**Guidelines for blood specimen** **collection, Processing and storage**

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

1. **Introduction**

The use of biomarkers detectable in blood 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 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 in this guideline update when sufficient data were 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 present blood biomarker guidelines reflect the outcome of committee review of the current 2025~~4~~ literature and other clinical chemistry resources regarding biofluid biomarkers that are routinely studied in the blood. The current best practices expand on the prior guidelines and address a number of additional biomarkers reported to be in use in ADRC surveys for which the following criteria could be demonstrated:

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

**1. Studied at ADRCs and assayed in blood biofluid (e.g., plasma or serum);**

**2. Qualified in multiple cohorts by independent centers**

Table 1, below, lists biomarkers in use at the 37 ADRCs that met the criteria and were reported during a survey of activities performed in 2024.

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

|  |
| --- |
| **Blood Biomarkers Currently Studied at ADRCs** |
| Amyloid β (Aβ 38, Aβ 40, Aβ 42) |
| Brain Derived Neurotrophic Factor (BDNF) |
| Cytokine and chemokine panels (e.g., Interleukins) |
| Glial Fibrillary Acidic Protein (GFAP) |
| Intercellular Adhesion Molecule 1 (ICAM-1, CD54) |
| Neurofilament Light Chain (NEFL, NfL) |
| Neurogranin (NRGN) |
| Neuron Specific Enolase (NSE, ENO2) |
| 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.) |
| TREM1, soluble |
| Vascular Cell Adhesion Molecule (VCAM-1) |
| Vascular Endothelial Growth Factor (VEGF) |
| VILIP-1 |
| YKL40 (Chitinase like 3) |

1. **Preparation and storage of blood biospecimens**

**Collection of blood samples for biomarker studies.**

1. **Biological factors affecting biofluid biomarkers detection.**

A number of biological factors related to the human research participant were demonstrated to relate to differences in blood biomarker results (*e.g*., age, sex, race/ethnicity, BMI or body composition, 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 blood draw)[[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 for cases from ADRC specimens, attempts should be made to record as much information related to these variables as possible for appropriate adjustments to be made during analysis of results[1, 2].

1. **Fasting versus non-fasting prior to blood draw.**

Many Alzheimer’s disease studies utilize fasting blood collection and this is recommended[3]. However, given patient and clinic needs, this is not always possible. Therefore, whether fasting or non-fasting, time of day and time since last meal should be recorded.

1. **Avoiding Errors.**

Estimates suggest that up to 46% of laboratory errors come from pre-analytic processing[[4](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_4)]. Factors related to blood collection devices (needle gauge, tube lubricants, tube walls) can impact blood biomarker levels[[1](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), [5](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_5), [6](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_6)]. The largest source of pre-analytical variation derives from the time delay to centrifugation and time from centrifugation to storage, during which the tubes are kept at RT or at 4° C[3]. Standardized and uniform techniques of sample processing are recommended[[1](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), [2](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_2), [7](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_7)]. Deviation from standard recommendations should be noted upon data entry.

1. **Order of use of blood draw tube type.**

Detailed step-by-step procedures for collection of blood samples are available in the CLSI H3-A6 [8]. Broad recommendations for standardization of sample collection are:

