PROGRAM Standard Operating Procedure – Laboratory Services	
Title: MIC34000 – Blood Culture	Policy Number:
Program Name: Laboratory Services	
Applicable Domain: Lab, DI and Pharmacy Services	
Additional Domain(s): NA	
Effective Date:	Next Review Date:
Issuing Authority: Director, Laboratory and Diagnostic Imaging Services	Date Approved:
Accreditation Canada Applicable Standard: NA	

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GUIDING PRINCIPLE:
Blood cultures are collected from patients with suspected sepsis or bacteremia. The isolation of any organism(s) from a blood culture must be considered significant and correlated with the clinical picture. Although primarily directed towards the processing of blood cultures, occasionally other specimen types (sterile fluids, abscess material, bone marrow, etc.) are received in blood culture bottles. These bottles may be processed in the same way as blood cultures. The BACTEC FX instrument continuously monitors routine blood cultures for evidence of growth for 5 days.

PURPOSE/RATIONALE:
This standard operating procedure describes how to determine the significance of growth in blood culture specimens.

SCOPE/APPLICABILITY:
This procedure applies to Medical Laboratory Technologists (MLTs) processing specimens for blood culture.

SAMPLE INFORMATION:

Special Precautions	Refer to MIC40100-Suspect High Risk Organism Workup
Type	• Blood • Sterile fluid received in blood culture bottle
Source	• Refer to SCM20800-Blood Culture Collection for blood culture collection procedure • If fluid is received in blood culture bottles, order as CXFBC, fluid in blood culture bottle

Volume	• Refer to SCM20800-Blood Culture Collection for blood culture bottle volumes
Stability	Adhere to the expiration date on the bottle
Storage Requirements	• Room temperature, do not cool or freeze • Transport of bottles after collection should always be done at room temperature • Frozen samples may affect the recovery of fastidious organisms
Criteria for rejection	1. Broken/cracked bottle 2. Blood cultures collected prior to antibiotics being given are considered an irretrievable specimen. Improperly collected, labeled, transported, or handled specimens should be processed. SCM40110-Waiver of responsibility form needs to be filled out by the responsible nurse

REAGENTS and/or MEDIA:

- BACTEC Plus Aerobic/F culture bottles, BACTEC Lytic/10 Anaerobic/F culture bottles and BACTEC Peds Plus/F culture bottles
- Blood agar (BA), Chocolate agar (CHO), MacConkey agar (MAC) and Brucella agar (BRU)
- Identification reagents: catalase, oxidase, Staph latex test, Strep latex test, etc.

SUPPLIES:

- Sub culturing/venting unit
- Alcohol pads
- Disposable inoculation needles
- Microscope slides
- Anaerobic jar and pouch
- Wooden sticks

EQUIPMENT:

- BACTEC FX
- Biosafety cabinet
- 35° ambient air and 35° CO_2 incubators
- VITEK 2 and supplies

SPECIAL SAFETY PRECAUTIONS:

Containment Level 2 facilities, equipment, and operational practices for work involving infectious or potentially infectious materials or cultures:

- Ensure that appropriate hand hygiene practices be used
- Lab gown must be worn when performing activities with potential pathogens
- Gloves must be worn when direct skin contact with infected materials is unavoidable
- Eye protection must be used when there is a known or potential risk of exposure of splashes
- All procedures that may produce aerosols, or involve high concentrations or large volumes should be conducted in a biological safety cabinet (BSC)
- The use of needles, syringes and other sharp objects should be strictly limited

All patient specimens are assumed to be potentially infectious. Routine Practices must be followed. Since viable micro-organisms are used, all cultures must be handled with appropriate precautions. All equipment in contact with cultures should be decontaminated by appropriate methods.

QUALITY CONTROL:

- Refer to Test Manual for reagent quality control procedures

PROCEDURE INSTRUCTIONS FOR NEGATIVE BLOOD CULTURE BOTTLES:

Step	Action
1	The BACTEC FX instrument continuously monitors routine blood cultures for growth for 5 days. Negative results are auto verified by the LIS as follows: • No growth after 48 hours of incubation (preliminary) • No growth after 5 days of incubation (final)
2	Refer to MIC71000-BACTEC FX Instrument to extend the incubation period if requested.

