PROVIDENCE Sacred Heart Medical Center & Children's Hospital

I. Principle

The presence of microorganisms in the circulatory system of humans is indicative of a life-threatening situation and is associated with a significant mortality rate. Blood culture is therefore one of the most important and critical procedures performed in the microbiology laboratory.

The BacT/Alert Microbial Detection system utilizes a colorimetric sensor and reflected light to monitor the presence of and production of carbon dioxide CO_2 that is dissolved in the culture medium. If microorganisms are present in the test sample, carbon dioxide 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.

II. Specimen Collection: See Blood Culture Collection Procedure

III. Materials

A. BacT/Alert Media: Aerobic (blue top), Anaerobic (purple top)
B. Supplies for positive bottles
   1. Alcohol prep - 70% isopropyl alcohol
   2. Transfer needles
   3. Glass slides
   4. Gram stain reagents

IV. Procedure

A. Processing Blood Cultures

Inpatient blood cultures may require accessioning through function OER or REI, 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)

B. Venting Blood Culture Bottles

Aerobic blood culture bottles no longer require venting.

C. Loading bottles into the BTA
   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.

D. 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.

E. Odd Bottles (Non-BTA bottles)

1. Accession bottles and label with printed labels.
2. Place a label on the "Odd Blood Culture Bottle Log." Indicate current date/time and which bottle(s) were received.
3. Incubate bottle(s) at 35 ± 2°C in the Blood bench incubator.
4. At the beginning of 1st shift, the Blood bench tech should:
   a. Examine <u>any</u> odd bottles for evidence growth (i.e., turbidity or hemolysis). New bottles should also be checked a second time at the end of 1st shift. If any visual changes suggest potential growth of microorganisms, perform and examine a Gram stain from the bottle. If the aerobic blood culture bottle has not shown evidence of growth after the initial 24 h incubation, perform Gram stain.
   b. If organisms are seen in the smear, refer to Section V. below.
   c. If no organisms are seen:
      - Perform a blind subculture to a chocolate agar (CHOC) and a blood agar plate (BAP). Incubate the CHOC plate in Blood bench CO_2 incubator and the BAP in an anaerobic jar.
      - Reincubate blood culture bottle at 35 ± 2°C in the Blood bench incubator.
      - Enter preliminary report in LIS: "Blood culture bottle received. Results pending further incubation." [BBRPEN]
   d. Examine plates for growth at 24 and 48 h incubation.
   e. Continue to visually monitor the bottle twice daily on its second day of incubation and then once daily thereafter.
5. If no growth occurs from blind subculture, continue visually monitoring the bottle(s) for up to 7 d. If any visual changes suggest growth of microorganisms, perform a gram stain. If no changes are noted on day 7 of incubation, perform another blind subculture of the aerobic bottle as previously described. Examine plates after 24 and 48 h incubation. If subcultures fail to grow finalize the culture as "No Growth."

   Note: It is not necessary to perform any blind subcultures on anaerobic bottles. If no visual changes occur in the bottle during incubation, perform a Gram stain on day 7. If no organisms are present, finalize report as "No Growth" for the anaerobic bottle.

V. Processing Positive Blood Cultures (BacT/Alert)

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

A. **Organisms Seen on Gram Stain**

1. Subculture according to the morphology and gram reaction of the organisms seen (see chart below).
   a. Write the subculture <u>date and time</u> 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.

2. 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.
3. Perform direct antimicrobial susceptibility tests of positive cultures on Gram-negative rods only.

B. Examine Gram Stain: **No Organisms Seen**

1. Perform a second smear for methanol fixation as outlined above.
2. If no organisms are seen on the methanol-fixed smear, perform blind subculture to BAP-ANA and CHOC-CO_2. The plates should be labeled with the date, time, and "Blind Sub."
3. Reload the bottle into the BacT/Alert incubator using the "LOAD BOTTLE" function as soon as possible.
4. If the BacT/Alert flags a reloaded bottle as positive a second time, repeat the gram stain. If no organisms are seen, perform blind sub and place the bottle in the blood incubator at 35 ± 2°C in the Blood bench incubator and proceed with testing as outlined above for non-BacT/Alert bottles.

Positive Blood Culture Set-up Chart

Gram Stain Morphology	CHOC	BAP	BAP*	MAC	Other
GPC (staph)	CO_2	CO_2	ANA		CHROM MRSA II O_2
GPC (strep)	CO_2	CO_2	ANA A & P disks		Bile Esculin O_2
GPR (diphtheroid)	CO_2	CO_2	ANA		Bile Esculin O_2
GPR (*Bacillus/Clostridium*)		CO_2	ANA		
GNR	CO_2	CO_2	ANA K disk	O_2	Kirby Bauer** O_2
GNDC	CO_2	CO_2	ANA	O_2	MTM CO_2
Mixed Gram +/-	CO_2	CO_2	ANA	O_2	CNA CO_2 CHROM/TSA O_2
Yeast					CHROM Candida & SAB O_2

*Anaerobic subcultures are only necessary for isolates growing in anaerobic bottles or for strep growing in aerobic or anaerobic bottles.

**Only one direct KB needs to be set up per patient in a 24 h period.

VI. Reporting and Entering Workload for Positive Blood Cultures

A. Verbal report "THIS IS A CRITICAL VALUE"!
   1. Telephone the patient's physician/or patient's charge nurse immediately after confirming a positive culture by gram stain. The person taking the report must repeat the patient name, ID number, and organism name back to you and understand that this is a Critical Value.
   2. Document the verbal report, including the name of the person called, the date, and time of notification with "rb" (read back), in the Positive Blood Book and the computer. Print labels in Misys under Function RE or REI.
   3. Subsequent positive blood cultures within 48 h of the first positive that contain the same organism, do not need to be called to the clinician. If a different organism morphology is observed in subsequent positive blood cultures, the gram stain results must be called as outlined above.

B. Computer
   1. In Micro Results Entry, enter the accession number.
   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, Aer, Ana, AerA, or AerB and which media were inoculated.

VII. Identification and Reporting of Isolates from Subcultures

A. Isolate Identification & Antimicrobial Susceptibility Testing
   Refer to the Isolate Work-up Charts for appropriate identification and antimicrobial susceptibility testing methods. For Gram-negative rods, only report the direct susceptibility results for isolates that are clearly lactose fermenters (coliforms).

B. *Staphylococcus* Isolates – MSSA vs. MRSA Determination
   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.

C. Computer Entry
   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.

VIII. References

A. Misys Microbiology Procedure Manual
B. BioMerieux 3D Operator Manual 2003
C. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.L Landry, M.A. Pfaller. 2007. Manual of Clinical Microbiology, 9th ed., Vol. 1, ASM Press, Washington, D.C.