



# STEM CELL LABORATORY (STCL)



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**STCL-SOP-052**  
**HEMATOPOIETIC PROGENITOR CELL ASSAY (HPCA) – CORD**  
**BLOOD BANK PRODUCTS**

**1 PURPOSE**

- 1.1 One surrogate measure of the hematopoietic cell number, engraftment potential, and overall survival after transplantation of a sample of cord blood is its content of hematopoietic colony forming cells. This assay is dependent on the ability of a single hematopoietic cell to divide and differentiate, forming clusters of cells (colonies) in a semi-solid media containing appropriate growth factors.

**2 INTRODUCTION**

- 2.1 This assay involves plating a single cell suspension at low cell density in media made semi-solid with methylcellulose and other cytokines or supplements that support the proliferation and differentiation of the hematopoietic progenitors for 14-16 days. The semi-solid media minimizes the movement of cells in the culture ensuring that the daughter cells derived from a single progenitor stay in close proximity to each other. The cellular composition and the size of the colony are used to classify the cell of origin. The growth of colony forming units, granulocyte-macrophage (**CFU-GM**), colony forming unit granulocyte-erythrocyte-macrophage and megakaryocyte (**CFU-GEMM**), and burst-forming unit erythroid (**BFU-E**), is enumerated after 14-16 days of incubation under specific conditions, and each colony or burst is believed to have resulted from one proliferating progenitor cell.
- 2.2 Sample Requirements
- 2.2.1 Umbilical Cord Blood (UCB), that has been red cell depleted and volume reduced according to the Processing CBU SOP. The sample must be sterile and provided with the nucleated cell count, additional adhesive barcode labels, Graft Characterization Form, Progenitor Assay Form and Flow Cytometry Worksheet.

**3 SCOPE AND RESPONSIBILITIES**

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

**4 DEFINITIONS/ACRONYMS**

- |     |          |   |
|-----|----------|---|
| 4.1 | STCL     | Stem Cell laboratory  |
| 4.2 | HPCA     | Hematopoietic Progenitor Cell Assay                                       |
| 4.3 | IMDM     | Iscoe's Modified Dulbecco's Medium with 2% FBS                            |
| 4.4 | CFU-GM   | Colony Forming Unit granulocyte/macrophage                                |
| 4.5 | CFU-GEMM | Colony Forming Units granulocyte-erythrocyte-macrophage and megakaryocyte |

|      |          |                              |
|------|----------|------------------------------|
| 4.6  | CFU-BFUE | Burst-forming unit erythroid |
| 4.7  | UCB      | Umbilical Cord Blood         |
| 4.8  | CCBB     | Carolinas Cord Blood Bank    |
| 4.9  | BSC      | Biological Safety Cabinet    |
| 4.10 | mL       | milliliter                   |

