Unit Procedure

Ficoll Separation of Mononuclear or Total White Cell Fraction from Peripheral Blood

SOP No./WI No.: CTSI-CRC-PL-206

Department: Processing Laboratory

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Date	06 JAN 2017	6 Jan 2017	Jan 6, 2017

1. OBJECTIVE

This Standard Operating Procedure (SOP) defines the procedures used in the Clinical and Translational Support Laboratory (CTSL) to ensure that white blood cells (WBC) or mononuclear white blood cells (MNC) are processed in a uniform manner. This procedure provides the basis for documenting compliance with accepted practices, such as Good Clinical Practices (GCP), and serves as a basis for application to protocols

2. SCOPE

2.1. The SOP applies to CTSL personnel conducting separation of White Blood Cells (WBCs) from whole blood with the use of Ficoll for Indiana Clinical and Translational Sciences Institute (CTSI) CTSL. It is intended to provide the basic procedure but does not over-ride protocol defined processing when provided.

2.2. All CTSL processing SOPs may be superseded by specific directives from the investigator as directed in CTSI-CRC-PL-151 Management of Requests for Sample Processing Support. Initial entry into the worksheet will define whether there are specific processing directives applicable to a specimen.

3. RESPONSIBILITIES

3.1. CTSL personnel are responsible for compliance with this procedure when processing WBCs and MNCs using Ficoll cell preparations.

4. DEFINITIONS

4.1. Processing variables may affect downstream analyses in unknown ways. Therefore, it is imperative that samples are processed uniformly according to protocol specific conditions

4.2. White blood cells (WBC) or mononuclear white blood cells (MNC) may be separated from other elements of whole blood by density gradient centrifugation. The density of the white cells differs from that of red cells, platelets, and plasma, and the density of mononuclear white cells differs from these elements and non-mononuclear white cells. Therefore, at equilibrium in a properly chosen density gradient, the desired cellular element will appear as a band visible in a transparent tube. This band can be selectively pipette to obtain a reasonably pure sample of those cells. The density of the medium used for total white blood cell isolation is 1.119 while the density of the medium used for mononuclear cell isolation is 1.077.

ACD: Acetate Citrate Dextrose	CRC: Clinical and Translational Science Institute
CTSL: Clinical and Translational Support Laboratory	DPBS: Dulbecco's Phosphate Buffered Saline
EDTA: Ethylenediaminetetraacetic acid	MNC: Mononuclear White Blood Cells
NaHEP: Sodium Heparin	PI: Principal Investigator
RCF: relative centrifugal force	RPMI: Roswell Park Memorial Institute
SOP: Standard Operating Procedure	WBC: White Blood Cells
WBC: White Blood Cells	BSC: Biological Safety Cabinet

5. ASSOCIATED DOCUMENTS

5.1. CTSI-CRC-QA-003 Document Control and Management
5.2. CTSI-CRC-CLN-030 Handling of SOP Deviations
5.3. CTSI-CRC-CLN-031 Handling of Protocol Deviations
5.4. CTSI-CRC-PL-121 General Safety
5.5. CTSI-CRC-PL-301 Mechanical Refrigeration Units
5.6. CTSI-CRC-PL-151 Management of Requests for Sample Processing Support
5.7. CTSI-CRC-PL-209 Sample Processing
5.8. CTSI-CRC-PL-160 Specimen Receipt, Tracking and Distribution
5.9. CTSI-CRC-PL-158 Data Sample Management System
5.10. CTSI-CRC-PL-207 Viable cell cryopreservation

6. PROCEDURE

6.1. Reagents
  6.1.1. Cell Culture Medium: RPMI 1640, sterile (Invitrogen) Cat # 22400
  6.1.2. Dulbecco's Phosphate Buffered Saline (DPBS), Sterile (Invitrogen) Cat# 14190-235
  6.1.3. Ethanol, 70% in a spray bottle
6.2. Supplies
  6.2.1. Cryovials, 2 mL
  6.2.2. Disposable Pipette(s)
  6.2.3. Alcohol Wipes
  6.2.4. 15ml and 50ml Conicals, sterile
  6.2.5. Cell strainer 100 µm Nylon, sterile (Fisher) Cat # 08-771-19
  6.2.6. Microcentrifuge tubes, 1.5mL
  6.2.7. Pipette tips (20ul, 200ul, and 1000ul), Sterile

