PURPOSE: To determine the presence or absence of bacterial pathogens in blood specimens. Occasionally other specimen types (sterile fluids, abscess material, bone marrow, etc.) are received in blood culture bottles and may be processed the same way.

SAMPLE INFORMATION:

Special Precautions	Refer to Policy B-0160: Specimens Containing Suspected Risk Group 3 Pathogens for Primary Specimen Handling Flow Chart.		
Type	• Blood • Sterile fluid received in blood culture bottles.		
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	**Bottle**	**Min. Volume**	**Max. Volume**
	Plus Aerobic/F and Anaerobic/F	3 mL	10 mL
	Peds Plus/F	0.5 mL	3 mL
Stability	Adhere to the expiration date on the bottles.		
Storage Requirements	Bottle storage before blood collection: • Room temperature. Bottle storage after blood collection: • Room temperature, do not cool or freeze. • Although organisms have been recovered from frozen blood culture bottles received in the laboratory, transport of bottles after collection should always be done at room temperature. • Frozen samples may affect the recovery of fastidious organisms.		
Criteria for rejection and follow up action	1. Unlabeled/mislabeled specimen 2. Broken/cracked vial **Please note:** Except for the above conditions, blood culture samples are not rejected regardless of delayed transport, if received frozen or if bottles are expired. Please ensure the appropriate specimen quality comments are attached to the specimen in OE and process blood culture specimen as per usual procedure.		

REAGENTS and/or MEDIA:

- BACTEC™ Plus Aerobic/F Culture Vials, BACTEC™ Lytic/10 Anaerobic/F Culture Vials and BACTEC™ Peds Plus™/F Culture Vials.
- Blood agar (BAP), Chocolate agar (CHOC), MacConkey agar (MAC) and Brucella agar (BRUC).
- Identification reagents: catalase, oxidase, rapid Staph, rapid Strep, etc.

SUPPLIES:

- Wooden applicator sticks
- Disposable inoculation needles
- Biosafety cabinet
- Microscope slides
- Alcohol pads
- Sub culturing/aerobic venting unit
- 35° ambient air and 37° CO_2 incubators
- Anaerobic jar, pack and indicator
- BD BACTEC FX
- Vitek 2 and supplies

SPECIAL SAFETY PRECAUTIONS:

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

- 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. Universal precautions 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 Quality Control 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 evidence of growth for 5 days. Negative results are auto verified as follows: • No growth after 48 hours of incubation (preliminary) • No growth after 5 days of incubation (final)
2	Refer to MIC70300 – BACTEC FX Instrument Procedures to extend the incubation period if requested by physician. Follow the instructions to extend the incubation time on the analyzer and in the LIS.

PROCEDURE INSTRUCTIONS FOR POSTITIVE BLOOD CULTURE BOTTLES:

Step	Action
1	Refer to MIC70300-BACTEC FX Instrument Procedures to remove positive bottle from the instrument.
2	Refer to MIC10230-Microbiology Specimen Processing for the handling of bottles when the BACTEC alarm sounds.
3	Allow smear to dry and perform gram stain. Refer to MIC20500 – Gram stain resulting in LIS – Blood Cultures procedure and interpretation of smear.
4	Interpret positive blood culture stains immediately. During the regular Microbiology lab hours of 08:00 to 20:00, turnaround time for these gram stains is <1 hour. Bottles that alarm positive outside these times will be processed first thing in the am by either the wound bench technologist at 08:00 or urine bench technologist when they return from rounds, whichever occurs first.
5	If gram stain results are gram negative coccobacilli or gram negative diplococci, apply the Risk Group 3 Organism Precaution sticker and parafilm to all plates to prevent exposure to possible *N.meningitidis*, *Brucella* spp. or *Francisella* spp.
6	Immediately phone results of any positive stain results for microorganisms and document the conversation within the LIS.
7	If no organisms are seen in the gram stain: • Perform an Acridine Orange stain to detect low numbers of bacteria. Refer to MIC20100. • Refer to MIC20500 - Gram stain resulting in LIS –Blood Cultures, for LIS procedures on how to result blood culture gram stains in LIS when no bacteria are seen. • Replace bottle into the BACTEC within 5 hours. If the 5 hour window for vial replacement into the BACTEC has expired, place the bottle in the O_2 incubator with a note attached with the date of the fifth day after collection. Add media **5DAY** to order media and gram stain.

