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Valley Laboratories	**Next Review:**	*2 years after approval*
	Owner:	*Lindsey Westerbeck: Dir, Lab*
	Policy Area:	*Lab - Microbiology*
	References:	
	Applicability:	*Valley Laboratories*

Processing CSF Specimens for Culture in the Hospital Lab

PURPOSE

This procedure describes how to process CSF specimens for culture.

POLICY

- All work on CSF specimens is to be done in the biological safety cabinet.
- Order Code: **CSFC** (Culture, CSF)
  - Includes routine bacterial culture and STAT Gram stain
- Order Code: **GS** (Gram Stain)
  - STAT Gram stain is to be manually ordered upon receipt in laboratory when a CSF culture is requested, regardless of culture order priority.
- STAT Gram stain is to be setup after the culture has been inoculated to avoid contamination.
- When there is insufficient specimen, the Gram stain is to be omitted rather than the culture.
- If aliquoting of specimen is performed, no aliquots are to ever be returned to the original container to avoid potential cross-contamination.
- CSF culture and STAT Gram stain should be setup ideally within 1 hour of collection.

EQUIPMENT, REAGENTS AND SUPPLIES

- Biological Safety Cabinet
- Centrifuge
- Vortex
- Chocolate Agar Plate
  - Store at 2 to 8° C, protect from direct sunlight and UV light. May use until expiration date when kept refrigerated.
  - Alternatively, store at room temperature (no more than 25° C) for up to 24 hours. Plate **must** be discarded if not used within 24 hours.
- CO_2 Bio-Bags - BD Bio-Bag Type C
- Sterile Pipette
- Disposable Inoculating Loop

SPECIMEN REQUIREMENTS

- CSF is the only specimen type that will be plated on-site prior to sending to Sutter Health Shared Lab

(SHSL) Microbiology for culture work-up.
    - All other culture specimen types will go direct to the SHSL Microbiology.
- To minimize contamination with normal skin flora, **culture** and **STAT gram stain** should be performed on **CSF tube 2 or 3** (unless otherwise specified in the order).

PROCEDURE

Organizing Specimens for Culture

Step	Action
1.	Print Sunquest labels for the specimen being set-up for culture.
2.	Obtain chocolate agar plate and make sure media is at room temperature prior to specimen inoculation.
3.	Place a barcode label on each specimen tube and plate.
4.	Prior to culture setup, it is important to check the following: • Patient label on specimen matches the Sunquest labels • Specimen received is acceptable for the test requested • Specimen source is appropriate for on-site culture setup
5.	**IMPORTANT**: Label plate with tech code and date/time of culture setup.
6.	Using a pencil, label a slide for STAT gram stain.

Processing Specimens for Culture

NOTE: Do not use a pipette to mix the sediment because the bacteria and cells may adhere to the sides of the tube resulting in false negative results.

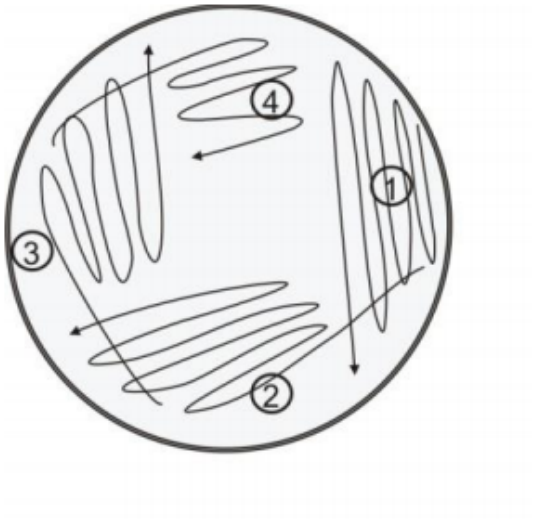
<table>
<tr><th>Specimen Type</th><th>Process</th></tr>
<tr><td>CSF</td><td>
<table>
<tr><th>If</th><th>Then</th></tr>
<tr><td>Specimen is thin and volume is >1cc</td><td>• Centrifuge CSF specimen for 15 minutes at 1000 x g.
• Decant supernatant into a labeled, sterile tube, leaving approximately 1ml at bottom of original tube.
• Vortex the sediment for about 30 seconds to resuspend the pellet.
• Using a sterile pipette place 1-2 drops of re-suspended sediment onto plate.
• Make smear for STAT gram stain - refer to Valley Laboratories procedure, Preparing a Gram Stain in the Hospital Laboratory.</td></tr>
<tr><td>Specimen is thin and volume is ≤1cc
or
Specimen is viscous or cloudy (regardless of</td><td>• Do not centrifuge.
• Using a sterile pipette, place 1-2 drops of well-mixed CSF specimen directly onto plate.
• Make smear for STAT gram stain - refer to Valley Laboratories procedure, Preparing a Gram Stain in the</td></tr>
</table>
</td></tr>
</table>

volume)	*Hospital Laboratory.*

Streaking Plates for Isolation

Step	Action
1.	Inoculate plate by placing 1-2 drops of well mixed specimen to **<u>one</u>** quadrant with sterile pipette.
2.	Streak plate for isolation.
3.	Using the disposable loop spread the initial inoculum back and forth over a fourth of the plate, or the 1st quadrant. • **NOTE:** Avoid touching the sides of the Petri dish.
4.	Turn the plate 90°. Pass the loop through the edge of the 1st quadrant approximately 2-3 times, while streaking into the 2nd quadrant.
5.	Flip the loop and continue streaking in the 2nd quadrant without going back to the 1st quadrant.
6.	Rotate the plate another 90° and repeat the above procedure until two additional quadrants are streaked, for a total of 4 quadrants. • **NOTE:** When streaking the additional quadrants, do not to go back into the 1st quadrant.

IMAGE: Streaking for Isolation - Total of 4 Quadrants



Preparing Samples for Transport

Step	Action
1.	Tape or parafilm the diameter of the plate to prevent any opening during transport.
2.	Place inoculated media into CO_2 Bio-Bag. Seal and crush ampule to generate the CO_2 environment.
3.	Place CO_2 bag containing plates into designated pink transport bag for SHSL Microbiology.
4.	Transport at room temperature to SHSL Microbiology.
5.	Retain remaining CSF specimen on-site, clearly documenting on label the type of sample (i.e. supernatant).

RELATED DOCUMENTS

- Valley Laboratories procedure, *Preparing a Gram Stain in the Hospital Laboratory*

REFERENCES

- Centers for Disease Control and Prevention, *Laboratory Methods for the Diagnosis of Meningitis*, Chapter 6, rev April 15, 2016.
- *Clinical Microbiology Procedures Handbook*, 4th ed., Leber et al, 2016.

All revision dates:

Attachments

No Attachments

Approval Signatures

Step Description	Approver	Date
Lab Medical Directors	Kristen Vandewalker: MD	pending
Lab Medical Directors	Andrea Ong: MD	pending
Lab Medical Directors	Hannah Wong: MD	pending
Lab Medical Directors	Rowberry Ron: MD	pending
Lab Medical Directors	Jamie Cassity: MD	pending
Lab Medical Directors	Mary Keohane: MD	pending
Lab Medical Directors	Marian Butcher: MD	pending
	Lindsey Westerbeck: Dir, Lab	10/2/2020