STEM CELL LABORATORY (STCL)

DOCUMENT NUMBER: STCL-PROC-035

DOCUMENT TITLE:

Manual Cell Differential \(Slide Method\), CBUs for CCBB Program

DOCUMENT NOTES:

Document Information

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

Date Information

Creation Date: 18 Dec 2012	**Release Date:** 21 Jan 2013
Effective Date: 21 Jan 2013	**Expiration Date:**

Control Information

Author: WATE02	**Owner:** WATE02
Previous Number: STCL-PROC-035 Rev 2	**Change Number:** FRM-CCR-168, FRM-CCR-11

STCL-PROC-035

Manual Cell Differential (Slide Method) - CBUs for CCBB Program

1. PURPOSE

1.1. Manual differential cell counts are required when automated methods fail, when instrument flags or possible interferences are suspected, or when nucleated erythrocytes (NRBCs), are not included in the automated differential. The manual differential count will identify the leukocytes, mononuclear cells and NRBC counts, as well as provide a platelet estimate as needed.

2. INTRODUCTION

2.1. A sample from all cord blood units (CBUs), both PRE (pre Hespan sedimentation), and POST (post plasma depletion/RBC reduction), will have slides prepared in duplicates. One slide form each duplicate will be stained using the WESCOR 7120 automated hematology slide stainer. All cord blood units will have an automated differential performed at the time of processing. If any of the WBC differential values form the Sysmex Hematology Series multi-channel instruments are "starred" out, a manual differential will be requested by CCBB personnel. In addition, any automated platelet value of $<100.0 \times 10^9$/L (100,000), will require a manual platelet estimate review. Proportions of leukocytes and nucleated erythrocytes will be determined from a 100 nucleated cell differential count. Mononuclear cells (defined as blasts, monocytes and lymphocytes), will be differentiated from other leukocytes and nucleated erythrocytes and the values will be recorded on the STCL Manual Differential (Slide Method) CBUs for CCBB Program (FRM1).

2.2. SPECIMEN REQUIREMENTS

2.2.1. Blood smears are to be prepared on two microscopes slides using the pre-Hespan product and on two microscope slides using the post-Hespan product. Only one slide from each group is to be stained. All slides are labeled, placed in a slide holder, and stored in the working packet for future studies.

3. SCOPE AND RESPONSIBILITIES

3.1. The Medical Director, Laboratory Manager, and designated STCL employees are responsible for ensuring that the requirements of this procedure are successfully met.

4. DEFINITIONS/ACROYMNS

4.1. MNCs	Mononuclear cells
4.2. CBU	Cord Blood Unit
4.3. RBC	Red Blood Cells
4.4. NRBCs	Nucleated Red Blood Cells
4.5. STCL	Stem Cell Laboratory
4.6. CCBB	Carolinas Cord Blood Bank
4.7. UCB	Umbilical Cord Blood
4.8. EMMES	Emmes Corporation, Inc. (Database)

5. MATERIALS

Reagents (stains) Wescor Company:

5.1. Reagent A Preservative and rinse
5.2. Reagent B Blue stain
5.3. Reagent C Red stain
5.4. Reagent D Methanol

Supplies:

5.5. 25 x 75 frosted slides
5.6. Slide holders
5.7. Yellow tips
5.8. 22 x 22 glass coverslips
5.9. Cytoseal 60 mounting medium

6. Equipment:

6.1. Wescor 7120 Aeropray Automated Slide Stainer (STCL-equip-012)
6.2. Olympus Microscope BH-2
6.3. American Tally III Electronic cell counter
6.4. 20λ Pipette

7. SAFETY

7.1. Wear appropriate personal protective equipment when handling any / all potentially hazardous blood and body fluids.

8. PROCEDURE

8.1. **Preparing and staining a slide:**

8.1.1. Obtain approximately 4µl of well mixed, unprocessed umbilical cord blood and place drop near the frosted end of a 25 x 75 mm slide, (perform in duplicate).

