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

STANDARD OPERATING PROCEDURE: Isolation of Peripheral Blood Mononuclear Cells using BD CPT tube

Date: 10-April-2007

Author: Matthew Cochran Approval: Sally Quataert

1. Purpose: This procedure defines the process for isolating peripheral blood mononuclear cells using a BD CPT tube.

2. Scope: This procedure applies to both citrate and heparin anti-coagulant treated BD CPT tubes and is used in the RHIC laboratory.

3. References:

BD Product Insert REF 362753 BD Vacutainer® CPT™ Cell Preparation Tube with sodium heparin
BD Product Insert REF 362761 BD Vacutainer® CPT™ Cell Preparation Tube with sodium citrate

4. Materials and Equipment:

Bovine Serum Albumin (BSA) Fraction V ICN Cat. # 160069 or equivalent
Hank's balanced salt solution (HBSS) sterile or sterile HBSS with 1% BSA
Beckman Coulter Allegra X-12R or X-15R centrifuge with SX4750 swinging bucket rotor and inserts with seals
Hemocytometer and microscope or Cellometer auto-cell counter with slides
0.4% Trypan blue exclusion dye in saline
Biological Safety Cabinet, Class II
Household bleach (5% sodium hypochlorite)
10% bleach solution
Absorbent towels
Sterile serological pipets 10 and 5 mL size
Pipet aide for serological pipets
Sterile pipet tips
Digital variable single channel air displacement pipet 5-50 μL
BD Vacutainer® CPT™ Cell Preparation tube Cat. # 362753 with heparin or equivalent with sodium citrate 8 mL draw capacity 16 X 125 mm tube size for blood collection and processing
Waste pan
sterile 15 mL and 50 mL conical polypropylene tube
Test tube racks for 12 X 75 mm tubes, 15 mL and 50 mL conical tubes
12 X 75 mm 5 mL plastic tubes with closures, VWR International Cat # 60818-500 or equivalent

5. General Procedure:

5.1 Blood is collected according to specific RSRB/IRB protocol using the procedure defined in the product insert. The blood should be inverted in the CPT tubes 8 to 10 times immediately and centrifuged within 2 hours of collection to maintain highest viability.

5.2 Gently invert the BD CPT tube containing 8 mLs of blood 8 to 10 times to mix before centrifuging. Place the tubes in the centrifuge cups for 16 X 125 mm tubes and seal with aerosol containment cover. Centrifuge the tubes for 30 minutes at 2500 rpm (1500 X g) at 20ºC. An equivalent weight balance tube is used as a counterbalance.

5.3 Open the sealed centrifuge cups inside a biological safety cabinet and remove the CPT tubes. The cap is removed from the tubes and placed in a disinfectant waste pan containing 10% bleach solution. Using a sterile 5 mL serological pipet, remove from 0 to 5 mL of plasma and platelets above the buffy coat layer and discard into waste pan.

5.4 With a fresh sterile 5 mL serological pipet remove the buffy coat into a sterile 15 or 50 mL conical tube depending on the number of CPT tubes per specimen. (* HBSS or HBSS/1%BSA is used to wash the PBMC depending upon assay requirements in end use. HBSS/1%BSA helps maintain monocyte health and is the preferred wash buffer.) Wash tube and gel surface with about 2 mL wash buffer and add to cells in 15 ml or 50 mL tube. Wash the cells by adding wash buffer to 15 mLs or 50 mLs as appropriate. Centrifuge for 10 minutes at 300 X g at 4ºC in sealed centrifuge cups with equivalent weight balance tube.

5.5 Decant the supernatant into a waste container with gauze toweling and 10% bleach solution being careful not to disturb the cell pellet. Gently resuspend the cell pellet in the residual buffer by tapping tube with finger. Repeat the wash as in step 5.4 two times to remove most platelets.

5.6 Resuspend cells in approximately one mL of the appropriate cell culture medium or assay buffer for every 8 to 10 mL of starting blood. Remove 5 µLs with a sterile tip and mix with 45 µLs of 0.4% trypan blue exclusion dye in PBS in a 12 X 75 mm tube. Load a hemocytometer or cell counter slide chamber with approximately 10 µLs of trypan blue/cell solution. Count viable and non-viable cells and record cell number either using a hemocytometer and microscope or an automated cell counter and slide system.

5.7 After removal of the aliquot for cell counting, the remaining cells may be centrifuged at 300 X g for 10 minutes at 4ºC and the cell concentration adjusted for the specific application.

Revision History

Version	Change	Impact	Justification	Change Date:
HIC-1-0019	New	Previous information in HIC-1-0001 versions	This has become the standard PBMC isolation method in the RHIC core lab. The procedure is used for a diverse number of applications in addition to HIC-1-0001 and versions	04/10/07