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

SAMPLE INFORMATION:

Special Precautions	Refer to Policy B-0160: Specimens Containing Suspected Risk Group 3 Pathogens for Primary Specimen Handling Flow Chart
Type	CSF collected into clean, sterile, leak-proof centrifuge tubes to be transported to the laboratory immediately.
Source	• Central nervous system shunt fluid • Fluid from Ommaya reservoirs • External ventricular drainage fluid • CSF from lumbar puncture
Volume	Generally, 1-3 mL of CSF is required for the Microbiology Laboratory. If viral, fungal or mycobacterial testing is required, then at least 3-4 mL should be sent.
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:

- Disposable inoculation needles
- Ringed cytology microscope slides
- Biosafety cabinet
- Sterile red top vacutainer tube
- 35° ambient air and 37° CO_2 incubators
- Wooden sticks
- 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 Test Manual for reagent quality control procedures.

COMMON BACTERIAL CAUSES OF ACUTE MENINGITIS:

Age Group	Organism(s)
Neonate	*E.coli, S.agalactiae, Listeria monocytogenes*
Infants and Children	*S.agalactiae, H.influenzae, S.pneumoniae, N.meningitidis*
Adolescents and Young Adults	*S.pneumoniae, N.meningitidis*
Older Adults	*N.meningitidis, S.pneumoniae, H.influenzae, S.agalactiae, L.monocytogenes*
Ventriculoperitoneal Shunt Infections	CNS, *S.aureus, Streptococcus* spp., Gram-negative bacilli, *Candida albicans, Corynebacterium* spp., *Propionibacterium acnes*

PROCEDURE INSTRUCTIONS:

Step	Action
Processing CSF Tube #4	
1	Do not centrifuge, regardless of amount received.
2	Using aseptic technique, transfer entire specimen into a labelled, sterile red top tube. Para film for transport.
3	Forward specimen to the Provincial Laboratory for any requested viral tests as per Prov. Lab protocol. If transport time will be > 24 hours, freeze at -70 for transport on dry ice.
4	If no viral testing is ordered, then freeze specimen in the -70 freezer.

Step	Action	
Processing CSF Tube #2		
1	> 1 mL received	• Centrifuge specimen at 3500 rpm for 10 minutes in the core laboratory. • Transfer supernatant to labeled red top tube.
	< 1 mL received	• Do not centrifuge.
2	In the biosafety cabinet, using a sterile pipette: • Aspirate the sediment from the bottom of the collection tube. • Place 1 - 2 drops each onto BA, CHO and MAC. • Streak for isolated growth using a disposable inoculation needle. Streak out to cover the whole plate. • Prepare smear by placing 1 or 2 drops of CSF onto a ringed cytology microscope slide. Allow the drop(s) to form one large drop. Clean slide with alcohol swab prior to inoculation. • Shunt fluids should be also planted to THIO broth and held for 14 days.	
3	Place specimen collection tube, supernatant tube and MAC in the O_2 incubator. Place BA and CHO plates in the CO_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 stains 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 results of any positive stain results for microorganisms to ordering location and document the conversation within the LIS. Positive gram stain results need to be copied to Chief Medical Officer of Health (HPU1) and Infection Control (SOHS) if inpatient.	
7	Examine plates after 24 hour incubation. Record observations in the LIS.	
8	Re-incubate CO_2 plate(s) for an additional 24 hours. Discard O_2 plate.	
9	At 48 hours, examine plates and record observations in the LIS.	
10	At 72 hours, examine plates and record observations in the LIS.	
11	If applicable, examine THIO on day 2, day 5, day 10 and day 14 for growth. Record observations in the LIS.	

INTERPRETATION OF RESULTS:

Step	Action
1	Confirm gram stain has been read prior to reading culture plates. Ensure growth on culture media correlates with gram stain results. If discordant results are found: • Re-examine smear and culture plates. • Check for anaerobic growth. • Re-incubate culture to resolve. • May need to inoculate special selective media. • Consider re-smearing or re-planting specimen to exclude the possibility of error.
2	If the Gram-stained smear is positive and there is no growth on the plates, re-incubate CO_2 plates for total of 7 days.
3	Observe plates at 24 hours, 48 hours and 72 hours for growth. Record all observations in the LIS.
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.

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"** • Report: **"No anaerobes isolated"** if specimen is from a shunt and THIO was processed**.**
Growth of organism	• Report organism identification under the isolates tab. • List quantitation. • Report susceptibility results as per ASTM. • Refer to Reportable Diseases – Public Health Act as of September 2009 for reporting to HPU1. • Refer to MIC35100 – Nosocomial Infection Notification Job Aid to determine if organism needs to be copied to Infection Control. • Refer to L-0910-Laboratory: Critical Values for results that need to be phoned to ordering location.
S.pyogenes, *H.influenzae* or *N.meningitidis* isolated	• Must to be phoned to the Chief Medical Officer of Health (HPU1) as per MIC35000 - Reportable Diseases Notification. • In Order Entry; copy report to Chief Medical Officer of Health (HPU1).
H. influenzae or N.meningitidis isolated	• Any CSF specimen positive for *H.influenzae* or *N.meningitidis* must be sent immediately to Provincial Lab for typing as soon as identification is confirmed. 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 MIC10510.
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 CSF specimens must be sent to NML for International Circumpolar Surveillance (ICS) program. Refer to MIC10520.

CULTURE NOTES:

- A positive culture generally indicates infection with the organism.
- Lack of pus cells in CSF does not rule out infection, especially in Listeriosis.
- The most common cause of community acquired bacterial meningitis is Streptococcus pneumoniae.
- Direct bacterial antigen testing is not recommended.
- 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.

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.

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	11 Jan 2017	Initial Release	L. Steven
2.0	30 Nov 2018	Updated to include new Vitek 2 instrument; Changed logo. Updated to include reporting if THIO processed.	L. Steven