PROCEDURE: BLOOD CULTURE – BENCH PROCEDURE

I. PRINCIPLE

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. The Microbiology Laboratory employs a blood culture detection system which monitors liquid emulsion sensors using solid state photo detectors. These sensors are 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: 24/7 from RIH, TMH & NH

III. 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

B. Recommended volumes
   1. Adults: 10-20 ml of blood per blood culture set
   2. Pediatric: 4-5 ml of blood per blood culture set

C. **DO NOT REFRIGERATE BOTTLES.**

D. Vials are inoculated by the person performing the venipuncture at the site of the drawing and sent to the lab. The initials of the person performing the blood culture draw must be on each bottle.

E. Each set is logged in and assigned to one order number.

F. Improperly collected specimens that are not cancelled (ie. Single blood bottle submitted) must include the following disclaimer in the report:

"Specimen improperly collected. Recovery of organisms will be compromised, re-collection of specimen suggested."

G. Refer to Procedure: BACT/Alert Virtuo for instrument procedure

H. Notify Director/Manager/Senior technologist regarding culture requests for any of the following potential pathogens:

Note: 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* (Melioidosis)
6. *Yersinia pestis* (Bubonic plague, pneumonic plague, black death)

Note: The following dimorphic fungi may require a Fungal blood culture be submitted to a Reference lab:

1. *Blastomyces* (Blastomycosis, Chicago Disease, Gilchrist's Disease)
2. *Coccidioides* (Coccidioidomycosis, Valley Fever)
3. *Histoplasma* (Histoplasmosis, Cave/Darling's/Ohio Valley Disease)
4. *Paracoccidioides* (Paracoccidioidomycosis, South American/Brazilian blastomycosis, Lutz's Disease)

I. 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.
**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.

J. Isolates that need to be sent to the RIDOH:
1. All suspected Select Agents (CATEGORY A)
2. *Campylobacter*
3. *Clostridium botulinum*
4. *E. coli (0157: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*
17. *Streptococcus pneumoniae* (only in patients <5 years old)

IV. QUALITY CONTROL

A. All media and biochemicals utilized should meet established QC parameters referenced in applicable IQCPs.

V. TEST PROCEDURE

A. Work-up of Positive Blood Cultures - Refer to *Organism ID/AST* Procedure for organism specific testing
   1. Incubate subculture plates and examine aerobic plates at 24 hours.
   2. All subcultured plates are incubated for 48 hours before a final report is set.
   3. Anaerobic subculture plates (BRU) are incubated a full 48 hours before examination.
   4. 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) Organisms with specific work-up and/or referral criteria:
         (1) Coagulase-negative *Staphylococcus*
            (a) Compare identifications between multiple positive sets
            (b) If identifications differ, document ID in worksheet and add isolate comment: &DID
            (c) If identifications match, work up according to *Organism ID/AST* Procedure and refer subsequent positive sets according to *Culture Referral Procedure*
            (d) Rule out *Staphylococcus lugdenensis* (DO NOT report identification unless identified as *Staphylococcus lugdenensis)*
         (2) Alpha hemolytic *Streptococcus*
            (a) Perform VITEK MS (MALDI) identification and report identification for all isolates.
               (i) Non-microaerophilic ahs
                  (a) If single set, identify organism and report with applicable comments
                  (b) If multiple sets and identification matches, work up according to *Organism ID/AST* Procedure and refer subsequent positive sets according to *Culture Referral Procedure*
               (ii) Microaerophilic ahs
                  (a) If single set, work up according to *Organism ID/AST* Procedure
                  (b) If multiple sets, work up according to *Organism ID/AST* Procedure and refer subsequent positive sets according to *Culture Referral Procedure*
         (3) Corynebacterium sp. are identified if isolated from multiple culture sets to rule-out *Corynebacterium jeikeium*
   5. Bring cultures with >3 organisms up on ROUNDS
   6. Isolates that are fully worked-up are stocked – Refer to *Isolate Stocking Procedure*
      a) Organisms that are not fully worked-up because of the possibility of contamination status should be held in the 7-day save pile.
   7. 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 *Organism ID AST* procedure and organisms ID charts)	STANDARD SUSCEPTIBILITY TESTING
GRAM NEGATIVE RODS	Gram stain	
Enteric	MALDI	astn812
Pseudomonas aeruginosa	Oxidase, MALDI	kbpa2
Non-Lactose Fermenters	Oxidase, MALDI	MICNX2F
No Growth on MAC	R/O Select Agent, MALDI (when ruled out)	
Haemophilus	Satellite, MALDI	Cefinase (*H. influenzae* only)
STREPTOCOCCUS	Gram stain, hemolysis, catalase	
Beta	Strep grouping, aerotolerance, MALDI	kbstrep
Alpha	PTAB, PYR, LAP, aerotolerance, MALDI	kbstrep, pen E
Streptococcus pneumoniae	PTAB, MALDI	STP8F
Gamma (not *Enterococcus*)	PYR, LAP, MALDI	kbstrep, pen E
Enterococcus	PYR, MALDI	astgp67
STAPHYLOCOCCUS	Gram stain, catalase	
Staphylococcus aureus	MRSA PCR (1st aerobic bottle), Staphaurex	astgp67
Staphylococcus lugdenensis	Staphaurex, PYR, MALDI	astgp67
Coagulase negative staph	Staphaurex, PYR	astgp67
Micrococcus	ATAB	NONE
YEAST	FPP (1st Bottle), MALDI	MICYO11
AEROBIC GRAM-POSITIVE RODS	Gram Stain, hemolysis, catalase, MALDI	NOT ROUTINE
ANAEROBES	Gram stain, aerotolerance, MALDI, discs	NOT ROUTINE