INTERPRETATION OF RESULTS:

Step	Action
Interpretation of cultures	
1	Examine aerobic plates after 24 hours incubation. Record your observations in the LIS.
2	Re-incubate CO_2 plates for an additional 48 hours. Re-incubate O_2 plate for an additional 24 hours.
3	Examine anaerobic plate after 48 hours incubation. Record observations in the LIS. If growth on direct gram smear and aerobic plates matches growth on BRUC, 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 BRUC anaerobically for an additional 72 hours.
2	If no growth is observed, you may subculture bottle to CHOC and incubate microaerobically for camplylobacters.
3	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.
4	Provide genus and species identification as soon as possible. Refer to critical values procedure and Schedule 3 – Reportable Diseases for culture identification results that need to be phoned.
5	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 *Staphylococcus*, viridans *Streptococcus*, cornyeform bacteria (diptheroid), *Propionibacterium* species, *Bacillus* species (not anthracis) or *Micrococcus* species are considered possible skin contaminants. Perform only minimal identification and do not perform AST. Add Isolate Comment: **&BC03** to state: **"Susceptibility testing not performed. The clinical significance of skin flora isolated from a single blood culture is undetermined. Please contact the Microbiology Laboratory if further work-up is required"**. Contaminants can be recognized from true pathogens if they are recovered in one blood culture set when multiple sets are taken or if isolated from a single bottle in a set.
7	Perform susceptibility testing as per DynaLIFE ASTM.
8	Freeze organism (including contaminants) in glycerol and record in patient isolate log.
9	For subsequent positive cultures, it is not necessary to repeat full biochemical testing if culture morphology is the same. Perform a few spot tests (catalase, coagulase, indole, PYR, etc.) to verify that it is the same organism.

10	Refer susceptibility results to subsequent positive cultures. Use Isolate Comment **&BC02** to state: **"Please refer to_____ for susceptibility results."** Add bottle type if referring additional bottle in same set or accession number if referring to additional set. Repeat susceptibility testing on persistently positive blood cultures after 3 days for gram negative organisms and 5 days for gram positive organisms.
11	A copy of all positive reports on inpatients, excluding ER, must be sent to Infection Control (SOHS) as per MIC35100 - Nosocomial Infection Notification Job Aid.
12	Any blood culture or sterile fluid positive for Group A *Streptococcus*, *Haemophilus influenzae* or *Neisseria meningitidis* must to be phoned to the Chief Medical Officer of Health (HPU1) as per MIC35000 - Reportable Diseases Notification. Additionally, a copy of the report must be sent.
13	Any blood culture positive for *Haemophilus influenzae* or *Neisseria meningitidis* must be sent immediately to the Provincial Lab Edmonton for typing as soon as identification is confirmed as per MIC35200 - Significant Organism Referral Job Aid. Assure there is a purity plate made that can be used for this purpose and can be sent out the day the identification is confirmed. Refer to MIC10520-Referral of Category B Specimens to Provincial Laboratory.
14	Any positive blood culture or sterile fluid positive for Group A *Streptococcus*, Group B *Streptococcus*, *Streptococcus pneumoniae, Haemophilus influenzae* and *Neisseria meningitidis* must be sent to NML Winnipeg for surveillance testing as per MIC35200 - Significant Organism Referral Job Aid. Refer to MIC10520-Referral of Category B specimens to NML for International Circumpolar Surveillance Program.
15	Any positive blood culture or sterile fluid positive for any organisms on Schedule 3 – Reportable Diseases needs to be reported to the Chief Medical Officer of Health (HPU1) as per MIC35000 – Reportable Diseases Notification. Refer to document to determine if results need to be phoned or a copy sent.

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.
- The specimen may contain an organism that will not grow in the culture media.
- *Streptococcus pneumoniae* may fail to grow in the aerobic medium.
- False negative readings may result when certain organisms are present which do not produce enough CO_2 to be detected by the BACTEC system.
- False negative readings may result when significant growth has occurred before placing the vial into the Bactec.
- False positive readings may occur when the white blood cell count is high.

REFERENCES:

- Clinical Microbiology Procedures Handbook, 4th edition, ASM Press, 2016
- 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, ASM Press, Washington, D.C.
- Bactec FX culture bottles package inserts
- Bactec FX Instrument User's Manual

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0		Initial Release	L. Steven