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Biobanking of Cerebrospinal Fluid

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Summary

Cerebrospinal fluid (CSF) is a physiologically essential fluid produced by the brain that is involved in protecting the brain and in the exchange of nutrients and waste products. CSF has long been utilized to confirm clinical suspicion of various infectious and inflammatory disorders, such as meningitis and multiple sclerosis. However, there has been increasing interest in collecting CSF in order to study the clinical significance of additional biomarkers. This chapter outlines the procedures necessary to collect, process, store, and utilize CSF obtained for the purposes of biobanking from both living and deceased patients.

Keywords

Cerebrospinal fluid (CSF); Collection; Centrifugation; Aliquoting; Storage; Biobanking; Transport

1. Introduction

Cerebrospinal fluid (CSF) is a physiologically essential fluid produced by the brain (3). It functions to protect the brain from impact with the surrounding rigid cranium and from intracranial pressure differences that may result from alterations in blood flow (3). In addition, it serves as a transport medium for nutrients and for elimination of waste products (3). It is predominantly secreted by the choroid plexus, which is derived from ependymal cells that line the ventricular system (4). CSF circulates within the ventricular system, passing through the lateral, third, and fourth ventricles before it is resorbed into the venous circulation by the arachnoid granulations on the superior aspects of the cerebral convexities.

CSF has long been utilized to confirm clinical suspicion of various neurological diseases. Its close proximity to the brain parenchyma makes it invaluable in assessing the condition of a patient's central nervous system. Evaluation of infectious and inflammatory disorders, such

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as meningitis and multiple sclerosis, respectively, is commonly performed by obtaining CSF from patients via a lumbar puncture (LP) (5). There has been increasing interest in collecting CSF in order to study the clinical significance of additional biomarkers. For example, tau proteins and amyloid beta peptides may be detected in patients with Alzheimer disease (3). Furthermore, novel biomarkers may provide clinicians with less invasive and cost-effective diagnostic answers and surveillance modalities in certain conditions that would require brain biopsies (3).

CSF may be procured from both living and non-living patients. In both instances, one must ensure that patient consent has been obtained prior to collecting bodily fluids for the express purposes of research and biobanking. Some of the guidelines and procedures detailed below may not be feasible in everyday clinical practice, but less stringent requirements may suffice for specific research protocols (6).

2. Collection & Storage Materials

1. Atraumatic needles (*see* Note 1).
2. Syringe (capable of containing at least 10 mL of fluid).
3. Polypropylene collection tubes (*see* Note 2).
4. Dry ice (for transportation, with at least three days' worth).
5. Personal protective equipment (including, but not limited to, a face shield, mask, gown, gloves, and shoe covers).

3. Specimen Collection Methods

3.1 Factors to Consider

A number of factors must be considered before a sample of CSF is withdrawn from a subject, the most important of which are the following.

1. Postmortem Interval: In patients who are non-viable, CSF should ideally be collected as soon as possible in order to minimize degradation of the components present within it. In our experience, under optimal circumstances, the postmortem interval should be under 24 hours.
2. Time of Day of Withdrawal: Under optimal conditions, procurement of CSF should occur at consistent times of the day in order to standardize concentrations of circadian-rhythm dependent biomarkers (6) (*see* Note 3).
3. Volume to Withdraw: Standardization of procurement volume is recommended, and accurate documentation is necessary. At least 10 mL is suggested, with 12 mL or more being optimal (*see* Note 4).

3.2 Collection Methods & Anatomic Locations

1. Review the consent form in order to thoroughly familiarize yourself with any restrictions on tissue sampling, if present. Make sure that the consent form has

been property signed by the appropriate next of kin. Biospecimens procured from living patients must be done so with informed consent (7).

2. Identify the correct patient by comparing the full name and medical record number present on the consent form with the information printed on the decedent identification tag, which is usually attached to the first digit of the foot, ankle, or wrist.

3. Wear the appropriate personal protective equipment. At least two layers of disposable gloves (nitrile, latex, etc.) are preferable to just one layer. It is highly recommended that cut resistant gloves be worn under the layers of disposable gloves.

