

Standard Operating Procedure IV: CSF Collection and Processing

**** NOTE: The following procedure is to be performed wearing laboratory coat, gloves, eye protection, and mask.**

1. If an external ventricular drain (EVD) is placed as part of the routine care, fresh cerebral spinal fluid (CSF) samples will be taken at the time of placement of the EVD and at indicated time points. CSF obtained by lumbar puncture is also acceptable.
2. Up to 5 ml of CSF per sample will be collected using standard sterile techniques in a sterile 10 mL conical bottom polypropylene tube. (CSF that has been sitting in the collection chamber has to be discarded and cannot be used for sampling).
3. Label the collection tube with PIN and time of collection.
4. Store on ice immediately following collection and until processing, which should occur as soon as possible after collection (1 hour maximum).
5. Transfer the CSF to a labeled and chilled 15 mL conical tube. Record the volume.
6. Centrifuge the sample at 750 x g for 10 minutes at 4°C (centrifuge program 3).
7. While centrifuging, label the appropriate number of cryotubes (SOP I). Take the volume in mL from step 5 and divide by 0.25 to get the number of tubes. There may be some loss of sample after centrifuging, depending on the amount of contaminants. Place the tubes on wet ice to chill.
8. Remove the tube from the centrifuge and keep on ice. Aliquot 250 µl into each cryovials.
9. Transport on ice to the freezer. Store samples frozen at ≤ -70 °C.

***Special Note 1.1:** If CSF flow through the EVD is sluggish or stops it may be impossible to obtain 5 ml of CSF. After establishing the reason for the CSF flow problems as much CSF as is possible should be obtained (up to 5 ml) over 5 - 10 minutes. Fluid should not be drawn out of the EVD using negative pressure since this may obstruct the tip of the catheter. CSF should be allowed to flow freely. Strict sterile technique should be used if a 3-way stopcock the closed EVD system is entered to obtain CSF samples. The collection of CSF must be performed by someone qualified to do so.*

***Special Note 1.2:** It is important to maintain the same volume of CSF collected to prevent bias that may arise from "diluted" specimens. See: Teunissen C.E. et al. A consensus protocol for the standardization of cerebrospinal fluid collection and biobanking. Neurology 2009;73:1914-1922.*