Processing Cerebral Spinal Fluid (CSF) Specimens

Purpose

This procedure provides instructions for RECEIVING AND PROCESSING CEREBRAL SPINAL FLUID (CSF) SPECIMENS in Core lab processing and Microbiology to maintain sterility and to have a central location for all excess CSF.

Policy Statement

CSF is collected by lumbar puncture and considered an irretrievable specimen.
This policy applies to all staff who work in Core lab processing and Microbiology.

Materials

- Aliquot tubes
- Sterile pipettes

Sample

Generally, three to four numbered tubes of CSF are obtained from one lumbar puncture collection. Tubes are numbered 1, 2, 3, and 4, with tube #1 representing the first portion of the sample collected.
Do not transport CSF through the pneumatic tube station.

CSF without orders should be kept at ambient (room) temperature until orders are received.

Sample Rejection

Reference the Unlabeled or Mislabeled Specimen Process/Form

Stability

CSF count
- Process immediately. Counts must be performed as soon as possible after the specimen has been received. Any cells present disintegrate within a short time.

CSF protein
- Cerebrospinal fluid is stable at 4°C for up to 3 days **OR** <-20°C for 6 months.

CSF glucose
- If testing is delayed, the specimen should be centrifuged and stored at 4°C.

CSF culture
- **DO NOT refrigerate.**
- If the specimen cannot be processed immediately, it should be kept at ambient temperature. (Refrigeration may prevent the recovery of *Neisseria meningitidis* and *Haemophilus influenzae).*

CSF molecular testing
EVPCR: cerebrospinal fluid is stable at 4°C for 3 daysHSV and MEPNL: cerebrospinal fluid is stable at 4°C for 7 days

Procedure

Follow the activities in the table below for RECEIVING CEREBRAL SPINAL FLUID (CSF) SPECIMENS in **Core Laboratory Processing**.

		Processing Action	Related Document

	1.	Verify all specimens are labeled per Children's specimen labeling policy.	Policy 630.00 Laboratory Specimen Labeling GL 2.0 Unlabeled/Mislabeled Specimen Challenge Form and Procedure
	2.	Receive orders using function ORM. • In ORM, enter 14 under Day(s) of activity. • Ensure unrestricted MBAT orders are received. These will have a "C" listed under specimen type. • See step 3 if there are no orders in ORM. NOTES: • If CSFC and CYTM are ordered, cancel CSFC as duplicate. • CSFC and CTP should be canceled if specimen is clotted. • If MEPNL is ordered, there **must** be a corresponding CSC order. If the CSC order is missing, call the provider to request a CSC order.	LIS 1.26 Order Receipt Modify (GUI ORM) PRC 1.17f1 CSF Processing & Aliquoting Guide
	3.	If there are orders present in ORM, receive them. If there are no orders in ORM, review patient's chart in Cerner to see if there are Future Orders to activate. If no orders present: • Contact the ordering provider or nursing unit and request for orders to be placed. • Specimen must be maintained at ambient temperature until orders are placed. • Notify the provider that CSF testing is time sensitive and that orders need to be placed immediately. ○ If the provider/nursing unit doesn't place orders within 15 minutes, notify the operations supervisor or the charge tech.	GL 1.20 Laboratory Escalation Policy GL 2.1 Irretrievable Specimens With No Orders LIS 1.22 Activating Future On-Hold Orders
	4.	Write the original volume on each tube below the tube number with a black Sharpie.	

	5.	In ORM, add CSFV to an accession number associated with the order.
		• Result Entry will pop-up
		  ○ Add the volume for each tube next to the corresponding tube number.
		  ○ For each tube not received, type 'SNR' (specimen not received).
		  ○ The CSFO box can be used if a different container type is received **OR** if more than four tubes are collected. This box is automatically hidden if not modified.
			LIS 1.26 Order Receipt Modify (GUI ORM)

	6.	If CSFV is a restricted test for the accession, add the tube volume in the SDES.	

	7.	Place all CSF tubes and labels into a biohazard bag. Walk specimen to Microbiology. Notify Microbiologist of CSF specimens.	
	8.	Day shift: Microbiology will aliquot testing and return Chemistry testing to Processing for centrifugation. Evening Shift: EP2 is responsible for aliquoting testing.	
	9.	Once chemistry aliquot is received: • Centrifuge the aliquot in either the STAT Spin Express centrifuge for 5 minutes at 5000 rpm OR the Eppendorf Centrifuge 5430 for 3 minutes at 7500 rcf. • Label pilot tube with corresponding primary barcode label.	
	10.	Pour off centrifuged CSF into labeled pilot tube and place in Alinity testing rack for the Auto Cell.	