* 1. Blood should be collected while seated from the median cubital vein as opposed to other, more fragile, veins
	2. Alcohol used to clean the skin should be allowed to evaporate before venipuncture.
	3. A tourniquet applied 3-4 inches above the site of venipuncture should be loosened once blood starts to flow.
	4. Blood is generally drawn with a vacutainer system.
	5. Tubes for plasma should be adequately filled with blood to ensure the optimal blood/additive ratio.
	6. For most studies, the needle gauge of 19-23 is preferable with 21g being the most common [[5](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_5), [8](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_8)].
	7. Order of blood draw should be as follows (skip tubes not being utilized) (CLSI H3-A6; Qiagen):
		1. Blood Culture Tube
		2. Coagulation tube
		3. Serum tube with or without clot activator or gel
		4. Heparin tube with or without gel separator
		5. EDTA Plasma tube with or without separator
		6. Glycolytic inhibitor tube
		7. PAXgene™ Blood RNA tube
1. **Processing of serum tubes.**
	1. Serum tubes include a clot separator gel or clot activator.
	2. After filling serum tubes, incubation to allow clot formation is needed.
	3. Serum should be clotted in a tube rack in a vertical position for at least 30 minutes and no more than 60 minutes, followed by centrifugation if the patient is not on anti-coagulant therapy.
	4. Relative centrifugal force (RCF; number x g; g-force) should be utilized rather than using revolutions per minute (RPM) in SOPs and publications, since RPMs vary by rotor size.
	5. Centrifugation at 2000 x g is performed for 10 min to separate serum and clotted material.
	6. Note: Horizontal rotors are preferable.
	7. Note: Centrifugation at room temperature versus refrigerated (4°C) can cause variation in downstream assay detection levels for certain markers in serum.
	8. Note: When processing DNA from plasma, see (G), below.
	9. Note: Refrigerated centrifuges are also recommended for platelet preparation.

1. **Processing of plasma tubes.**
	1. Plasma tubes are vacutainers with different additives, including EDTA and heparin.
	2. Plasma tubes should be filled and then gently inverted 8-10 times to mix the additives.
	3. Plasma tubes are incubated at room temperature for 30 minutes prior to centrifugation.
	4. Relative centrifugal force (RCF; number x g; g-force) should be utilized rather than using revolutions per minute (RPM) in SOPs and publications, since these vary by rotor size.
	5. Centrifugation at 2000 x g is performed for 10 min to separate serum and clotted material.
	6. Note: Horizontal rotors are preferable.
	7. Note: Centrifugation at room temperature versus a refrigerated (4°C) can cause variation in downstream assay detection levels for certain markers in plasma.
	8. Note: Refrigerated centrifuges are also recommended for platelet preparation.
	9. Note: When processing DNA from plasma, see (G), below.
2. **Processing of DNA from plasma tubes**.
	1. During preparation of plasma, an interphase between red cells and clear serum fluid forms, containing buffy coat, a layer of white blood cells.
	2. After the plasma is carefully pipetted into destination tubes, aliquots of buffy coat cells should be frozen at – 80° C until DNA extraction. Generally, 5 ml plasma will produce 0.1-0.2 mL of buffy coat volume.
3. **Processing of tubes with glycolytic inhibitors.**
	1. **Mixing**: Immediately after collection, gently invert the tube 8-10 times to ensure proper mixing of the blood with the glycolytic inhibitor.
	2. **Centrifugation**: Process the samples promptly. Centrifuge the tubes at the recommended speed and duration to separate the plasma or serum from the cells.
	3. **Storage**: Store the processed samples at the appropriate temperature to maintain sample stability. Glycolytic inhibitor tubes should be stored at 4° C if analysis is delayed.
4. **Processing of PAXgeneTM RNA tubes.**
5. Immediately after blood collection, gently invert the PAXgene RNA tubes 8-10 times.
6. Place tubes in a rack to stand in the vertical position at RT for a minimum of two (2) hours and a maximum of 72 hours.
7. Place the PAXgene RNA tubes in a 5” cryobox and store cryobox in a -20° C freezer.
8. After 24 hours, transfer to -80° C storage
9. Record the specimen location and the time the specimens are placed in freezer
10. Further processing of PAXgene RNA tubes should follow the manufacturer’s protocol for RNA purification, quantification and integrity evaluations.

1. **Additional sample processing considerations.**
	1. Rapid processing of samples is optimal (total processing time < 2hrs from “stick-to-freezer”). Detailed procedures for processing blood specimens are provided by CLSI H18-A4 [7]. General recommendations follow, although individual steps may need modification for specific markers:
	2. Serum/plasma should be physically separated from contact with cells as soon as possible (<2hrs). Do not store aliquots from serum/plasma that were in contact with cells for > 2hrs.
	3. Other factors that should be documented:
		1. Type of collection tube (manufacturer’s name, type of anticoagulant, etc.)
		2. Time from collection to centrifugation
		3. Temperature between collection and centrifugation
		4. Presence and type of separator
		5. Temperature of centrifugation
		6. Number of centrifugations (single or double)
2. **Other considerations for additives.**
	1. Whether it is necessary to add protease inhibitors to samples after aliquoting is not certain and depends upon the nature of the study. This may be worth consideration if plasma or serum samples are to be used for proteomic analyses and must be noted.
3. **Post centrifugation considerations.**
	1. The following should be documented:
		1. Type of secondary container (tube, straw)
		2. Time between centrifugation and freezing
		3. Storage temperature
		4. Number of freeze/thaw cycles
		5. Duration of storage
		6. Storage location of aliquot vials
		7. Degree of hemolysis and lipemia (see section P, below)

