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**PRINCIPLE**

Identification of bacterial meningitis is one of the most important functions of the diagnostic microbiology laboratory because acute meningitis is life-threatening. CSF from a patient suspected of meningitis is an emergency specimen that requires immediate processing to determine the etiologic agent. CSF is obtained by transcutaneous aspiration, and therefore, all organisms recovered from the culture are potential pathogens and must be reported to the physician immediately.

Because the number of organisms in the CSF can be as low as 103 CFU/ml, concentration of the Gram stain by cytocentrifugation is important for rapid diagnosis. Cytospin concentration can increase the sensitivity up to 100-fold compared with both uncentrifuged and conventional centrifuged fluid. Aerobic bacteria commonly cause bacterial meningitis, but anaerobes may be present in CSF when a meningeal abscess or a similar infectious process is adjacent to the meninges. These include traumatic head injury or prostheses, such as metal cranial plates and shunt drains. Inoculation of anaerobic media is not recommended for diagnosis of community-acquired meningitis. For shunt infections a backup broth that will grow anaerobes and aerobes in low numbers is recommended.

Direct antigen testing is not recommeneded and not performed at this institution because the cost-benefit is low and the sensitivity of testing for certain serogroups of *Neisseria meningitidis* is poor. In a study of 103 episodes of meningitis, antigen tests had a sensitivity of 9% for Gram stain-negative specimens, with a 33% sensitivity overall.

PCR is becoming the method of choice for rapid, sensitive diagnosis of meningitis, especially for organisms that are present in low numbers or for organisms that are difficult to grow, particularly when the patient is partially treated with antimicrobial agents, e.g., *Borrelia, Ehrlichia, Mycobacterium tuberculosis, Mycoplasma,*and *Streptococcus pneumoniae*). Unfortunately, many of these tests are not available commercially.

**Specimen collection**

**NOTE:** This is a medical procedure that is performed by a physician guided by appropriate precautions.

1. Lumbar puncture
	1. Disinfect the puncture site with antiseptic solution and alcohol in a manner identical to phebotomy skin preparation for blood culture to prevent specimen contamination and introduction of infection.
	2. Insert a needle with stylet at the L3-L4, L4-L5, or L5-S1 interspace. When the subarachnoid space is reached, remove the stylet; spinal fluid will appear in the needle hub.
	3. Measure the hydrostatic pressure with a manometer.
	4. Sequentially collect the CSF into up to five calibrated sterile tubes labeled no. 1 to no. 5.
	5. Physicians should be instructed to sequentially collect 2.0 ml of CSF into three sterile calibrated tubes if only routine chemistry (total protein and glucose), bacteriology (C&S), and hematology (cell count) are required.
	6. Table 3.7–2 outlines the collection and test ordering guidelines that should be used by physicians who collect CSF samples from adults, children, and neonates. The laboratory should include specific collection guidelines for CSF samples in its guide to services, including the minimal volume of samples required to perform specific tests, including those that will be referred out. Up to five sequentially collected sterile calibrated tubes should be collected depending on the extent of CSF testing required. Physicians should be instructed to collect CSF into a fifth tube if a portion of the sample needs to be sent to a reference laboratory for specialized microbiology tests.
2. Ventricular shunt fluid
	1. Clean the reservoir site with antiseptic solution and alcohol prior to removal of fluid to prevent introduction of infection.
	2. Remove fluid by aspiration of CSF from the Ommaya reservoir or by collection from the ventricular drain or shunt. Sequentially collect CSF into a minimum of three sterile calibrated tubes if only routine chemistry (total protein and glucose) (tube no. 1), bacteriology (C&S) (tube no. 2), and hematology (cell count) (tube no. 3) are required.
3. An initial CSF sample should be collected prior to antimicrobial therapy for highest diagnostic sensitivity. Subsequent CSF samples are then collected every 2 to 3 days once antimicrobial therapy is started to monitor for resolution of the infection.

**Specimen transport**

1. Submit to laboratory as soon as possible.
2. Do not refrigerate.
3. Each sterile calibrated tube containing CSF must be properly labeled with the patient’s name, birthdate, and/or unique identification number, and the date and time of collection as well as the method of collection.
4. Limit requests to those reflecting patient’s condition.

**Rejection criteria**

1. Call physician to prioritize requests if there is insufficient volume.
2. Fungal and acid-fast bacillus (AFB) cultures of the CSF are infrequently indicated in acute community-acquired meningitis. Refer to Appendix 3.7–1 for a sample laboratory policy to communicate to caregivers. Since the fungal pathogens in CSF (*Cryptococcus neoformans, Coccidioides immitis,* and *Histoplasma capsulatum*) are best and most rapidly diagnosed by serologic methods or cultures of other sites, fungal CSF culture should be discouraged. However, the fungi that cause CSF disease grow well on the media inoculated for routine culture. For fungal requests, incubate the routine culture plates for a longer period and inoculate a fungal broth (Sabouraud) with a large volume of specimen to increase the yield of *Cryptococcus* and *Coccidioides. M. tuberculosis* is best diagnosed by PCR.
3. Specimens in leaky containers must be processed, but alert the physician of the possibility of contamination.
4. Direct antigen testing is not recommended and is not available for ordering at this institution.