4. Label the syringe(s) with the appropriate identification labels.

5. The location from which CSF may be collected is contingent primarily upon whether the patient is alive or not.

    a. Viable Patients:

        i. In living patients, lumbar puncture is the most common method of CSF procurement (3). In order to minimize the risk of iatrogenically induced traumatic injuries to the spinal cord, lumbar punctures are generally performed in the lower lumbar segments (specifically from vertebral bodies L3 to L5) (6), where the spinal cord eventually tapers off into the conus medullaris and the components of the cauda equina originate. A detailed explanation of how to perform a lumbar puncture is beyond the scope of this chapter.

        ii. CSF may be obtained directly from access ports on external ventricular drains, which are medical devices that are placed within the lateral ventricles to control rising intracranial pressure in neurological and neurosurgical patients with traumatic brain injuries, subarachnoid hematomas, hydrocephalus, space occupying lesions such as neoplasms, or neurological disease (8).

    b. Deceased Patients:

        The collection of CSF from deceased patients is typically conducted during a postmortem examination, if one has been requested.

        i. One approach is direct procurement from the lateral ventricles upon removal of the calvarium in patients for which a complete postmortem examination has been requested, or in situations limiting examination to the central nervous system (with or without additional organ system and/or body cavity restrictions). The needle is inserted into the dorsal aspect of the cerebral hemispheres in the parasagittal plane (immediately lateral to midline) for a depth of several

centimeters and manipulated until drawing back on the plunger of the syringe results in extraction of fluid. Fluid samples may be collected only after the pathologist has examined the brain in detail and documented its characteristics and any abnormalities.

ii. Should a postmortem examination exclude evaluation of the intracranial contents (e.g., cranium and skull base, dura mater, leptomeninges, and brain, pituitary gland, cranial nerves), one may still collect CSF via spinal tap. Because the patient is no longer viable, the location of the spinal tap need not be restricted to the lumbar region of the spinal cord.

6. Thoroughly clean the workstation and rinse the used surgical instruments. Disposable scalpel blades must be placed within a designated sharps container.

7. Remove all personal protective equipment and dispose of in biohazard receptacles.

8. Wash your hands and wrists with warm water and soap.

9. Transport the syringe to the laboratory by.

4. Processing & Storage of Cerebrospinal Fluid

4.1. Temporary Storage Prior to Processing

Attempts should be made to minimize the pre-processing period during which samples may be stored at room temperature. A processing delay of no longer than approximately one to two hours after collection is suggested (*see* Note 5).

4.2. Centrifugation

The specimen should be centrifuged in order to separate out higher density particles and contaminants (“precipitate”) from the less dense CSF (“supernatant”). Centrifugation should be conducted prior to freezing and storage (6) (see Note 6).

4.2.1. Centrifugation Times—Standardized centrifugation spinning conditions are recommended by the consensus guidelines proposed by Teunissen *et al* depending upon whether a sample requires that delicate cells be preserved for RNA isolation or not (6).

1. Situations Requiring RNA Isolation: In such situations, centrifugation at 400 g for 10 minutes at room temperature is suggested (6).

2. All Other Situations: A 2000 g spin for 10 minutes at room temperature is appropriate for all other situations (6).

4.3. Aliquoting

Aliquoting is an important step in the process of biobanking CSF and is performed after centrifugation is completed. Splitting a large sample into multiple smaller volumes and storing each smaller sample in a separate tube distributes the risk of specimen degradation

that occurs with repeated freezing and thawing cycles (6, 7). Suspected or confirmed freeze/thaw cycles should be logged.

1. Tubes manufactured with polypropylene are recommended for the purposes of aliquoting and storage of CSF for the same reasons as described above for the instruments for CSF collection (6) (see Note 2). The collection tubes should have screw top caps to ensure a secure seal and prevent unintentional sample loss (6, 7).
2. The specimen is aliquoted with a calibrated pipette into plastic storage tubes, with each tube receiving exactly a very small amount of fluid. Teunissen *et al* recommends volumes of 0.2 mL, 0.5 mL, and 1.0 mL (6). Ideally, each aliquot should be drawn with an unused pipette tip in order to minimize contamination between storage tubes. Storage tubes should not be filled to greater than 75% of capacity in order to prevent freeze-drying.

4.4. Specimen Storage:

The storage tubes must be labeled appropriately, preferably with a barcode system (7), and placed in a −80° C freezer (see Note 7), where they may be kept indefinitely for the purposes of future testing (6, 7). Labels should be water and frost resistant and must be designed to withstand the conditions present within a −80° C freezer (6, 7). If additional samples are available, storage in liquid nitrogen may also be performed (7). This method of storage serves as a backup once the samples in the −80° C freezer have been exhausted (7).

Information specific to each sample, including sample identifying information, sample type, patient demographics, and clinical data, and well as freezer location, freezer identification, and sample location within freezer, must all be recorded and kept on a secure, centralized computer-based database system (6, 7). This information should be backed up regularly and frequently (7).