6.2.8. Serological pipettes (2mL, 5mL, 10mL), Sterile
6.2.9. Gauze sponges, 4x4

6.3. Equipment
6.3.1. 4°C/-20°C/-80°C Mechanical Refrigerators/Freezers
6.3.2. Centrifuge - refrigerated
6.3.3. Biological Safety Cabinet (BSC)
6.3.4. Pipettes-adjustable volume
6.3.5. Pipette-aid

6.4. Record on the protocol specific form CTSI-CRC-PL-FM508 Lab Processing Sheet if exceptions to this SOP are applicable per CTSI-CRC-PL-151 Management of Requests for Sample Processing Support

6.5. Receive samples per CTSI-CRC-PL-160 Specimen Receipt, Tracking and Distribution
6.5.1. Record any exceptions or issues on the protocol specific form CTSI-CRC-PL-FM508 Lab Processing Sheet.
6.5.2. If samples cannot be processed immediately, maintain sample temperature conditions as received.
6.5.3. Unlabeled/mislabeled specimens will be held until identification can be determined per CTSI-CRC-PL-157 Unlabeled/Mislabeled Specimens.

6.6. Notify study personnel if sample(s) are not received as directed per protocol specific form CTSI-CRC-PL-FM508 Lab Processing Sheet and attach email or document communication on the form

6.7. Specimen required
6.7.1. Anti-coagulated Whole Peripheral Blood, Cord Blood or Bone Marrow.
6.7.2. Acceptable anticoagulants include ACD or heparins as preferred anticoagulants and EDTA is acceptable but will not provide optimal purity.

6.8. If applicable, follow CTSI-CRC-PL-209 General Sample Processing through plasma aliquot step, if applicable.

6.9. Use aseptic technique and perform all of the following open vial manipulations in a BSC
6.9.1. Diluting the peripheral blood
6.9.2. Dispense peripheral blood (1-10 ml) into a sterile 50 ml conical tube using sterile technique.
6.9.3. Measure total specimen volume and record on the protocol specific form CTSI-CRC-PL-FM508 Lab Processing Sheet if requested.
6.9.4. Add DPBS by first rinsing the original container, then adding DPBS to equal 2X the total volume of blood per 50ml conical tube, yielding a dilution of 1:3. *Example: 10mL of blood with 2mLl of DPBS= 30mL total diluted blood.*
6.9.5. Mix the cell suspension gently by rotating 8-10 times.

6.10. Separation Technique
6.10.1. Overlayer Technique:

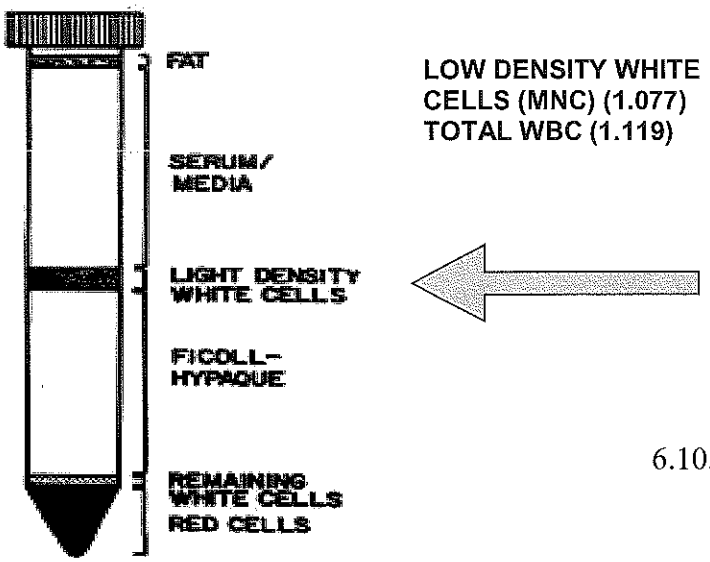
6.10.1.1. Layer the Lymphocyte Separation Medium with the diluted blood into a 1:2 ratio respectively.
6.10.1.2. Example: Use 15 mL of Lymphocyte Separation Medium to 30 mL of the diluted blood.
6.10.1.3. Dispense the correct volume of the Lymphocyte Separation Medium into a 50 ml conical tube.
6.10.1.4. Slowly layer the diluted cell suspension on top of the Lymphocyte Separation Medium without allowing the two layers to intermix.
6.10.1.5. The initial 1-2 ml is pipette down the side of the tube at an approximate 45° angle.
6.10.1.6. The remaining volume may be pipette at an increasing angle.
6.10.1.7. Repeat for remainder of the diluted sample.
6.10.1.8. Securely cap the tubes and transport to the centrifuge.

