

# Framingham Heart Study

## Manual of Procedures

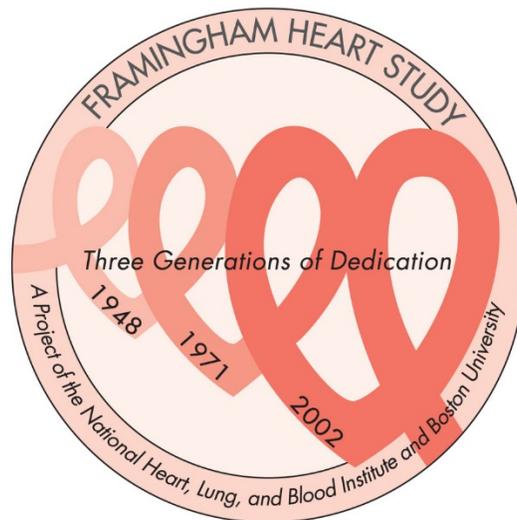
**MOP-version 1.0**

August 22, 2018

### Research Examination Center

Generation 3, Omni 2, NOS Cohorts Examination 3

### Section #9 Phlebotomy





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## 1.0 Gen3/Omni G2/NOS Exam 3 Laboratory Protocol

Blood samples are collected from participants in a supine position, after a 12-hour fast.

The following tubes are drawn:

- 4 x 10 mL EDTA blood collection tubes
- 2 x 10 mL Serum blood collection tubes
- 1 x 8 mL CPT [Cell Preparation Tube]
- 1 x 4 mL CPT [Cell Preparation Tube]
- 4 x 4.5 mL Citrate blood collection tubes
- 1 x 3 mL Hirudin blood collection tube

*Total volume of fasting blood draw is 93 mL (6.3 T/3.1 ounces).*

A second blood sample is collected from participants at peak exercise, in a sitting position.

The following tubes are drawn:

- 1 x 10 mL EDTA blood collection tubes
- 1 x 10 mL Serum blood collection tubes

*Total volume of peak exercise blood drawn is 20 mL (1.4 T/0.7 ounces).*

**Total volume of blood draw at exam is 113 mL (7.7 T/3.8 ounces)**

\*\*A small number of participants (N<50), need a cell line blood draw.  
If a participant needs a cell line, an additional 8 ml CPT tube is drawn.

*Total volume of blood drawn is 121 ml (8.2/4.1 ounces).*

### **EDTA**

1. EDTA whole blood is used for HbA1c, which is measured daily in the FHS laboratory.
2. EDTA plasma is used for Cholesterol, HDL cholesterol, Triglycerides and Glucose which are measured daily in the FHS laboratory.
3. EDTA plasma and red cells are saved in several aliquots for future measurements; stored at -80 C.
4. Buffy coat specimens are collected from the EDTA blood collection tubes. These aliquots are transferred periodically to the Framingham Heart Study Genetics Laboratory, at Boston University Medical Center.

## **Serum**

1. Serum is used for Creatinine, Albumin, ALT and AST, which are measured daily in the FHS laboratory.
2. Serum is saved in several aliquots for future measurements; stored at –80 C.

## **CPT**

CPT blood collection tubes are transferred daily to the Framingham Heart Study Genetics Laboratory at Boston University Medical Center. Peripheral blood mononuclear cells are isolated and cryopreserved for future generation of induced pluripotent stem cells and an immune response study.

## **Citrate**

1. Citrate whole blood and plasma are used for platelet function testing, which is performed daily in the FHS laboratory.
2. Citrate plasma is saved in several aliquots for future measurements; stored at –80 C.

## **Hirudin**

Hirudin whole blood is used for platelet function testing, which is performed daily in the FHS laboratory.

## **Urine** [Random, spot urine]

1. Urine is used for pregnancy testing, which is performed daily in the FHS laboratory. Bone Study personnel identify the subset of female participants who require pregnancy testing.
2. Urine is saved in several aliquots for future measurements; stored at –80 C.

## 2.0 CReM Protocol for PBMCs for FHS Adult Participants

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IPS CORE

### Isolation of Peripheral Blood Mononuclear Cells (PBMCs) for Framingham Heart Study (FHS) Adult Participants

Induced pluripotent stem cells (iPSCs) can be generated from freshly collected or frozen PBMCs

- Clean venipuncture site and top of blood draw tube with 70% isopropyl alcohol pads
- Draw 4 mL of peripheral blood into one BD Vacutainer CPT Cell Preparation Tube (BD 362760). Invert tube 8-10x and keep upright at room temperature (RT).  
The CPT Vacutainers are barcoded and scanned into the FHS inventory tracking system. An excel file is created that serves as the daily shipping file that lists all specimens included in that day's delivery and is emailed for uploading into the inventory system of FHS Genetics Laboratory at Boston University Medical Center (BUMC)
- Samples are packaged according to Category B Biological Substances and are picked up and transported by Breakaway Courier Systems to Boston University Medical Center, FHS Genetics Laboratory, 75 E. Newton Street, Evans 301, Boston, MA 02118.
- Centrifuge samples at 1,800 RCF for 30 min at RT (ideally within 2 hrs of collection).
- Use a 1 mL pipet tip to collect buffy coat (cell layer between gel barrier and plasma) into a sterile 15 mL conical centrifuge tube and bring volume up to 10 mL with sterile PBS; invert several times.
- Centrifuge 300 RCF for 15 min and aspirate supernatant.
- Resuspend cell pellet in 10 mL of sterile PBS.
- Centrifuge 300 RCF for 10 min and aspirate supernatant.
- Resuspend cell pellet in 2 mL freezing medium (90% FBS/10% DMSO) and aliquot into 2 vials @1 mL/cryovial, ~ 2x10<sup>6</sup> cells/vial. Typical yield is approx. 3-4x10<sup>6</sup> cells in a 4 mL blood draw.
- Slow freeze vials at -80°C overnight and transfer to liquid nitrogen storage the following day.

#### Materials and reagents:

- Sterile Alcohol Prep Pads (Dynarex 1103; Medplusmedicalsupply.com; Model: DYN-1103)
- BD Vacutainer™ CPT™ Cell Preparation Tubes with Sodium Citrate; Manufacturer: BD 362760; Vendor, Fisher Scientific; [http://www.bd.com/vacutainer/pdfs/bd\\_cpt\\_VDP40104.pdf](http://www.bd.com/vacutainer/pdfs/bd_cpt_VDP40104.pdf)
- Dulbecco's PBS (DPBS) (Fisher Cat # 14-190-144)
- FBS: Hyclone FBS Characterized (Cat # SH30071.03; LOT# AYF161496), 0.22 micron filtered
- CellTreat Filter systems (MedSupply Partners Cat # CT-229706; CT-229705)
- DMSO (Sigma Cat # D2650)
- Nalgene 2.0 mL cryovials (Fisher Cat # 03-337-7D)

Reference: Sommer AG, Rozelle SS, Sullivan S, Mills JA, Park SM, Smith BW, Iyer AM, French DL, Kotton DN, Gadue P, Murphy GJ, Mostoslavsky G. *J Vis Exp.* 2012 Oct 31;(68).

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