## 5 MATERIALS

|       |  |  |
|-------|--|--|
| 5.1   | <b>Reagents</b>                          | <b>Manufacturer / Catalog #</b>  |
| 5.1.1 | MethoCult 4434 Medium                    | Stem Cell Technologies/ Cat# 4434  |
| 5.1.2 | IMDM with 2% FBS                         | Stem Cell Technologies/ Cat# 7700  |
| 5.1.3 | Sterile water 1L                         | Sigma/ Cat# W3500-1L (or equivalent)   |
| 5.2   | <b>Supplies</b>                          |  |
| 5.2.1 | 3 ml sterile syringes with luer lock tip | BD Biosciences/ Cat# 309657  |
| 5.2.2 | Blunt-end needle, 16G                    | Stem Cell Technologies/ Cat# 28110   |
| 5.2.3 | 24-well Costar cell culture plates       | Corning Inc./ Cat# 3524  |
| 5.2.4 | Sample ID barcodes                       | Computype/ Cat# 1018184  |
| 5.2.5 | Permanent marker                         | Sharpie/ Cat# 30001  |
| 5.2.6 | Sterile 12 x 75 polystyrene tubes        | Port City Diagnostics/ Cat# T2063STR   |
| 5.2.7 | Sterile 15 ml conical tubes              | Port City Diagnostics/ Cat# U1100SRGFT   |
| 5.2.8 | Sterile 200µl pipette tips               | Port City Diagnostics/Cat# 7509-96RS   |
| 5.2.9 | Sterile serological pipettes             | Port City Diagnostics/ 1ml Cat# SER-0010-S01<br>2ml Cat#SER-0020-S01<br>5ml Cat#SER-0050-S01<br>10ml Cat#SER-0100-S01                          |
| 5.3   | <b>Equipment</b>                         |  |
| 5.3.1 | Barcode scanner                          | Zebra/ Model ZM400   |
| 5.3.2 | Inverted Microscope                      | Olympus IMT-2  |
| 5.3.3 | Thermo Scientific CO2 Incubators         | HERAcell 150 SN# 225658 & SN# 225659<br>Isotemp Plus SN# Z01J464990ZJ  |
| 5.3.4 | Vortex Mixer                             | VWR/ Mini Vortexer MV1   |
| 5.3.5 | DIFFCOUNT electronic cell counter        | Modulus Data Systems/ SN# 319806   |
| 5.3.6 | Stamping Clock                           | Lathem 1000E/ SN# 1E014032   |
| 5.3.7 | 3 channel traceable timer                | Fisher Scientific/ SN# 111878606<br>/ SN# 111878753  |
| 5.3.8 | Class II Biological Safety Cabinet       | NUAIRE 425-600/ SN# 123044050508<br>Baker SG400/ SN# SL29877V  |
| 5.3.9 | Micropipettes                            | Rainin/ 20µl : SN# M11689G<br>20µl: SN# I0985053K<br>200µl: SN# I0984537K<br>200µl: SN# M10263E<br>1000µl: SN# R73215A<br>1000µl: SN#H0962198K |

## 6 EQUIPMENT

6.1 Refer to Section 5.3.

## 7 SAFETY

7.1 All procedures for cell processing and set-up of cell culture assays should be performed using sterile technique under a biohazard safety cabinet certified for Level II handling of biological materials, and universal handling precautions. When handling a biological hazardous substance, such as umbilical cord blood, appropriate personal protective equipment (PPE), must be worn as the primary barrier of protection. PPE may include, but is not limited to face protection, lab coats and gloves. Appropriate PPE should be donned before handling potentially hazardous biological materials and removed immediately and replaced if gross contamination of the equipment occurs.

## 8 PROCEDURE

### 8.1 Cord Blood

8.1.1 For optimal time management, at the beginning of your shift, the technologist may prepare IMDM tubes, MethoCult syringes and plates ahead of time for later use. (*For detailed instructions, refer to **Section 8.3, Preparation and Storage notes on MethoCult and IMDM.***)

8.1.2 Cord Blood samples are delivered to the STCL by CCB staff in a designated container along with a UCB packet of information which includes the Graft Characterization Form, the Progenitor Assay Form, and the Flow Cytometry Worksheet. Slides to be stained, extra barcodes, and a manual differential worksheet, if applicable, will also accompany the sample(s). CCB staff will date / time stamp the top page of each UCB packet of information in an effort to track the delivery time in the STCL. More than one cord blood sample may be delivered at one time.

8.1.3 Remove reagent supplies from the refrigerator at this time to allow them to come to room temperature. Set a timer for 15 minutes to alert you when the reagents are ready for use.

**NOTE:** All media should be allowed to come to room temperature before use (**at least 15 minutes**).

8.1.4 The HPCA technologist will verify sample ID with extra barcodes, and pre-labeled UCB packet of information. For internal labeling purposes, a letter will be assigned to each cord blood sample beginning with the first letter of the alphabet. This letter will be transcribed onto all the extra barcode labels (including the one on the sample tube) and the forms provided for that sample. If more than 26 samples are received in one day, the alphabet ID resets using a double lettering system (*ie. A, B, C.....AA, BB, etc*). Record the Study ID of the technician responsible for plating on the Progenitor Assay Form.

