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):	
Effective Date:	Next Review Date:
Issuing Authority: Director of Health Services	Date Approved:
Accreditation Canada Applicable Standard:	

GUIDING PRINCIPLE:
Infection of normally sterile body fluids often results in severe morbidity and mortality. Rapid and accurate microbiological assessment of these specimens is essential for successful patient management. Any microorganism found in a normally sterile site must be considered significant, and all isolates must be reported. 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:
To determine the presence or absence of bacterial pathogens in body fluid specimens.

SCOPE/APPLICABILITY:
This procedure applies to Medical Laboratory Technologists processing specimens for body fluid culture.

SAMPLE INFORMATION:
Commonly submitted 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 • Arthrocentesis • 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
- Refer tissue or biopsy specimens for culture to *Dyna*LIFE

SAMPLE INFORMATION:

Special Precautions	Refer to Policy 17-02-V1: Specimens Containing Suspected Risk Group 3 Pathogens
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 Blood Culture-Fluid and process as blood culture • If swab is received, add Specimen Quality comment **SWBFL**
Source	Refer to chart on page 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 potential infectious materials or cultures.

- Ensure that appropriate hang 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:

Step	Action	
Processing specimens for body fluid culture		
1	>1 mL received	• Centrifuge specimen at 3500 rpm for 10 minutes • Transfer supernatant to labeled red top tube
	<1 mL received	• Do not centrifuge
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	

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 **NOTE:** If specimen is from the neck or above, label with day 10 date • 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:** 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, Microbiology Technologist may be called in if 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		
• *Actinomyces* spp. • *Arcanobacterium* • *Aeromonas* • *Bacillus anthracis**+ • *Bacteriodes fragalis* • β-hemolytic strep. • *Brucella* spp.*+ • *Campylobacter* • *Candida* spp. • *Capnocytophaga* spp. • *Eikenella corrodens*	• Enterobacteriacea • *Erysipelothrix* • *Francisella**+ • Molds • *H.influenzae* • *Helicobacter* • *Kingella kingae* • *Listeria* spp. • *Moraxella catarrhalis* • *N.gonorrhoeae* • *N.meningitides**+	• *Nocardia* spp. • *Pasteurella multocida* • *Pseudomonas aeruginosa* • *Staphylococcus aureus* • *S.intermedius* • *S.lugdunensis* • *Streptococcus anginosis* grp. • *S.pneumoniae* • *Vibrio* spp.
Potential Pathogens		
• *Aggregatibacter* spp. • Anaerobes other than *Bacteriodes fragilis* • *Bacillus* spp. • Coagulase-negative *Staphylococcus*	• *Corynebacterium* spp. • *Enterococcus* spp. • *Haemophilus* spp. • *Lactobacillus* spp. • *Micrococcus* spp. • *Moraxella* spp.	• Gram-negative, non-fermenters other than *P.aeruginosa* • *Staphylococcus* spp. other than those listed as "pathogens"

* Risk group 3 organism. If suspected, refer to 17-02-V1: Specimens Containing Suspected Risk Group 3 Pathogens

\+ All work-up should be performed in the BSC

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 **NOTE:** If specimen is from the neck or above, re-incubate BRU and THIO for a total of 10 days. Observe plates and broth at days 5, 8 and 10
4	**If there are ≥3 organism growing on any media:** • Consult *Dyna*LIFE microbiologist
5	**If there are 1 to 3 organisms growing on >1 media:** • <u>If organism(s) is a pathogen</u>: ➢ Perform identification and susceptibility testing • <u>If organism(s) is a potential pathogen</u>: ➢ Perform identification and susceptibility testing if ANY of the following are true: ○ Organism is intracellular in direct smear ○ Organism is pure in direct smear ○ Organism is predominant in direct smear ○ Organism pure on culture ○ Multiple or previous cultures are positive for the same organism(s) ➢ If NONE of the above is true, perform identification and list
6	**If there are 1 to 3 organisms growing on 1 medium only, THIO broth:** • <u>If organism(s) is aerobic</u>: ➢ Perform identification and susceptibility testing if ANY of the following are true: ○ Organism is intracellular in direct smear ○ Organism is pure in direct smear ○ Organism is predominant in direct smear ○ Organism pure on culture ○ Multiple or previous cultures are positive for the same organism(s) ➢ If NONE of the above is true, perform identification and list