PROCEDURE INSTRUCTIONS FOR POSITIVE BLOOD CULTURE BOTTLES:

Step	Action
1	Refer to MIC71000-BACTEC FX Instrument to remove the positive bottle(s) from the instrument.
2	Refer to MIC10100-Microbiology Specimen Processing for the handling of positive blood culture bottles in the LIS when the BACTEC alarm sounds.
3	In the biosafety cabinet, using a sub culturing/aerobic vent: • Place 1 to 2 drops of blood onto BA, CHO, MAC and BRU • Streak for isolated growth using a disposable inoculation needle • Prepare smear by placing 1 to 2 drops of blood onto a clean microscope slide and spread out with an inoculation needle to form a thin smear
4	Incubate all media: • Place BA and CHO in the CO_2 incubator • Place the blood culture bottle and MAC in the O_2 incubator • Place BRU in anaerobic jar with anaerobic pouch and indicator as soon as possible after inoculation. Label jar with date of 48 hour read **NOTE:** Anaerobes should not be exposed to air for 42 to 48 hours after inoculation
5	Allow smear to dry and perform gram stain. Gram stain must be read before culture plates. Refer to MIC20115-Gram Stain.
6	Interpret positive blood culture smears immediately. During the regular Microbiology lab hours of 08:00 to 20:00, turnaround time for these gram stains is <1 hour. Outside the regular Microbiology lab hours, positive blood culture smears will be read the following morning at 08:00.
7	Immediately phone positive blood culture gram stain results to ordering location and document in the LIS.
8	If no organisms are seen in the gram stain: • Refer to MIC20500-Gram stain resulting in LIS-Blood Cultures to result in the LIS when no bacteria are seen

INTERPRETATION OF RESULTS:

Step	Action
1	Ensure growth on culture media correlates with gram stain results. If discordant results are found between the gram stain and growth: • Re-examine smear and culture plates • Check for anaerobic growth • Re-incubate media to resolve • Consider re-smearing or re-planting specimen
2	• Observe BA and CHO plates at 24 hours and 48 hours • Observe MAC plate at 24 hours. Re-incubate if required
3	• Observe BRU after 48 hours • If organisms seen on the direct gram smear and aerobic plate match growth on BRU, plate can be discarded after 48 hours • If no growth is seen on aerobic plates or aerobic growth does not correlate with direct gram smear, re-incubate BRU for an additional 72 hours
4	If growth is observed, perform biochemical testing to report preliminary ID of the isolate. Refer to the Microbiology Bacteriology Manual organism ID charts to guide work-up.
5	Provide genus and species identification as soon as possible. If a preliminary identification cannot be made after 24 hours, release a preliminary culture report using the gram stain morphology.
6	Growth of a coagulase-negative *Staphylococci*, viridans *Streptococcus*, cornyeform bacteria (diptheroid), *Bacillus* spp. (not anthracis), *Micrococcus* spp., *Propionebacterium* spp. and *Neisseria* spp., (other than *meningitidis* or *gonorrhoeae*) are considered possible skin contaminants: • Perform only minimal identification and do not perform susceptibility testing. Add Isolate Comment: **&BC03** • Contaminants can be recognized from true pathogens if they are recovered in only one bottle in a series of blood culture sets
7	When identification of organism is confirmed and determined to not be a contaminant, perform susceptibility testing as per ASTM.
8	If growth of same pathogen is found in subsequent positive blood culture bottles: • If morphology is the same, perform spot tests (Staph. Latex test, spot indole, etc.) to verify organisms' identity • If no spot tests are applicable, perform full identification • Repeat susceptibility testing on persistently positive blood cultures after 3 days for gram negative organisms and 5 days for gram positive organisms

REPORTING INSTRUCTIONS:

