

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

**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 monitor routine blood cultures for evidence of growth for 5 days.

**PURPOSE/RATIONALE:**

To determine the presence or absence of bacterial pathogens in blood culture specimens.

**SCOPE/APPLICABILITY:**

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

**SAMPLE INFORMATION:**

<b>Special Precautions</b>	Refer to Policy 17-02-V1: Specimens Containing Suspected Risk Group 3 Pathogens
<b>Type</b>	<ul style="list-style-type: none"> <li>• Blood</li> <li>• Sterile fluid received in blood culture bottle</li> </ul>
<b>Source</b>	<ul style="list-style-type: none"> <li>• Refer to SCM20800-Blood Culture Collection for blood culture collection procedure</li> <li>• If fluid is received in blood culture bottles, order as CXFBC, fluid in blood culture bottle</li> </ul>

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<b>Volume</b>	<ul style="list-style-type: none"> <li>Refer to SCM20800-Blood Culture Collection for blood culture bottle volumes</li> </ul>
<b>Stability</b>	Adhere to the expiration date on the bottles
<b>Storage Requirements</b>	<ul style="list-style-type: none"> <li>Room temperature, do not cool or freeze</li> <li>Transport of bottles after collection should always be done at room temperature</li> <li>Frozen samples may affect the recovery of fastidious organisms</li> </ul>
<b>Criteria for rejection</b>	<ol style="list-style-type: none"> <li>Broken/cracked bottle</li> <li>Blood cultures collected prior to antibiotics 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 physician or nurse</li> </ol>

**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:**

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|--|---|
| <ul style="list-style-type: none"> <li>Sub culturing/venting unit</li> <li>Alcohol pads</li> <li>Disposable inoculation needles</li> </ul> | <ul style="list-style-type: none"> <li>Microscope slides</li> <li>Anaerobic jar and pouch</li> <li>Wooden sticks</li> </ul> |
|--|---|

**EQUIPMENT:**

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| <ul style="list-style-type: none"> <li>BACTEC FX</li> <li>Biosafety cabinet</li> <li>35° ambient air and 35° CO<sub>2</sub></li> </ul> | <ul style="list-style-type: none"> <li>incubators</li> <li>Vitek 2 and supplies</li> </ul> |
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**SPECIAL SAFETY PRECAUTIONS:**

Containment Level 2 facilities, equipment, and operational practices for work involving infectious or potential 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.

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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 as follows: <ul style="list-style-type: none"> <li>• No growth after 48 hours of incubation (preliminary)</li> <li>• No growth after 5 days of incubation (final)</li> <li>• No growth after 10 days if extended culture is requested</li> </ul>
2	Refer to MIC71000-BACTEC FX Instrument Procedures to extend the incubation period if requested.

**PROCEDURE INSTRUCTIONS FOR POSITIVE BLOOD CULTURE BOTTLES:**

Step	Action
1	Refer to MIC71000-BACTEC FX Instrument Procedures to remove the positive bottle(s) from the instrument.
2	Refer to MIC10100-Microbiology Specimen Processing for the handling of positive bottles in the LIS when the BACTEC alarm sounds.
3	In the biosafety cabinet, using a sub culturing/aerobic vent: <ul style="list-style-type: none"> <li>• Place 1 to 2 drops of blood onto BA, CHO, MAC and BRU</li> <li>• Streak for isolated growth using a disposable inoculation needle</li> <li>• 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</li> </ul>
4	Incubate all media: <ul style="list-style-type: none"> <li>• Place BA and CHO in the CO<sub>2</sub> incubator</li> <li>• Place the blood culture bottle and MAC in the O<sub>2</sub> incubator</li> <li>• Place BRU in anaerobic jar with anaerobic pouch and indicator as soon as possible after inoculation. Label jar with date of 48 hour read</li> </ul> <p><b>NOTE:</b> Anaerobes should not be exposed to air for 42 to 48 hours after inoculation</p>
5	Allow smear to dry and perform gram stain. Gram stain must be read before culture plates. Refer to MIC20115-Gram Stain Procedure.
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: <ul style="list-style-type: none"> <li>• Refer to MIC20500-Gram stain resulting in LIS-Blood Cultures to result in the LIS when no bacteria are seen</li> </ul>

