Standard Operating Procedure (SOP) 002V7.0
Acquisition of DNA from Buffy Coat
SPREC BLD PED A A N F A R [2]

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Author: Rana German, M.S., Biospecimen Manager

Approved by:

[Signature]
Michele Cote, PhD., 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 K₃ 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 The plasma is withdrawn (SOP 004V8.0) and the blood tube is recapped and logged into storage box.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. CheMagic magnetic bead extraction platform is used at IUGB.

**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 Biomatrix® 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:


Bibliography


Electronic Resources

- Holland Lab/Berkeley https://www.hollandlabucb.org/
- WebPath Phlebotomy Tutorials http://library.med.utah.edu/WebPath/TUTORIAL/PHLEB/PHLEB.html
- https://www.biomatrica.com/