

## Standard Operating Procedure (SOP) 004V7.0

Acquisition of Plasma from Whole Blood  
SPREC PL1-PED-A-A-N-B-A [2]

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Author: Pamela Rockey, M.S., Biospecimen Manager

Approved by:



Anna Maria Storniolo, M.D., KTB Executive Director

### Materials:

**Blood collection sets:** BD (Becton, Dickinson and Company) Vacutainer® Blood Collection Set, 21 gauge butterfly (Fisher cat. # 02-664-1)

**EDTA 9 ml Collection tube:** Greiner Bio-One Hematology K<sub>3</sub> EDTA Evacuated Tubes 9ml (Fisher cat. # 22-040-037)

**Centrifuges:** Eppendorf 5702 or 5702R

**Transfer Pipets:** Disposable Graduated Transfer Pipette (Fisher cat. # 13-711-9AM)

**Cryostorage tubes:** Corning 2.0ml Cryogenic Vials. (Fisher cat. # 0337421)

**Repeater Pipet:** Eppendorf Repeater Plus Pipette (Fisher cat. # 21-380-9)

**Combitips:** (Fisher cat. # 13-683-705)

**Glass Culture Tubes:** Fisher 16x100mm disposable culture tubes (Fisher cat. # 14-961-29)

**Labelling:** All blood tubes are to be pre-labeled with bar code stickers prior to venipuncture. Bar code packets are assigned during the donor registration process.

**Position for venipuncture:** sitting

**Order of the Blood Draw:** Blood collection tubes must be drawn in a specific order to avoid cross-contamination of additives between tubes [4]. The order of draw is 1) SST (SOP 001V8.0), 2) EDTA 9ml, and 3) EDTA 2ml (SOP 001V8.0). A total of three tubes of blood are drawn during the collection process.

**Temperature for collection and processing:** Cold temperatures around 4°C activate platelets and may therefore lead to the release of peptides and enzymes into the plasma. Later removal of platelets leaves the platelet-associated peptides and enzymes in the plasma sample [3], therefore all steps in the plasma processing are carried out at room temperature.

**Processing:** Blood is drawn into the blood collection tube (EDTA 9ml) and gently mixed by inverting the tube eight times immediately after drawing. Centrifugation begins immediately after the blood is drawn and plasma is obtained by centrifugation for 15 min. at 2000rcf. Using a disposable transfer pipette the plasma layer is aspirated, being careful not to disturb the buffy coat layer, and transferred to a glass culture tube. A repeater pipet is used to aliquot 750µl of recovered plasma into each of five pre-labeled cryogenic vials. If plasma volume is low, fewer aliquots are collected. If plasma volume exceeds 3.75ml, existing 5 vials are topped off. Vials are capped and immediately placed into cryoboxes on dry ice. Blood tube with undisturbed buffy coat is recapped and stored temporarily at room temperature for subsequent DNA extraction (SOP 002V6.0).

**Storage of Plasma:**

Freeze-thaw is not optimal [1] and therefore, plasma should be aliquoted. Plasma aliquots are logged into cryoboxes and placed on dry ice for transport to the storage facility. Plasma is stored at -80°C.

**Standardization:** All variables including the time the whole blood is at room temperature prior to separation, time plasma is stored at -80°C prior to shipment and/or utilization, volume of aliquots and color of plasma will be entered into the database.

**Oversight:** All adverse and unexpected events will be recorded in the database and will be addressed by the Executive Committee. This includes all phases of the process: donation, storage and retrieval, processing, and utilization.

## **References:**

1. Mitchell B.L., et al., *Impact of Freeze-thaw Cycles and Storage Time on Plasma Samples Used in Mass Spectrometry Based Biomarker Discovery Projects*. Cancer Informatics 2005. 1: p. 98-104
2. Sabine Lehmann et.al. International Society for Biological and Environmental Repositories (ISBER) Working Group on Biospecimen Science. Standard preanalytical Coding for Biospecimens: Review and Implementation of the Sample PREanalytical code (SPREC).Biopreservation and Biobanking Vol. 10 No.4, 2012
3. Tammen, H., et al., *Peptidomic analysis of human blood specimens: comparison between plasma specimens and serum by differential peptide display*. Proteomics, 2005. 5(13): p. 3414-22.
4. WHO Guidelines on Drawing Blood: Best Practices in Phlebotomy. Geneva: World Health Organization; 2010. 2, Best practices in phlebotomy. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK138665/>

## **Bibliography**

- Ayache, S., et al., Effects of Storage Time and Exogenous Protease Inhibitors on Plasma Protein Levels. Am J Clin Pathol. 126(2):174-184,2006
- Elliott P., Peakman T.C.; UK Biobank. The UK Biobank sample handling and storage protocol for the collection, processing and archiving of human blood and urine. Int J Epidemiol. 2008 Apr;37(2):234-44.
- Lam, N.Y.L., et al., EDTA is a Better Anticoagulant than Heparin or Citrate for Delayed Blood Processing for Plasma DNA Analysis. Clinical Chemistry 50:256-257, 2004
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- Rai, A.J., et al., HUPO Plasma Proteome Project Specimen Collection and Handling: Towards the Standardization of Parameters for Plasma Proteome Samples. Proteomics 5:3262-3277, 2005
- Tammen, H., et al., Peptidomic Analysis of Human Blood Specimens: Comparison between Plasma Specimens and Serum by Differential Peptide Display. Proteomics 5:3414-3422, 2005
- Tuck, Melissa K et al. "Standard operating procedures for serum and plasma collection: early detection research network consensus statement standard operating procedure integration working group." Journal of proteome research vol. 8,1 (2009): 113-7. doi:10.1021/pr800545q

## **Electronic Resources**

- <http://library.med.utah.edu/WebPath/TUTORIAL/PHLEB/PHLEB.html>
- [http://www.geisingermedicallabs.com/catalog/blood\\_specimens.shtml](http://www.geisingermedicallabs.com/catalog/blood_specimens.shtml)