PROCEDURE: POSITIVE BLOOD CULTURES – GRAM STAIN PROCEDURE

I. PRINCIPLE

Blood cultures are one of the most important cultures performed by the laboratory. Diagnosis and interpretation of bacteremia and fungemia depends on appropriate volume per blood culture and number of venipunctures drawn per episode. Automated continuously monitoring blood culture methods will vary depending on instrumentation. However, all systems are highly sensitive and detect organism growth for almost all organisms within 5 days. Both culture procedures and interpretation of significance of an organism must be carefully controlled to avoid misinterpretation of skin or contaminating flora vs. an agent of infection.

II. SPECIMENS

A. Blood
   1. Blood may be peripheral, arterial, or drawn through a CVL.
   2. Autopsy bloods are usually heart blood.
   3. Blood Transfusion Bag – *See Transfusion Reaction Cultures Procedure*
   4. Recommended volumes:
      a) Adults: 10-20 ml of blood per blood culture set
      b) Children: 1-5 ml of blood per blood culture set
   5. Blood volume should be evenly divided between 2 bottles per set; 1 aerobic and 1 anaerobic formulation. If the volume of blood obtained is less than 2 ml, as in the case of an infant, inject the total volume into a single aerobic bottle. Note that only one aerobic bottle was received.
   6. The recommended inoculation volume for each bottle is 0.5 ml to 10 ml of blood. A minimum of 0.5 ml of blood is required for the recovery of *Haemophilus influenzae*. The bottle labels display 5 ml sample fill increments. When using a closed collection system, such as a butterfly setup for direct draw into the bottle, observe the blood flow carefully and remove the fill needle from the bottle when the 10-ml mark is reached**. Do not overfill the bottles.**
   7. Vials are inoculated by the person performing the venipuncture at the site of the draw and sent to the lab. The initials of the person performing the blood culture draw should be on each bottle.
   8. Each set is logged in and assigned one order number.
   9. Notify Director/Manager/tech-in-charge regarding:
      a) Specimens requesting *Brucella* or *Leptospira*
   10. Improperly collected specimens that are not canceled must include the following disclaimer in the report:
      a) Improperly collected-Recovery of organisms will be compromised, recollection of specimen suggested.

B. Body Fluids
   1. Sterile body fluids can be inoculated directly into the blood culture bottles. It is also recommended that the original body fluid specimen be sent for a gram stain and anaerobic culture.

C. AFB & Fungal Blood Isolators

III. PROCEDURE

A. Pull positive vial from instrument. Check order number to see if bottle already has a positive counterpart. A counterpart is defined as the aerobic or anaerobic bottle of same order.

   1. IF BOTTLE IS A NEW POSITIVE ORDER NUMBER:
      a) When a Blood culture is positive on the Instrument, unload the bottle from the Instrument

b) Check off the bottle on the Verstrek PC and hit [*SEND TO LIS*] button. The significant flag in Soft will be set and can be seen in the Status field as "+". This holds it up from getting any new Negative auto results.
c) Perform methanol fixed gram stain.
d) Check in LIS for number of blood cultures sent on patient and note number of previous positives.
e) Subculture to appropriate media based on gram stain as stated in subculture protocol.
f) Positive blood culture gram stains will be reported as a Test Comment
g) A gram stain charge needs to be added at Media Level but only once per order number. Use "One GS charge per set ^$GS" for first bottle of each set. Use the no charge "Second pos bot ^GS' when second bottle of same set comes up positive.
h) When a gram stain is resulted as a Test Comment, Set the Significant flag √ , then set Status to INTERIM. (This sets the report in red on Lifelinks)
i) Refer to Reporting Results for Notification requirements.

2. IF GRAM STAIN IS NEGATIVE/ NO ORGANISMS SEEN:
   a) Repeat using an air-dried Methanol-fixed method or heat fixed method (no methanol).
   b) Sub according to protocol and put back on instrument within 3 hours.
   c) Return bottle to instrument, and in Soft unset the Significant button by clicking on it twice**.**
   d) The red √ in the " + " column should appear and then disappear along with the "+" in the Status field. It will now qualify again for Negative Auto resulting Worklist.
   e) If Blood bottle flags as positive a second time and is still 'No organisms seen" do an acridine orange stain. If acridine orange stain is negative repeat steps 2, 3,and 4. If acridine orange is positive subculture appropriately.
   f) For acridine orange stain, prepare a slide of NOS positive Blood Culture.
   g) Allow to air dry.
   h) Place slide on Blood Bench for Blood Bench Technologist to stain.
   i) Protocol for staining acridine orange slide is found in Processing Manual in Stains section located in Room 1136 Processing area.