**Media Setup**

Blood Agar Plate

Chocolate Agar (CO2)

Enriched Thio (Shunt collections only)

Gram Stain via Cytospin

**Procedure**

**Inoculation**

1. Process specimen as soon as received in the Biological Safety Cabinet.
2. Verify patient name on both label and requisition.
3. Spin specimen at 1500 G for 15 minutes for culture inoculation.
4. Inoculate media from concentrated specimen.
	1. Using a sterile pipette, aspirate fluid from the bottom of the collection tube.
	2. Place 2 or 3 drops each onto BAP and CHOC. Streak in quadrants.
	3. If greater than 1 ml is available for routine culture from a ventricular source or shunt, inoculate to broth. If large volumes of specimen are received, they usually are from shunts or reservoirs.
5. If a CSF specimen tube appears to be empty or contains only 1 to 5 drops of fluid and if more specimen cannot be obtained sterilely from other laboratory departments, proceed as follows.
	1. If 1 drop is available, use a sterile Pasteur pipette and prepare a smear for Gram stain from a portion of the specimen.
	2. Using a sterile Pasteur pipette, place about 0.5 ml of broth medium into the specimen tube. Recap the tube, and invert it to mix the contents.
	3. Use all the broth to inoculate media and do smear.
	4. Note on report the volume received.

**Gram stain**

1. Place 100ul of sample into a cytospin specimen chamber. Follow procedure for operation of centrifuge from manufacturer.
2. Air dry the slide in a biosafety cabinet or covered on a slide warmer.
3. Fix smear with methanol.
4. Interpret CSF Gram stains immediately.
	1. Any bacteria seen are considered significant. However, confirm low numbers only seen in one or two fields with a second smear. If positive, notify the physician immediately.
5. Save some unspun CSF at 4°C and/or at -20°C for 1 week for PCR or other subsequent requests.

**Incubation**

1. Incubate Chocolate plate at 35 to 37°C in 5% CO2.
2. Incubate TSA BAP plate and broth as applicable at 35 to 37°C in ambient air.

**Culture examination**

1. Examine all plate and broth media for macroscopic evidence of growth at 24 h.
2. If no visible growth is observed on the culture media, reincubate.
	1. Read aerobic plates daily for 3 days.
3. Examine broth media daily for 4 days and hold for 7 days before discarding.
4. Cultures with growth
	1. Notify physician of positive culture findings in accordance with Critical Value Policy.
	2. Identify all organisms by rapid methods as applicable.
		1. Perform Pneumoslide test on all alphahemolytic streptococci to identify *S. pneumoniae.* If positive, report *S. pneumoniae.*
		2. Perform catalase and Gram stain of organisms growing on BAP and/ or CHOC. Identify further according to Gram stain and rapid tests in Table 3.3.2–5. *Listeria* and group B streptococci are significant CSF pathogens.
		3. Perform oxidase test on gram-negative diplococci. If positive and colony is grayish to white, perform commercial kit identification to identify Neisseria meningitides. If positive, report *N. meningitidis.*
		4. For all staphylococci, rule out/in Staph aureus using rapid techniques.
		5. *Generally determine the probable genus and usually the species identification of most CSF pathogens within 2 h of visible growth on the plates.*
	3. Do not perform complete identification or antimicrobial susceptibility testing (AST) if the isolate is clearly a plate contaminant or the isolate is a coagulase-negative staphylococcus (CoNS) isolated from broth only. **NOTE:** Isolates of CoNS and *Corynebacterium* are probably contaminants in community-acquired infection but may or may not be a cause of infection in shunt infections and those with head injuries. A few colonies of catalase-positive, gram-positive rods growing only on CHOC should be subcultured to BAP to check hemolysis and rule out *Listeria* before being reported as corynebacteria.
	4. Perform AST on enteric and nonfermenting gram-negative rods, enterococci, *S. pneumoniae, Staphylococcus aureus,* and other significant staphylococci.
		1. For *H. influenzae,* perform and report beta-lactamase test and perform AST.
		2. For *Listeria, Streptococcus agalactiae,* and *N. meningitidis,* do not perform AST or beta-lactamase testing, which can lead to erroneous results. Resistance to penicillin in these isolates is rare; confirm any non-penicillin-susceptible isolates in a reference laboratory. For the penicillin-allergic patient, consult with the physician to guide AST.
	5. Hold positive culture plates for at least 7 days.

**REPORTING RESULTS**

1. Report the Gram stain results as soon as possible, usually within 1 h of receipt.
2. Report probable genus and species as soon as preliminary tests are completed.
3. Document critical value notification of positive findings.

**INTERPRETATION**

1. Generally a positive culture indicates infection with the organism.
2. Lack of WBCs in CSF does not rule out infection, especially in listeriosis
3. The most common cause of community-acquired bacterial meningitis is *S. pneumoniae.* Performance of the Pneumoslide test on all alpha-hemolytic streptococci seen in Gram stain is key to rapid diagnosis.
4. Isolation of enterococci from CSF is always a cause for concern. The presence of the organism may be an indication of strongyloidiasis.

**LIMITATIONS**

1. False-positive results can result from contamination of the specimen or the culture with skin microbiota.
2. False-negative results can be caused by low numbers of organisms, prior antimicrobial treatment, or the fastidious nature of the infective organism.

**REFERENCE**

1. Clinical Microbiology Procedures Handbook. 3.7. CSF Cultures. March 2007 Update.