, the information must be corrected. The person assigned to the blood bench on first shift should check for loading errors each day. These can be visualized on the main screen of the instrument or will show up on the load list reports that are automatically printed each day.
3. Cell Calibration
   The cells in the BacT/Alert instrument require calibration annually. The instrument will alert the user when calibration is due. The calibration wands are stored in one of the cabinets

under the instrument. Refer to the BacT/Alert Operator Manual for instructions on cell calibration.

11.0 Quality Assurance

11.1 Culture Contamination Rate

The Laboratory Quality Assurance department tracks the rate of blood culture contamination each month. Refer to the Quality Management and Performance Improvement Plan for additional information.

11.2 Specimen Volume

The Microbiology laboratory performs biannual monitoring of 100 sets of blood cultures for adequate volume. Measurement is performed by visually comparing patient culture bottles with a reference bottle. Optimally, 20 mL of blood per culture set (2 bottles) should be collected for culture. Sets with less than 17 mL of blood are considered suboptimal. The following comment is added to the culture report:

A suboptimal volume of blood was submitted and may lead to a false negative result. A blood culture set collected from an adult should contain 20 mL of blood split between the two bottles. [BLDL]

12.0 Limitations

1. Low levels of organisms may not be detected in the incubation interval of the culture.
2. The media may not support the growth of some organisms.
3. SPS may inhibit the growth and viability of the organism.
4. Bacterial metabolism may not produce sufficient CO_2 for detection in automated systems.

13.0 References

1. Clinical Microbiology Procedures Handbook, 3rd ed. and 2007 update, Vol. 2. Garcia, L.S., editor in chief. ASM Press, Washington, D.C.

14.0 Document Control History

Microbiology Director Approval: Dr. Ann Robinson 03/08/2010, Blood Culture Collection 03/2000, revised 11/2002

Medical Director Approval: J. Schappert 03/10/10

Microbiology Supervisor Reviews: Jerry Claridge

- Collection Procedure: 06/2001, 11/2002, 03/2003, 04/2004, 11/2005, 05/30/2006, 10/2006, 10/2007, 10/2008, 10/2009, 04/01/2011, 03/2013
- Blood Culture Procedure: 05/2007, 05/2008, 06/2009, 04/01/2011, 03/2013
- Combined Procedure: 3/11/14

Procedure updates: 03/05/2010 Combined previously separate section: media selection. 04/14/2010 Combined previously separate section: odd bottle procedure. Simplified accessioning section. 03/11/2011 Update for HFH false-positive bottles. 10/11/2011 Modified blood culture for fungus to order CBF, eliminated section for body fluid inoculation (see Specimen Processing Procedures), added guidelines for distributing blood between bottles when < 20 mL is collected. 02/01/2012 Changed media for yeast CHOC to SAB. 04/12/2012 Added CHROMagar MRSA to staph set-up. 03/11/2014 Added safety information, volume collection guidelines, collection timing, detailed protocol for collection, specimen transport, rejection criteria, references to FilmArray and subsequent ID testing with culture plates, directions for direct AST testing, combined skin contaminant comments into this document, added no-growth batch reporting, maintenance & QC, QA and Limitations sections. 3/18/14 Added NICU to pediatric oncology workup and reporting for skin contaminants vs. potential opportunistic pathogens. 3/19/14 Clarified to report AST results on each set for multiple sets positive with an opportunistic pathogen. 7/22/2014 Changed protocol for calling positives. Previously, subsequent positive

cultures within a 48-h window were not called unless a new organism morphology was seen. Now, each new set is called. Added instructions to use only one drop of blood culture to inoculate plates. 04/16/2015 Updated reporting for instances where a bottle is mixed with mecA-positive coagulase-negative staph and MSSA. 05/19/2015 Added instructions for checking the bottom of the bottles for indications of microbial growth upon receipt.