**M. Aliquoting Recommendations:**

1. If possible, it is recommended to use low protein binding versions of these materials
2. Aliquots should be made in siliconized polypropylene tubes (or straws) using polypropylene tips for pipets.
3. Small aliquots (generally not larger than 0.5 ml) are recommended for storage. Avoid unnecessary freeze/thaw cycles of samples. For plasma or serum samples, consider aliquoting in small volumes, *e.g*., 100 to 200 microliters, if these are to be used for a large number of analyses if such storage sizes does not have a known impact on the assay results.

**N. Storage and shipping recommendations:**

* + 1. Long-term storage should be at -80°C or in liquid nitrogen.
		2. If storage on dry ice is utilized for shipment, the headspace should be vented or the sample should be allowed to sit in -80°C freezer for 9 hr prior to thaw[[9](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_9)].
		3. Consider using disposable thermometers to keep track of temperature during transportation.

**O. Document the volume of plasma or serum that was obtained.**

1. Record total volume of plasma or serum collected
2. Record individual aliquot volumes for plasma or serum

**P. Factors influencing the quality of serum or plasma:**

* 1. **Hemolysis**: Red or pink tingeing of plasma or serum is an indicator that significant hemolysis has occurred (scored on a depth of color scale (denoted H1-H6, with grade H3 and higher representing significant hemolysis- see Appendix for example), and can be quantified using a correction factor by measuring hemoglobin level (Hb) by spectrophotometry (several methods have been published).
	2. **Lipemia**: A milky white substance floating in the plasma or serum, which may render the samples less useful for many biomarker studies should be determined on case-by-case basis [Reference Needed].

**Q. Important points for PAXgene collection and processing.**

1. Store PAXgene™ Blood RNA Tubes at RT (18° C to 25° C) before use.
2. If the PAXgene ™ Blood RNA Tube is the only tube to be drawn, a small amount of blood should be drawn into a “Discard Tube” prior to drawing blood into the PAXgene ™ Blood RNA Tube. Otherwise, the PAXgene ™ Blood RNA Tube should be the last tube drawn in the phlebotomy procedure.
3. Allow at least 10 seconds for a complete blood draw to take place in each tube. Ensure that the blood has stopped flowing into the tube before removing the tube from the holder. The PAXgene™ Blood RNA Tube with its vacuum is designed to draw 2.5 mL of blood into the tube.
4. Immediately after blood collection, gently invert/mix (180 degree turns) the PAXgene™ Blood RNA Tube 8 – 10 times.
5. Incubate the PAXgene™ Blood RNA Tube UPRIGHT at RT (18°C to 25° C) for 24 hours. Record time/date of draw.
6. REPEAT STEPS a-e for each PAXgene™ Blood RNA Tube to be collected per subject.
7. After 24 hours at RT, transfer the PAXgene tubes to -80°C (or -20° C) freezer. Record time and date of freezing.

**R. Long term storage recommendations.**

Centers should refer to OBBR [2] guidelines maintenance and long-term storage recommendations.[2]

