

**PROCEDURE: BLOOD CULTURE – BENCH PROCEDURE****I. PRINCIPLE**

Blood cultures are one of the most important cultures performed by the microbiology laboratory. Diagnosis and interpretation of bacteremia and fungemia depends on the appropriate volume being drawn per blood culture and the correct number of venipunctures drawn per episode. Lifespan's Microbiology Laboratory employs a blood culture detection system which monitors liquid emulsion sensors using solid state photo detectors. These sensors are located in each blood culture bottle used for specimen collection. This detection feature, along with the special enriched blood culture media detects most organisms (including fastidious species) within the 5 day incubation on the instrument. This allows detection of a wider range of common and fastidious organisms and eliminates the need to extend blood cultures, except for rare exceptions. Blood culture procedures and the subsequent interpretation of significance must be carefully controlled to avoid misinterpretation of skin/contaminating flora as an agent of infection. The physician needs to consider the clinical presentation and patient specific factors to ultimately determine the significance of the blood culture results.

**II. AVAILABILITY**

Submission of specimen: 1<sup>st</sup>, 2<sup>nd</sup> & 3<sup>rd</sup> shift at RIH, TMH & NH

**III. TEST CODE**

- A. CXBLD
- B. CXBL2
- C. CXBL3
- D. ISFUN (testing performed only at RIH in mycology lab)
- E. ISAFB (testing performed only at RIH in AFB lab)

**IV. SPECIMENS**

- A. Blood
  - 1. Peripheral
  - 2. Arterial Line
  - 3. Central Line
  - 4. Portacath
  - 5. PICC Line
  - 6. Hickman
  - 7. Subclavian
  - 8. Midline
  - 9. Heel Stick
  - 10. TrRxn Unit – [Refer to Transfusion Reaction Cultures Procedure](#)
  - 11. Autopsy (usually heart blood)

**V. MATERIALS AND EQUIPMENT**

- A. Materials
  - 1. Sterile airway venting needles
  - 2. Routine media and stains
  - 3. Blood culture bottles
  - 4. Alcohol pads
  - 5. Isolator tubes and supplies

- B. Equipment
  1. Biosafety Hood
  2. Heating Block
  3. Automated Blood Culture Instrument
  4. Anoxomat System

VI. **STORAGE AND HANDLING** – Refer to [Procedure: BACT/Alert Virtuo](#) for instrument procedure

- A. Recommended volumes
  1. Adults: 10-20 ml of blood per blood culture set
  2. Children: 1-5 ml of blood per blood culture set
- B. 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.
- C. A minimum of 0.5 ml of blood is required for the recovery of *Haemophilus influenzae* and *Neisseria spp.* 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.
- D. Do not overfill the bottles.
- E. **DO NOT REFRIGERATE BOTTLES.**
- F. 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 must be on each bottle.
- G. Each set is logged in and assigned one order number.
- H. Notify Director/Manager/Senior technologist regarding culture requests for:  
[Refer to Sentinel Laboratory Documents for select agents.](#)
  1. *Brucella* (Brucellosis, Mediterranean fever, Malta fever)
  2. *Francisella* (Tularemia, rabbit fever, hare fever, deerfly fever)
  3. *Bacillus anthracis* (Anthrax, Woolsorters' Disease, Splenic Fever)
  4. *Burkholderia mallei* (Glanders, Malleus)
  5. *Burkholderia pseudomallei* (Meliodiosis)
  6. *Yersinia pestis* (Bubonic plague, pneumonic plague, black death)

*The following dimorphic fungi require a Blood Isolator Cultures:*

  7. *Blastomyces* (Blastomycosis, Chicago Disease, Gilchrist's Disease)
  8. *Coccidioides* (Coccidioidomycosis, Valley Fever)
  9. *Histoplasma* (Histoplasmosis, Cave/Darling's/Ohio Valley Disease)
  10. *Paracoccidioides* (Paracoccidioidomycosis, South American/Brazilian blastomycosis, Lutz's Disease)
- I. Inadequately collected specimens (not canceled) must include the following disclaimer in the report:  
*"Specimen improperly collected. Recovery of organisms will be compromised, re-collection of specimen suggested."*
- J. Extended Blood cultures are not required for the recovery of most\* fastidious organisms, including HACEK organisms. The automated blood instrument readily grows these organisms within the routine 5 days incubation length.
  1. *\*It is acceptable to extend blood culture incubation time if the suspected organism is: Bartonella (Cat Scratch Fever) or Brucella it requires 10-day blood culture incubation.*

