Cerebral Spinal Fluid (CSF) Collection and Storage

Supplies

For the Lumbar Puncture (LP):

Atraumatic 24G Sprotte Spinal Needle	(Fisher Scientific: NC1085924)
Lumbar Puncture Kit	(Owens & Minor: 4824-20)
Sterile 3 cc luer-lock syringes	(BD: 00382903096572)
Sterile polypropylene 13 cc collection tube	(Sarstedt: 60.540.108)
Sterile gloves	(Owens & Minor: varying sizes)
Underpad	(Westnet: 1340)

For CSF processing after the procedure:

2.0 ml polypropylene tubes with screw caps	(Sarstedt: 72.694.600)
Freezer box	(Fisher Scientific: 03-395-465)
Pipette(s)	
Tube rack	
Centrifuge	
Dry ice	

Enrolling subjects

Print consent forms for study participation including patient name, birthdate, and medical record number (MRN) on each page. The consent form is also mailed to patients along with a recruitment letter prior to their visit.

Approach patients about participation while still in the waiting area or in the exam room before the procedure is started. Explain the study and associated risks as well as the steps taken to mitigate these risks. Emphasize that their participation is entirely optional and will not affect their medical care.

If the patient is interested, they will sign the consent form after speaking with an attending physician who is associated with the study. Informed consent must be obtained by this physician. Provide the patient with a copy of their signed consent form before the end of their visit.

Should the patient decline to participate, dispose of the consent form in a secure waste container for PHI. If the patient enrolls in the study but the LP is unsuccessful or no research CSF is collected, do not dispose of their consent forms and ensure they still receive a copy.

Consent: Special Cases
There are several cases in which the subject may not be able to give informed consent in the usual manner.

If the patient is not cognitively able to provide informed consent, a guardian or representative authorized to represent the patient may provide consent instead.

If the patient does not speak English well enough to provide informed consent, the study may be explained in the patient's primary language instead. Additionally, the patient would be provided with and sign a "short form" for research participation written in their primary language. The interpreter would sign the English consent form.

If the patient cannot read or write, the consent form can be presented orally and the subject can mark it to signify informed consent. An additional signature from a witness must also be obtained.

Set-up

Arrange LP supplies as normal. Ensure that the atraumatic needle and an extra collection tube are carefully added to the sterile field with the other supplies. Collect research samples only after all clinical samples are collected. If dropwise collection is used, research CSF may be collected in a 13 cc polypropylene collection tube (Sarstedt: 60.540.108). Collection tubes for clinical samples are included in the LP kit.

During the procedure

The lumbar puncture is performed with an atraumatic Sprotte spinal needle (Fisher Scientific: NC1085924). CSF is collected by aspiration using polypropylene syringes (BD: 00382903096572). Alternatively, dropwise collection directly into the clinical and research collection tubes may be performed.

Draw Order

As CSF is collected, note the order in which syringes/collection tubes are filled. This information may be necessary for proteins or cell counts that show a lumbar-thoracic gradient effect.

Processing and Storing Samples

CSF is centrifuged at 800g x 5min at 4°C before aliquoting equally (0.25mL per aliquot) into ten 2.0 mL polypropylene tubes (Fisherbrand: 02-682-557). CSF can be aliquoted directly using each syringe or with a pipette if dropwise collection was done. Aliquot samples as soon as possible so that they can be frozen quickly. Avoid thawing/re-freezing samples.

Note that polypropylene specifically is used to store research samples since polystyrene and other plastics can lower various protein concentrations.

Traumatic lumbar puncture

If CSF has a red coloration (rather than the normal clear and colorless appearance) the sample may be contaminated with blood. In this case, centrifuge samples at 1000 g for 10 minutes before aliquoting. Avoid disturbing the pellet when pipetting centrifuged samples.

Database Management

Samples may be tracked using a secure data storage service such as REDCap. Samples from each patient are assigned a unique Subject ID (e.g. “v0032”). Once the subject completes the consent forms, they can be assigned an ID and their birthdate and MRN may be associated with it in the database.

The number of tubes/syringes collected for research, the number of aliquots, aliquot volume (0.25 ml), and aliquot location are each recorded, among other variables. Aliquots are labeled to indicate the draw order of their original syringe/collection tube. For instance, if an aliquot is from the first tube collected for research, its label might be “v0032.1”.

If a patient donates CSF from multiple LPs, the same Subject ID is used. Aliquots from later LPs are differentiated by adding the LP number to the Subject ID. For instance, an aliquot from the first tube of the second LP could be labeled “v0032-2.1”.

Labelling tubes

Labels are added to the side of the tubes and use purple caps. The side label contains the Subject ID, sample type, and collection date and the date of collection (i.e. v0032.1 / CSF / DOS_SAGES II). Aliquot samples quickly and carefully. Samples should not be at room temperature for more than 2 hours. Once tubes are labeled, transfer them into a freezer box on dry ice and keep frozen until they are moved to a -80 C freezer.