IF	REPORT
Growth of pathogen	• Report organism full identification • List quantitation as **"Isolated"** • Report susceptibility results as per ASTM • Freeze isolate and log into stored isolates log
Growth of same pathogen in subsequent bottles	• Report organism full identification • List quantitation as **"Isolated"** • Refer susceptibility results to subsequent positive cultures. Add isolate comment **&BC02**. Add bottle type if referring additional bottle in same set or accession number if referring to additional set
Growth of contaminant	• Report the minimal identification (i.e., Coagulase negative Staphylococci) **NOTE:** Full identification does not need to be made and should not be reported • Add isolate comment **&BC03** • Do not perform or report susceptibility • Freeze isolate and log into stored isolates log
H. influenzae or *N.meningitidis* isolated	• Must be sent immediately to Alberta Precision Laboratories for typing • Refer to MIC36600-Microbiology Organism Referral • Freeze isolate and log into stored isolates log
S.pyogenes, S.agalactiae, S.pneumoniae, H. influenzae or *N.meningitidis* isolated	• Any *S.pyogenes*, *S.agalactiae, S.pneumoniae, H.influenzae* or *N.meningitidis* isolated from blood culture specimens must be sent to NML for International Circumpolar Surveillance (ICS) program • Refer to MIC36600-Microbiology Organism Referral • Freeze isolate and log into stored isolates log

NOTE:

- Refer to Reportable Diseases-Public Health Act as of September 2009 for reporting to HPU1
- Refer to LQM70620-Laboratory Critical Results List-Microbiology for results that need to be phoned to ordering location
- Refer to MIC36100-Nosocomial Infection Notification Job Aid to determine if organism needs to be copied to Infection Prevention and Control
- Refer to MIC36200-Referral of Category A Specimens to APL for sending category A isolates to APL
- Refer to MIC36300-Referral of Category B Specimens to APL for sending category B isolates to APL
- Refer to MIC36500-Referral of Category B Specimens to NML for sending category B isolates to NML
- Refer to MIC36600-Microbiology Organism Referral

LIMITATIONS:

1. A negative blood culture does not eliminate the possibility of bacteremia or sepsis.
2. Inadequate specimen collection, improper specimen handling and low organism levels in the specimen may yield false negative results.
3. A contaminated specimen will give a positive reading but will not indicate a clinically relevant result.
4. If less than 5mL or more than 10mL of blood is inoculated into an aerobic or anaerobic BACTEC bottle, SPS sensitive organisms, such as some *Neisseria* species, may fail to grow.
5. If less than 3mL of blood is inoculated into an aerobic or anaerobic BACTEC bottle, there may not be enough blood present to provide NAD for certain *Haemophilus* species.
6. The specimen may contain an organism that will not grow in the culture. *Streptococcus pneumoniae* may fail to grow in the aerobic medium.
7. False negative readings may result when certain organisms are present which do not produce enough CO_2 to be detected by the BACTEC FX system.
8. False negative readings may result when significant growth has occurred before placing the bottle into the BACTEC FX.
9. False positive readings may occur when the white blood cell count is high.

CROSS-REFERENCES:

- LQM70620-Laboratory Critical Results List-Microbiology
- MIC10100-Microbiology Specimen Processing
- MIC20115-Gram Stain Procedure
- MIC20500-Gram stain resulting in LIS-Blood Cultures
- MIC36100-Nosocomial Infection Notification Job Aid
- MIC36200-Referral of Category A Specimens to APL
- MIC36300-Referral of Category B Specimens to APL
- MIC36500-Referral of Category B Specimens to NML
- MIC36600-Microbiology Organism Referral
- MIC40100-Suspect High Risk Organism Workup
- MIC71000-BACTEC FX Instrument Procedures
- SCM20800-Blood Culture Collection
- SCM40110-Waiver of responsibility form

REFERENCES:

1. Leber, A. (2016). *Clinical microbiology procedures handbook.* (4th ed.) Washington, D.C.: ASM Press
2. Jorgensen J.H., Pfaller M.A., Carroll K.C., Funke G., Landry M.L., Richter S.S., Warnock D.W. (2015). *Manual of Clinical Microbiology, 11th edition*. Washington, D.C: ASM Press
3. Policy B-0160: *Specimens Containing Suspected Risk Group 3 Pathogens for Primary Specimen Handling Flow Chart*

APPROVAL:

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Date

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REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	28 May 18	Initial Release	L. Steven
2.0	22 Feb 21	Procedure reviewed and added to NTHSSA policy template	L. Steven
3.0	27 Feb 23	Procedure reviewed	L. Steven