



National Institutes of Health

National Institute of Neurological Disorders and Stroke
National Institute on Aging

MarkVCID2 Fluid Sample Best Practices

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MarkVCID Consortium

By the MarkVCID1 Fluid-based Biomarkers Subcommittee (Co-chairs Donna Wilcock, PhD and Pia K. Webb, MD, PhD) and Coordinating Center (PI Steven M. Greenberg, MD, PhD).

Based in substantial part on the Biospecimen Best Practice Guidelines for the Alzheimer's Disease Centers, v3.0. Reproduced with permission. 24 June 2014 National Institute on Aging. Created and published by the NIA Biospecimen Task Force (Co-chairs Tatiana Foroud, PhD and Thomas J. Montine, MD, PhD).

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1. Blood Guidelines

General Guidelines

Several factors related to the physiology of the human research participant have been demonstrated to impact blood biomarker results (e.g., age, gender, ethnicity, exercise, overall health, food and beverages consumed prior to collection, medications, time of day of blood draw).

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. At a minimum, age, sex, ethnicity, medications and time of blood draw will be recorded.

Many Alzheimer's disease studies globally utilize fasting blood collection and this is preferred and strongly encouraged. Whether fasting or non-fasting, time since last meal should be collected.

Blood Collection and Processing Procedures

Follow the MarkVCID standardized sample collection consumables and procedures below.

A. Collection Tubes, Storage Cryovials and Biosample Volumes

1. To ensure the consistency and quality of the samples across sites, the MarkVCID Consortium adopted the following collection tubes and cryovial for storage:

- a. [Serum SST collection tube 8.5 ml BD Vacutainer 367988](#)
- b. [Plasma EDTA collection tube 10 ml BD Vacutainer 366643](#)
- c. [Cryovials for plasma/serum 0.5ml DWK W985874](#)
- d. [Cryovial for packed cells for DNA collection 1.2ml DWK W985862](#)

2. Based on the tube volumes above, consortium sites are expected to collect and store for consortium use between 4-5ml plasma, 4-5ml serum (5ml preferred for both sample types when possible) and 3-4ml packed cells for DNA extraction.

3. Sites unable to acquire the above cryovial for plasma and serum due to the temporary plastic shortage may use the following approved alternative low-protein binding backup cryovial:

- a. [Micrewtube \(low adhesion surface\) 0.5 ml Simport T341TLST](#)
with [Colored closure T340](#)

If a site is unable to acquire any of the tubes and cryovials above, please reach out to the Coordinating Center (Carissa.Tuozzo@mgh.harvard.edu) for additional support.

B. Blood Collection

1. Using the required blood collection tubes noted above, blood should be collected 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 (see required collection tubes noted above).
5. Tubes should be adequately filled with blood to ensure the optimal blood/additive ratio and maximum availability of stored biosamples.
6. Order of blood draw should follow the procedures in CLSI H3-A6 (Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture). Serum tubes (with or without clot activator or gel) should be drawn before EDTA tubes (with or without separator).

Further blood sample collection procedures are available in the CLSI H3-A6.

C. Processing of Samples

All Biosamples

1. Rapid processing of biosamples is optimal (total processing time < 2hrs from “stick-to-freezer”). Do not store aliquots from serum/plasma that have been in contact with cells >2hrs.
2. Aliquots should be made on ice in polypropylene tubes using polypropylene tips for pipets.
3. Complete the MarkVCID case report form for each sample type and document the number of aliquots that were obtained by scanning them into the MarkVCID Virtual Biorepository.
4. Long-term storage should be at -80°C or liquid nitrogen with temperature monitoring.
5. Avoid unnecessary thawing and refreezing of samples.
6. Factors that should be monitored and documented when necessary:
 - a. Time from collection to centrifugation
 - b. Temperature between collection and centrifugation
 - c. Temperature of centrifugation
7. Post centrifugation considerations that should be monitored and documented when necessary:
 - a. Type of secondary container (tube, straw)
 - b. Storage temperature
 - c. Number of freeze/thaw cycles
 - d. Duration of storage
 - e. Storage location of aliquot vials

Follow steps specific to each biosample type below:

Serum Sample Collection

1. Serum SST tube should be gently inverted 5 times immediately after drawing the blood and clotted for 30-60 min in vertical position before centrifugation.
2. Centrifuge at 2000g for 10 min with horizontal rotors. Centrifugation will take place at room temperature.
3. After centrifugation, remove serum from tube and transfer 0.25ml aliquots into the 0.5ml cryovials noted above.
4. Freeze in an upright position at -80°C.

