CHARM - Plasma and Buffy Coat Separation from Whole Blood Protocol

Last Modified: May 7, 2021

SOP #: 2021.05.07 **Approved By: Trevor Pugh** **Date:**

1.0 Introduction & Purpose

Whole blood samples are subjected to a density gradient centrifugation allowing the separation of whole blood into three distinct layers: plasma (top layer containing cell free DNA), "buffy coat" (middle leukocytes and thrombocytes layer, used for genomic DNA extraction or DNA/RNA co-isolation), and erythrocytes (bottom layer) (see Figure 1). Both the plasma and buffy coat are stored at -80°C for future processing, while the red blood cells are discarded.

This protocol is designed to separate plasma and the "buffy coat" layer from whole blood, without the use of a high-speed centrifuge.

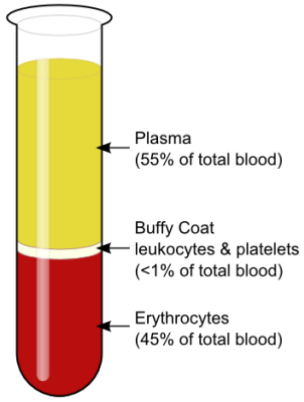

Figure 1: Blood Separates into three distinct layers upon centrifugation under specific conditions

2.0 Equipment & Materials

Equipment	Materials
Biosafety Cabinet Autopipettor and 5-10mL serological pipettes Low-speed swinging rotor centrifuge	100% Bleach 1.5 mL microcentrifuge tubes 1.5 mL low-binding tubes P1000 Pipette and filter tips Crushed ice

3.0 Protocol

3.1 Preparation

- Cool the low-speed swinging rotor centrifuge to 4°C prior to use (20-30 minutes before procedure).
- Set the centrifuge to 1900 xg for 10 min
  - Ensure centrifuge brake speed is set to 1. If the centrifuge breaks too quickly, the red blood cells will rupture, contaminating your separated plasma.

4.2 Separation

1. It is important to keep samples cool after the initial centrifugation. Plasma cfDNA in particular degrades quickly at room temperature. Ensure to have a supply of crushed ice available, or keep phase separated samples in the cooled centrifuge until ready to process, especially if processing multiple samples simultaneously.
2. Note the following information for each sample for ATiM input:

- Date and time of sample collection
- Date and time of sample received in lab
- Number of blood collection tubes
- Sample ID
- Volume of plasma and # of tubes (upon completion of protocol)
- Volume of buffy coat and # of tubes (filled in upon completion of protocol)

3. Create labels for end product with the following information:
    - Sample ID
    - Sample Type (Buffy coat/plasma) – blood collection tube type (STRECK)
    - Collection Date/Time
    - Separation Date/Time
4. Decontaminate Biological Safety Cabinet and pipettes (with Virox wipes and 70% ethanol).
5. Set out and label the appropriate number of 1.5 mL low-binding centrifuge tubes per mL of plasma. Generally, the volume of plasma is ~35-45% of the total volume of blood. Ex: 30 mL of blood, usually results in ~10-14 mL of plasma.
6. Set out and label one 1.5 mL microcentrifuge tube (does not need to be low binding) per 10mL of blood for buffy coat samples. Extras may be needed if the sample has a thick buffy coat layer.
7. Place vacutainer tubes containing blood into the pre-cooled swing rotor cell centrifuge. Make sure the centrifuge is balanced. Double check the spin and break speeds are correct, and press START.
8. Once spin is complete and the blood forms three layers, carefully remove the tubes from the centrifuge without disturbing the sample.
9. Using a 1000 µL pipette and corresponding filter tip, carefully transfer the plasma layer from the vacutainer tubes into low-binding tubes, 1 mL at a time, leaving a layer of approximately 0.25 mL of plasma behind to ensure that the residual buffy coat fraction remains undisturbed.
    - Blood or plasma contaminated tips should be disposed in a receptacle containing 100% bleach.
10. Once transferred, place tubes on ice. When all plasma has been aliquoted, record the total number of tubes and move samples to -80°C ASAP.
11. Using a 1000 µL pipette and corresponding filter tip, carefully transfer the buffy coat into prepared 1.5 mL tubes; try to minimize disruption of the red blood cell layer.
    - Hovering the pipette tip right above the middle of the buffy coat during aspiration should lead to the majority of the layer being easily collected on the first try. Collect the whole buffy coat until no white is visible on the surface of the RBC layer.
12. Once the buffy coat is collected and transferred to 1.5 mL tubes, record volume and number of tubes, and store at -80°C.
13. Add 0.5-1 mL of bleach to each vacutainer tube for decontamination; close the cap, shake and dispose of the tube in a biohazard waste container.

14. Fully decontaminate BSC when finished, and update ATiM and any spreadsheets with appropriate information.

4.0 Safety Information

It is advised that proper PPE (gloves, lab coat and goggles) is worn when following this SOP. Check MSDS for all chemicals used while performing this procedure. Ensure that all tips that touched the buffy coat and RBC layers are ejected into a container with 100% bleach and disposed of only after remaining in contact with bleach for several hours.

5.0 References

- **Biological Safety Cabinet User Guide:** https://www.thermofisher.com/document-connect/document-connect.html?url=https%3A%2F%2Fassets.thermofisher.com%2FTFS-Assets%2FLED%2Fmanuals%2FD20891~.pdf&title=MTMwMCBTZXJpZXMgQTIgQlNDIC0gT3BlcmF0aW5nIE1hbnVhbA==
- **Refrigerated Centrifuge User Guide**: https://www.eppendorf.com/product-media/doc/en/330816/Centrifugation_Operating-manual_Centrifuge-58XX-family.pdf?_ga=2.133759374.1845285415.1585939161-669600888.1585939161

6.0 Additional Resources

- Refer to SOP# 2020.02.002: **Qiagen Gentra Puregene Blood Extraction** Protocol for extraction of DNA.
- Refer to SOP# 2020.02.007: **Gentra PureGene/AllPrep DNA/RNA/miRNA Universal** Protocol for extraction of DNA and RNA from buffy coat samples. ***STRECK tube samples must follow this protocol*
- SOP # 2019.02.003: **Purification of Circulating Nucleic Acids from Plasma** Extraction Protocol for directions on further processing of the separated blood sample layers.

7.0 Modifications of Protocol

Date	Section	Initial & Change
May 7, 2021	All Sections	Updated Pugh Lab SOP 2020.01.005 to be specific for CHARM study at sites without high-speed centrifuge.