**GUIDELINE FOR CEREBROSPINAL FLUID collection, Processing and storage**

**NOTE: An international working group provided detailed guidelines and recommendations for the collection and processing of CSF samples (pre-analytic methods), which should be reviewed and incorporated as much as possible. The guidelines below are consistent with those guidelines.**

**I. Introduction.**

The use of biomarkers detectable in CSF for diagnostic testing and disease monitoring has grown considerably in the past years, both in the number of biomarkers in broad use in Alzheimer’s Disease research and in the level of validation data available. A major goal across research consortia is to standardize methods of collection, storage and other preanalytical factors that will affect data sharing and multicenter initiatives where data is combined in large datasets. The previous release of this guideline was released by the NIA/ADRC.NACC in 2014. Over the past ten years, numerous publications have specifically addressed validation of biomarker assays and characterized cross platform performance across large multicenter cohorts. The characterization of preanalytical factors that can affect the determination of physiological levels of circulating biomarkers has been increasingly reported in such studies. Similarly, there is a need to combine data runs from cohort studies that differ in time and geography, even for the same assay and technology platform. Factors affecting stability of analytes under laboratory conditions and collection vehicles were studied and are summarized here. These factors are incorporated into updated guidelines when sufficient data are available. The science of preanalytical factors should inform research networks on appropriate methods to use in evaluating individual biomarkers and facilitate the standardization and comparability of assay performance to avoid site or operator-specific impact on the data obtained.

The following recommendations are intended to be guiding principles for Alzheimer’s Disease Research Centers (ADRCs) for the collection, processing, and storage of cerebrospinal fluid (CSF) for Alzheimer’s disease biomarker research purposes. Such guidelines will foster data harmonization across ADRCs while still encouraging Center investigators to explore novel CSF Alzheimer's disease biomarkers and study the optimization of pre-analytic CSF collection and storage methods. These guidelines were developed with consideration of available evidence to date on optimal pre-analytic procedures with the understanding that many areas require more research. Clinical tips and related resources on best practices for lumbar puncture procedures are described in a separate document ([ADNI3\_Biomarker\_Sample\_Collection\_Processing](https://adni.loni.usc.edu/wp-content/themes/freshnews-dev-v2/documents/bio/ADNI3_Biomarker_Sample_Collection_Processing_and_Shipment.pdf)). Example CSF collection, handling, processing, and storage supply lists are also described here ([CDC\_CSF\_Guidelines](https://www.cdc.gov/acute-flaccid-myelitis/hcp/diagnosis-testing/specimen-collection-for-afm.html)) for ADRCs initiating their CSF collection program.

The present CSF biomarker guidelines reflect the outcome of committee review of the current 2025 literature and other clinical chemistry resources regarding biofluid biomarkers that are routinely or exclusively studied in the CSF. The current best practices expand on the beta-amyloid (Aβ) guidelines and address a number of additional biomarkers in ADRC surveys for which the following criteria could be demonstrated:

**II. Criteria for biomarker inclusion in the current guidelines**

1. **Studied at ADRCs and assayed in CSF**
2. **Qualified in multiple cohorts by independent centers**

**Table 1. Current Biomarkers Assayed in CSF at ADRCs and Considered for this Guideline.**

|  |
| --- |
| **CSF Biomarkers Currently Studied at ADRCs** |
| Amyloid β (Aβ 38, Aβ 40, Aβ 42) |
| Cytokine and chemokine panels (e.g., Interleukins) |
| Glial Fibrillary Acidic Protein (GFAP) |
| Neurofilament Light Chain (NEFL, NfL) |
| Neurogranin (NRGN) |
| Synaptosome Associated Protein-25 (SNAP-25) |
| Synuclein α (SNCA) |
| Synuclein α (SNCA, p-Ser129-SNCA)  |
| Synuclein Beta (SNCB) |
| TAR DNA Binding Protein 43 (TDP-43) |
| Tau, Brain Derived Isoform or total (BD-Tau) |
| Tau, Microtubule Binding Region fragment (MTBR) |
| Tau, Phosphorylated at T181, T205, S212, S217, T231, S396 (p181-Tau, p205-Tau, p217-Tau, p231-Tau, etc.) |
| TREM2, soluble |
| Vascular Endothelial Growth Factor (VEGF) |
| VILIP-1 |
| YKL40 (Chitinase like 3) |

