

## Cerebrospinal Fluid Culture

**Purpose** This procedure provides instruction for Cerebrospinal Fluid Culture for the Microbiology laboratory.

**Principal and Clinical Significance** Bacterial meningitis is the result of infection of the meninges. Acute meningitis is a very serious infection. CSF from a patient that has meningitis is an emergency specimen that requires immediate processing to determine the infecting organism. **Positive Gram stain results and positive culture results must be reported to the physician immediately.**

**Policy Statements** This procedure applies to Microbiologists who perform culture set-up and plate reading.

**Test Code** CSC

**Materials**

Reagents	Supplies	Equipment	Media
<ul style="list-style-type: none"> <li>Gram Stain reagents</li> <li>Catalase</li> <li>Staphaurex™</li> </ul>	<ul style="list-style-type: none"> <li>Sterile disposable pipettes</li> <li>Glass slide</li> <li>Sterile screw-cap container/tube</li> <li>Saline tubes</li> <li>Inoculating loop</li> </ul>	<ul style="list-style-type: none"> <li>Ambient air incubator</li> <li>Cytocentrifuge</li> <li>CO<sub>2</sub> incubator</li> <li>Incinerator</li> <li>Microscope</li> <li>Vortex mixer</li> </ul>	Refer to the Sunquest specimen label for media information <ul style="list-style-type: none"> <li>Chocolate agar (CHOC)</li> <li>Sheep blood agar (SB)</li> <li>Thioglycolate (THIO)</li> </ul>

- Specimen**
- A. Acceptable specimens: SDES codes/Specimen type
- CSF – Cerebrospinal fluid
  - LCSF – Lumbar puncture CSF
  - RST – Reservoir tap CSF
  - SHF – VP shunt fluid
  - VF – Ventricular fluid
  - VEN – Ventriculostomy
  - SUB – Subdural fluid
  - SHU – Shunt fluid (shunts other than VP shunts)
  - Append code CLTD for “Clotted specimen” to SDES
  - Use free text to note “low volume” to SDES

Refer to the Lab Test Directory for Specimen Collection, Transport and Specimen assessment, – [CSF Culture and Gram Stain](#)

- B. Special instructions
- Handle CSF as a STAT specimen.
  - Report the Gram stain within 60 minutes of receipt.

**Special Safety Precautions**

Microbiologists are subject to occupational risks associated with specimen handling.

- [Biohazard Containment](#)
- [Biohazardous Spills](#)
- [Safety in the Microbiology Laboratory](#)

## Procedure

### A. Specimen Processing

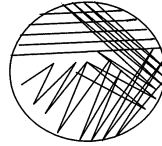
1. Use tube 2 for culture and PCR testing. Tube 1 should not be used because of possible contamination.
2. Working in the Biosafety cabinet, aliquot 1 mL from original tube or syringe to labeled sterile screw top or snap cap tube.
3. Place the original tube or syringe into fridge for saving.
4. Prepare Gram Stain from aliquot.
  - i. **Cytospin smear method:** Using a sterile disposable plastic transfer pipette, transfer 2-3 drops (250 µl) of CSF into a Cytospin specimen chamber. Refer to the Cytocentrifuge procedure for operation. MCVI 6.1 [Cytocentrifuge](#)
  - ii. **If volume is insufficient for Cytospin, (<500 µl total volume),** place 1 drop of CSF specimen on the slide, allowing the drop to form a heap. Do not spread.
  - iii. **If clotted, see section 6 and 7 below.**
  - iv. **Diluted specimens are not acceptable for CSF gram stains.** The BD Normal Saline (0.5 ml tubes) and THIO periodically contain non-viable gram staining organisms, leading to misinterpretation and false positive gram stains.
5. Dry and heat fix slide in bio-safety hood on the slide heater.
6. If **1-5 drops total volume** (<250 µl total volume) of specimen is received:
  - i. Note "low volume" in SDES.
  - ii. Use 1 drop for the Gram stain, directly onto a slide, as directed above under 4 ii.
  - iii. Using a sterile pipette, place 1-2 drops onto a CHOC and SB. Do not inoculate THIO.
7. If **clotted** CSF specimen is received in **Minneapolis: Note "clotted specimen" in SDES**
  - i. Use a sterile swab, "wring out" the clot to express any caught up CSF and remove the swab and clot from the CSF tube.
  - ii. Inoculate the plates with the swab (clot).
  - iii. If there is expressed CSF remaining in the tube, aspirate with a sterile pipette and inoculate each plate in the first quadrant with a small drop.
  - iv. Inoculate the THIO broth with the clot from the swab. Do not invert.
  - v. Use a second swab (or sterile pipette, if there is expressed CSF) to make the gram with the remaining specimen in the CSF tube.
8. If **clotted** CSF specimen is received in **St Paul: Note "clotted specimen" in SDES**
  - i. Use swab or pipette to sample any liquid if present for the Gram Stain (liquid may be under the clot). Place drop or rub sample onto slide.
  - ii. If the clot is solid, swab the top portion of the clot, leaving the clot in the tube. Rub swab on slide.
  - iii. Send sample STAT to Minneapolis for plate inoculation.