VI. INTERPRETATION & REPORTING RESULTS

A. **NO QUANTITATION IS REPORTED FOR BLOOD CULTURES**

B. Alpha hemolytic strep, coagulase negative staph (CNS), *Micrococcus*, *Bacillus*, and *Propionibacterium* are common skin contaminants associated with improper collection techniques.

C. *Bacillus* species should be screened for anthrax. *B. anthracis* is non-hemolytic and non-motile and has typical ground-glass colony morphology.

D. Positive blood cultures with CNS should also be correlated with Catheter tip cultures collected within 5 days of each other.

E. 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

F. Preliminaries and Final “No Growth” cultures will be updated daily by auto resulting processes.

G. Refer to Procedure: Critical Results Notification for positive blood culture notification requirements.

H. 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.

I. 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.
&SING	AHS & CNS when there is only one culture drawn in a 5-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 5-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: *Aerococcus urinae*	Susceptibilities not routinely performed.
&DOC	Provider has requested workup beyond the laboratory's routine protocol.	Doctor Requested workup
&NFW	Examples: *Corynebacterium, Micrococcus, Bacillus*	No further workup
&PROC	Growing in one set out of multiple sets drawn: *Micrococcus, Propionibacterium, Bacillus*	Probable Contamination

VII. LIMITATIONS

A. Low levels of organisms may not be detected.
B. Improper collection may lead to erroneous results being reported
C. A minimum of 0.5 ml blood is required for the recovery of *Haemophilus influenzae* and *Neisseria spp.*
D. There are fastidious microorganisms that infect the blood that cannot be grown in routine culture of blood. Refer to Planting Manual for processing.
E. Some bacteria do not produce enough CO_2 gas for detection in automated systems.
F. Providers must use culture results in conjunction with clinical presentation and medical history of the patient.
G. Gram-stained smears from uninoculated culture medium may contain small numbers of non-viable but stainable bacteria from media constituents, staining reagents and devices.
H. 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.
I. It is possible to have 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.
J. Although some aerobes may be recovered from anaerobic broth, strict aerobes may not be detected because of the highly reduced nature of the medium.

K. Overfilled blood culture bottles may cause false positive results.

VIII. REFERENCES

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IX. 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

C. 1/12/2026 Updated work-up protocol for CoNS and AHS and increased referral periods from 3 to 5 days. Removed specific information and referenced corresponding procedures regarding specimen collection, instrument operation, and organism work-up.