6.10.2. Under layer Technique:
6.10.2.1. Layer the Lymphocyte Separation Medium with the diluted blood into a 1:2 ratio respectively.
6.10.2.2. Example: Use 15 mL of Lymphocyte Separation Medium to 30 mL of the diluted blood.
6.10.2.3. Dispense the diluted cell suspension into a 50 ml conical tube.
6.10.2.4. Slowly dispense the Lymphocyte Separation Medium underneath the cell suspension by placing the tip of the pipette containing the gradient density at the bottom of the sample tube.
6.10.2.5. The conical tube is held at an approximate 45° angle so that the two layers do not intermix.
6.10.2.6. Repeat for remainder of the diluted sample.

6.10.3. Centrifuge following the chart below:

	Parameter	Setting
1.077 DENSITY GRADIENT	RCF	**400 x g**
	Time	30 minutes
	Temperature	Room Temperature (20-24°C)
	Brake	**Off**
	Acceleration	3
1.119 DENSITY GRADIENT	RCF	**700 x g**
	Time	30 minutes
	Temperature	Room Temperature (20-24°C)
	Brake	**Off**
	Acceleration	3

6.10.3.1. Do **NOT** use the brake on the centrifuge, allow it to stop gradually.
6.10.3.2. After the centrifugation is finished, using care to not disrupt the cell layers, remove the tubes from the centrifuge and place in the BSC.
6.10.3.3. Using sterile technique, aspirate the top plasma/media portion to within 1 cm of the desired band of cells without disturbing the mononuclear layer.
6.10.3.4. Discard the aspirate.
6.10.3.5. With a serological pipette sterilely collect the desired band of cells slowly in a circular motion (noted with arrow in Figure in the section 6.6.3.6) and transfer to a sterile 15 or 50 ml conical tube.
6.10.3.6. Ensure that the cells are collected near the sides of the conical tube. Aspirate minimal volume of the total Lymphocyte Separation Medium (Ficoll-Hypaque).



6.10.3.7. Add DPBS to the tube containing the aspirated cells and gently mix (do not vortex) and transport to the centrifuge. **Minimum dilution of 1:5** (aspirated cells: DPBS).
6.10.3.7.1. Example: For 1 ml of the aspirated cells add 4 ml of DPBS.
6.10.3.7.2. Use the appropriate program parameters as specified below.

6.10.3.8. Remove the conical tubes from the centrifuge and transport to the BSC. Aspirate the supernatant and re-suspend the cells in 5-50 ml DPBS or RPMI media.

6.10.3.9. If applicable, follow CTSI-CRC-PL-207 Viable Cell Cryopreservation or further processing per CTSI-CRC-PL-151 for Management of Requests for Sample Processing Support

6.11. File the original completed protocol specific CTSI-CRC-PL-FM508 Lab Processing Sheet after retaining and storing an electronic copy in the protocol specific source document return folder located in the Clinical Research Center. Documents shall be maintained per CTSI-CRC-QA-003 Document Control and Management.

6.12. Protocol deviations are managed per CTSI-CRC-CLN-031 Handling of Protocol Deviations.

6.13. SOP deviations are managed per CTSI-CRC-CLN-030 Handling of SOP Deviations

6.14. Of special note, per scope of this SOP, complying with special investigator specific directives that may or may not be prescribed on the protocol specific form CTSI-CRC-PL-FM508 Lab Processing Sheet is not a deviation to this SOP but is noted on the applicable worksheet and documentation of the special directives is maintained in the appropriate study folder in the CTSL.

7. REFERENCES

7.1. Lympholyte® H., Cedarlane Laboratories. 08 May 2006.

7.2. Sigma–Aldrich Leukocyte Separation, Sigma–Aldrich, November 2009.

8. APPENDICES

8.1. None

9. AMENDMENT HISTORY

Date of Amendment:	05 Jan 2017
Amendment Request by:	Robert Orr
Change Control No, if applicable:	CTSI-CRC-CLN-DC-2016-081
Details of Amendment:	Updated with new template Version 02; Revised for clarity; Updated SOP references in section 5 and steps 6.5, 6.8, 6.12 and 6.13; Removed reference to obsolete SOP in step 6.65