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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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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_3 EDTA Evacuated Tubes 9ml (Fisher cat. # 22-040-037)
Centrifuges: Eppendorf 5702 or 5702R
Transfer Pipets: Disposable Graduated Transfer Pipettte (Fisher cat. # 13-711-9AM)
Cryostorage tubes: Corning 2.0ml Cryogenic Vials. (Fisher cat. # 0337421)
Microcentrifuge tubes: 2.0ml sterile (Celltreat cat. # 229446)
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. With the same disposable transfer pipette that plasma was aspirated, 1ml of the buffy coats layer is aspired into a pre-labeled 2ml microcentrifuge. If some red blood cells from bottom layer is aspired with the buffy coats, that is fine. The microcentrifuge tubes with buffy coats are placed in a storage box and temporarily kept at room temperature until a box of 81 tubes is filled, then placed on dry ice at the event and later transferred to -80 freezer to be stored for DNA isolation (SOP 002V7.0).

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/

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- 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