Rochester Human Immunology Center Core Laboratory
David H. Smith Center for Vaccine Biology and Immunology
Aab Institute of Biomedical Sciences

STANDARD OPERATING PROCEDURE: Isolation of Human Blood Mononuclear Cells using Ficoll-Hypaque Density Gradient Centrifugation

Date: 09-September-2007

Authors: Sally A. Quataert **Approved: Sally A. Quataert**

Purpose: This procedure is used for the separation of blood mononuclear cells from human peripheral blood, cord blood or tissues collected in anticoagulant using Ficoll-Hypaque density gradient centrifugation.

Scope: This procedure is used for the isolation of mononuclear cells from peripheral or cord blood, tissues from adults and infants when Ficoll-Hypaque density gradient method is used within the Rochester Human Immunology Center core laboratory.

Principle: Human Mononuclear cells and platelets have a lower density than Ficoll-Hypaque (1.077 g/L) and are separated from higher density granulocytes and red blood cells when centrifuged after either under or overlaying the diluted blood on the Ficoll-Hypaque layer. Blood from young infants may contain immature red blood cells that may contaminate the upper buffy coat layer after centrifugation. The contaminating RBC may be removed from the infant buffy coat by either a second Ficoll-Hypaque centrifugation step or by hypotonic lysis with ammonium chloride solution.

References:
Current protocols in Immunology (1998) 7.1.1-7.1.3 John Wiley & Sons, Inc.
Recommendations for Prevention of HIV Transmission in Health-Care Settings: Universal Blood and Body Fluid Precautions Guideline CDC 1987
CDC Biosafety in Microbiological and Biomedical Laboratories 4th edition U.S.H&HS, Public Heath Service

Safety: Personnel will adhere to safe work processes outlined in U.S. Public Health Universal Precautions Guidelines for use of human blood and body fluids and follow biosafety level 2 (BSL2) practices.

Reagents and Material:
Bovine serum albumin (BSA) Fraction V ICN Cat. # 160069 or equivalent
BD Allegra X-12 centrifuge with SX4750 swinging bucket rotor and inserts with seals
Hemocytometer or Automated Cellometer
0.4% Trypan blue exclusion dye in saline
Biological Safety Cabinet, Class II
Household bleach (5% sodium hypochlorite
Absorbent towels
Sterile serological pipets 50, 25,10, 5, 2, 1 mL size
Pipet aide for serological pipets

STANDARD OPERATING PROCEDURE: Isolation of Human Blood Mononuclear Cells using Ficoll-Hypaque Density Gradient Centrifugation
Sterile pipet tips
Digital variable single channel air displacement pipets 5-50 µL, and 30-300 µL
Waste pan
10% bleach solution
sterile 15 mL or 50 mL conical polypropylene tube
Test tube racks for 15 mL or 50 mL conical tubes
Hanks' balanced salt solution without phenol red, calcium or magnesium (HBSS)(Cellgro Catalog #21-022-CM) or Dulbecco's PBS.
Ficoll-Paque™ Plus (Amersham Pharmacia Biotech Catalog # 17-1440-03) or Lymphocyte Separation Medium (Cellgro Cat# 25-072-CV) density of 1.077 g/liter at 20^0 C.
sterile containers for dilution of blood
ice and bucket

General Procedure for Blood Processing:
* All work is performed using BSL 2 procedures and following universal precautions for handling human blood and body fluids.

Blood is collected in anti-coagulant and stored at room temperature for processing preferably within 2 hours of collection. Blood may still be processed up to 24 hours after collection if stored at room temperature. Store other tissues at optimal temperature to minimize degradation. The top layer containing the mononuclear cells and platelets is washed and centrifuged to remove platelets after harvesting. The cells may be stored up to 48 hours at 4 degrees C in HBSS with 1 % BSA or at 37^0 C in complete RPMI medium.

Specific Procedure:

1. Dilute adult blood with an equal volume of 1X Dulbecco's phosphate buffered saline or 1X HBSS in a sterile container. Infant blood should be diluted 1:4 in DPBS or HBSS. Layer 10 mLs diluted blood over or under 3 mLs of Ficoll-Hypaque in a sterile 15 mL conical tube or 35 mL over 17.5 mL of Ficoll-Paque solution. Layer over the layer by dribbling slowly down the side of the tube using a pipet or under the layer by introducing the pipet tip below the ficoll surface and slowly releasing the diluted blood.
2. Place tubes in centrifuge cups in balanced positions at 20^0 C and attach aerosol containment lids. Centrifuge at 900 X g (2500 RPM in the Allegra centrifuge) for 30 minutes at 20^0 C with no brake.
3. After centrifuge has stopped, place sealed cups carefully within a BSL2 hood and remove tubes being careful not to disturb the white cell layer containing the mononuclear cells on top of the ficoll layer. Remove the white cells by siphoning with a 2 or 5 mL pipet. Place the cells in either a sterile 15 or 50 mL polypropylene tube depending on the number of cells. Add HBSS to either 10 to 15 or 35 to 50 mL line and centrifuge the harvested cells at 300 X g (1100 RPM) for 10 minutes at 4^0 C. Pour off supernatant through gauze (anti-splash) into waste container. Tap up cells in the pellet and wash 2 additional times by resuspending cells in 10 to 15 to 20 to 50

STANDARD OPERATING PROCEDURE: Isolation of Human Blood Mononuclear Cells using Ficoll-Hypaque Density Gradient Centrifugation

mL HBSS and centrifuging at 300 X g (1100 RPM) for 10 minutes at 4 ^{0} C to remove platelets. Cells are keep at 4^0 C following harvesting in order to maximize cell viability.

4. Resuspend cells by adding HBSS with 1% BSA or complete RPMI medium with FBS at a ratio of 1 mL medium per 8 ml original blood volume. Remove 5 µLs with a sterile tip and mix with 45 µLs of 0.4% trypan blue exclusion dye. Count viable and non-viable cells and record cell number.

Revision History

Version	Change	Impact	Justification	Change Date:
HIC-1-0020	New		Stand alone for training of personnel	09/06/07