SOP Title:

Plasma and Buffy Coat Separation from Whole Blood Protocol

1.0 Purpose and Scope

This standard operating procedure (SOP) is for the separation of plasma and buffy coat from whole blood samples at PM-OICR TGL.

This protocol consists of centrifugation of whole blood samples in order to obtain plasma for further processing. Figure 1 shows the layers of whole blood.

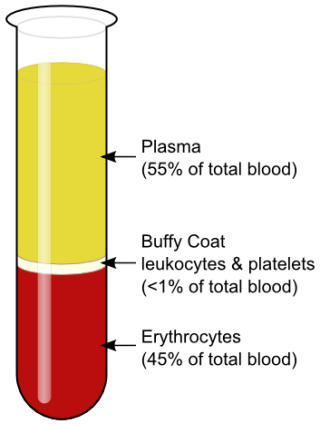

Figure 1: The three layers of whole blood after centrifugation.

Related TGL documents:

- YYYY_MM_DD_SAMPLE SUBMISSION FORM_PI_Lastname_Firstname_TGL.xls
- Purification_of_Circulating_Nucleic_Acids_from_Plasma_Protocol_v1.0.2

Refer to section 5.0 Appendix for information regarding the modifications and adjustments to this SOP.

2.0 Materials

Workspace
Sample prep room: biosafety cabinet

Equipment and associated consumables		
Medstore	Serological Pipettes, 10mL, sterile, individually wrapped, 200/cs	357551
	Serological Pipettes, 5mL, sterile, individually wrapped, 200/cs	357529
Eppendorf	Eppendorf Conical Tubes, 15 mL, Sterile, pyrogen-, DNase-, RNase-, human and bacterial DNA-free, colorless, 500 tubes (10 bags × 50 tubes) (Do not substitute, high g force tubes)	0030122151
	Eppendorf Centrifuge 5424, non-refrigerated, without rotor, rotary knobs, 230 V/50 – 60 Hz	022620401
	Eppendorf Centrifuge 5810 R, refrigerated, (use rotor FA-45-6-30)	022625501
	ROTOR 6x50ML CON. WITH A-T LID (5804/10)-for plasma isolation-FA-45-6-30	5820715006
	ADAP 15ML CON F/50ML CON BOREHOLES PK/2	5820717009
VWR	TUBE MICRO CLR 1500UL PK250	22234-044
Commonly used reagents		
MedStore/Greenfield Specialty Alcohols	LAVO PRO 6 BLEACH 6% 5L (3 bottles per case), (Sodium Hypochlorite 6%)	1952B
	Ethanol anhydrous 100%, 4X4L white jugs (cleaning only)	P016EAAN

3.0 General Guidance:

- De-identified study codes must be used on all documentation.
- Whole blood may contain infectious agents. Wear safety glasses and a lab coat in case of splattering. Immediately stop work if you experience a poke injury or ocular exposure; seek help! If ocular exposure occurs, proceed immediately to eye wash station and rinse eye for a minimum of 5 minutes. Report exposure to Debbie Kolozsvari, OICR Health and Safety (X7933), and/or Manager.
- Ensure proper protective equipment is worn during this protocol. Gloves, lab coat and goggles are required.
- Change gloves if they become soiled with blood.
- Never store blood collection vials (EDTA, STRECK) in freezers, they will crack!
- Use Eppendorf brand centrifuge tubes (PN0030122151) rated for 19,000g. Other 15ml tube brands may fracture/warp at high speed!
- If whole blood is in EDTA tubes, ensure the blood is being processed within two hours of being drawn. If whole blood is in STRECK tubes (ct/cfDNA assays), blood may be processed up to 24 hours later.
- Before beginning work every day, wipe down all pipetors and bench surfaces with peroxide wipes, then wipe with 70% ethanol (made from bulk ethanol, 4L). Wipe down work surface after the protocol is complete.
- Record all initial blood volumes as received, all information on blood vacutainers, and final volume yields of isolated plasma/ buffy coat in the sample submission tracking sheet. Photo documentation is encouraged. Record all samples in MISO LIMS including external identifiers. Record protocol version and include your name in sample submission sheets so that user/tech can be traced.
- Record and highlight all unusual observations, errors, or other issues in sample sheet.
- All tips and tubes that come in contact with blood should be decontaminated in a bleach solution for a minimum of 30 minutes, or overnight before disposal.