- K. Isolates that need to be sent to the RIDOH:
1. ALL suspected Select Agents (CATEGORY A)
  2. *Campylobacter*
  3. *Clostridium botulinum*
  4. *E. coli (O157:H7)*
  5. *Haemophilus influenzae*
  6. *Listeria monocytogenes*
  7. *Mycobacterium tuberculosis* (CATEGORY A)
  8. *Mycobacterium* species
  9. *Neisseria meningitidis*
  10. *Salmonella*
  11. *Shigella*
  12. VISA/VRSA
  13. *Streptococcus pyogenes* (Group A Strep)
  14. *Vibrio*
  15. *Yersinia*
  16. *Candida auris*

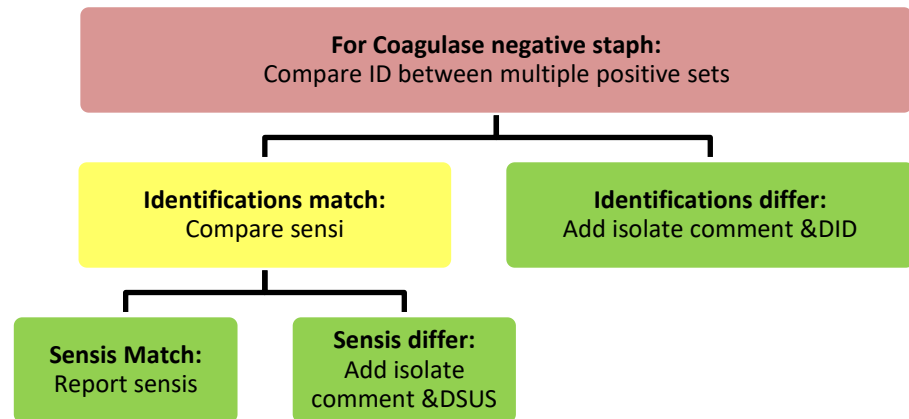
## VII. QUALITY CONTROL

- A. All biochemical testing should meet QC parameters per the CAP requirements. Refer to IQCP for specific tests/instruments

## VIII. TEST PROCEDURE

- A. Gram stains from the previous evening/night are reviewed/assessed for accuracy
1. Smears that are correct are documented by ordering and resulting GS Review and entering media comment: No change – review date: (insert date and your initials).
  2. Notify provider of all incorrect Gram stain results. [Refer to Appendix AP11 for reporting instructions.](#)
- B. Work-up of Positive Blood Cultures - [Refer to Organism ID/AST Procedure](#) for organism specific testing
1. Blood Culture Referral and Work-up - [See Table 1](#)
    - a) Refer identification and susceptibility results according to [Culture Referral Procedure](#)
    - b) Save a representative plate in the 7-day save pile for any culture that is referred
    - c) When multiple blood culture sets are positive, organisms that CANNOT be referred are:
      - (1) Coagulase negative *Staphylococcus* - [See Figure 1](#)
        - (a) Compare identifications between multiple positive sets
        - (b) If identifications differ, document ID in worksheet and add isolate comment: &DID
        - (c) If identifications match, compare susceptibility patterns to determine significance
        - (d) If susceptibility results differ, suppress results and add isolate comment: &DSUS
        - (e) Release susceptibilities if multiple sets are the same
        - (f) Rule out *Staphylococcus lugdenensis* DO NOT report Identification unless identified as *Staphylococcus lugdenensis*

Figure 1.



