MarkVCID Fluid-Sample Best Practice Guidelines

Version Feb 12, 2020
MarkVCID Consortium

By the MarkVCID Fluid-based Biomarkers Subcommittee (Donna Wilcock, PhD, Chair) 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).

The MarkVCID Consortium is funded by the National Institutes of Health through the National Institute of Neurological Disorders and Stroke and National Institute on Aging (Cooperative Agreement U24NS100591).

MarkVCID
Fluid-Sample Best Practice Guidelines
(v2.12.20)

Contents

I. 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. Platelet Poor Plasma (PPP) ACD collection 6ml tube BD Vacutainer 364816
    d. Cryovials for plasma/serum 0.5ml DWK W985874
    e. 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 PPP, 4-5ml plasma, 4-5ml serum (5ml preferred for all 3 types when possible) and 3-4ml packed cells for DNA extraction.

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 be as follows:
    1. Serum with or without clot activator or gel
    2. Heparin with or without gel separator
    3. EDTA with or without separator
    4. Acid Citrate Dextrose (ACD)

Further blood sample collection procedures are available in the CLSI H3-A6 (Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture).

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/PPP that have been in contact with cells >2hrs.
2. Aliquots should be made on ice in siliconized O-ring containing 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.

Platelet Poor Plasma (PPP) Collection
(Goetzl Protocol adapted for MarkVCID Fluid Best Practice Guidelines)

1. PPP ACD tube should be gently inverted 5-10 times immediately after drawing the blood.
2. Centrifuge at 500g for 20 min. Centrifugation will take place at room temperature.
3. Using a plastic syringe transfer 3 ml of the supernatant containing platelet-rich plasma (PRP) to a 15 ml plastic test tube containing 3 ml of calcium- and magnesium-free Dulbecco's balanced salt solution with 2 mM EDTA and 2 µM PGE1* and mix (see consumables list for product information). Adjust the volume of Dulbecco's salt solution if less than 3 ml of PRP is collected to obtain a 1:1 (v/v) ratio.
4. Centrifuge the Dulbecco's salt solution/PRP mixture at 2200 g for 20 min. Centrifugation will take place at room temperature.
5. After centrifugation, remove supernatant containing PPP from tube and transfer 0.25ml aliquots into the 0.5ml cryovials noted above.
6. Freeze in an upright position at -80°C.

*A large batch of Dulbecco's solution can be prepared, aliquoted and stored at -80°C.

II. Cerebrospinal Fluid Guideline

1. A minimum of 5ml of CSF must be collected and stored for consortium use.
2. CSF should be collected at a consistent time in the morning under fasting conditions (*e.g.*, 0800-1100h).
3. Use 25 g needle for deep local anesthesia rather than the needle provided in kit.
4. Atraumatic spinal needle (*e.g.*, Sprotte 24 g atraumatic spinal needle) is recommended for the LP to minimize risk of post-LP headache (<1%). Other spinal needles that have been used are a 22 g Sprotte atraumatic needle or a 25 gauge Quincke needle, although their use is associated with a somewhat higher post-LP headache risk (up to 5%).
5. To ensure the consistency and quality of the samples across sites, the MarkVCID Consortium has adopted the following CSF collection tube and cryovial for storage:
    a. Rose Scientific Collection Tube 17022 (individually wrapped)
    b. Cryovial DWK W985874
6. 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. Using a 22-gauge needle permits CSF to flow under gravity. The extension tubing provided in LP kits should not be used.
7. There is some controversy concerning the use of negative pressure for withdrawal of CSF. Clinical judgment should be used in evaluating risk of exceedingly rare vs. more common adverse events.
8. Clinical Exemplar – to avoid a post-lumbar puncture headache, the following actions are strongly recommended:
    a. Participant rest in recumbent position for one hour post-LP (a common clinical practice)
    b. Encourage liberal fluid intake
    c. Subject should avoid exertion (exercise, housework, gardening, sexual activity, lifting/bending, etc.) for 24-48 hours following lumbar puncture
    d. Stress importance that participant maintain usual caffeine intake to prevent caffeine-withdrawal headache
9. Standardized processing techniques using ONLY polypropylene tubes are recommended for all samples.
10. CSF for research purposes should NEVER come in contact with polystyrene (clear hard plastic) or glass, since this could result in falsely low measurement levels of various proteins.
11. After gently mixing the full volume of CSF drawn, spin CSF samples to remove RBCs.
12. Aliquot 0.25ml of CSF into 0.5ml cryovials noted above. Reduce air space in tubes as much as possible.
13. Uniform, non-redundant annotation of samples is recommended.
14. Document the number of aliquots obtained.
15. Appropriate and complete documentation surrounding biospecimen collection, processing, and storage are essential and relevant to the quality of research data to be obtained.
16. Avoid unnecessary thawing and refreezing of samples.

