

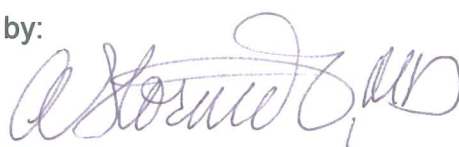
Standard Operating Procedure (SOP) 002V5.0

Acquisition of DNA from Whole Blood
SPREC BLD PED A A N F A R (2)

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Approved by:

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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)

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

Processing instrument: AutogenFlex Star (SN 401033)

Reagents Kit: Flexigene AGF3000 blood kit for DNA extractions from whole fresh and frozen blood.

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. [3] 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: DNA is stable in blood at 23-25⁰ 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 kept 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 The plasma is withdrawn (SOP 004 V6.0) and the remaining red cells and buffy coat are kept at room temperature until the end of the collection event. Blood cells are stored at -80°C until ready for DNA extraction.

Extraction: DNA is extracted from the blood cells at the Indiana CTSI Specimen Storage Facility (ICTSI-SSF) lab using an AutogenFlex Star (SN 401033) instrument and the Flexigene AGF3000 blood kit for DNA extractions from whole fresh and frozen blood. All manufacturer's guidelines are employed.

Storage of DNA: DNA is stored both frozen (-80°C) and at ambient temperature. One 900ul aliquot of DNA in Tris-HCl is received from ICTSI-SSF and handled in the following way:

Four 50ul aliquots of each sample are pipeted into pre-labeled DNASTable® tubes following the protocol outlined in the Biomatrica® DNASTable® Handbook and SOP008V4.0 The tubes are allowed to dry uncovered for three days in a laboratory fume hood. Tubes are then covered, logged into the KTB database and stored at ambient temperature and a relative humidity of <20%. The amount of DNA in each tube is calculated and recorded by multiplying the concentration by the volume added to the tube. Concentration is measured at the SSF Lab using a nanodrop spectrophotometer ND-1000 (Thermo Scientific). The sample taken for the spectrophotometer is pipeted from the middle of the tube following vigorous vortexing.

The remaining 700ul of DNA is stored as two 350ul aliquots at -80°C in FG3 medium, (10 mM Tris-Cl solution at pH 8.5. Qiagen)

Standardization: All variables including the time the whole blood is at room temperature prior to freezing, time stored at -80°C as whole blood prior to processing, processing time, time stored at -80°C as DNA prior to shipment and/or utilization 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. 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. http://lab.healthalliance.com/pdfs/collection/Order_of_Draw_for_Blood_Specimens.pdf

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

First-Generation Guidelines for NCI-Supported Biorespositories

http://biospecimens.cancer.gov/biorepositories/guidelines_full_formatted.asp

Holland Lab/Berkely

<http://ehs.sph.berkeley.edu/holland/protocollibrary.html>

<http://library.med.utah.edu/WebPath/TUTORIAL/PHLEB/PHLEB.html>

. http://lab.healthalliance.com/pdfs/collection/Order_of_Draw_for_Blood_Specimens.pdf

http://www.geisingermedicallabs.com/catalog/blood_specimens.shtml

www.quiagen.com FlexiGene® DNA AGF3000 Handbook

www.biomatrica.com