8.1.2. Hold a second (pusher), slide at a 45° angle next to the drop of blood.

8.1.3. Allow the drop of blood to spread along the end of the pusher slide and then push forward in one rapid, smooth motion, creating a "feathered" edge.

8.1.4. Allow blood to air dry.

8.1.5. Place a barcode corresponding to the cord blood unit being processed on the frosted end of the slide and identify slide by handwriting PRE on both slides.

8.1.6. Repeat steps above with the post Hespan depleted cord blood sample.

8.1.7. Place all 4 slides on a slide holder and deliver to STCL slide staining area.

8.1.8. Stain one slide form each group according to Wescor staining procedure guidelines.

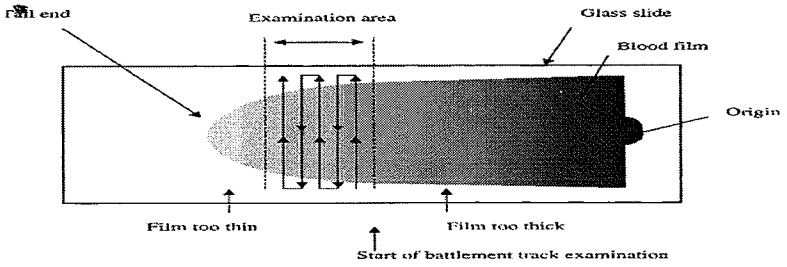
8.1.9. Mount a 22 x 22 glass cover slip by placing a drop on Cytoseal 60 mounting medium on the stained slide feathered edge. Top with a cover slip and allow to completely dry.

8.2. **Counting:**

8.2.1. Review the feather end of the blood smear for uniformity of staining and cell distribution. A feathered edge should not contain a large percentage of WBCs.

- Neutrophils should have a light pink cytoplasm with small, numerous and evenly distributed light pink to bluish purple granules. The nucleus should have a dark blue to purple color.
- RBC's should have a pink to light red color with a lighter central parlor
- Platelets exhibit a cytoplasm with a light blue stain and contain variable numbers of small, blue granules.

8.2.2. Select a good area to count near the feathered edge where the red cells are evenly distributed in a monolayer and only occasionally touching or overlapping. Move from lateral edge to lateral edge using the battlement method.



8.2.3. Proportions of leukocytes (blasts, promyelocytes, metamyelocytes, myelocytes, bands, neutrophils, lymphocytes, monocytes, eosinophils, basophils and nucleated erythrocytes (NRBCs), will be scored from a 100 nucleated cell differential count for a cord blood unit as requested.

8.2.4. Mononuclear cells, (blasts, lymphocytes and monocytes), will be differentiated from other leukocytes and NRBCs. The raw percentage of each cell identified will be recorded on

the STCL differential form (9D.211.03 FRM1), on the appropriate portion of the recording columns.

8.2.5. Document the percentage of NRBCs for both PRE and POST in the corresponding space of the differential form, as well as the percentage of mononuclear cells (add lymphocyte, monocyte and blast raw % counts).

8.2.6. If a platelet count was indicated, perform at this time and record values in the designated area the differential form.

8.2.7. Place slides and differential form in file box near the stainer area for later pick up from CCBB personnel.

8.3. **Platelet Count Estimation:**

8.3.1. The current platelet estimation criteria, for an average 100x oil immersion field which represents approximately 200 cells/field, is that each platelet represents approximately 15 x 10^9/L.

8.3.2. Using the 100x objective, count the number of platelets in 10 fields.

8.3.3. Calculate an average number of platelets.

8.3.4. Multiply the average number of platelets by 15 to arrive at the platelet count estimate.

8.3.5. The estimate should equal the automated platelet count ± 20% for counts >50 x 10^9/L and ±50% for counts <50 x 10^9/L. This is an estimate ONLY.

8.3.6. A platelet count may be falsely decreased when a significant number of large platelets are present or if platelet clumping is present. These can be noted during the evaluation of the blood film.

8.3.7. The platelet count may be falsely increased by schistocytes of other microcytic RBCs. These can be noted during the evaluation of the blood film.