III. 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.

IV. Appendix

MarkVCID Fluid Biosample Requirements
(version 2/12/2020)

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. (https://markvcid.partners.org/4-virtual-biorepository-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 and CSF Collection and Storage
Sites must use the following tubes for collection:

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
Platelet Poor Plasma	6ml	BD Vacutainer 364816	4-5ml	0.25ml	0.5ml	
Total Blood Volume	**24.5ml**					

Sample Type	Tube Vol Max Draw	Product Number & Link	Stored Sample Amount	Aliquot Size	Cryovial Size	Cryovial Product Number & Link
CSF (if collecting)	30ml	Rose Sci wrapped 17022	5ml	0.25ml	0.5ml	DWK W985874

Required Procedures for Instrumental Validation (Test-Retest of Subjects)
Each site must collect and store plasma, PPP, and serum as follows:

- Common biosamples (including plasma, PPP, and serum) at the same time of day on 10 subjects at 3 timepoints, at least 5 days apart from one another and completed within 30 days
- Subjects should be a minimum of 50% diseased patients. Remaining can be healthy controls or additional patients

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.

V. 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.
2. National Cancer Institute, NCI best practices for biospecimen resources, 2011 (NCI Best Practices website: http://biospecimens.cancer.gov/practices/; PDF of the NCI Biospecimens Best Practice: http://biospecimens.cancer.gov/bestpractices/2011-NCIBestPractices.pdf
3. Vanderstichele, H., et al., *Standardization of measurement of β-amyloid((1-42)) in cerebrospinal fluid and plasma.* Amyloid, 2000. **7**(4): p. 245-258.
4. Becan-McBride, K., *Laboratory sampling: Does the process affect the outcome?* Journal of Intravenous Nursing, 1999. **22**(3): p. 137-142.
5. Bowen, R.A.R., et al., *Impact of blood collection devices on clinical chemistry assays.* Clinical Biochemistry, 2010. **43**(1-2): p. 4-25.
6. Apple, F.S., et al., *National Academy of Clinical Biochemistry and IFCC Committee for Standardization of Markers of Cardiac Damage Laboratory Medicine Practice Guidelines: Analytical issues for biochemical markers of acute coronary syndromes.* Circulation, 2007. **115**(13): p. e352-e355.
7. CLSI, *Procedures for handling and processing of blood specimens for common laboratory tests; Approved Guideline - Fourth Edition.* H18-A4. **30**(10).
8. CLSI, *Procedures for the collection of diagnostic blood specimens by venipuncture; Approved Standard - Sixth Edition.* H3-A6. **27**(26).
9. Murphy BM, S.S., Mueller BM, van der Geer P, Manning MC, & Fitchmum MI, *Protein instability following transport on try ice.* Nature Methods, 2013. **10**(4): p. 278-98.

CSF Guidelines and Sharing and Dissemination of Fluid Samples

1. Evans RW, Armon C, Frohman EM, and Goodin DS. Assessment: Prevention of post–lumbar puncture headaches: Report of the Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology. Neurology 2000;55;909-914.
2. Armon C, and Evans RW. Addendum to assessment: Prevention of post–lumbar puncture headaches: Report of the Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology. Neurology 2005;65;510-512.
3. Peskind ER, Riekse R, Quinn JF, Kaye J, Clark CM, Farlow MR, Decarli C, Chabal C, Vavrek D, Raskind MA, Galasko D. Safety and acceptability of the research lumbar puncture. Alz Dis Assoc Disord 2005;19;220-225.
4. Hesse C, Larsson H, Fredman P, Minthon L, Andreasen N, Davidsson P, Blennow K. Measurement of Apolipoprotein E (apoE) in cerebrospinal fluid. Neurochem Res. 2000;25:511-7.
5. Alcolea D, Martinez-Lage P, Izagirre A, Clerique M, Carmona-Iragui M, Alvarez RM, Fortea J, Balasa M, Morenas-Rodriguez E, Llado A, Grau O, Blennow K, Lleo A, Molinuevo JL. Feasibility of lumbar puncture in the study of cerebrospinal fluid biomarkers for Alzheimer's disease: A multicenter study in Spain. J Alz Dis 2013, Nov 19 (Epub ahead of print).
6. National Cancer Institute, NCI best practices for biospecimen resources, 2011 (NCI Best Practices website: http://biospecimens.cancer.gov/practices/; PDF of the NCI Biospecimens Best Practice: http://biospecimens.cancer.gov/bestpractices/2011-NCIBestPractices.pdf