**Table 2. Literature support addressing collection issues (tube type, processing, freeze-thaw cycles).**

|  |  |  |  |
| --- | --- | --- | --- |
| **Biomarker**  | **Issue of Concern** | **Specific Consideration** | **References** |
| Aβ1-40, 1-42  | Tube material and fill volumeDifferences in measurable quantity | Adsorption of Aβto the recipients. Re: fill volume, some studies find no impact (summary table on Hansson 2018).Loss in clot formation in serum, lower levels in Plasma EDTA vs. Citrate  | [3, 9, 10] |
| BDNF | Differences in measurable quantity. | EDTA Plasma tubes decrease measurable BDNF. | [11,12] |
| Cytokines | Tube type/Additive | Certain cytokines shown to be less stable in serum. Recommend EDTA plasma tubes. | [13-14] |
| GFAP | Freeze/thaw cycles | After 2 freeze/thaw cycles, concentration decreases | [9, 15, 16] |
| NfL | Freeze/haw cycles | Loss of 8% activity after 3 Freeze-thaw cycles | [15] |
| NRGN | Stability | Stable at -20° C | [14] |
| pTau217 | Centrifugation prior to measurement | Better correlations of plasma pTau217 with CSF pTau217 and Aβ42/Aβ40 for samples centrifuged at 2000 x g at 4° C for 10 min compared to non-centrifuged. | [12] |
| pTau, Tau, MTBR-Tau | Freeze/haw cycles | After 2 freeze/thaw cycles, concentration starts to significantly decrease.  | [9, 12, 15, 1] |

**S. Note on processing of 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 plasma 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.

**Table 3. Notes on storage and stability issues: Evidence for specific biomarkers.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Biomarker** | **Issue of concern** | **Specific considerations** | **References** |
| Aβ1-40, 1-42 | Quantity- see above | Decreased detection level | [3,10] |
| BDNF | Quantity- see above | Decreased detection level | [11-12] |
| Cytokines/chemokines | Stability | Stable frozen, storage at 4 C affects IL‑8,VEGF, TNFα and EGF | [18] |
| GFAP | Freeze/thaw cycles | After 2 freeze/thaw cycles, concentration decreases | [9, 13, 15] |
| NfL | Freeze/haw cycles | Loss of 8% activity after 3 Freeze-thaw cycles | [13,14] |
| NRGN | Stability | Stable at -20° C, though plasma/serum fragment patterns exist. | [14] |
| pTau, Tau, MTBR-Tau | Freeze/haw cycles | After 2 freeze/thaw cycles, concentration starts to significantly decrease.  | [9, 12, 13, 15] |
| pTau217 | Thawing Temperatures | Thawing conditions (RT or on ice) does not affect performance of plasma pTau217. | [12] |

1. **Sharing and Dissemination of Plasma and Serum Samples.**
2. 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 sample to be collected per visit to reserve specimen material for internal and/or future studies, balanced with a transparent resource sharing plan.
3. Specific evaluation criteria for specimen requests should be documented and consistently applied by a Center-designated committee.
4. Plasma or serum 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.
5. Use of sample management software can assist with sample tracking and dissemination.
6. 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 plasma or serum samples domestically and internationally to optimize successful transport of these valuable biospecimens.

**V. References**

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12. Wessels, J.M., Agarwal, R.K., Somani, A. et al. *Factors affecting stability of plasma brain-derived neurotrophic factor.* Sci Rep 10, 20232 (2020). https://doi.org/10.1038/s41598-020-77046-6Hviid et al. *Reference interval and preanalytical properties of serum neurofilament light chain in Scandinavian adults.* Scand J Clin Lab Invest, 2020, Vol. 80, Page 291-295.
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**Additional manuals as resources:**

**Extracellular Vesicle Preparation**

[**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)

**Greiner Vacuaette SOP**

[**https://www.gbo.com/fileadmin/media/GBO-International/02\_Downloads\_Preanalytics/TECHNICAL\_Instructions\_for\_Use/English/980200B\_09\_EN.pdf**](https://www.gbo.com/fileadmin/media/GBO-International/02_Downloads_Preanalytics/TECHNICAL_Instructions_for_Use/English/980200B_09_EN.pdf)

**PAXgene RNA Tube SOP**

[**https://www.bdbiosciences.com/content/dam/bdb/products/global/blood-collection/blood-collection-tubes/762xxx/7621xx/762165\_base/pdf/VDP40400.pdf**](https://www.bdbiosciences.com/content/dam/bdb/products/global/blood-collection/blood-collection-tubes/762xxx/7621xx/762165_base/pdf/VDP40400.pdf)