Document Number:	**SASoM/METHOD/104.v2**
Title:	**Isolation of PBMCs from whole blood samples**
Version:	**v2**
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Effective from:	25/05/2020
Valid to:	24/05/2022

SOP History		
Number	Date	Reason for Change
v1	25/05/2018	Original
v2	25/05/2020	Update

1.0 Purpose –

This SOP describes the current procedure for Isolating PBMCs (Peripheral Blood Mononuclear Cells) in Laboratory 248 at the St Andrews School of Medicine (SASoM).

2.0 Scope –

This SOP applies to the staff in the SASoM involved in primary PBMC cell culture.

3.0 Responsibilities –

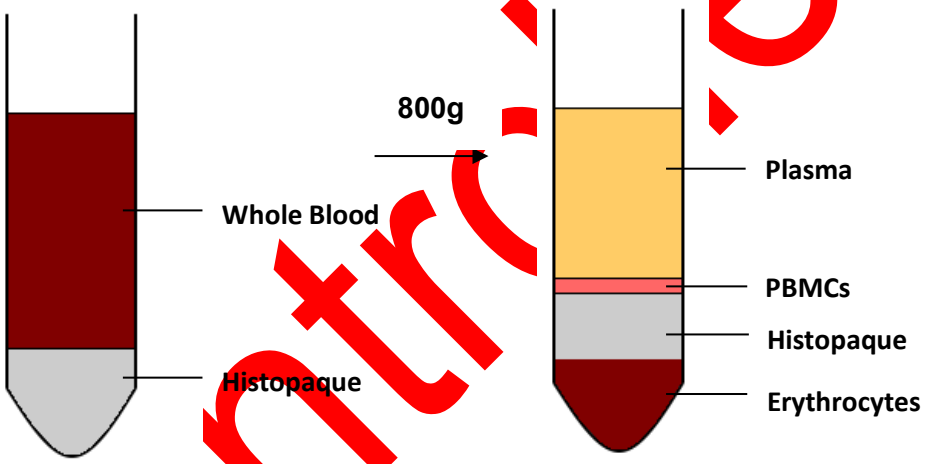
All staff involved in PBMC isolation are responsible for ensuring that the methods are followed in accordance with this SOP.

All staff must have read and signed the relevant risk assessment documents before performing this procedure.

4.0 Procedure –

ALL BLOOD-CONTAMINATED WORK TO BE DONE IN THE POWIS HOOD IN LAB 249

1. Whole blood should be collected by a qualified phlebotomist. Collect blood in a falcon tube containing 10% EDTA (10ul EDTA per 1ml of blood as an approximate ratio).
2. Dilute blood 1:1 with cold PBS in a 50ml tube
3. Add 10ml Histopaque (Sigma) to a clean 50ml tube
4. Carefully layer the blood over the Histopaque using a sterile glass pipette, pressing the tip against the side of the tube and slowly dispensing the blood. This is to prevent the blood and the Histopaque layer from mixing.
5. Transfer the tube very slowly to not disturb the layers to a centrifuge and spin at 800g for 30 minutes (no brake).
6. After centifugation the blood should have split into distinct layers:



7. Using a plastic Pasteur pipette, carefully transfer the whitish/pink layer of PBMCs (formed at the interface between the plasma and Histopaque layer), making sure to avoid the Histopaque layer, and transfer to a clean 50ml tube.
8. Add 10ml of cold PBS to the PBMCs and then spin at 300g for 10 minutes (can have the brake on at this point). This is the first wash step.

   At this point all subsequent work can be done in a regularly tissue culture hood.

9. Remove supernatant and re-suspend pellet in 10-12ml cold PBS and transfer to a clean 15ml tube.
10. Spin once again at 300g for 10 minutes. Repeat wash steps if necessary.
11. Count cells whilst they are suspended in PBS if immediately required for cell culture. 1ml of blood roughly equates to 1x10e6 PBMCs. PBMCs should be incubated at 37°C with 5% CO_2
12. At this point application of PBMC use may vary. For differentiation studies make sure to use appropriate media with the necessary cytokines.

PBMCs can be frozen down and stored in cryovials for future use. In this after the second or third wash step, spin down again and re-suspend PBMCs in freeze medium (10% DMSO, 90%FCS) at the desired cell density.

5.0 Personal protection -
A Howie laboratory coat and lab gloves must be worn at all times. Sharps should be disposed of in sharp-bins

6.0 Spillages -
Always clean up any spills immediately after use, only you know what you have spilt and are aware of its hazard.

Blood spillages should be immediately sprayed with 70% ethanol, mopped up then thoroughly cleaned again with 70% ethanol.

All blood-contaminated material should be disposed of in the yellow incineration buckets.

7.0 Training –
All personnel using this method need to have received cell-culture and sterile lab-practice training

Drawing of blood only to be performed by a qualified phlebotomist.

8.0 Related documents –
8.1 Risk assessments –

9.0 Approval and sign off –

Author:

Name: Oliver Read

Position: Post Doc

Signature: Date:

Management Approval:

Name: Peter Mullen

Position: Research Fellow

Signature: Date:

QA release by:

Name: Alex MacLellan

Position: QA Manager

Signature: Date:

STANDARD OPERATING PROCEDURE

Please sign below to indicate you have read this S.O.P and understand the procedures involved.

NAME	POSITION HELD	SIGNATURE	DATE