### B. Specimen inoculation

1. Allow all media to come to room temperature before inoculation.
2. Label all plates, tubes and slides properly with the patients name, accession number and date.
3. Inoculate the media from the aliquot tube. Refer to the Sunquest specimen label for the order of inoculation.
4. Using a sterile pipette, place 2-3 drops onto a CHOC and SB.
5. Inoculate THIO with remaining CSF by displacing the air from the pipette, placing the tip half way down into the broth and gently releasing the fluid without introducing air bubbles. Do not invert.
6. Always inoculate the culture media first before preparing the slide when using the same pipette.
7. Aliquot tube should be discarded when empty.
8. Streak plates semi-quantitatively for primary isolation.
  - a. Sterilize the inoculating loop in the incinerator for 5 s to 10 s. Allow the loop to cool.
  - b. Pass the loop back and forth through the inoculum in the first quadrant several times, covering approximately ¼ of the plate.
  - c. Flame the loop, turn the plate a quarter turn and pass the loop through the edge of the first quadrant approximately 4 times while streaking into the second quadrant.

Continue streaking in the second quadrant without going back into the first quadrant 3-4 times.

- d. Flame loop again, turn the plate another quarter of a turn, and pass the loop through the edge of the second quadrant approximately four times while streaking into the third quadrant. Continue streaking in the third quadrant without going back into the second quadrant 3-4 times.



### C. Incubation

1. Incubate CHOC and SB in 4-10% CO<sub>2</sub> at 35°C.
2. Place THIO in ambient air incubator at 35°C.

### D. Gram stain examination

1. Perform and interpret all CSF Gram stains within 60 minutes of receipt.
2. Quantitate WBC and bacterial morphotypes.
3. Any bacteria are considered significant. However, confirm low numbers only seen in one or two fields with a second smear.
4. **Critical Value:** Report positive CSF Gram stains immediately to the physician or nursing unit by telephone.
5. **Day Shift:** Document results and notification information when positive in Sunquest under the Direct Exam tab.
6. **Evening, Nights and St Paul:** Document results and notification information when positive in Sunquest under the Direct Exam tab AND on the Gram stain log sheet.
7. Blot excess oil from slide. Hold slide for one week.
8. If a Gram stain QA failure should occur, review slide and culture.
  - As slides are discarded at 7 days, do not wait until culture is finalized on day 7 to review the Gram stain.

### E. Culture examination

#### 1. Day #1

- a. Examine aerobic plates and THIO.
  - a. Examine negative plates daily for 4 days before discarding.
  - b. Examine THIO daily for 7 days before discarding.
- c. Plated media- **Notify physician or patient's nurse of positive culture results.**
  1. Gram stain each colony type and perform initial identification procedures (i.e., catalase, oxidase, etc.).
  2. Semi-quantitate growth on plate.
  3. Correlate colony types with the direct Gram stain.
  4. Set up definitive biochemical or identification procedures on all organisms if well isolated (i.e., VITEK MS, VITEK 2).
  5. Perform antimicrobial susceptibility testing on significant organisms if well isolated.
  6. If an organism is determined to be a contaminant by the physician, a complete identification or AST may not be required. Consult physician as needed. Document in culture work-up, if AST not performed per physician request.
  7. Subculture organisms that are not well isolated to appropriate media for further work-up.
  8. Perform  $\beta$ - lactamase testing on *Haemophilus influenzae*.
  9. MRSA isolation requires a "Called to" if not from E.D. (disc), or it is a repeat isolate.
  10. Re-incubate primary plates and subcultures for an additional days.
  11. Report preliminary results.