- (2) Alpha hemolytic *Streptococcus*
  - (a) Perform VITEK MS (MALDI) identification and report identification for all isolates.
    - (i) Non-microaerophilic ahs
      - (a) If multiple sets and identification matches, proceed to compare susceptibility pattern to determine significance
      - (b) Release susceptibilities if multiple sets are the same
      - (c) If susceptibility results differ, suppress results and add isolate comment: &DSUS
    - (ii) Microaerophilic ahs
      - (a) Perform susceptibility testing and release results for each specimen submitted
  - (3) *Corynebacterium* are identified if isolated from multiple culture sets. *Corynebacterium jeikeium* is ruled out in multiple sets.
2. Incubate subculture plates and examine aerobic plates at 24 hours.
3. Incubate all subcultured plates full 48 hours
4. Anaerobic subculture plates (BRU) is incubated a full 48 hours before examination.
5. Bring cultures with >3 organisms up on ROUNDS
6. *Neisseria meningitidis* does not require routine susceptibility testing. Notify Infection Control.
7. All Isolates are saved – [Refer to Isolate Stocking Procedure](#)
  - a) Organisms that are not worked-up fully because of the possibility of contamination status should be held in the 7-day save pile.
  - b) ALL Autopsy isolates are stocked
  - c) Fastidious organisms are stocked by freezing in broth medium at -70°C
  - d) Non-fastidious organisms are stocked in motility tubes at room temperature
  - e) Stocked organisms are saved for 4 months.
8. Organisms of questionable significance should be saved and discussed at rounds.

TABLE 1

COMMON ORGANISMS	KEY ELEMENTS of IDENTIFICATION (not an exhaustive list, refer to <i>Organism ID AST</i> procedure and organisms ID charts)	STANDARD SUSCEPTIBILITY TESTING
<b>GRAM NEGATIVE RODS</b>	Gram stain	
Enteric	MALDI	astgn84
<i>Pseudomonas aeruginosa</i>	Oxidase, MALDI	kbpa1
Non-Lactose Fermenters	Oxidase, MALDI	MICNX2F
No Growth on MAC	R/O Select Agent, MALDI (when ruled out)	
<i>Haemophilus</i>	Satellite, MALDI	Cefinase ONLY
<b>STREPTOCOCCUS</b>	Gram stain, hemolysis, catalase	
Beta	Strep grouping, aerotolerance, MALDI	kbstrep
Alpha	PTAB, PYR, LAP, aerotolerance, MALDI	kbstrep, pen E
<i>Streptococcus pneumoniae</i>	PTAB, MALDI	STP6F
Gamma (not <i>Enterococcus</i> )	PYR, LAP, MALDI	kbstrep, pen E
<i>Enterococcus</i>	PYR, MALDI	astgp67
<b>STAPHYLOCOCCUS</b>	Gram stain, catalase	
<i>Staphylococcus aureus</i>	MRSA PCR (1st aerobic bottle), Staphaurex	astgp67
<i>Staphylococcus lugdenensis</i>	Staphaurex, PYR, MALDI	astgp67
Coagulase negative staph	Staphaurex, PYR	astgp67
<i>Micrococcus</i>	ATAB	NONE
<b>YEAST</b>	PNA FISH (1st Bottle), MALDI	MICY09
<b>AEROBIC GRAM-POSITIVE RODS</b>	Gram Stain, hemolysis, catalase, MALDI	NOT ROUTINE
<b>ANAEROBES</b>	Gram stain, aerotolerance, MALDI, discs	NOT ROUTINE

## IX. INTERPRETATION & REPORTING RESULTS

- A. **NO QUANTITATION IS REPORTED FOR BLOOD CULTURES**
- B. A positive blood culture generally means that a person is bacteremic.
- C. All positive blood cultures should get a workup with identification and susceptibility when appropriate.
- D. Alpha hemolytic strep, coagulase negative staph (CNS), micrococcus, bacillus, Propionibacteria are common skins contaminants associated with improper collection techniques.
- E. *Bacillus* species should be screened for anthrax. *B. anthracis* is non-hemolytic and non-motile and have typical ground-glass colony morphology.
- F. Positive Blood cultures with CNS should also be correlated with Catheter tip cultures collected within 3 days of each other.
- G. [Refer to APPENDIX AP12 Updating blood cultures with preliminary identification via Cepheid® Xpert® MRSA/SA](#) for appropriate result procedure when MRSA/SA BC PCR is performed
- H. Preliminaries and Final “No Growth” cultures will be updated daily by auto resulting processes.
- I. Positive Blood Culture Notification Requirements:
  1. Refer to: *Notification Scheme for Test Results of Clinical Significance*.  
**NOTE:** All cultures that are positive for an organism that was not seen in the initial Gram stain are to be called. Calls made after the first occurrence are done as a courtesy to the clinicians.