Follow the activities in the table below for PROCESSING CEREBRAL SPINAL FLUID (CSF) SPECIMENS in **Microbiology Department**.

	Microbiology Action	**Related Document**
1.	Place tubes into numerical order (Tubes are labeled #1-4). • If the labels on the CSF tubes do not correspond to the correct testing, relabel the CSF tubes with the correct testing. • Ensure that the patient identifiers are visible on the original label.	PRC 1.17f1 CSF Processing & Aliquoting Guide
2.	Decant specimen in SMART. Aliquot labels will be printed for each test. • Decanting is not required for the CSC test code.	LIS 1.27 Decanting Specimens
3.	Aliquot CSF per instructions below. When CSF is aliquoted, use aseptic technique to maintain sterility within a biosafety cabinet. • If the corresponding tube has insufficient volume, aliquot from the lower numbered tube to obtain minimum volume. ○ EXCEPTION: Tube #1 should not be used for microbiology testing due to risk of contamination with skin flora. • If any testing has insufficient volume, contact the provider to determine priority of testing.	LIS 1.2 Order Entry in Sunquest
4.	Prepare gram stain & culture directly from tube #2. • St Paul Microbiology: Prepare tracker and send culture to Minneapolis Microbiology ○ When sending aliquots for culture or any molecular testing of CSF, use the sterile 5mL red-screw capped tubes. Do not use 15mL conical tubes.	MC 1.11 Cerebrospinal Fluid Culture LIS 1.6 Transport Batches in Sunquest
5.	Place all original CSF tubes into the designated rack. • Minneapolis: Micro Fridge #3 (Culture Desk) • St Paul: Lab Micro Small Media (REF)	
6.	Distribute testing to corresponding departments. CSF should not be transported in the pneumatic tube system.	

	7.	Set up culture and read Gram stain (or notify tech to read, if necessary). If a Gram stain is received from St. Paul, it must be reviewed by a Minneapolis Microbiology technologist and documented in the Gram stain log.	MC 1.11 Cerebrospinal Fluid Culture MC 2.0 Gram Stain
	8.	Specimen will be racked in batches on the night shift. See the section “Sample Storage” below for instructions.	LIS 1.28 Racking, Unracking, Tracking Specimen
	See below for processing of common CSF tests		

IF	Then (if test is ordered)
4 tubes received	Tube 1-Glucose, Protein, Tube 2-Gram Stain, CSF Culture, Molecular Tube 3-Cell Count Tube 4-CSF Save, Other
3 tubes received	Tube 1-Glucose, Protein Tube 2-Gram Stain, CSF Culture, Molecular Tube 3-Cell Count, Other
1-2 tubes received with minimal volume or QNS	Contact ordering provider or unit to prioritize testing.

Glucose (CGL)/Protein (CTP)

- Label bullet tube with foot label containing CTP/CGL accession number.
- Aliquot 0.2 mL from tube #1 into labeled bullet tube.
- Place bullet tube and large barcode label in biohazard bag.
- Place in Core lab processing bin for centrifugation (notification light automatically turns on). Verbally confirm drop-off.

NOTE: If CTP and CGL are ordered together, 0.2 mL of CSF is sufficient for both tests.

Culture CSF (CSC) and Gram Stain

- Prepare Gram stain and inoculate plates using CSF from tube #2 according to MC 1.11 Cerebrospinal Fluid Culture

MEPNL

- Aliquot 0.5 mL (Minimum 0.3 mL) of CSF from tube #2 into labeled sterile snap capped tube or sterile red screw capped tube. Affix large CID label to the aliquot tube.

EVPCR

- Aliquot 0.5 mL (Minimum 0.3 mL) of CSF from tube #2 into labeled sterile snap capped tube or sterile red screw capped tube. Affix large CID label to the aliquot tube.

HSVPP

- Aliquot 0.5 mL (Minimum 0.3 mL) of CSF from tube #2 into labeled sterile snap capped tube or sterile red screw capped tube. Affix large CID label to the aliquot tube.

CSF Count/Diff (CSFC)

- Label screw cap tube with foot label containing CSFC accession number.
- Aliquot 2 mL (Minimum 1.0 mL) from tube #3 into labeled screw cap tube.
- Place in Hematology specimen receiving rack and turn on notification light. Verbally confirm drop-off.

