PROGRAM Standard Operating Procedure – Laboratory Services	
Title: MIC34200 – CSF 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:
Bacterial meningitis is the result of infection of the meninges (lining around the brain). Specimens include central nervous system shunt fluid, external ventricular drainage fluid and cerebro-spinal fluid (CSF). The examination of CSF from patients suspected of having meningitis is always considered a STAT procedure.

PURPOSE/RATIONALE:
To determine the presence or absence of bacterial pathogens in CSF specimens.

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

SAMPLE INFORMATION:

Special Precautions	Refer to Policy 17-02-V1: Specimens Containing Suspected Risk Group 3 Pathogens
Type	• CSF collected into clean, sterile, leak-proof tube
Source	• Central nervous system shunt fluid • Fluid from Ommaya reservoirs • External ventricular drainage fluid • CSF from lumbar puncture
Volume	• Generally, 1 to 3 mL of CSF is required for the bacterial culture • If viral, fungal or mycobacterial testing is required, then at least 3 to 4 mL should be sent for referral
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. Waiver of responsibility form SCM40110 needs to be filled out by the responsible nurse

REAGENTS and/or MEDIA:

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

SUPPLIES:

- Sterile red top vacutainer tube
- Disposable inoculation needles
- Alcohol pads
- Ringed cytology microscope slides
- Wooden sticks

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 CSF 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 CSF onto BA, CHO and MAC • Streak for isolated growth using a disposable inoculation needle • Prepare smear by placing 1 to 2 drops of CSF onto a clean ringed cytology microscope slide and allow the drop(s) to form one large drop **NOTE:** Shunt fluids are also planted to THIO broth and held for 14 days	
3	Incubate all media: • Place BA and CHO in the CO_2 incubator • Place specimen collection tube, supernatant tube, MAC and THIO (if applicable) in the O_2 incubator	
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 CSF smear 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 CSF gram stain results to ordering location and document in the LIS.
7	Positive CSF gram stain results need to be copied to OCPHO (HPU1) and Stanton Infection Prevention and Control (SIPAC) if ER or In-patient.

Common bacterial causes of acute meningitis by age group	
Neonate	• *Esherichia coli* • *Streptococcus agalactiae* • *Listeria monocytogenes*
Infants/Children	• *Streptococcus agalactiae* • *Haemophilus influenzae* • *Streptococcus pneumoniae* • *Neisseria meningitides**+
Adolescents and Young Adults	• *Streptococcus pneumoniae* • *Neisseria meningitidis**+
Older Adults	• *Streptococcus pneumoniae* • *Neisseria meningitides**+ • *Haemophilus influenzae* • *Streptococcus agalactiae* • *Listeria monocytogenes*
Ventriculoperitoneal Shunt Infections	• Coagulase-negative *Staphylococcus* • *Staphylococcus aureus* • *Streptococcus* spp. • Gram-negative bacilli • *Candida albicans* • *Corynebacterium* spp. • *Propionibacterium acnes*

* Risk group 3 organism. If suspected, refer to Policy B-0160: "Specimens Containing Suspected Risk Group 3 Pathogens" for Primary Specimen Handling Flow Chart

\+ 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 • May need to inoculate special selective media • 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 • Observe THIO on day 2, 5, 10 and 14 if applicable

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. If a preliminary identification cannot be made after 24 hours, release a preliminary culture report using the gram stain morphology.

REPORTING RESULTS:

IF	REPORT
No growth after 1 day	**PRELIM:** • Report: **"No Growth after 1 Day. Further report to follow"**
No growth after 3 days	**FINAL:** • Report: **"No growth aerobically after 3 days"**
Shunt fluid: No growth after 3 days	**INTERIM:** • Report: **"No growth aerobically after 3 days"**
Shunt fluid: No growth after 14 days	**FINAL:** • Report: **"No growth anaerobically after 14 days"**
Growth of organism	• Report organism identification • List quantitation as **"Isolated"** • Report susceptibility results as per ASTM • Freeze isolate(s) and log into stored isolates log
S.pyogenes, *H.influenzae* or *N.meningitidis* isolated	• In order entry, copy report to OCPHO (HPU1) and Stanton Infection Prevention and Control (SIPAC) if ER or In-patient
H. influenzae or N.meningitidis isolated	• Must be sent immediately to Alberta Precision Laboratories for typing • Add test ?REFE and finalize with "." • Freeze organism and record in patient isolate 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 specimens must be sent to NML for International Circumpolar Surveillance (ICS) program • Add test ?REFN and finalize with "." • Freeze organism and record in patient isolate log

NOTE:

- Refer to Reportable Diseases – Public Health Act as of September 2009 for reporting to OCPHO (HPU1)
- Refer to 17-02-V1: Specimens Containing Suspected Risk Group 3 Pathogens for specimens containing risk group 3 pathogens
- Refer to 15-10-V1 Laboratory Critical Results Procedure for results that need to be phoned to ordering location
- Refer to MIC10510-Referral of Category B Specimens to *Dyna*LIFE and Alberta Precision Laboratories for sending isolates to *Dyna*LIFE and APL
- Refer to MIC10520-Referral of Category B Specimens to NML for sending isolates to NML
- Refer to MIC35100-Nosocomial Infection Notification Job Aid to determine if organism needs to be copied to Stanton Infection Prevention and Control

LIMITATIONS:

1. A positive culture generally indicates infection with the organism.
2. Lack of pus cells in CSF does not rule out infection, especially in Listeriosis.
3. The most common cause of community acquired bacterial meningitis is Streptococcus pneumoniae.
4. Direct bacterial antigen testing is not recommended.
5. Since THIO is mainly a broth for anaerobes and does not support the growth of the most common pathogens in CSF, it is not recommended for routine CSF culture but should be used when Shunt Infection is suspected.

CROSS-REFERENCES:

- MIC10510-Referral of Category B Specimens to *Dyna*LIFE and Alberta Precision Laboratories
- MIC10520-Referral of Category B Specimens to NML
- MIC20115-Gram Stain Procedure
- MIC35000-Reportable Diseases Notification
- MIC35100-Nosocomial Infection Notification Job Aid

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	11 Jan 17	Initial Release	L. Steven
2.0	04 Dec 18	Updated to include new Vitek 2 instrument	L. Steven
3.0	30 Jan 21	Procedure reviewed and added to NTHSSA policy template	L. Steven