7	• <u>If organism is pure growth and anaerobic</u>: ➢ Perform identification and refer to *Dyna*LIFE for susceptibility testing if ANY of the following are true: ○ Organism is a pathogen ○ Organism is intracellular in direct smear ○ Organism is pure or predominant in direct smear ○ Multiple or previous cultures are positive for the same organism ➢ If NONE of the above are true, perform identification and list • <u>If there are ≥2 anaerobic organisms</u>: ➢ Perform identification and consult *Dyna*LIFE microbiologist regarding susceptibility testing if ANY of the following are true: ○ Organisms are a pathogens ○ Organisms are intracellular in direct smear ○ Organisms are pure or predominant in direct smear ○ Multiple or previous cultures are positive for the same organism ➢ If NONE of the above are true, perform identification and list
8	**If there are 1 to 3 organisms growing on 1 solid medium only (THIO clear):** • <u>If organism(s) is present in the direct smear</u>: ➢ Perform identification and list organism(s) ➢ Consult *Dyna*LIFE microbiologist regarding susceptibility testing • <u>If organism(s) is not present in the direct smear (possible lab contaminant)</u>: ➢ Report culture as "No growth" if ALL the following are true: ○ Organism(s) is not a pathogen or potential pathogen ○ Organism(s) colony distribution if suggestive of contaminant ○ No current or previous cultures are positive for the same organism(s) ➢ Consult *Dyna*LIFE microbiologist if ANY of the following are true: ○ Organism(s) is a pathogen or potential pathogen ○ Colonies are on the streak line or inoculum ○ Multiple or previous cultures are positive for the same organism(s)

REPORTING INSTRUCTIONS:

IF	REPORT
No growth after 1 day	**PRELIM:** • Report: "**No Growth after 1 Day. Further report to follow"**
No aerobic growth after 3 days	**INTERIM:** • Report: **"No growth aerobically after 3 days"** • 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	**FINAL:** • Report: **"No anaerobes isolated after 5 days"** • Add test comment **}AC10**

Growth of pathogen(s)	• Report organism(s) identification • List quantitation as **"Isolated"** • Report susceptibility results as per ASTM • Freeze isolate(s) and log into stored isolates log
Growth of potential pathogen(s)	• Report organisms(s) identification • List quantitation as **"Isolated"** • Report susceptibility as per microbiologist • Freeze isolate(s) and log into stored isolates log
Growth of significant organism(s) in THIO broth only	• Report organism(s) identification • List quantitation as **"Isolated from Enrichment Broth"** • Report susceptibility as per interpretation of results • Freeze isolate(s) and log into stored isolates log
Growth of non-significant organism(s) in THIO broth only	• Report organism(s) identification • List quantitation as **"Isolated from Enrichment Broth"** • Add isolate comment **&THIO** • Freeze isolate(s) and log into stored isolates log
H. influenzae or N.meningitidis isolated	• Must be sent immediately to Alberta Precision Laboratories for typing • Freeze isolate(s) 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 • Freeze isolate(s) 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 isolates to APL
- Refer to MIC36400-Referral of Category B Specimens to DL for sending isolates to *Dyna*LIFE
- Refer to MIC36500-Referral of Category B Specimens to NML for sending isolates to NML

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:

- 17-02-V1: Specimens Containing Suspected Risk Group 3 Pathogens
- LQM70620-Laboratory Critical Results List-Microbiology
- 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
- MIC36400-Referral of Category B Specimens to *Dyna*LIFE
- MIC36500-Referral of Category B Specimens to *NML*
- MIC53700-Aerotolerance Test
- SCM40100-Specimen Acceptance and Rejection Policy
- SCM40110-Waiver of Responsibility

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	30 Nov 18	Updated to include new Vitek 2 instrument	L. Steven
3.0	11 Jan 21	Procedure reviewed and added to NTHSSA policy template	L. Steven