CAUTION: Film quality, area selected for evaluation, RBC count level and platelet distribution are critical factors in performing and obtaining a valid platelet estimate.

8.4. **Calculations performed by EMMES**:

8.4.1. Total viable leukocyte count in the umbilical cord blood unit (CBU), PRE and POST Hespan sedimentation will be determined by calculating the automated nucleated cell count x 10^6/ml times the volume of the product times the percentage of viable cells.

8.4.2. The total viable mononuclear cell count in the CBU PRE and POST sedimentation will be determined by the following calculation and recorded on the CCBB differential form.

Total viable MNC= (total viable nucleated cell count) x (% mononuclear cells)

8.4.3. Viable mononuclear cell recovery after Hespan sedimentation will be determined by the following calculation and recorded on the CCBB differential form:

$$\text{Total viable MNCs Recovered} = \frac{\text{total viable MNC}_{(post)} \times 100}{\text{total viable MNC}_{(pre)}}$$

8.5. **Quality Control:**

8.5.1. Slides from only one CBU will be made at any time

8.5.2. Barcode numbers on the slide will be verified against the CBU barcodes PRE and POST Hespan sedimentation.

8.5.3. Barcode numbers on the CBU Processing form will be verified against barcode numbers on the microscope slides.

8.5.4. The technologist's ability to perform manual differentials on umbilical cord blood specimens will be documented in their respective training folders (or in Master Control, if applicable) on an annual basis. Proficiency samples are also distributed throughout the year by the College of American Pathologists (CAP) and evaluated by designated staff.

8.5.5. Stained and unstained slides will be filed in the CBU working packet and will be available for future use in the CCBB Program.

9. RELATED FORMS/DOCUMENTS

(FRM1) – STCL Manual Differential (Slide Method) - CBUs for CCBB Program

10. REFERENCES

- American Association of Blood Banks. Standards for Hematopoietic Progenitor Cell and Cellular Product. Current Edition.
- Foundation for the Accreditation of Hematopoietic Cell Therapy (FACT) and Netcord. International Standards for Cord Blood Collection, Processing, Testing, Banking, Selection and Release. Current Edition.
- Sandoz Altas of Hematology.1988 edition.
- Clinical Laboratories Bone Marrow Transplant. Hematology/Blood Film Exam Manual Differential Procedure (LTR19230). Current Revision.

11. Revision History

Revision No.	Author	Description of Change(s)
04	Barbara Waters-Pick	Reformatted

Signature Manifest

Document Number: STCL-PROC-035 **Revision:** 04
Title: Manual Cell Differential (Slide Method), CBUs for CCBB Program

STCL-PROC-035 Manual Diff CCBB

Author Approval

Name/Signature	Title	Date	Meaning/Reason
Barbara Waters-Pick (WATE02)		18 Dec 2012, 09:39:21 PM	Approved

Manager Approval

Name/Signature	Title	Date	Meaning/Reason
Barbara Waters-Pick (WATE02)		18 Dec 2012, 09:39:41 PM	Approved

Medical Director Approval

Name/Signature	Title	Date	Meaning/Reason
Joanne Kurtzberg (KURTZ001)		18 Dec 2012, 11:05:23 PM	Approved

QA Approval

Name/Signature	Title	Date	Meaning/Reason
Linda Sledge (SLEDG006)		19 Dec 2012, 08:15:15 AM	Approved

Document Release

Name/Signature	Title	Date	Meaning/Reason
Sandy Mulligan (MULLI026)		18 Jan 2013, 12:14:32 AM	Approved

Notification

Name/Signature	Title	Date	Meaning/Reason
Margie Rodriguez-Lopez (RODRI014)		18 Jan 2013, 12:14:32 AM	Email Sent
Sophia Avrutsky (AVRUT001)		18 Jan 2013, 12:14:32 AM	Email Sent
Barbara Waters-Pick (WATE02)		18 Jan 2013, 12:14:32 AM	Email Sent