**II. Acquisition of Cerebrospinal Fluid (CSF) Biospecimens.**

1. Fasting is not necessary for CSF Aβ collection[1, 2]; Centers may determine if fasting is recommended for other novel biomarker assessments.
2. CSF should preferably be collected at a consistent time of day, in the morning (e.g., 0800-1100h)[3-5].
3. Consider taking matching plasma and/or serum samples for simultaneous measurement of CSF and blood biomarkers.
4. Use of an atraumatic spinal needle (e.g., Sprotte 25, 24, or 22 gauge needle) is recommended for the LP to minimize risk of post-LP headache (<5%) [6]. Having spinal needles of various lengths (e.g., 90-mm, 103-mm, 120-mm, etc.) available at the time of the procedure will increase the success of CSF acquisition in individuals of varying size.
5. The gravity drip method is preferred for fresh CSF analyses and measurement of Aβ and tau[2] . CSF may be withdrawn under negative pressure with sterile polypropylene syringes. Up to 30 mL CSF may be withdrawn without increased risk of adverse events[7]. The extension tubing/siphon provided in LP kits should **not** be used.

**Table 2. Literature Support Addressing Collection Issues (time of day, fasting, tube type).**

|  |  |  |  |
| --- | --- | --- | --- |
| **Biomarker** | **Issue of Concern** | **Specific Consideration** | **References** |
| Analytes, various | Time of day sampling  | Diurnal variation shown with 6.2-12.2% variation for several (e.g., NRGN, SNCB, SNCG, but not GFAP, NfL or pTau/Tau) | [8] |
|  Aβ1-42 | Tube material and fill volume | Adsorption of Aβ to the recipients. Re: fill volume, some studies find no impact (summary table in [9] Hansson 2018). | [9, 10] |
| Aβ1-42 | Tube Surface Absorption During Transportation of Fresh Samples | Aβ1‐42 levels were lower with horizontal versus upright transportation | [2] |
| Aβ1-42 | Tube Surface Absorption During Transportation/ Storage Conditions | Aβ1‐42 levels were higher with maximal tube filling | [2] |
| Aβ1-42 | Tube Surface Absorption | Amyloid beta (Aβ)1‐42 levels varied by tube type, using a low-bind, polypropylene, e.g., Sarstedt 2.5 mL False Bottom Tube (FBT) reduced variation. | [2] |
| Aβ1-42 | Tube Surface Absorption During Mixing | For fresh CSF, Aβ1‐42 levels were higher with no mixing versus roller/inversion mixing. Roller mixing after thawing is recommended for frozen samples. | [2, 13] |
| Aβ1-42 | Hemolysis and Blood Borne Proteases | In frozen CSF samples, 0.25% blood contamination decreased Aβ1–42 concentrations | [1] |
| Aβ, pTau, tTau, | Time and temperature collection – permanent storage | Optimal storage is at -80° C | [9] |

**\*Based on available information in published guidelines and unpublished working knowledge from experienced research centers.**

**III. Preparation and Storage of Cerebrospinal Fluid Biospecimens.**

**A. Fresh and frozen CSF collection for Aβ and Tau measures[2].**

1. All tubes handled by a clinician during the lumbar puncture must be sterile.
2. Using gravity drip, discard first 1-2 mL CSF or until clear to limit potential blood contamination.
3. Continue to use the gravity drip method to collect 2.5 mL directly into a 3 mL polypropylene low protein binding tube (e.g., Sarstedt false-bottom tube). The number of tubes drawn in this manner will be determined by each Center. While no specific vendor is endorsed, tubes must be validated (by published report or Center experiments) to ensure they are low binding for Aβ.
4. For fresh CSF samples, there should be no further handling (no centrifugation, freezing, mixing/inverting or tube transfers).
5. Transport and store fresh CSF sample at 2-8° C up to 14 days (if not feasible, transport and store at room temperature [20-25° C] for up to 2 days). Samples should be kept upright to avoid interaction with tube caps, which are not polypropylene.
6. Once fresh CSF arrives at the testing site, there should be no further handling. Measure Aβ40, Aβ42, tTau, and pTau immediately, when possible.
7. If some CSF samples collected for Aβ and tau analyses (using methods above) are going to be stored in a biorepository, these samples may be frozen at -80° C for long-term storage. CSF aliquot storage tubes must be validated (by published report or Center experiments) for freezing and storing at -80° C[2].