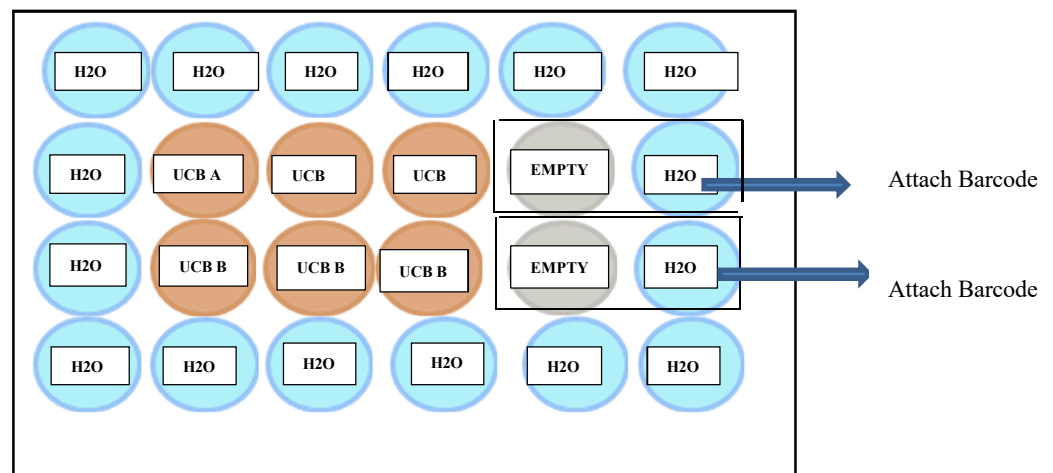
- ### Example:

### 35.1 Bank UCB @ 1600

- Page 4 of 10

- 8.1.12 Mix the contents thoroughly by gently aspirating the cell mixture into the syringe and dispensing it back into the tube (minimizing the formation of bubbles). Repeat this step 5 times or until cell mixture looks homogeneous. After the last mixing cycle, allow the empty syringe to remain inside the tube.
- 8.1.13 Allow the cell mixture to stand for at least 5 minutes to allow the bubbles to rise to the top. Set a timer for 5 minutes; the sample will be ready for plating at that time. Avoid letting the cell mixture sit for longer than 15 minutes before plating.
- 8.1.14 At this time, you may begin preparing the next sample for plating. (Refer to step 8.1.7 – 8.1.13).
- 8.1.15 At the end of the 5 minutes, slowly draw up the MethoCult mixture containing cells into the 3 mL syringe. Remove the blunt needle from the syringe by twisting the needle with the help of the needle cover. Lift off the lid of the plate and place ~ 0.5 mL of the cell suspension into each of the 3 wells of the culture plate assigned to the sample. Dispense 1 drop at a time into each well, beginning with your far left, until all of the mixture is gone. Replace the lid on the plate. Discard the IMDM dilution tubes and syringe at this time. Repeat steps if more samples are in-progress.
- 8.1.16 Swirl the plate gently to evenly distribute the sample to cover the bottom of each well and remove any visible bubbles, using aseptic technique. Please note that each plate can only accommodate 2 UCB unit samples.

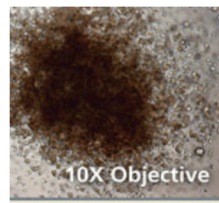
**Cell culture plate containing 2 UCB samples plated in triplicate.**



- 8.1.17 Deliver worksheets, labels and fresh UCB samples to the Flow Cytometry area.

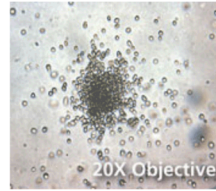
**NOTE:** UCB slides may be delivered to the Manual Differential Station for staining at any given time during the day, as time allows.

- 8.1.18 Place the tissue culture plate into a 37° C humidified incubator in 5% CO<sub>2</sub> for 14-16 days.
- 8.1.19 At the end of the incubation period, remove the plates to be scored from the incubator and recover the worksheet packet with the Progenitor Assay Form. Take only the number of plates that can be scored within one hour.
- 8.1.20 Confirm unit identity by comparing the barcode number on the plate to the barcode number on the form.
- 8.1.21 On the Progenitor Assay Form, record the number of cells plated per well, ( $1.25 \times 10^4$ ), the date and the study ID of the technologist reading the plate.
- 8.1.22 Colony growth is enumerated using a high quality inverted microscope with a blue filter to enhance the color of hemoglobinized erythroblasts. Use the following criteria when scoring a sample:
- 8.1.22.1 Place the plate on the inverted microscope stage and adjust the focus under low power (2X objective). Consider the overall colony appearance, distribution, background, plane of view and general morphology.
- 8.1.22.2 Count the colonies in each well and differentiate as follows:
- 8.1.22.2.1 BFU-E (erythroid)\*
- Bright red or brown
  - $\geq 200$  cells/burst
  - A multi-centric burst is counted as a single entity
  - Cells in each portion of a burst are tightly packed
  - Colonies can appear as one compact cluster or with multiple clusters
  - Cells from different individual centers of a burst that are closest to the center of the mass of the whole BFU-E tend to be in the same focal plane as those from adjoining centers.



