PROGRAM Standard Operating Procedure – Laboratory Services	
Title: MIC34100 – Body Fluid 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:
Infections of normally sterile body fluids often result in severe morbidity and mortality. Rapid and accurate microbiological assessment of these specimens is essential for successful patient management. With increased usage of prostheses, immunosuppressive therapy, and long-term care of individuals with chronic conditions, the likelihood of infection with commensal skin flora has increased.

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

SCOPE/APPLICABILITY:
This standard operating procedure applies to Medical Laboratory Technologists (MLTs) processing specimens for body fluid culture.

SAMPLE INFORMATION:
Common types of body fluids submitted for culture:

Fluid	Synonym	Location
Pleural	• Empyema • Thoracentesis	Fluid within the membrane surrounding the lungs and the chest wall
Peritoneal	• Abdominal • Ascites • Paracentesis	Fluid within the membrane lining the abdominal cavity
Joint	• Synovial • Bursa fluid • Prosthetic joint	Fluid at the union of two bones

Pericardial		Fluid within the membrane lining of the cavity of the heart
Cul-de-sac	• Culdocentesis	Fluid within the pouch between the wall of the rectum and the wall of the uterus
Amniotic	• Amniocentesis	Fluid within the membrane of the fetus
Other Fluids	Infection of normally sterile body fluids may result in severe morbidity and mortality. Any organism isolated must be considered significant. Specimens include: tympanocentesis fluid, intraocular fluid, hydrocele fluid, cyst fluid, etc.	

NOTE:

- Refer to MIC34300-Blood Products Culture for blood products

SAMPLE INFORMATION:

Special Precautions	Refer to MIC40100-Suspect High Risk Organism Workup
Type	• Fluid should be collected in a sterile specimen container or tube and/or into blood culture bottles • If fluid is received in blood culture bottles, order as CXFBC, fluid in blood culture bottle • If swab is received, add Specimen Quality comment **SWBFL**
Source	Refer to chart on pages 1 and 2
Stability	Transport to the laboratory immediately
Storage Requirements	If a delay in processing is anticipated, hold specimens at room temperature, do **NOT** refrigerate
Criteria for rejection	1. Insufficient volume for tests requested: contact the physician to prioritize requests 2. Leaking specimens should be processed, but alert the physician of the possibility of contamination 3. Improperly collected, labeled, transported, or handled specimens should be processed. SCM40110-Waiver of Responsibility form needs to be filled out by the responsible nurse 4. Specimens received in the laboratory in a syringe with the needle still attached will be rejected. In addition, an RL6 will be filed outlining the hazard. Refer to SCM40100 - Specimen Acceptance and Rejection Policy 5. If only blood culture bottles are received, a gram stain cannot be performed

REAGENTS and/or MEDIA:

- Blood agar (BA), Chocolate agar (CHO), MacConkey agar (MAC), Brucella agar (BRU) and Thioglycollate broth (THIO)
- Identification reagents: catalase, oxidase, Staph latex test, Strep latex test, etc.

SUPPLIES:

- Sterile red top vacutainer tube
- Disposable inoculation needles
- Microscope slides
- Anaerobic jar and pouch
- Wooden stick

EQUIPMENT:

- 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:

<table>
<tr><th>Step</th><th colspan="2">Action</th></tr>
<tr><td colspan="3">Processing specimens for body fluid culture</td></tr>
<tr><td rowspan="2">1</td><td>>1 mL received</td><td>• Centrifuge specimen at 3500 rpm for 10 minutes
• Transfer supernatant to labeled red top tube</td></tr>
<tr><td><1 mL received</td><td>• Do not centrifuge
• Add specimen quality comment NOSPI</td></tr>
<tr><td>2</td><td colspan="2">In the biosafety cabinet, using a sterile pipette:
• Place 1 to 2 drops of sediment or mixed fluid onto BA, CHO, MAC and BRU. Add 2 to 5 drops into THIO broth
• Streak for isolated growth using a disposable inoculation needle
• Prepare smear by placing 1 to 2 drops of fluid on a clean microscope slide and spread out with an inoculation needle to form a thin smear</td></tr>
</table>