**B. Additional CSF analytes and for biobanking.**

1. After discarding the first 1-2 mL and collecting Ab/tau samples via gravity drip method, proceed with either continued drip technique or syringe suction technique as determined by Center investigators.
2. Collect a total of up to 30 mL CSF in 5 or 10 mL polypropylene tubes and/or syringes.
3. A volume of 1 mL of CSF (or the minimum amount needed) should be sent to the local clinical laboratory for analysis of cell count to assess sample quality for blood contamination. Other local laboratory measurements, such as CSF protein or glucose, should be determined by Center investigators based on study needs. The local laboratory sample may be placed in the plastic (polystyrene) tubes that are included in some commercial LP kits. The polystyrene tubes can ONLY be used for the sample sent to the clinical laboratory.
4. If CSF was collected in syringes, transfer remaining CSF into one 30 mL polypropylene tube and do not mix.
5. Centrifuge for 2000 g x 10 minutes in a temperature-controlled room (20-25° C).
6. Remove CSF with a pipette and transfer to a second 30 mL polypropylene tube, being careful not to disturb the pellet.
7. Invert briefly to mix the 30 mL tube.
8. Divide the CSF into 0.5 mL aliquots in 1 mL polypropylene tubes (or fill tubes to at least 75% of tube capacity to reduce variability caused by CSF volume to tube surface area differences).
9. Use of screw-cap tubes with rubber O-rings are recommended to reduce evaporation.
10. Freeze at -80° C (no need to flash freeze on dry ice).

**C. Note on processing CSF for extracellular vesicles.**

Processing parameters for preparation, isolation, gradient centrifugation and volume considerations for extracellular vesicle (EV) research is beyond the scope of these guidelines and should be developed according to research needs of individual labs. Generally, 1-2 mL volumes of CSF are required for isolation of EVs, and therefore should be isolated from specific research protocols at individual centers to preserve curated ADRC specimen volumes for the larger community of users. Some guidelines for EV isolation are available from vendors. For example, see protocol link below in section V.

**D. Uniform, non-redundant, HIPAA-compliant annotation of samples is recommended.**

1. **Use of sample management software is recommended**.

Document the exact volume of fluid obtained at each CSF collection, as this can be variable.

1. **Documentation.**

Appropriate and complete documentation surrounding biospecimen collection, processing, and storage are essential and relevant to the quality of research data to be obtained. Note date and details of any changes to or deviations from the pre-analytic procedure.

1. **Freeze-Thaw Recommendations.**

Thawing and refreezing of samples is strongly discouraged as the number of freeze/thaw cycles affects analyte recovery for Ab. Freeze-Thaw stability should be determined for each biomarker studied, if unknown.

1. **Alarm systems.**

A monitored back-up alarm system and plan for freezer failure is recommended. Centers are encouraged to have an open freezer available in case of freezer failure. These can also be rented from specialized appliance repair companies if an extra freezer is not available. Annual or semi-annual maintenance is recommended, depending on the frequency of entry, tendency for ice build-up and the manufacturer’s recommendations.

**Table 3. Notes on Storage and Preanalytical Processing Issues Affecting Biomarker Stability.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Biomarker** | **Issue of concern** | **Specific considerations** | **References** |
| Aβ1-40 | Freeze-Thaw Cycles | Aβ1-40 significantly decreased after **4** freeze-thaw cycles, | [9] |
| Aβ1-42 | Freeze-Thaw Cycles | Aβ1-42 significantly decreased after **2** freeze-thaw cycles. | [14,17,19] |
| Aβ1-42/ Aβ1-40 ratio | Freeze-Thaw Cycles | Ratios stable over 4 freeze-thaw cycles  | [12] |
| GFAP | Freeze-Thaw Cycles | Average decline of 188.21 pg/ml beyond the first freeze-thaw cycle. | [15] |
| NfL | Stability | Not stable in neat CSF during 1 day at RT. Store at 4° C or freeze in aliquots immediately  | [15,16] |
| pTau, total Tau | Stability, Freeze-thaw | Tau was table long term at – 80° C, and for 6 freeze thaw cycles. | [17,19] |
| TREM2 | Freeze-thaw | TREM2 takes 5 freeze-thaw cycles to be impacted | [12] |

\*Considering the multitude of research initiatives and biomarkers the ADRC will support, the most stringent storage criteria are recommended for all sites.