- 8.1.22.2.2 CFU-GM (granulocyte and macrophage)\*
- Colorless, sometimes granular or “glossy”
  - Uniform in size and usually tightly packed
  - Individual cells can be distinguished, particularly at the edge of the colony
  - $> 40$  cells/ colony

- Macrophage colonies are larger and more spread out than granulocyte colonies
- Cells are typically spread out but may have macrophages clustered together in the center of the colony, making it appear dark.



#### 8.1.22.2.3 CFU-GEMM (granulocyte, erythroid, macrophage and megakaryocyte)\*

- Often large and have a larger capacity to proliferate
- Erythroid cells tend to be in the center and surrounded by non-erythroid cells



**\* For assistance in recognition for various colony types, refer to the *Atlas of Hematopoietic Colonies from Cord Blood* located in the HPC area in the STCL.**

**NOTE:** If the colony count is greater than 100 colonies per plate, score the plate as >100.

- 8.1.23 The technician reading the plate records the counts for each colony type, their Study ID, and the date the plate was read on the Progenitor Assay Form. The total nucleated cell count (TNCC) and the average colony count (from each triplicate plating) will be used to calculate the total number of CFU-GM ( $\times 10^5$ ), CFU-GEMM ( $\times 10^5$ ), and BFU-E ( $\times 10^5$ ) colonies. Verify the calculations by recording the date and initials on the back of the Progenitor Assay Form.
- 8.1.24 The completed UCB packet of information (*which includes the Graft Characterization Form, Progenitor Assay Form, and the Flow Cytometry worksheet*) will be delivered to designated STCL staff (*located in the Receiving Area*) so results can be entered in the CCBB Emmes database.

## 8.2 Quality Control

### 8.2.1 Proficiency Testing

- 8.2.1.1 Fresh or frozen cord blood proficiency testing samples will be performed quarterly by the delegated technicians trained in plate enumeration and plating. These samples will be



submitted to Stem Cell Technologies or College of American Pathologists (CAP) for review and grading. These blinded samples will be used to determine reproducibility between the technicians and all participating centers.

#### 8.2.2 Quarterly

- 8.2.2.1 Choose one cord blood sample from the daily workload and have all technicians trained in HPCA, plate this sample according to SOP. To minimize variables, all techs must plate within a one-hour time and store in the same incubator.
- 8.2.2.2 At the end of the 14-16 day incubation period, plates will be read and scored. The same technologist will read all plates for standardization purposes.
- 8.2.2.3 Each technologist's plate readings should agree within 80% of the scoring technician's.
- 8.2.2.4 If the counts exceed a 20% difference, the scoring technician and delegated plating technician will undergo a review of technique and a new sample will be repeated.
- 8.2.2.5 One plate will be chosen and read by both scoring technicians to determine reproducibility between them. The total colony count must agree within 80% from each other.

#### 8.3 Notes on Preparation and storage of MethoCult and IMDM Media

- 8.3.1 Follow this procedure when thawing an entire bottle of 100 mL MethoCult or IMDM media.
- 8.3.2 Thaw media bottle overnight under refrigeration (2 - 8°) or at room temperature until thawed.

**NOTE:** Do **NOT** thaw medium at 37°C. The methylcellulose will not be homogeneous in frozen MethoCult products and small lumps may be present if the product is thawed rapidly at 37°C.
- 8.3.3 **Shake the MethoCult bottle vigorously** until the media becomes opaque with bubbles. Due to the viscosity of the MethoCult media, it is necessary to shake the bottle vigorously before aliquoting.
- 8.3.4 Let the bottle stand for at least 5 minutes before aliquoting.
- 8.3.5 Label each tube with the name of media, aliquot date, expiration date, and tech initials.
- 8.3.6 Aliquot complete media into 15mL conical tubes and make sure the tubes are capped tightly to maintain sterility.
  - 8.3.6.1 Media is stable until the expiration date on the manufacturer's label when stored at -20°C or for one month at 2-8°C.