3. FOR PATIENTS WITH MULTIPLE POSITIVE BLOOD CULTURES (WITHIN 3 DAYS OF COLLECTION):

1ST Positive Blood Culture Set:

1ST BOTTLE:

1. GRAM STAIN
2. CALL R.N.
3. SUBCULTURE
4. DIRECT BIOCHEMICALS (IF APPROPRIATE).

2nd BOTTLE:

1. GRAM STAIN
2. SUBCULTURE

2ND, 3RD, 4TH, etc. Positive Blood Culture Set:

1ST BOTTLE:

1. GRAM STAIN
2. CALL R.N.
3. SUBCULTURE

2ND BOTTLE:

1. GRAM STAIN
2. SUBCULTURE

B. SUBCULTURE PROTOCOL

Gram stain:	Subculture to:	Set up direct from bottle:
GNR	BAP W/SS ANABRU MAC CNA CHOC (if small gram-negative rod, consistent with Haemophilus – **Also, tape plates closed**)	
Curved GNR	CHOC in Campy jar at **37 °(** (in (addition to all media above for GNR)	
STREP	BAP W/SS ANABRU	PTAB
STAPH	BAP ANABRU	ATAB *ALIQUOT *MRSA/SA pcr
GPR	BAP ANABRU	
YEAST	BAP SAB Add IMA if gs mixed w/ bacteria	PNA slides
MIXED	ANABRU BAP W/SS MAC CNA CHOC (IF GNCB)	
NO ORGANISM SEEN	BAP W/ SS CHOC ANA BRU	Acridine Orange Stain if NOS after bottle flagged as positive twice Hold plates for 48 hours
FUNGAL ELEMENTS	BAP ANABRU SAB IMA	
POS CRYPTO AG	Put Tape on Bottles indicating they should be given to Mycology on Day 5	Mycology tech sub to SAB and IMA

*Aliquot and MRSA/SA PCR needed **ONLY IF** first aerobic bottle that is positive showing pure Gram-positive cocci in clusters or Gram-positive cocci in singles: per each admission **OR** specimen collected on pt <6 months of age from HASBRO ED (including discharged patients)

IV. QUALITY CONTROL

A. All biochemicals and testing should meet QC parameters per the current CLSI document M22-A3.

V. REPORTING RESULTS

A. Refer to *Appendix C* for additional LIS codes and comments
B. "No Growth" Cultures—Preliminaries and Finals will be updated daily by auto resulting.
C. Positive Blood Culture Notification Requirements:
   1. Call must be made to R.N. or physician.
   2. The Verbal report must indicate # of positive sets, the total sets drawn, and the gram morphology. Refer to: *Notification Scheme for Test Results of Clinical Significance.*

      Example:
      "2 sets positive for 4 sets drawn on Wednesday (date) with GNR's"

   3. Be sure to document in LIS, the name of the person spoken to, location/floor or area call is made to, the date and time call is made. The person taking the report must read it back to the technologist. This also should be documented in the same phone report by using Soft '}CLGS' code not Soft "PHONE" code.
   4. All positive Blood Cultures must be called to appropriate person, even if a positive culture was called previously.
   5. Epidemiology need not be notified of any gram stains showing gram negative diplococci or positive meningococcal cultures. They will receive an automatic alert from Theradoc Information Systems.
   6. The RI Department of Health should be notified of any suspicious gram stains or cultures that have possible bioterrorism agents. Refer to list from RI Department of Health for all other notification requirements that pertain to positive blood cultures.
   7. When referring culture results to a previous culture, be sure to include the isolate name, and all isolate comments from the culture worked up.

VI. INTERPRETATION

A. A positive blood culture generally means that a person is bacteremic. All positive blood cultures should be plated to appropriate media for work-up.

VII. LIMITATIONS

A. Low level of organisms may not be detected at all times.
B. There are fastidious microorganisms that infect the blood that cannot be grown in routine culture of blood. Refer to Planting Manual for processing.
C. Some bacteria do not produce enough CO2 gas for detection in automated systems.

VIII. REFERENCES

A. Isenberg, Henry D., Clinical Microbiology Procedures Handbook, 2004. vol 1, Aerobic Bacteriology.
B. Blood Culture IV, Cumitech 1C.EJ Baron coordinating editor, 2003. ASM Press.