Plasma/Packed cells for DNA Collection

1. Plasma EDTA tube should be gently inverted 5-10 times immediately after drawing the blood.
2. Centrifuge at 2000g for 10 min with horizontal rotors. Centrifugation will take place at room temperature.
3. After centrifugation, carefully remove 4-5ml of plasma from the tube and transfer 0.25ml aliquots into 0.5ml cryovials noted above.
4. After the plasma is removed from the draw tube, gently mix the remaining 3-4ml of packed cells and transfer three 1ml aliquots into the cryovials noted above. A large transfer pipet is recommended to accomplish the transfer.
5. Freeze plasma and packed cell aliquots in an upright position at -80°C.

2. Sharing and Dissemination of Fluid Samples

1. Biospecimens
 - a. It is recommended that a disclaimer accompany all biospecimen disbursements, even if tested negative for HIV and hepatitis B and C, which PIs sign. The disclaimer would indicate that they understand that absence of infectivity of biospecimens cannot be guaranteed, that laboratory personnel have been trained in procedures related to handling of human tissue, and that universal precautions will be observed.
 - b. It is recommended that frozen brain, blood, and DNA not be distributed from cases positive for hepatitis or HIV, unless a study specifically requires this type of tissue. These may be kept and labeled as either hepatitis or HIV positive for such needs. Fixed tissue may be distributed with specific hepatitis and HIV warnings as above.
2. It is required that all biospecimens for sharing beyond the site at which they are obtained be de-identified and given a unique identifier that follows the specimen from acquisition through processing and storage to retrieval and distribution.
3. Biospecimen requests must be approved by the appropriate decision-making body
4. Effective annotation that results in minimal effort expenditure to retrieve samples is recommended.
5. Tracking and storage methods that minimize disruption of stable state during retrieval to ensure biospecimen quality are recommended.
6. Inventory database is recommended to track specific position of each biospecimen.
7. Utilize the [MarkVCID Shipping Human Biospecimens Guideline](#) for shipping.

3. Appendix

MarkVCID Fluid Biosample Requirements

(version 1.24.22)

MarkVCID Consortium sites are required to follow the agreed upon sample collection requirements listed below. For more details, please refer to the MarkVCID Fluid Sample Best Practices on the internal MarkVCID website.

Required Trainings

All site staff who receive and process samples, print labels, scan and input information into the MarkVCID virtual repository must complete the virtual biorepository training via SkyPrep. Please contact Carissa.Tuozzo@mg.harvard.edu for account setup and access to the training.

All staff who ship biosamples must maintain current training and certification in the shipping and handling of biological specimens as mandated by their site.

Biosamples

The Consortium has mandated biosample types to be collected and stored for MarkVCID Consortium use.

Blood Collection and Storage

Sample Type	Tube Volume	Product Number & Link	Total Stored Sample Amount	Aliquot Size	Cryovial Size	Cryovial Product Number & Link
Serum	8.5ml	BD Vacutainer 367988	4-5ml	0.25ml	0.5ml	DWK W985874
Plasma & Packed Cells for DNA	10ml	BD Vacutainer 366643	Plasma: 4-5ml Packed cells: 3ml	Plasma: 0.25ml Packed cells: 1.0ml	Plasma: 0.5ml Packed cells: 1.2ml	Plasma: DWK W985874 Packed Cells: DWK W985862
Total Blood Volume	18.5ml					

Supplies and Shipping

Sites must purchase all biosample handling and storage supplies outlined above.

Sites are responsible for shipping biosamples to other sites when necessary. The Coordinating Center will cover shipping costs and has set up a FedEx account for site use see MarkVCID Shipping Human Biospecimens Guideline for waybill and account information).

Brady Printer Supplies

The Coordinating Center will cover the cost of the Brady IP i5100 300dpi printer ribbon spool and label roll. Please email the Coordinating Center with a request to re-order these supplies and allow a 4-6 week window for processing and delivery.

4. References

Blood Guidelines

1. Rai, A.J., et al., *HUPO Plasma Proteome Project specimen collection and handling: Towards the standardization of parameters for plasma proteome samples*. *Proteomics*, 2005. **5**(13): p. 3262-3277.
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Document History

Summary of Changes MarkVCID2 Fluid Sample Best Practices			
Version	Description of Changes	Reason for Change	Version Date
1.0	N/A – original version	N/A	1.24.2022
2.0	<ul style="list-style-type: none">p.3: Removed reference to ACDp.4: Removed reference to PPP	Revised language to reflect MarkVCID2 study procedures	6.8.2022
3.0	<ul style="list-style-type: none">p.3: Removed reference to drawing heparin tubes and updated order of draw language	Revised language to reflect MarkVCID2 study procedures	6.23.2022
4.0	<ul style="list-style-type: none">p. 3: Removed polypropylene VWR cryovial as an alternative option	This is not a low-protein binding cryovial and can affect biomarker analyses	5.20.2024