Cytology for Malignant Cells (CYTM)

- Label screw cap tube with foot label containing CSFC accession number.
- Aliquot 1 mL from tube #3 into labeled screw cap tube.
- Place in Hematology specimen receiving rack and turn on notification light. Verbally confirm drop-off.

Referral Testing/MBAT/Other

- Tubes 3 or 4 are used for referral testing, MBATs, and all other testing.
- See Lab Test Directory for proper processing of orderable test codes.
- If MBAT is ordered and no clear instructions are provided with the order, consult with a Referral Testing Tech before processing.
    - If add-to orders are placed, Micro and Referral staff must work together to determine requirements (i.e. minimum volume needed, storage conditions)
    - Micro staff will then aliquot add-to testing and hand-off to Referral staff
    - Referral testing staff are responsible for communicating with the provider if sample volumes are QNS or if additional problems with add-to testing arise.

CSF Save (SFS):

- Receive SFS if tube #4 is not used for testing.
- Place SFS primary barcode label on tube #4.
- If no testing is done on tube #4 but a SFS was not ordered, order a SFS and place SFS primary barcode label on tube #4.
- These should be stored in the same manner as all other CSF.

If testing was done on all tubes but a SFS was ordered, cancel the order as QNS.

- To “Answer” a CSF save in Sunquest:
    - Function: MEM
    - Enter through to “Test-1”
    - Type SFS
    - Accept
    - Enter the accession number
    - Press Enter
    - Type ‘CSAVE’ and then press Enter
    - Type ‘A’ and then press Enter to accept the manual result

Sample Storage

Store CSF for 7 days in the refrigerator. Move to freezer and store for 21 days. Discard CSF 29 days after collection.

- Original CSF containers must be racked daily using SMART on the night shift.
  - Minneapolis: Store in MPLS VIRO FRIDGE #2 using SMART rack location MINCSF. Insert Rack label into plastic label holder.
  - St. Paul: Store in Lab Micro Small Media (REF) using SMART rack location STPCSF.
  - Each rack can hold four Rack ID labels.
  - Start a new Rack ID at the next row marked with a black line.
  - Insert Rack label into plastic label holder attached to the rack.
  - If the day's batch includes more than 18 specimen containers, create new Rack ID and continue racking the rest of the specimen.
- Original CSF containers must be moved from storage refrigerator to storage freezer on the 8th day after collection. Place specimen containers in biohazard bag and place Rack label in pocket of biohazard bag. Put new bag at the front of the storage bin. **DO NOT DISCARD RACK IN SMART.**
  - Minneapolis: Store frozen samples in MPLS VIRO ULTRA LOW (FRZ)
  - St Paul: Store frozen samples on the second shelf of Lab Micro/Chem Freezer.
- Original CSF containers must be disposed of on the 29th day after collection. The last bag in the storage bin should be placed in biohazard waste after the new bag is added. Discard Rack ID in SMART.

References

Bishop, Fody and Schoeff. *Clinical Chemistry Principles, Procedures, Correlations*. 5th edition ©2005

CLSI. *Body Fluid Analysis for Cellular Composition; Approved Guideline.* CLSI document H56-A. Wayne, PA: Clinical Laboratory Standards Institute; 2006.

Historical Record

Version	Written/Revised by	Effective Date	Summary of Revisions
1	Daniel Gebrekidan	07/10/2009	
2	Daniel Gebrekidan & Jennifer Johnson	08/11/2011	
3	Lisa Kappenman & Daniel Shaw	12/01/2015	Major revisions: added tests and processing of each; clarified distribution of tubes; included volumes
4	Dawit Getachew	06/10/2019	Added step 2, minor revisions
5	Dawit Getachew	11/22/2021	Biennial Review: Minor revisions and changes. Removed OER and CVIS from steps 2, 3, and 4.
6.	Miranda Berry	02/08/2024	Major revisions: Updated processing to follow CLSI guidelines. Processing performed in micro dept within biosafety cabinet. Racking specimens and storage instructions added.
7	Andrew Fangel, Katie Bina, Tara Hollerich, Jude	09/27/2024	Revisions and re-formatting:

		Moss, Claire Lyden, Sarah Wadd		Added stability for Molecular testing. Order CSC when MEPNL ordered alone. If CSFV is a restricted test for the accession, add the tube volume in the SDES. Clarified aliquoting responsibility by shift. Use sterile 5mL red capped tubes. Gram stains from St. Paul must be reviewed by Micro tech. Added clarification on referral testing add-tos. Added direction on SFS orders.