5. Utilization of Cerebrospinal Fluid

5.1. Specimen Analysis

Basic CSF analysis includes routine diagnostic procedures such as evaluation of protein content and cell counts (including the presence of erythrocytes). More specific testing may also be performed (for example, isoelectric focusing followed by immunoblotting and staining for IgG for the detection of oligoclonal bands in patients with multiple sclerosis) (5, 6).

5.2. Sample Shipment

In the event that a sample must be transported, it must be done so on dry ice and preferably initiated on a Monday so that the sample arrives at its destination during the same week (6). The amount of dry ice utilized must be adequate to ensure that the specimen remains at an appropriate temperature for at least three days. Excessive thawing temperatures must be avoided.

6. Notes

1. There is no data to indicate that biomarker concentrations are affected by the instrument that is utilized during the procurement process (6). Therefore, the type and gauge of needle that is selected for the purpose of CSF withdrawal is solely dependent upon whether the patient is alive or deceased. Clearly, the safety and comfort of a living patient is of paramount importance, and studies have shown that atraumatic needles are tolerated best in patients undergoing lumbar punctures (6). The incidence of post-procedural headaches is approximately six times greater (70% vs. 12%) if large gauge needles (i.e., 20–22 gauge) are utilized over smaller gauge needles (i.e., 16–19 gauge) (6, 9, 10).

2. Unlike needles, the type of collection tube that is utilized has been shown to affect the concentrations of CSF biomarkers (e.g., total tau proteins and amyloid beta peptides) (6, 11). The consensus guidelines proposed by Teunissen et al recommend collection tubes manufactured with polypropylene due to its low protein binding potential (6). Glass collection tubes should be avoided due to the potential sharps injury hazards that they pose to laboratory personnel (6). Ideally, only one tube should be utilized, but if more than one is necessary, the entire amount should be combined after centrifugation in order to minimize concentration gradient effects (6) (please refer to the section entitled “Centrifugation” (section 4.2) under the “Processing & Storage of Cerebrospinal Fluid” in this chapter). Additives should not be placed within these containers.

3. Certain biomarkers that are present within CSF have been shown to vary in concentration at different times of the day as a consequence of the effects of the circadian rhythm on their production and release (12). However, since collection is often logistically difficult to coordinate and perform at regular time periods because of several variables in day to day clinical practice, documentation of collection time is crucial so that collaborators may select the most appropriate samples from the repository at a later date (6).

4. Many biomolecular and cellular constituents differ in concentration depending on anatomic location, such that a rostrocaudal concentration gradient exists for the majority of CSF components (5). As a consequence, the volume of CSF collected may impact the concentration of several biomarkers of interest (6). In addition to the anatomic site from which CSF is procured, one must also factor in the volume that is obtained; larger volumes correlate with more thorough sampling. The greater the volume of CSF collected, the greater the chance that variation in constituent biomarker concentrations is minimized (6). If a lumbar puncture is the method of choice for living patients, the first two milliliters may be utilized for the purposes of basic CSF analysis, while the remainder is retained for the purposes of biobanking (6). In these patients, the volume of CSF that is withdrawn has not been shown to correlate with increased risk of post-procedural headache (6, 13, 14).

5. In most situations, the storage of withdrawn CSF samples may be kept at room temperature during the pre-processing period immediately following sample collection, which includes the time before, during, and after centrifugation (6). In all cases, documentation of collection time and of storage time is necessary so that samples with uniform pre-processing periods may be selected for study at a future date. There is no data that indicate an advantage to temporarily storing CSF samples at 4° C until processing (6). However, it should be noted that the information available about this specific issue is limited (6).

6. Generally, when CSF is collected, contamination with blood and/or brain parenchyma is unavoidable. Even if the sample appears clear to the naked eye, minute amounts of these contaminants (especially serum compounds with high concentration levels such as coagulation factors) are almost always present and may contribute to erroneous results (6). Centrifugation virtually separates contaminants from samples of interest. If possible, an erythrocyte count should be obtained for each instance, and samples with a count greater than 500/μL are not recommended for use for biomarker studies (6).

7. Biosamples are frozen at −80° C in order to retain a high degree of nucleic acid and protein integrity (7).

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Precautions

As with all instances of tissue and bodily fluid collection from central nervous system (CNS) sources, appropriate universal precautions must strictly be adhered to in order to minimize the possibility of exposure to infectious agents. This is especially true in patients with confirmed or suspected Creutzfeldt-Jakob disease (CJD) and other human prion disorders (transmissible spongiform encephalopathies), which are a group of rare, untreatable, and invariably fatal neurodegenerative diseases (1). Direct inoculation is the main risk when handling tissues and bodily fluids from these patients. As such, contamination of mucosal surfaces and the eyes must be avoided (2).