



# Proficiency Testing

## Data Submission Worksheet For Fresh Cord Blood Program

**Please Note:** All participants are asked to plate the same volume by creating the same 10X dilution in the instructions sent by STEMCELL Technologies Inc. These session-specific instructions will be e-mailed to participants when the fresh cord blood sample is shipped out. Participants will perform a total nucleated cell (TNC) count **after** inoculating the cultures and report this value along with the rest of their data.

For detailed instructions on colony assay set-up refer to the Technical Manual for Human Colony-Forming Cell Assays Using MethoCult™ (Manual Catalog #28404), available upon request and on our website at [www.proficiencytesting.com](http://www.proficiencytesting.com).

You can submit data in one of three ways:

- **Online** with the Proficiency Testing Data Submission Forms available on our website at [www.proficiencytesting.com](http://www.proficiencytesting.com)
- **E-mail** this completed worksheet to [proficiency@stemcell.com](mailto:proficiency@stemcell.com)
- **Fax** this completed worksheet to 1.604.877.0703 or 1.800.567.2899 (North America) (Attention Education Department)

Name: \_\_\_\_\_

E-mail: \_\_\_\_\_

Institution: \_\_\_\_\_

Participant ID: \_\_\_\_\_

### Part 1: Cell Inoculation

1. Thaw the IMDM with 2% FBS and the tube of MethoCult™ GF by placing them at 4°C the day before the cord blood sample is expected to arrive. (You will receive this session-specific information in an e-mail sent by STEMCELL Technologies.)
2. Measure the volume of the CB sample received and record this information in box **A**.
3. Prepare the 10X cell dilution as directed by the e-mailed instructions from STEMCELL Technologies. Record the volume of CB sample used in box **B** and the volume of IMDM with 2% FBS added in box **C**. Mix the cell suspension by gently pipetting up and down three times.
4. Add 0.5 mL of the 10X cell dilution to the 5 mL tube of MethoCult™ GF and vortex vigorously for at least four seconds. Let stand until the bubbles rise to the surface (at least five minutes).
5. Plate 1.1 mL of MethoCult™ GF + cells into each of four 35 mm culture dishes.
6. Place two 35 mm dishes in each of the 100 mm dishes. Add a 35 mm dish containing sterile water without a lid to each 100 mm dish to ensure adequate humidity.
7. Incubate at 37°C, 5% CO<sub>2</sub>, >95% humidity for fourteen days.

<b>A</b>	Volume of CB sample	mL
<b>B</b>	Volume of CB sample used in 10X cell dilution	mL
<b>C</b>	Volume of IMDM + 2% FBS used in 10X cell dilution	mL

### Part 2: Cell Counting

Determine the total nucleated cell count (TNC) using the method you normally use in your lab. If you do not have a procedure, refer to the Technical Manual for Human Colony-Forming Cell Assays Using MethoCult™ (Manual Catalog #28404). Record the total number of nucleated cells per mL in box **D**. Indicate if this value was determined by manual or automated methods in box **E**.

<b>D</b>	Nucleated cell conc.	Nucleated cells/mL
<b>E</b>	Manual or automated cell count	

# Proficiency Testing

Data Submission Worksheet for Fresh Cord Blood Program

## Part 3: CD34 Enumeration

(CD34 Enumeration is optional for non-NMDP members.)

Instrument used to perform flow cytometric analysis:

Supplier: \_\_\_\_\_  
(e.g. BD, Coulter)

Model: \_\_\_\_\_  
(e.g. FACS Calibur, etc.)

Methodology used:

- a.  ISHAGE Protocol
- b.  Modified ISHAGE
- c.  Double Platform
- d.  Single Platform
- e.  Other (describe): \_\_\_\_\_

Reagents (list antibodies used for phenotyping):

CD34 \_\_\_\_\_

CD45 \_\_\_\_\_

Other \_\_\_\_\_

% CD34<sup>+</sup> = \_\_\_\_\_ gated on viable cells

% CD45<sup>+</sup> CD34<sup>+</sup> = \_\_\_\_\_ gated on viable cells

## Part 4: Colony Enumeration

Count colonies on day 14 and record results below. If your facility does not discriminate between colony types and you would only like to report total colonies, record your counts in the "TOTAL" row of the table below.

Date Counted:

COLONY	DISH			
	1	2	3	4
BFU-E				
CFU-GM				
CFU-GEMM				
<b>TOTAL</b>				

## Part 5: Colony Identification

Refer to the photographs located on our website at [www.proficiencytesting.com](http://www.proficiencytesting.com) and identify the colonies.

PHOTO	COLONY	PHOTO	COLONY
A		E	
B		F	
C		G	
D		H	