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 and follow up action	• Insufficient volume for tests requested: contact the physician to prioritize requests. • Leaking specimens should be processed, but alert the physician of the possibility of contamination. • Improperly collected, labeled, transported or handled specimens should be processed. Waver of responsibility form SCM40110 needs to be filled out by the responsible nurse.

REAGENTS and/or MEDIA:

- Blood Agar, Chocolate Agar and MacConkey Agar , THIO broth
- Identification reagents: catalase, rapid staph, tube coagulase, oxidase, spot indole, etc.

SUPPLIES:

- Wooden applicator sticks
- Disposable inoculation needles
- Biosafety cabinet
- Microscope slides
- 35° ambient air and 37° CO_2 incubators
- Vitek 2 Compact and supplies

SPECIAL SAFETY PRECAUTIONS:

Containment Level 2 facilities, equipment, and operational practices for work involving infectious or potentially 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 where there is a known or potential risk of exposure to 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.

QUALITY CONTROL:

Refer to Quality Control 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:

Follow the steps in the table below to process CSF TUBE #4:

Step	Action
Processing 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 hrs. freeze at -70 for transport on dry ice.
4	If no viral testing is ordered, then freeze specimen in the -70 freezer.

Follow the steps in the table below to process CSF TUBE #2

Step	Action	
Processing Tube #2		
1	If >1 mL received	• Centrifuge tube #2 at 3500 rpm for 10 minutes. • Aseptically transfer the supernatant to a labeled sterile glass tube and set aside. • Label 2 sterile ringed slides and plates with LIS plate labels.
	If < 1 mL received	• Do not centrifuge • Label 2 sterile ringed slides and plates with LIS plate labels.
2	In the biosafety cabinet, using a sterile pipette: • Aspirate fluid from the bottom of the collection tube. • Place 1 - 2 drops each onto BAP, CHOC and MAC. Streak for isolated growth using a disposable inoculation needle. Streak out to cover the whole plate.  • Prepare smears by placing 1 or 2 drops of CSF on microscope slides. Allow the drop(s) to form one large drop. Do not spread the fluid. • Shunt fluids should be also planted to THIO broth and held for 14 days.	
3	Place the remaining sample sediment, supernatant tube, and MAC plate in the O_2 incubator.	
4	Place BAP and CHOC plates in the CO_2 incubator in the designated tray.	
5	Allow smears to dry and perform Acridine Orange and Gram Stain. (refer to MIC20100 and MIC20115 for procedure and interpretation of stains)	
6	Interpret CSF stains immediately. During the regular Microbiology lab hours of 07:00 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.	
7	Immediately phone results of any positive stain results for microorganisms and document the conversation within the LIS.	
8	Send a pending report for all gram stains.	

INTERPRETATION OF RESULTS:

Step	Action
Interpretation of cultures	
1	Examine plates after 24h incubation. Record your observations in the LIS. Preliminary report: "~No Growth After 1 Day. Further report to follow"
2	If no growth is observed, reincubate and read plates for up to 3 days (minimum incubation time is full 72h). If the Gram-stained smear is positive and there is no growth on the plates, re-incubate plates for total of 7 days.
3	Identify and perform susceptibility testing as per Dynalife ASTM
4	Freeze organism in glycerol and record in patient isolate log.
5	A copy of all positive reports must be sent to Chief Medical Officer of Health (HPU1).
5	A copy of all positive reports on inpatients must be sent to Infection Control (SOHS).
7	Any positive CSF for Group A Streptococcus or Streptococcus pneumoniae must be sent to Streptococcus Unit at NML Winnipeg for surveillance testing.
8	Any positive CSF for Haemophilus influenza or Neisseria meningitis must be sent immediately to the Provincial Lab Edmonton 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. Provincial Lab will then forward the specimen to NML for surveillance testing.

PROCEDURE NOTES:

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.

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