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- d. THIO broth
    1. Visually inspect THIO.
    2. If growth is observed, perform gram stain on THIO.
    3. Correlate the culture result with the Gram stain of the THIO. Do not subculture the THIO if the smear correlates with the growth on the plates. Discard after 2 days.
    4. If there appears to be additional organisms in the THIO that are not on the plates, determine if Anaerobic Culture has been ordered.
      - If Anaerobic Culture has been ordered, subculture to appropriate aerobic media. Identify appropriate organisms. If organism in THIO appears to be an anaerobe, confirm isolation of organism in Anaerobic Culture before finalizing culture.
      - If Anaerobic Culture has not been ordered, subculture to appropriate aerobic and anaerobic media. Identify appropriate organisms. Add bill code ANAID.
2. Day #2
- a. Examine primary plates from the previous day for additional microorganisms.
  - b. Read and record identification tests and susceptibilities from the previous day.
    - Refer to [MC 6.00 Susceptibility Testing Guidelines](#) for instructions regarding the CLSI CSF Reporting Rule
  - c. Set up additional tests as needed.
  - d. Visually inspect THIO. If growth is observed, perform gram stain on THIO. Refer to section '4' above for further instructions.
  - e. Ensure THIO with growth was gram stained for 2 consecutive days.
  - f. Send updated report.
  - g. Send *S. pneumoniae*, *H. influenzae*, *N. meningitidis*, *S. agalactiae* and *Listeria* isolates to MDH as EIP organisms. [MCVI 4.1 Infection Prevention Notification and MDH Submission](#).
3. Additional Days
- a. Complete identification and susceptibility testing procedures until all significant isolates are finished.
  - b. If there is no growth on the plates, they can be tossed at 4 days. Culture is held open while THIO continues to incubate.
  - c. Hold negative THIO for 7 days. If no growth in THIO, final the report as "No Growth, 7 days".
  - d. If THIO was not set up due to low volume, culture can be finalized at 4 days.
  - e. Save a representative primary plate, whether a complete work-up was performed or not, at room temperature for 14 days in case a physician calls for further studies.
  - f. Save a representative primary plate for anaerobes in an anaerobic jar or bag, whether a complete work-up was performed or not, at room temperature for 14 days in case a physician calls for further studies.
  - g. Send updated report and finalize.
  - h. Send invasive pathogens cultured from sterile sites to MDH for the EIP program. Refer to procedure [MCVI 4.1 Infection Prevention Notification and MDH Submission](#) for further information.**
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**Critical Results**

Critical results are reported within 60 minutes to the provider. Additional identifications also require notification. Refer to [MCVI 4.0 Critical Results](#) procedure for full list. Identification of organisms to watch for include:

- MRSA
- ESBL or Carbapenemase producers
- Agents of Bioterrorism--*Bacillus anthracis*, *Brucella*, *Burkholderia mallei/pseudomallei*, *Francisella tularensis*, or *Yersinia pestis*
- Fungal or Yeast isolates:
  - Mucorales-all genera included in this Order, including those listed below
    - *Mucor spp.*
    - *Rhizomucor spp.*
    - *Rhizopus sp.*
    - *Syncephalastrum spp.*
    - *Lichtheimia spp.*
    - *Cunninghamella spp.*
  - *Cryptococcus neoformans*
  - *Coccidioides immitis*
  - *Histoplasma capsulatum*
  - *Blastomyces dermatitidis*
  - *Sporothrix schenkii*
- VRE
- *Corynebacterium diphtheriae*
- *Neisseria meningitidis*

**Method  
Performance  
Specifications**

1. Lack of WBCs in CSF does not rule out infection, especially in Listeriosis.
2. The most common cause of community acquired bacterial meningitis is *S. pneumoniae*.
3. Isolation of Enterococcus from CSF may be an indication of strongyloidiasis.

**Result  
Reporting**

1. **Critical Values:** Report positive Gram stains and positive culture results by telephone to the physician or patient's nurse. Document in the computer, the person called, first name, first initial of last name, appropriate credentials and the date/time of the call.
2. Culture results: Record culture results and culture work-ups in Sunquest MRE. Report results semi-quantitatively, i.e. 1+, 2+, 3+ or 4+.
3. **No growth cultures:** Update NG cultures daily in *Microbiology Automatic No-Growth Result Entry*. If cultures remain negative, culture will be automatically finalized in the computer on day 7. Alternatively, manually update culture daily and manually final on day 7.

Enter worksheet in worksheet box and click **Add**

Worksheet entry: CSC LAST UPDATE COMPLETED 07/07/2006 AT 1302

Selected worksheets

CSC CEREBROSPINAL CULTURES

Click **Start Update**

4. **Positive cultures:** Record culture results and culture work-ups in Sunquest MRE *Culture Entry* tab in Observations or Workups by using customized keyboards or by entering a code in the result box.