4.0 Procedure

1. Preparation

1) Remove the standard rotor (A-4-81) in the Eppendorf 5810R centrifuge and replace with rotor FA-45-6-30. Insert 15ml adapters PN5820717009 into rotor. Pre-cool the centrifuge to 4°C. Use of the centrifuge break may disrupt blood layers at end of spin.

2. Separation of Sample

2) Record the following when available: initial blood draw date/time stamp; date/time extraction is started; volume of whole blood; any information listed on the sample vacutainer. Record in the sample tracking sheet, photo documentation is encouraged.
3) Label two 15 mL tubes (Eppendorf PN0030122151 only; rated for 19,000g!) and one 1.5 mL microcentrifuge tube for every tube of whole blood.
4) Invert whole blood tube (Streck/EDTA) 8-10 times to ensure thoroughly mixed.
5) Place the vacutainer tubes containing whole blood into the pre-cooled Eppendorf centrifuge 5810 R, making sure it's balanced. Perform Spin 1: 4°C, 1900 x g for 10 min (3763 RPMs).
6) When complete, the sample should be in three layers (see figure 1). Carefully remove the tubes from the centrifuge without disturbing the sample layers. Should you disrupt the layers by accident, repeat step 5 before proceeding.
7) Using a 5 mL serological pipette, carefully transfer the plasma layer (top layer, figure 1) from the vacutainer to a labeled 15 mL tube (PN0030122151). You may **combine** two vacutainer plasma aliquots from the same sample into one 15 mL tube (you may receive up to 4 vacutainers per sample).

Leave approximately 0.5 mL of plasma behind! The buffy coat fraction must remain undisturbed.

8) Place the 15 mL tubes with plasma into the pre-cooled Eppendorf centrifuge 5810 R, making sure it's balanced. Perform Plasma Spin 2: 4°C, 16000 x g for 10 min (10,921RPM).
9) During the second spin, use a P1000 or P200 pipette to carefully remove the buffy coat layer from the vacutainer into a labeled 1.5 mL tube. One vacutainer tube will yield one aliquot of buffy coat. Collect the whole buffy coat layer until no white is visible on the surface of the red blood cell layer. The buffy coat layer, or peripheral blood mononuclear cells (PBMCs) should be immediately frozen at -80°C, or proceed to DNA extraction from buffy coat SOP.

Minimize disruption of the red blood cell layer.

10) Once Plasma Spin 2 is complete, carefully remove the tubes from the centrifuge; avoid disturbing any cellular debris (pellet) that may have formed on the bottom or side of the tube.

11) Using a 10 mL serological pipette, carefully transfer the plasma into a second, clean, labelled 15 mL tube, leaving approximately 0.5 mL behind to avoid disturbing pelleted debris. Aliquot plasma to 1.5ml tubes for long term storage. Proceed to cfMeDIP or ctDNA extraction SOPs.

A fine layer of white precipitate on the top of the plasma may be observed and is a lipid layer, it may be transferred with the plasma.

12) Store all labeled tubes of buffy coat and plasma in -80°C until further processing.

13) Decontaminate used 15 mL tubes and vacutainers by adding 1 ml of 100% bleach and inverting; decontaminate accumulated tips in a bleach solution prior to disposal in biohazardous waste.

5.0 Revision History

Version Number	Date *(yyyy-mm-dd)*	History of change
2016.01.005	2017-06-27	Working SOP for Pugh lab (created by IC, edits by YH & TL)
1.0	2018-03-13	Edits and formatting for TGL by Kayla
1.0.1	2018-03-23	Edits by DT