# STEM CELL LABORATORY (STCL)

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**DOCUMENT TITLE:**

Separation of Cells Using a Ficol-gradient

**DOCUMENT NOTES:**

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# STCL-SOP-022 JA2
# SEPARATION OF CELLS USING A FICOL-GRADIENT

## 1. PURPOSE

1.1. The purpose of this procedure is to isolate leukocytes from bone marrow or peripheral blood samples using a LSM (lymphocyte separation medium). LSM, when added to bone marrow or peripheral blood specimens, will aggregate to the erythrocytes resulting in rapid separation of the cellular layers during centrifugation.

## 2. INTRODUCTION

2.1. LSM is a sterile-filtered solution of 6.2g Ficoll and 9.4g sodium diatrizoate per 100ml. The use of this solution is intended for the isolation of mononuclear cells from heparinized whole human blood or heparinized or ACD processed bone marrow. Centrifugation allows for the migration of cells through the ficol-gradient, separating mononuclear lymphocytes from erythrocytes, polynuclear lymphocytes, and most platelets.

## 3. MATERIALS

3.1. 15cc conical tubes

3.2. LSM

3.3. Sterile Pasteur pipettes

3.4. IMDM ( Iscove's Modified Dulbecco Media w/ FBS)

3.5. Sterile 5ml volumetric pipettes

## 4. EQUIPMENT

4.1. Sysmex XS-1000ι automated hematology instrument or equivalent

4.2. Sorvall RT-7 refrigerated centrifuge or equivalent

4.3. Pipet-Aid electronic control pipettor or equivalent

## 5. SCOPE

5.1. Using aseptic technique when performing the assay, the technologist is responsible for following the SOP as written. The Medical Director, Laboratory Manager, and Quality Manager are responsible for ensuring that the requirements of this procedure are successfully met.

## 6. DEFINITIONS AND ACRONYMS

6.1. LSM Lymphocyte Separation Medium

| | | |
|---|---|---|
| 6.2. | IMDM | Iscove's Modified Dulbecco Media w/ FBS |
| 6.3. | BSC | Biological Safety Cabinet |
| 6.4. | RBC | Red blood cells |
| 6.5. | PB | peripheral blood |
| 6.6. | BM | bone marrow |
| 6.7. | mls | milliliters |
| 6.8. | mins | minutes |
| 6.9. | rpms | revolutions per minute |
| 6.10. | ul | microliter |
| 6.11. | PPE | personal protective equipment |

## 7. SAFETY

7.1. Use appropriate personal protective equipment (PPE) when handling all potentially infectious blood and body fluids. PPE includes, but is not limited to, lab coats, gloves, googles, etc.

## 8. PROCEDURE

8.1. Label two (2) 15cc conical tubes with the patient's name and Duke History number. Add 3 - 4mls of IMDM to each tube.

8.2. Gently mix PB or BM and aspirate 2 - 5mls of specimen using a sterile 5ml volumetric pipette. Dispense the sample into one of the tubes containing the IMDM solution. Gently mix with the pipette using an up/down motion.

8.3. Using aseptic technique, place a sterile Pasteur pipette into the 15cc conical tube containing the cell suspension. Using a sterile 5ml volumetric pipette, aspirate 3 – 4 mls of LSM and slowly dispense it into the conical tube by allowing it to flow from the top of the volumetric pipette.

8.4. Carefully, remove the Pasteur pipette from the solution without disturbing the blood-LSM interface. Cap tube securely.

**DO NOT MIX OR DISTURB THE CELL-LSM LAYERS**

8.5. Place conical tube in refrigerated centrifuge and spin for 15min at 3,000 rpms.

8.6. Upon completion, the following layers should be visible from top to bottom:

8.6.1. Layer 1 - Plasma

8.6.2. Layer 2 - Mononuclear cells

8.6.3. Layer 3 - LSM

8.6.4. Layer 4 – RBCs

8.7. Working in the BSC, aspirate the mononuclear layer with a sterile 5 ml volumetric pipette and transfer it into the second 15cc tube containing IMDM. Mix gently with the pipette using an up/down motion. Cap tube securely.

8.8. Place conical tube in refrigerated centrifuge and spin for 10 mins at 1800 rpms.

8.9. Remove conical tube from centrifuge; a pellet should be visible at bottom of tube.

8.10. Working in the BSC, discard supernatant by quickly inverting tube with a one move motion into discard container. DO NOT SHAKE OR TAP.

8.11. Add 1ml of IMDM to the pellet at the bottom of the conical tube and mix thoroughly.

8.12. Place a 200µl aliquot into a 12 x 75 test tube and perform a white blood cell count using an automated hematology analyzer or equivalent.

8.13. Attach the instrument print out to the sample worksheet and record all applicable data. Calculate the amount of sample to plate a final density of 1 x 10e4 cells/well. (Refer to SOP for Hematopoeitic Progenitor Assay for Clinical Specimens for additional details).

## Signature Manifest

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### STCL-PROC-022 JA2 Ficol

### Author Approval

| Name/Signature | Title | Date | Meaning/Reason |
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### Manager Approval

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| Barbara Waters-Pick (WATE02) | | 19 Feb 2012, 12:16:35 PM | Approved |

### Medical Director Approval

| Name/Signature | Title | Date | Meaning/Reason |
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### QA Approval

| Name/Signature | Title | Date | Meaning/Reason |
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| Linda Sledge (SLEDG006) | | 19 Feb 2012, 09:36:25 PM | Approved |

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