2. Call must be made to R.N. or provider.
  3. The verbal report must indicate # of positive sets, the total sets drawn, and the Gram morphology.
  4. The verbal report must be read-back to the reporting technologist.
  5. Documentation of the positive blood culture phone call must be documented as a test comment: (JCLGS) Gram Stain result Called to and Readback By: *NAME, TITLE* on *LOCATION, DATE TIME*
  6. Every new set that is positive and/or new organism type must be called - even if a previously positive culture was called.
  7. 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.
  8. The significant flag should ALWAYS be set at the time when the Gram stain is reported
- J. 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.
- K. When referring culture results to a previous culture, report the isolate identification and all isolate comments included in the original work-up.
- L. Use appropriate isolate comments - See Table 2

TABLE 2

ISOLATE COMMENT	COMMON USES	STATEMENT
&DID	CNS w/different identifications	Identification varies from second set drawn at this time. Probable contaminant.
&DSUS	AHS & CNS w/different susceptibility patterns	Susceptibilities vary from second set drawn at this time. Probable contaminant.
&SING	AHS & CNS when there is only one culture drawn in a 3-day time frame	Single Blood Culture set collected, Unable to determine significance. Consult with Microbiology required for further workup.
&MULB	AHS & CNS when there is more than one culture drawn in a 3-day time frame but only one set is positive	Multiple Blood Culture sets drawn, Susceptibilities are not performed when this organism is isolated from a single blood culture. Consult with Microbiology required for further workup.
&NOSU	There are interpretations in CLSI (either M100 or M45) but we don't usually set them up. Example: <i>Aerococcus urinae</i>	Susceptibilities not routinely performed.
&DOC	Provider has requested workup beyond the laboratory's routine protocol.	Doctor Requested workup
&NFW	Examples: <i>Corynebacterium</i> , <i>Micrococcus</i> , <i>Bacillus</i>	No further workup
&PROC	Growing in one set out of multiple sets drawn: <i>Micrococcus</i> , <i>Propionibacterium</i> , <i>Bacillus</i>	Probable Contamination

**X. LIMITATIONS**

- A. Low level of organisms may not be detected at all times.
- B. Improper collection may lead to erroneous results being reported
- C. There are fastidious microorganisms that infect the blood that cannot be grown in routine culture of blood. Refer to Planting Manual for processing.
- D. Some bacteria do not produce enough CO<sub>2</sub> gas for detection in automated systems.
- E. Providers must use culture results in conjunction with clinical presentation and medical history of the patient.
- F. Gram-stained smears from uninoculated culture medium may contain small numbers of non-viable but stainable bacteria from media constituents, staining reagents and devices.
- G. It is difficult to avoid an occasional contaminant in a blood culture. The situation is further complicated by the fact that some common contaminants (i.e., *Staphylococcus epidermidis*, *Propionibacterium acnes*) have been reported as etiological agents of endocarditis and septicemia. Finding the organism repetitively in multiple blood sets from a patient is the best evidence that the organism is not a contaminant.
- H. It is possible to have a septicemia caused by an organism that will not grow or grow and not be detected by the automated blood instrument. If such an organism is suspected, additional, alternative methods for recovery or detection should be considered. VersaTREK Myco medium is recommended for cultivating and detecting *Mycobacterium* species.
- I. Although some aerobes have been recovered from anaerobic broth, strict aerobes may not be detected because of the highly reduced nature of the medium.
- J. Overfilling the bottle may cause a false positive result.

**XI. REFERENCES**

- A. Baron, E.J., Scott, J.D., Tompkins, L.S. 2005. Prolonged incubation and extensive subculturing do not increase recovery of clinically significant microorganisms from standard automated blood cultures. *Clinical Infectious Diseases*. Dec 1;41(11):1677-80. Epub 2005 Oct 28.
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- F. Potula, Raghava, Dadhania, Vipul, Truant, Allan. 2015. Automated blood culture testing: A retrospective study indicates that a three-day incubation period is sufficient. *Medical Laboratory Observer*. Sep;47(9):

**XII. REVISIONS**

- A. 1/20/2022 Updated automated blood culture instrument information and procedure reference
- B. 9/26/2022 Updated procedure to include full work up of microaerophilic ahs