STEM CELL LABORATORY (STCL)

DOCUMENT NUMBER: STCL-PROC-024

DOCUMENT TITLE:

Coverslip Preparation

DOCUMENT NOTES:

Document Information

Revision: 04	**Vault:** STCL-Processing-rel
Status: Release	**Document Type:** Processing

Date Information

Creation Date: 28 May 2014	**Release Date:** 01 Jul 2014
Effective Date: 01 Jul 2014	**Expiration Date:**

Control Information

Author: WATE02	**Owner:** WATE02
Previous Number: STCL-PROC-024 Rev 03	**Change Number:** STCL-CCR-204

STCL-PROC-024
COVER SLIP PREPARATION

1. PURPOSE

1.1. The purpose of this procedure is to describe the process by which cover slips are properly prepared for bone marrow harvest samples.

2. INTRODUCTION

2.1. A drop of bone marrow (from the collection bag pre-processing) is placed on one cover slip. A second cover slip is placed on top of the first; the drop of bone marrow spreads between the two cover slips and then the cover slips are pulled apart. After the films have air dried, they are ready to be stained and cellular elements examined microscopically in the marrow.

2.2. The primary advantage of the cover slip method is it accommodates a more even distribution of the white cells. A few disadvantages of the cover slip method include a higher skill level and the fact that cover slips are more fragile.

3. SCOPE and RESPONSIBILITIES

3.1. The Stem Cell Laboratory medical director, Stem Cell Laboratory manager, and designated Stem Cell Laboratory staff are responsible for ensuring that the requirements of this procedure are successfully met.

4. DEFINITIONS / ACRONYMS

4.1.	N/A	Not Applicable
4.2.	BMH OR BAG	Bone Marrow Harvest Operating Room Bag (pre-processing)

5. MATERIALS

5.1. Supplies

5.1.1. Yellow pipet tips

5.1.2. Clean cover slips

5.1.3. Cover slip holders

5.1.4. Microscope slide

5.1.5. Microscope slide holder

5.1.6. Pencil

5.2. Reagents

5.2.1. Mounting Media

5.2.2. Wescor Reagents A, B, C, D and Aerofix Additive

6. EQUIPMENT

6.1. Pipet

6.2. Wescor Hematology Stainer

7. SAFETY

7.1. Wear all appropriate personal protective equipment when handling potentially hazardous blood and body fluids to include, but not limited to, gloves, lab coats, goggles, etc.

NOTE: Use EXTREME CARE when handling glass coverslips to avoid cuts.

8. PROCEDURE

8.1. Using a pipet and yellow pipet tip, place a small drop of bone marrow on a clean cover slip.

8.2. Place a second cover slip upon the first, allowing 3/4 of the cover slips to overlap and the drop of bone marrow to be in the center of the overlapping area. The two cover slips must be pulled apart just before the bone marrow stops spreading. The two cover slips must remain in the same plane during separating and must be pulled apart with a smooth sliding motion.

NOTE: Judging the size of the drop of bone marrow and the timing for the separation of the cover slips requires practice.

8.3. The films are allowed to air dry, at which time, they are ready to be placed into cover slip holders.

8.4. Stain the cover slips per *STCL-EQUIP-012 WESCOR Aerospray Hematology Stainer.*

8.5. Label the frosted end of a microscope slide by handwriting with a pencil the recipient's name, history number, "BMH OR BAG" and date.

8.6. After staining is complete, mount the 2 coverslips to a microscope slide (stain side down) with mounting media.

8.7. Allow the media to completely dry prior to reading by microscopy.

NOTE: A monolayer of cells can be evaluated by placing an unstained cover slip on a slide (without mounting media) and viewing it under low power.

8.8. Proportions of leukocytes (blasts, promyelocytes, metamyelocytes, myelocytes, bands, neutrophils, lymphocytes, monocytes, eosinophils, basophils and nucleated erythrocytes) will be determined when a 100 nucleated cell differential count has been completed.

8.9. **Good films must:**

8.9.1. have an even distribution of cellular elements.

8.9.2. have a monolayer of cellular elements.

8.9.3. not contain strings of fibrin.

8.9.4. not contain holes or streaks.

8.10. **Sources of Error:**

8.10.1. Dirty or greasy cover slips.

8.10.2. Squeezing pressure between the two cover slips.

8.10.3. Pulling the cover slips apart after the blood stops spreading.

8.10.4. Pulling the cover slips apart too soon results in a thick film.

8.10.5. A lifting motion during the separation of the cover slips.

8.11. Place slide(s) in a microscope slide holder, labeled with the recipient's name, and file slide in slide cabinet.

9. RELATED DOCUMENTS/FORMS

9.1. STCL-EQUIP-012 *WESCOR Aerospray Hematology Stainer*

10. REFERENCES

10.1. N/A

11. REVISION HISTORY

Revision No.	Author	Description of Change(s)
04	Barbara Waters-Pick	Section 1.1 added "for bone marrow harvest samples" at the end of the sentence. Section 2.1 added "from the collection bag, pre-processing" to the 1st sentence; references bone marrow instead of blood. Section 3.1 – updated Scope and Responsibilities Section 5.1 – deleted: capillary tube, pipetman®, needle; added: microscope slide, microscope slide holder, pencil Section 5.2 – Added reagents to include: mounting media, wescor reagents A, B, C, D, and Aerofix Additive Section 6.1 – added Pipet and Wescor Hematology Stainer Section 8.1 – added "Using a pipet and yello pipet tip, place a" small drop of "bone marrow" on a clean cover slip. Section 8.2 – references only "bone marrow" Section 8.3 – added "placed into cover slip holders" at the end of the sentence. Sections 8.4 thru 8.8 – Added new steps to provide more detail. Section 8.10.4 & 8.10.5 – Added new steps. Section 8.11 – Added new step. Section 9.1 – Added STCL-EQUIP-012 to this section. Section 11 – Added Revision History to this document Changed footer from "STCL, DUMC" to "Stem Cell Laboratory, DUMC".

Signature Manifest

Document Number: STCL-PROC-024 **Revision:** 04

Title: Coverslip Preparation

All dates and times are in Eastern Time.

STCL-PROC-024 Coverslip Preparation

Author

Name/Signature	Title	Date	Meaning/Reason
Barbara Waters-Pick (WATE02)		04 Jun 2014, 03:41:37 PM	Approved

Manager

Name/Signature	Title	Date	Meaning/Reason
Barbara Waters-Pick (WATE02)		04 Jun 2014, 03:41:53 PM	Approved

Medical Director

Name/Signature	Title	Date	Meaning/Reason
Joanne Kurtzberg (KURTZ001)		05 Jun 2014, 09:20:26 AM	Approved

Quality

Name/Signature	Title	Date	Meaning/Reason
John Carpenter (JPC27)		10 Jun 2014, 10:55:14 AM	Approved

Document Release

Name/Signature	Title	Date	Meaning/Reason
Sandy Mulligan (MULLI026)		11 Jun 2014, 04:41:48 PM	Approved