STEM CELL LABORATORY (STCL)

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Procedure for Operating Hemocytometers

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STCL-PROC-023
PROCEDURE FOR OPERATING HEMACYTOMETERS

1 PURPOSE

1.1 This document describes how to use and care for a hemacytometer.

2 INTRODUCTION

2.1 The improved Neubauer hemacytometer (also spelled hemocytometer) is a thick glass slide with a microscopic grid etched on the glass surface. Hemacytometers have two counting chambers with raised sides that will hold a quartz cover slip exactly 0.1 mm above the chamber floor. Cover slips for counting chambers are thicker than those for conventional microscopy. Each chamber is divided into nine large squares delineated by triple white lines.

3 SCOPE AND RESPONSIBILITIES

3.1 This procedure applies to the use of the hemacytometer in the determination of the concentration of cells in a cell suspension visualized under the microscope when automated testing is not possible.

4 DEFINITIONS/ACRONYMS

4.1 N/A

5 MATERIAL

5.1 Specimens

5.1.1 Cells counted in the hemacytometer are either WBCs obtained following collections of umbilical cord blood, bone marrow, peripheral stem cells, peripheral blood or cell lines maintained in tissue culture.

5.2 Supplies

5.2.1	Cryogenic vials 1.5ml	Sarstedt Brand Products
5.2.2	Quartz cover slips	Sigma Co
5.2.3	Trypan Blue	Sigma Co
5.2.4	Phosphate Buffered Saline	Gibco BRL life Technologies

6 EQUIPMENT

6.1	Improved Neubauer Hemacytometer:	Reichert-Jung, Hausser Scientific Co.
6.2	Microscope	American Optical, Olympus.
6.3	Tally counter	Sigma Co.

7 SAFETY

7.1 Wear all appropriate personal protective equipment (PPE) when handling any potentially hazardous blood or body fluids to include, but not limited to, gloves, lab coats, etc.

8 PROCEDURE

8.1 Place the cover slip over the hemacytometer counting chamber.

8.2 Cell clumps can be re-suspended by gently pipetting the cell suspension.

8.3 Using a small pipette, place a small drop (12ul) of the cell suspension on the edge of the "V"-shaped groove in the chamber; the hemacytometer will fill via capillary action.

8.3.1 It is important to avoid overloading the chamber or introducing air bubbles into the chamber as this may produce inaccurate results. Do NOT move the cover slip once the sample has been loaded in the chamber.

8.4 The sample should be allowed to settle for approximately 30 seconds to allow time for the cells to stop drifting around the chamber putting most of the cells in the same plane of focus.

8.5 Place the hemacytometer on the microscope stage and focus on the cells.

8.6 METHOD FOR COUNTING CELLS

8.6.1 Use a tally counter to count the number of cells in the 4 outer corner squares (each corner square has 16 smaller squares), and average the results.

8.6.2 Take into consideration that some cells may be resting directly over the outside edges of the large corner squares.

8.6.3 By common convention count the cells that lie on the top and left outer borders of the large corner squares (this prevents cells from being counted twice, therefore, giving a more accurate cell count).

8.6.4 Cells outside the counting areas are NOT to be counted.

8.7 CELL VIABILITY

8.7.1 Staining of cells often facilitates visualization and counting. Some procedures require the use of a viability determinate dye. Dyes such as Trypan Blue or Erythrosin B are among the several stains recommended for use to distinguish viable cells from non-viable cells; only non-viable cells absorb the dye and appear blue or red, depending on the dye used. Conversely, live healthy cells appear round and shiny without absorbing the blue or red-dye. The use of these stains, however, is time sensitive; therefore, mix the cells with the dye prior to counting to avoid false data.

8.7.2 Mix cells with an equal volume of Trypan Blue at concentration of 0.4% (w/v in DH_2O) to determine live/dead count (dead cells are blue). By common convention count a total of 100 cells (live/dead).

8.8 CALCULATING CONCENTRATION PER MILLILITER

The calculation of cell concentration is based on the volume underneath the cover slip. One large square equals a volume of 10^4cells/ml.

8.8.1 The cell concentration is calculated as follows: # of cells per ml= [# of cells counted x dilution factor (if used) x 10,000].

8.8.2 To adjust to a desired concentration apply the following equation: V1 x C1 = V2 x C2

8.8.3 V1 is the unknown volume needed to be removed from the flask containing total number of cells/ml.

8.8.4 C1 is the total number of cells/ml.

8.8.5 V2 is the total volume needed.

8.8.6 C2 is the desired concentration.

8.9 DILUTION FACTOR

8.9.1 Sometimes it is necessary to dilute a cell suspension to get a cell density low enough for counting.

8.9.2 Based on the cell density of the suspension, e.g.: 1 part of cell suspension with 5 parts of diluent medium will make 1:5 dilution in which 5 is the dilution factor.

8.10 MAINTENANCE AND CLEANING

8.10.1 Handle the hemacytometer and the quartz cover slip with extreme care.

8.10.2 After each use, soak the hemacytometer and cover slip in a 10% Clorox solution for at least 30 minutes. Rinse with tap water followed by a rinse with 70% ethanol and then dry using compressed air or lens paper.

8.10.3 Keep the hemacytometer covered at all times when not in use. If the hemacytometer or cover slip breaks or the glass surface becomes scratched, discard the damaged items in a sharps container and replace as needed.

9 RELATED DOCUMENTS/FORMS

9.1 N/A

10 REFERENCES

10.1 BD Biosciences Discovery Labware: http://www.bdbiosciences.com

11 REVISION HISTORY

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