3	Incubate all media: • Place BA and CHO in the CO_2 incubator • Place specimen, supernatant tube, and MAC in the O_2 incubator • Label THIO with day 2 date and day 5 date and place in the THIO rack in the O_2 incubator • Place BRU in anaerobic jar with anaerobic pouch and indicator as soon as possible after inoculation. Label jar with day 2 date and place in the O_2 incubator **NOTE:** If specimen is from the neck or above, label BRU and THIO with day 10 date **NOTE:** Anaerobes should not be exposed to air for 42 to 48 hours after inoculation
4	Allow smear to dry and perform gram stain. Gram stain must be read before culture plates. Refer to MIC20115-Gram Stain Procedure.
5	Interpret body fluid 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, a Microbiology Technologist may be called in if the ordering physician determines the stain must be read immediately.
6	Immediately phone positive fluid gram stain results to ordering location and document in the LIS.

Probable Pathogens^

GNB Aerobic:
- *Aeromonas* spp.
- *Brucella* spp.*+
- *Chromobacterium* spp.
- *Eikenella corrodens*
- *Pasteurella multocida*
- *Pseudomonas aeruginosa*
- *Salmonella* spp.
- *Shigella* spp.
- *Sphingobacterium* spp.
- *Vibrio* spp.
- *Yersinia* spp.

GNB Anaerobic:
- *Bacteroides fragilis*
- *Capnocytophaga* spp.

GNC/CB Aerobic:
- *Francisella tularensis*+
- *Haemophilus influenzae*
- *Kingella kingae*
- *Moraxella catarrhalis*
- *Neisseria gonorrhoeae*
- *Neisseria meningitidis*+

GPC Aerobic:
- β-hemolytic *Streptococci*
- *Staphylococcus aureus*
- *Streptococcus anginosis* grp.
- *Streptococcus pneumoniae*

GPB Aerobic:
- *Bacillus anthracis*+
- *Bacillus cereus*
- *Erysipelothrix* spp.
- *Listeria* spp.
- *Nocardia* spp.

GPB Anaerobic:
- *Actinomyces* spp.
- *Arcanobacterium* spp.
- *Clostridium perfringens*

Others:
- *Candida* spp.
- Molds

Potential Pathogens^	
• *Aggregatibacter* spp. • Anaerobes not listed above • *Bacillus* spp. not listed above • Coagulase-negative *Staphylococci* • *Corynebacterium* spp. • Enteric GNB not listed above • *Enterococcus* spp.	• *Haemophilus* spp. • *Lactobacillus* spp. • *Micrococcus* spp. • *Moraxella* spp. • Non-enteric GNB not listed above • *Staphylococcus* spp. not listed above

* Risk group 3 organisms. If suspected, refer to MIC40100-Suspect High Risk Organism Workup
+ All work-up should be performed in the BSC
^ For organisms not listed, consult the Microbiology Technical Supervisor, or refer to the *Manual of Clinical Microbiology*

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, 48 hours, and 72 hours • Observe MAC plate at 24 hours and 48 hours
3	• Observe BRU and THIO after 48 hours • Re-incubate BRU and THIO for an additional 72 hours • If anaerobic growth is suspected, perform gram stain: ➢ If gram stain resembles growth on aerobic plates, further workup is not indicated ➢ If growth does not resemble growth on aerobic plates, perform aerotolerance test. Refer to MIC53700-Aerotolerance Test • If specimen is from the neck or above, re-incubate BRU and THIO for a total of 10 days
4	**If there are ≥3 organisms growing on any media:** • Consult APL microbiologist
5	**If there are 1 to 3 organisms growing on >1 media:** • <u>If organism is a probable pathogen</u>: ➢ Perform and report full identification ➢ Perform and report susceptibility testing as per ASTM • <u>If organism is a potential pathogen</u>: ➢ Perform and report full identification ➢ Perform and report susceptibility testing if ANY of the following are true: o Organism is intracellular in direct smear o Organism is predominant in direct smear o Organism is pure on culture o Multiple or previous cultures are positive for the same organism