**IV. Sharing and Dissemination of Cerebrospinal Fluid Samples.**

1. The repository is a national resource to be shared for the purpose of answering valid scientific questions related to cognitive aging and dementia. Center investigators should discuss in advance the amount of CSF to be collected per visit to reserve specimen material for internal and/or future studies, balanced with a transparent resource sharing plan.
2. Specific evaluation criteria for specimen requests should be documented and consistently applied by a Center-designated committee.
3. CSF biospecimen sharing is recommended to be limited to the smallest number of samples and sample volume required to adequately answer the research question under investigation, as defined by power calculations.
4. Use of sample management software to assist with sample tracking and dissemination.
5. Each Center should develop operating procedures to facilitate timely resource sharing, including IRB/HIPAA approval of resource sharing, institutional material transfer agreements, institutional data use agreements, Center-specific resource use agreements (Center acknowledgement and what can be done with sample), specific federal human sample shipping training, and shipping manifests related to shipping CSF samples domestically and internationally to optimize successful transport of these valuable biospecimens.

**V. Additional Considerations.**

Processing cells from CSF is beyond the scope of this guideline but can be accomplished with standard centrifugation of 300 x g (= 300 RCF) for 10 min, and the cell pellet saved in appropriate freezing medium after carefully decanting CSF. CSF derived cell pellets can be stored in appropriate freezing medium in cryovials at -80° C. (see CDC guideline, below for more information; 18).

**Biological factors affecting biofluid biomarkers detection:**

A number of biological factors related to the human research participant were demonstrated to relate to differences in CSF biomarker results (*e.g*., age, sex, race/ethnicity, BMI, exercise, medical conditions and comorbidities, food and beverages consumed prior to collection, medications-including OTC and supplements, circadian and diurnal cycle relative to time of lumbar puncture)[[1-3](file:///C%3A/Users/elliottce/AppData/Local/Microsoft/Windows/Temporary%20Internet%20Files/Content.IE5/DMP8FZQ8/Updated%20Blood%20and%20Urine%20sections%2012.5.13.docx#_ENREF_1)]. To support the full spectrum of use cases from ADRC specimens, attempts should be made to record as much information related to these variables as possible in order for appropriate adjustments to be made during analysis of results.

**V. References**

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14. Ho, S., et al., *Assessment of Preanalytical Cerebrospinal Fluid Handling and Storage Factors on Measurement of Aβ1-42, Aβ1-40, and pTau181 Using an Automated Chemiluminescent Platform.* J Appl Lab Med, 2024. **9**(4): p. 789-802.

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16. Koel-Simmelink, M.J., et al., *The neurofilament light chain is not stable in vitro.* Ann Neurol, 2011. **69**(6): p. 1065-6; author reply 1066-7.

17. Schoonenboom et al. 2005 *Effects of Processing and Storage Conditions on Amyloid β (1–42) and Tau Concentrations in Cerebrospinal Fluid: Implications for Use in Clinical Practice*, Clinical Chemistry, Volume 51, Issue 1, 1 January 2005, Pages 189–195, <https://doi.org/10.1373/clinchem.2004.039735>

18. Centers for Disease Control and Prevention (2018). *Acute Flaccid Myelitis: Specimen Collection Instructions.* Retrieved January 27, 2019, from <https://www.cdc.gov/acute-flaccid-myelitis/hcp/instructions.html>

19. Strand et al. *β-Amyloid in Cerebrospinal Fluid: How to Keep It Floating (Not Sticking) by Standardization of Preanalytic Processes and Collection Tubes,* The Journal of Applied Laboratory Medicine, Volume 6, Issue 5, September 2021, Pages 1155–1164, <https://doi.org/10.1093/jalm/jfab024.>

**Additional manuals as resources:**

[**https://www.beckman.com/resources/sample-type/extracellular-vesicles/getting-started/isolation/isolation-methods**](https://www.beckman.com/resources/sample-type/extracellular-vesicles/getting-started/isolation/isolation-methods)