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**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: <ul style="list-style-type: none"> <li>• Re-examine smear and culture plates</li> <li>• Check for anaerobic growth</li> <li>• Re-incubate media to resolve</li> <li>• Consider re-smearing or re-planting specimen</li> </ul>
2	<ul style="list-style-type: none"> <li>• Observe BA and CHO plates at 24 hours and 48 hours</li> <li>• Observe MAC plate at 24 hours. Re-incubate if needed</li> </ul>
3	<ul style="list-style-type: none"> <li>• Observe BRU after 48 hours</li> <li>• If organisms seen on the direct gram smear and aerobic plate match growth on BRU, plate can be discarded after 48 hours</li> <li>• 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</li> </ul>
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 <i>Staphylococcus</i> , viridans <i>Streptococcus</i> , coryneform bacteria (diphtheroid), <i>Bacillus</i> spp. (not anthracis), <i>Micrococcus</i> spp., <i>Propionibacterium</i> spp. and <i>Neisseria</i> spp., (other than <i>meningitidis</i> or <i>gonorrhoeae</i> ) are considered possible skin contaminants: <ul style="list-style-type: none"> <li>• Perform only minimal identification and do not perform susceptibility testing. Add Isolate Comment: <b>&amp;BC03</b></li> <li>• Contaminants can be recognized from true pathogens if they are recovered in only one of a series of blood culture sets</li> </ul>

**REPORTING INSTRUCTIONS:**

IF	REPORT
Growth of pathogen	<ul style="list-style-type: none"> <li>• Report organisms identification</li> <li>• List quantitation as <b>"Isolated"</b></li> <li>• Report susceptibility results as per ASTM</li> <li>• Freeze isolate and log into stored isolates log</li> </ul>
Growth of same pathogen in subsequent bottles	<ul style="list-style-type: none"> <li>• If morphology is the same, perform spot tests (catalase, coagulase, indole, PYR, etc.) to verify organism identity</li> <li>• Refer susceptibility results to subsequent positive cultures. Use Isolate Comment <b>&amp;BC02</b>. Add bottle type if referring additional bottle in same set or accession number if referring to additional set</li> <li>• Repeat susceptibility testing on persistently positive blood cultures after 3 days for gram negative organisms and 5 days for gram positive organisms</li> </ul>

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Growth of contaminant	<ul style="list-style-type: none"><li>Report organisms identification</li></ul> <b>NOTE:</b> Full identification does not need to be made. Only minimal identification needs to be listed <ul style="list-style-type: none"><li>Add isolate comment: <b>&amp;BC03</b></li><li>Do not perform or report susceptibility</li><li>Freeze isolate and log into stored isolates log</li></ul>
<i>H. influenzae</i> or <i>N.meningitidis</i> isolated	<ul style="list-style-type: none"><li>Must be sent immediately to Alberta Precision Laboratories for typing</li><li>Refer to MIC36600-Microbiology Organism Referral</li><li>Freeze isolate and log into stored isolates log</li></ul>
<i>S.pyogenes</i> , <i>S.agalactiae</i> , <i>S.pneumoniae</i> , <i>H. influenzae</i> or <i>N.meningitidis</i> isolated	<ul style="list-style-type: none"><li>Any <i>S.pyogenes</i>, <i>S.agalactiae</i>, <i>S.pneumoniae</i>, <i>H.influenzae</i> or <i>N.meningitidis</i> isolated from blood culture specimens must be sent to NML for International Circumpolar Surveillance (ICS) program</li><li>Refer to MIC36600-Microbiology Organism Referral</li><li>Freeze isolate and log into stored isolates log</li></ul>

**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 isolates to APL
- Refer to MIC36400-Referral of Category B Specimens to DL for sending isolates to DynaLIFE
- Refer to MIC36500-Referral of Category B Specimens to NML for sending isolates to NML
- Refer to MIC36600-Microbiology Organism Referral

**LIMITATIONS:**

- A negative blood culture does not eliminate the possibility of bacteremia or sepsis.
- Inadequate specimen collection, improper specimen handling and low organism levels in the specimen may yield false negative results.
- A contaminated specimen will give a positive reading but will not indicate a clinically relevant result.
- 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.
- 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.

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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<sub>2</sub> 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:**

- 17-02-V1: Specimens Containing Suspected Risk Group 3 Pathogens
- 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
- MIC36400-Referral of Category B Specimens to *DynaLIFE*
- MIC36500-Referral of Category B Specimens to *NML*
- MIC36600-Microbiology Organism Referral
- MIC71000-BACTEC FX Instrument Procedures
- SCM20800-Blood Culture Collection
- SCM40110-Waiver of responsibility form

#### **REFERENCES:**

1. Leber, A. (2016). *Clinical microbiology procedures handbook*. (4<sup>th</sup>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*, 11<sup>th</sup> 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

**REVISION HISTORY:**

REVISION	DATE	Description of Change	REQUESTED BY
1.0	28 May 18	Initial Release	L. Steven
2.0	30 Jan 21	Procedure reviewed and added to NTHSSA policy template	L. Steven

DRAFT

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