STEM CELL LABORATORY (STCL)

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DOCUMENT TITLE:

Viability Counts via Trypan Blue Dye Exclusion

DOCUMENT NOTES:

Document required for the BLA.

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STCL-SOP-022
VIABILITY COUNTS VIA TRYPAN BLUE DYE EXCLUSION

1 PURPOSE

1.1 Viability counts are performed routinely in the laboratory using trypan blue dye exclusion staining. Capillary samples using a smaller volume for pediatric patients and/or umbilical cord blood are used to conserve the primary product. This procedure outlines steps for the use of trypan blue dye exclusion staining for the determination of cell viability in fresh and thawed peripheral blood stem cells (PBSC), bone marrow (BM) and umbilical cord blood (UCB). This procedure also covers samples that have been thawed with and without the addition of dextran/albumin.

2 INTRODUCTION

2.1 Trypan blue is a vital stain that is normally only taken up by dead cells; since live cells possess intact cell membranes, living cells do not take up trypan blue stain into the cells.

3 SCOPE AND RESPONSIBILITIES

3.1 The program Medical Director, Laboratory Manager, and respective laboratory staff are responsible for ensuring that the requirements of this procedure are successfully met.

4 DEFINITIONS/ACRONYMS

4.1	PBSC	peripheral blood stem cells
4.2	UCB	umbilical cord blood
4.3	BM	bone marrow
4.4	ISBT	International Society Blood Transfusion (A global standard for the identification, labeling, and information transfer
4.5	DMSO	dimethyl sulfoxide

5 MATERIALS

5.1 Trypan blue dye (0.4%)

5.2 Normal saline (0.9%)

5.3 Albumin (Human) U.S.P. 25%

5.4 Microscope slide

5.5 Cover slip

5.6 Polystyrene tubes, 12x75

5.7 Pipette tips 0-200µL

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5.8 Pipette tips 200-1000µL

6 EQUIPMENT

6.1 Automatic Pipettor 0-1000µL

6.2 Automatic Pipettor 200-1000µL

6.3 Microscope

6.4 Automated Timer

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 coat, etc.

8 PROCEDURE

8.1 Label 12x75 test tube(s) with designated ISBT barcode, or patient name and date.

8.2 Prepare the appropriate dilution of cellular products and mix with trypan blue dye

8.2.1 For small volume, fresh, cellular products or cellular products which have been thawed using the dextran/albumin procedure:

8.2.1.1 Obtain residual sample used for counting on the automated machine (160µL cell pack and 40µL test sample for fresh UCB samples or 200µL of thawed product).

NOTE: There will be approximately 180µL remaining in the tube after count if using the XS-1000i.

8.2.1.2 For samples run on either the XE-2100 or the XE-5000, add 15µL of trypan blue dye to the remaining sample in the tube and mix.

8.2.1.3 For samples run on the XS-1000i, add 45µL of trypan blue dye to the remaining sample in the tube and mix.

8.2.2 For fresh BM or PBSC:

8.2.2.1 Pipette 0.8 ml of normal saline into the tube

8.2.2.2 Mix the cell suspension well and pipette 0.1 ml of hematopoietic cells into the tube with the saline

8.2.2.3 Add 225µL of trypan blue to the tube thereby creating a 5% suspension of trypan blue. Mix well.

8.2.3 For BM or PBSC thawed at 37° without the use of dextran/albumin:

8.2.3.1 At the time of infusion, remove a 0.1 ml aliquot from each bag at thawing and hold on ice in a combined aliquot tube until all bags have been thawed

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8.2.3.2 Prepare a tube containing 0.1 ml of 25% human albumin and 0.4 ml saline (5% albumin solution)

8.2.3.3 Note: DMSO is toxic to cells at temperatures above 10° C (50° F). Albumin absorbs out the intracellular DMSO, improving significantly the post thaw viability.

8.2.3.4 Mix the cell suspension well and pipette 0.1 ml of hematopoietic cells into the saline/albumin tube. Mix well.

8.2.3.5 Add 0.15 ml of trypan blue dye to the tube and mix well.

8.2.4 Incubate for five minutes.

NOTE: The trypan blue dye should not incubate longer than 5 minutes with the cell suspension because with longer incubations viable cells will begin to take up the dye. If viable cells take up the dye, a falsely low percent viability will result.

8.3 Dispense 10µL of the cell suspension on a microscope slide. Place a cover slip over the cells.

8.4 Under the microscope (minimum magnification 40X) count 100 white blood cells, noting dead (blue) versus live (unstained) white blood cells. Report sample viability as the percent of live cells ($\frac{\text{\# live cells}}{100}$).

Example: 5 blue cells, 95 unstained cells would be 95%.

8.5 Refer to specific processing procedure for viability exclusion.

8.6 Anticipated RESULTS

8.6.1 The viability of a fresh sample should be $\geq$ 85%.

8.6.2 The viability of a thawed cell sample should always be $\geq$ 70%.

8.6.2.1 If the viability is $\leq$ 70%, alert the attending physician immediately.

8.6.2.2 If the viability is $\leq$ 70%, have a second technologist repeat the viability.

9 RELATED DOCUMENTS/ FORMS

9.1 STCL-SOP-022 FRM 1 Trypan Blue Stain Quality Control

10 REFERENCES

10.1 www.cellgro.com, Mediatech Technical Information

10.2 American Association of Blood Banks. Standards for Hematopoietic Progenitor Cell and Cellular Product. Current edition.

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10.3 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.

10.4 Areman, EM, Deeg, HJ, Sacher, RA, Bone Marrow and Stem Cell Processing: A Manual of Current Techniques. Philadelphia, PA, 1993.

11 REVISION HISTORY

Revision No.	Author	Description of Change(s)
05	Barbara Waters-Pick	Referenced new form in Section 9.1

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