- 8.3.6.2 You may choose to prepare MethoCult syringes and IMDM tubes for daily use from a 15mL conical tube and allow them to sit in the refrigerator until ready for use.
- 8.3.6.3 Preparation of **MethoCult** syringes for fresh UCB plating:
  - 8.3.6.3.1 Remove a 12mL MethoCult aliquot tube from the refrigerator and shake well. Let tube sit for 5 min to allow bubbles to rise to the top.
  - 8.3.6.3.2 Using aseptic technique, attach a blunt-end needle to a 3 mL sterile syringe. To remove the air from the syringe, place the needle below the surface of the MethoCult medium and draw up approximately 0.5 mL, gently depress the plunger and expel the medium completely.
  - 8.3.6.3.3 Draw up the desired amount for plating, **1.5 mL**, until no air space is visible. Recap syringe and store at 2-8°C for later use. Label the rack with preparation date, expiration date (post thaw), and tech initials.
- 8.3.6.4 Preparation of **IMDM** tubes for fresh UCB plating:
  - 8.3.6.4.1 Remove a 12mL IMDM aliquot tube from the refrigerator.
  - 8.3.6.4.2 Using a sterile 1mL or 2mL volumetric pipette, prepare sets of 12 x 75 sterile tubes, in pairs, by adding 0.5 mL of IMDM to the first tube and 0.4 mL to the second tube. Label each tube with the amount of IMDM dispensed and store at 2-8°C until ready for use. Label the rack with the preparation date, expiration date (post thaw), and tech initials.

**NOTE:** It is important to communicate with CCBB personnel whenever possible to estimate the arrival time of samples being delivered to the STCL. This will allow the STCL enough time to bring the media to room temperature before it is used (at least 15 min.). Based on manufacturer's recommendations, the media should NOT remain at room temperature for a prolonged period of time.

## 9 RELATED DOCUMENTS / FORMS

- 9.1 STCL-SOP-052 FRM1 Progenitor Cell Assay Form
- 9.2 FLOW-FORM-012 Graft Characterization Form
- 9.3 FLOW-GEN-012 FRM5 Stem Cell Laboratory Flow Cytometry Worksheet

- 9.4 STCL-FORM-064 Manual Differential Worksheet – Clinical Products
- 9.5 STCL-SOP-052 (JA1) HPC Processing of Fresh UCB Samples – Flow Chart
- 9.6 STCL-SOP-052 (JA2) Media Lot-to-Lot Testing

## 10 REFERENCES

- 10.1 Human Colony-Forming Assays Using MethoCult, Stem Cell Technologies, Version 3.1.0, Oct 2009 or current version
- 10.2 Atlas of Human Hematopoietic Colonies, Stem Cell Technologies, Version 2.0.0, March 2010 or current version
- 10.3 Areman E. Deeg HJ Sacher R. Peripheral Blood Stem Cell Processing Protocol. IN: Bone Marrow and Stem Cell Processing: Manual of Current Techniques. F.A. Davis Company, Philadelphia, PA, 1992.
- 10.4 American Association of Blood Banks. Standards for Hematopoietic Progenitor Cell and Cellular Products. Current Edition.
- 10.5 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.

## 11 REVISION HISTORY

| Revision No. | Author         | Description of Change(s)  |
|--------------|----------------|---|
| 08           | B. Waters-Pick | <ul style="list-style-type: none"> <li>Added mL to Section 4 and corrected “cc” to “mL” throughout the document</li> <li>Corrected numbering in Section 5</li> <li>Corrected Section 6.1 to read “Refer to equipment in Section 5.3”</li> <li>Updated STCL to Stem Cell Laboratory in footer</li> <li>Added College of American Pathologists (CAP) to Section 8.2.1.1</li> <li>Updated document number in Section 9</li> <li>Updated 10.1 and 10.2 to add “ or current version” to entries</li> </ul> |

## Signature Manifest

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#### Management

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| Barbara Waters-Pick<br>(WATER002) |       | 27 Aug 2020, 11:03:40 AM | Approved       |

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