6	**If there are 1 to 3 aerobic organisms growing in THIO broth only:** • <u>If organism is a probable pathogen</u>: ➢ Perform and report full identification ➢ Perform and report susceptibility testing as per ASTM • <u>If organism is a potential pathogen</u>: ➢ Perform and report full identification ➢ Perform and report susceptibility testing if ANY of the following are true: o Organism is intracellular in direct smear o Organism is predominant in direct smear o Organism is pure on culture o Multiple or previous cultures are positive for the same organism
7	**If there are 1 to 3 anaerobic organisms growing in THIO broth only:** • <u>If organism is pure growth</u>: ➢ Perform and report full identification ➢ Refer to APL for susceptibility testing if ANY of the following are true: o Organism is a pathogen o Organism is intracellular in direct smear o Multiple or previous cultures are positive for the same organism • <u>If there are ≥2 anaerobic organisms</u>: ➢ Perform and report full identification ➢ Consult APL microbiologist regarding susceptibility testing if ANY of the following are true: o Organisms are pathogens o Organisms are intracellular in direct smear o Organisms are pure or predominant in direct smear o Multiple or previous cultures are positive for the same organisms
8	**If there are 1 to 3 organisms growing on 1 solid medium only:** • <u>If organism is present in the direct smear</u>: ➢ If organism is a probable pathogen: o Perform and report full identification o Consult APL microbiologist regarding susceptibility testing ➢ If organism is a potential pathogen: o Perform and report full identification o Consult APL microbiologist regarding susceptibility testing • <u>If organism is not present in the direct smear (possible lab contaminant)</u>: ➢ Report culture as "No growth" if ALL the following are true: o Organism is not a probable pathogen or potential pathogen o Organism colony distribution is suggestive of contaminant o No current or previous cultures are positive for the same organism ➢ Consult APL microbiologist if ANY of the following are true: o Organism is a probable pathogen or potential pathogen o Colonies are on the streak line or inoculum o Multiple or previous cultures are positive for the same organism

REPORTING INSTRUCTIONS:

IF	REPORT
No growth after 1 day	**PRELIM:** • Report: **"No growth after 1 Day"** • Report: **"Further report to follow"**
No aerobic or anaerobic growth at 3 days	**INTERIM:** • Report: **"No aerobic growth at 3 days"** • Report: **"@Anaerobic culture to follow"**
Aerobic growth at 2 or 3 days and no anaerobic growth	**INTERIM:** • Report aerobic growth as per procedure • Report: **"@Anaerobic culture to follow"**
No anaerobic growth after 5 days	**FINAL:** • Report: **"No anaerobes isolated after 5 days"**
No anaerobic growth after 5 days and specimen source is neck or above	**FINAL:** • Report: **"No anaerobes isolated after 5 days"** • Add test comment **}AC10**
Growth of probable 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 potential pathogen	• Report organisms' full identification • List quantitation as **"Isolated"** • Report susceptibility as per interpretation of results • Freeze isolate and log into stored isolates log
Growth of probable pathogen in THIO broth only	• Report organisms' full identification • List quantitation as **"Isolated from Enrichment Broth"** • Report susceptibility results as per ASTM • Freeze isolate and log into stored isolates log
Growth of potential pathogen in THIO broth only	• Report organisms' full identification • List quantitation as **"Isolated from Enrichment Broth"** • Report susceptibility as per interpretation of results • Freeze isolate and log into stored isolates log
Growth of anaerobes in THIO broth only	• Report organisms' full identification • List quantitation as **"Isolated from Enrichment Broth"** • Refer to APL for susceptibility testing as per interpretation of results • 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 body fluid 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. False-positive cultures can result from contamination of the specimen with skin flora.
2. False-negative results can be caused by low numbers of organisms, prior antimicrobial treatment, or the fastidious nature of the infective organism.
3. Body fluid swabs are not ideal specimens and should be noted in the specimen quality section of order entry.

CROSS-REFERENCES:

- MIC20115-Gram Stain Procedure
- MIC34300-Blood Products Culture for blood products
- 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
- MIC53700-Aerotolerance Test
- SCM40110-Waiver of Responsibility
- LQM70620-Laboratory Critical Results List-Microbiology

REFERENCES:

1. Clinical Microbiology Procedures Handbook, 4th edition, ASM Press, 2016
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, ASM Press, Washington, D.C.
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	12 Apr 17	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