

Standard Operating Procedure (SOP) 004V6.0

Acquisition of Plasma from Whole Blood
SPREC PL1-PED-A-A-N-B-A (3)

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Approved by:

Materials:

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

Collection tube: Greiner Bio-One VACUETTE™ Hematology K3 EDTA 9ml, 16 x 100 mm. (Fisher cat. # 22-040-037).

Centrifuges: Eppendorf 5702 or 5702R

Transfer Pipets: Disposable Graduated Transfer Pipet (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)

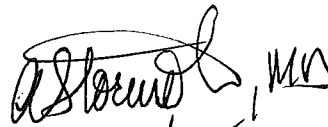
Glass Culture Tubes: Fisherbrand 16 x100mm disposable culture tubes (Fisher cat. # 14-961-29)

Labelling:All tubes are to have bar code stickers placed on the tube prior to venipuncture. Bar code stickers will be generated during the process of registration of the volunteer donor.

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, 2) EDTA 9ml, and 3) EDTA 2ml. 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.[1] Therefore all steps in the plasma processing are carried out at room temperature.


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Processing: Blood is drawn into the blood collection tubes (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. The Plasma is then transferred to a glass culture tube using a transfer pipet. 750 µl aliquots are pipetted into cryogenic vials using a repeating pipet and immediately placed on dry ice.

Storage of Plasma:

Freeze-thaw is not optimal [2] 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.


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References:

1. 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.
2. 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

Bibliography

1. 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
2. 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 1:98-104, 2005
3. 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
4. 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
5. 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
6. 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.

Online Resources: <http://www.metamatrix.com/content/HowToOrder/SpecimenCollection>
<http://library.med.utah.edu/WebPath/TUTORIAL/PHLEB/PHLEB.html>
http://www.geisingermedicallabs.com/catalog/blood_specimens.shtml


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