Observations: 1. 4+ HAEMOPHILUS SPECIES Further identification to follow

Workups:	Wkup # 1	Workup Components
	Med : CHOC	SC : CHOC
	Desc : TAN	GMS : HAE
	ID : HAEM	BL : POS

5. If growth is only in the THIO, report as:  
 Observations: 1. CUTIBACTERIUM ACNES ISOLATED FROM BROTH ONLY  
**PACN-BO**  
  

Workups:	Wkup # 10	Workup Components
	Med : THIO	SC : SB ASB2
	Desc : CLDY	GMS : PACN
	ID : PACN	
  
6. **Gram stains:** Report Gram stain results by selecting the *Direct Exam tab*. Follow Gram stain procedure for interpretation and resulting. Quantitate WBCS and bacteria.
  - o If no WBC seen, report NWBC
  - o If no bacteria seen, report NOS

Observations:        1. 2+ GRAM POSITIVE COCCI  
                              2. 4+ WBC'S
  
7. Review **Culture Summary** for accuracy before filing report.
  
8. **Call Infection Control with Gram stain results that appear to be gram-negative cocci/gram-negative diplococci. Also, call if *Neisseria meningitidis* is isolated.** Document date and time called in the computer.
  
9. If growth should occur or additional testing should be requested after the culture has been finalized, remove the final status and send out a supplementary report. The code SRPT (supplementary report) must be used in SREQ or *Culture Observations* as follows:
  - Updated or new culture information: In the *Culture Entry* tab, enter SRPT on an observation line followed by new results.
  - Requests for additional testing: In the *Misc. Updates* tab, enter SRPT in SREQ followed by the request.
  - Re-final the culture when identifications and/or testing are complete.
  
10. If a culture requires a correction, the code **CORR** (corrected report) must be reported on an observation line in the *Direct Exam* or *Culture Entry* tab. Refer to the procedure [MCVI 5.1 Mislabeled & Unlabeled Specimens](#)

<b>References</b>	<ol style="list-style-type: none"> <li>1. Leber, Amy Section 3, Aerobic bacteriology, 3.7, <i>Clinical Microbiology Procedures Handbook</i>, 2016 American Society for Microbiology, Washington, D.C.</li> <li>2. Isenberg, Henry D., <i>Essential Procedures for Clinical Microbiology</i>, American Society for Microbiology, D.C., 1998, pg.67-71</li> </ol>	
<b>Appendices</b>	WORKLABEL MEDIA-FORM DEFINITION BATTERY: CSC SPEC        MEDIA 0            CHOC, SB, THIO, GMST	
<b>Training Plan/Competency Assessment</b>	<b>Training Plan</b>	<b>Initial Competency Assessment</b>
	<ol style="list-style-type: none"> <li>1. Employee must read the procedure.</li> <li>2. Employee will observe trainer performing the procedure.</li> <li>3. Employee will demonstrate the ability to perform procedure, record results and document corrective action after instruction by the trainer.</li> </ol>	<ol style="list-style-type: none"> <li>1. Direct observation.</li> </ol>

**Historical Record**

	<b>Version</b>	<b>Written/Revised by:</b>	<b>Effective Date:</b>	<b>Summary of Revisions</b>
	1.0	Pat Ackerman	11/01/1982	Initial Version
	1.1	Pat Ackerman	01/18/1992	
	1.2	Pat Ackerman	12/25/1995	
	1.3	Pat Ackerman	09/10/2001	
	1.4	Pat Ackerman	07/10/2003	
	1.5	Pat Ackerman	09/11/2004	
	1.6	Pat Ackerman	07/16/2007	Updated Sunquest 6.2 reporting and recording. Revised SRPT and CORR reporting statement. Added hyperlinks.
	1.7	Becky Carlson	02/29/2008	Revised critical value to include negative gram stain to correlate to Critical Value Policy.
	1.8	Becky Carlson	10/2/2013	Updated into CMS online format.
	1.9	Becky Carlson	8/14/2014	Revised WBC reporting
	2	Becky Carlson	4/15/2015	Re-numbered from MC 412 for CMS formatting
	3	Becky Carlson	5/17/2016	Added clarification and instructions regarding clotted specimens.
	4	Susan DeMeyere	11/14/2018	Removed anaerobic culturing
	5	Susan DeMeyere	11/2/2020	Changed hold negative plates for 4 days. Removed perform B-lactamase on Neisseria sp. Removed SCANT reporting with growth only in THIO.
	6	Susan DeMeyere	1/3/2023	Add instructions for processing clotted samples in St Paul.
	7	Susan DeMeyere	9/15/2023	Removed calling negative Gram Stain results to provider.
	8	Susan DeMeyere	2/26/2024	Removed centrifugation of CSF, add Critical Results section.
	9	Susan DeMeyere	9/23/2024	Revised low volume instructions. Added instructions for Gram QA failure.