TISSUE BANK at the IU SIMON CANCER CENTER

Standard Operating Procedure (SOP) 002V6.0

Acquisition of DNA from Buffy Coat
SPREC BLD PED A A N F A R [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)
Centrifuge: Eppendorf 5702 or 5702R
MicroTubes: Micrewtube® 2.0ml microtubes sterile (Dot Scientific T332-7S)
PCR workstation Flow Hood: Air Science® PURAIR PCR80884
Barcode labels: Brady® Thremal Transfer labels THT-68. (Fisher 11-877-51)

Labelling: All blood tubes are to have bar code labels placed on the tube prior to venipuncture. Barcode 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 [3]. 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: DNA is stable in blood at 23-25°C. However, best practice recommends that the specimens be left at this temperature for as short a period as possible [1]. For the purpose of standardization, specimens should remain at room temperature until all specimens have been obtained.

Temperature for storage prior to processing: As the blood will be processed into DNA at a later date, the blood should be stored at -80°C. Blood collected at off-site collections should be transported in dry ice and placed in -80°C as soon as they arrive on campus.

Processing: Blood is drawn into the blood collection tube (EDTA 9ml) and gently mixed by inverting the tube eight times immediately after drawing. Centrifugation (15 min. at 2000rcf) for plasma separation begins immediately after the blood is drawn. The plasma is withdrawn (SOP 004V7.0) and the blood tube is recapped and logged into storage box. Remaining red cells and buffy coat are kept at room temperature until the end of the collection event. Following collection event blood cells are held on dry ice for transport then transferred to -80°C until ready for DNA extraction.

Extraction: DNA is manually extracted from the buffy coat cells at the Indiana University Genetics Biobank (IUGB) lab on the IUPUI campus. (SOP IUGB-3-11.03 Sample Processing)

Storage of DNA: Purified DNA in 1X TE buffer is received from IUGB on gel packs and kept at 2-8°C until ready for aliquoting and storage preparation. After removal of 1ug for genetic ancestry genotyping (SNP analysis), four aliquots of each DNA sample are air dried and banked at ambient temperature in Biomatrica® DNAstable® tubes per SOP 008V6.0. Remaining DNA in 1X TE buffer solution is stored at -80°C in one or two aliquots depending on volume and biospecimen manager discretion.

Standardization: All variables including the time of whole blood collection, time stored at -80°C as whole blood cells prior to processing, processing time, and time banked at -80°C or ambient temperature prior to shipment and/or utilization will be recorded 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. Farkas, D.H., et al., *Specimen collection and storage for diagnostic molecular pathology investigation.* Arch Pathol Lab Med, 1996. **120**(6): p. 591-6.
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. 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

- Austin, M.A., et al., *Guidelines of the National Heart, Lung, and Blood Institute Working Group on Blood Drawing, Processing, and Storage for Genetic Studies.* Am J Epidemiol, 1996. **144**(5): p. 437-41.
- Farkas, D.H., et al., *Specimen collection and storage for diagnostic molecular pathology investigation.* Arch Pathol Lab Med, 1996. **120**(6): p. 591-6.
- Holland, N.T., et al., *Molecular epidemiology biomarkers--sample collection and processing considerations.* Toxicol Appl Pharmacol, 2005. **206**(2): p. 261-8.
- Holland, N.T., et al., *Biological sample collection and processing for molecular epidemiological studies.* Mutat Res, 2003. **543**(3): p. 217-34.
- Steinberg, K., et al., *DNA banking for epidemiologic studies: a review of current practices.* Epidemiology, 2002. **13**(3): p. 246-54.
- Steinberg, K.K., et al., *DNA banking in epidemiologic studies.* Epidemiol Rev, 1997. **19**(1): p. 156-62.
- DNAstable Handbook. Biomatrica®, The Biostability Company. Aug. 2009.

Electronic Resources

- NCI-BBRB Biorepositories & Biospecimen Research Branch https://biospecimens.cancer.gov/default.asp
- Holland Lab/Berkeley https://www.hollandlabucb.org/
- WebPath Phlebotomy Tutorials http://library.med.utah.edu/WebPath/TUTORIAL/PHLEB/PHLEB.html
- Geisinger Venipuncture Procedure http://www.geisingermedicallabs.com/catalog/blood_specimens.shtml
- https://www.biomatrica.com/