# **Title: Hemacytometer SOP**

## Approvals

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## 1. Purpose:

1.1. Operation of a hemacytometer for cell count determination.

## 2. Scope:

2.1. Instrument used for determining the concentration of cells in a suspension.

## 3. Responsibilities

- 3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as directed and to update the procedure when necessary.
- 3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.

## 4. References

- 4.1. Cascade Biologics, Inc, www.cascadebio.com
- 5. Definitions: N/A

## 6. Precautions:

- 6.1. Be sure not to overfill chamber when loading cells.
- 6.2. Clean immediately after use (according to instructions).
- 6.3. Save the coverslips after finish.

## 7. Materials :

- 7.1. Hemacytometer (with coverslips)
- 7.2. Trypan Blue
- 7.3. 100x Microscope
- 7.4. Samples
- 7.5. Pipette (P20 or P200)
- 7.6. Sterile pipette tips (small)
- 7.7. P.P.E. (gloves & eyewear)
- 7.8. Microcentrifuge Tube

## 8. Procedure

## 8.1. Prepare cells & Trypan blue

- 8.1.1. Approximately 20 microliters of cell suspension will be required to charge the chambers of the hemacytometer. Therefore preparing 30-50 microliters of trypan blue-diluted suspension is generally convenient and sufficient.
- 8.1.2. It is not necessary for the tube used for trypan blue dilution to be sterile. However, if non-sterile tubes are used, make sure that all pipettes and pipette tips that come in contact with the cell suspension are sterile and that these do not come in contact with the cell suspension once they have been exposed to a non-sterile environment.
- 8.1.3. In a conical microfuge tube, add 15 microliters of trypan blue solution.

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- 8.1.4. Gently flick (finger vortex) the cell suspension and remove 15 microliters of cell suspension using sterile technique.
- 8.1.5. Combine the 15 microliters of cell suspension with the 15 microliters of trypan blue in the microfuge tube.
- 8.1.6. Pipette up and down several times to ensure a uniform cell suspension using the same pipette tip.

Note: You might need to dilute the cells 1/10 <u>before</u> adding trypan blue if cells are too dense.

## 8.2. Load the hemacytometer

- 8.2.1. Place the coverslip over the counting chambers. Use only the coverslip provided by the manufacturer of the chamber.
- 8.2.2. Load both counting chamber with the diluted cell suspension using a micropipette and tip. Approximately 10 microliters will be required per side (chamber).
- 8.2.3. Place the pipette tip at the edge of the coverslip, and allow the cell suspension to fill the space by capillary action. Fill the entire volume of the chamber but *do not overfill*.

## 8.3. Determine cell count (total and viable)

- 8.3.1. View cells under a microscope at 100x total magnification. The cells should be visible above the grid of the counting chamber (see Figure 1).
- 8.3.2. Determine the number of cells (total and non-viable) overlying four x 1 mm<sup>2</sup> areas of the counting chamber (labeled A-D in Figure 1).
- 8.3.3. For an accurate determination, the total number of cells overlying one  $1 \text{ mm}^2$  should be between 15 and 50. If the number of cells per  $1 \text{ mm}^2$  exceeds 50, dilute the sample and count again. If less dilute samples are not available, count cells on both sides of hemacytometer (8x 1 mm<sup>2</sup> areas).

## 8.4. Calculate cell concentration

8.4.1. The total (or viable) number of cells can be converted to concentration by using the calculations below. If a dilution of cells was made before Trypan blue addition, that must be factored into the calculation.

Total (or viable) Cells counted in 4 mm <sup>2</sup>	Divided by 4 = cells per mm <sup>2</sup>	Multiply by dilution factor (= volume of trypan blue)	= cells/10 <sup>-4</sup> ml	X 10 <sup>4</sup> = cells/ml	X total volume of cell suspension = total (or viable) cells recovered
101 (92)	25 (23)	2	50 (46)	5.0 (4.6) x 10 <sup>5</sup> cells/ml	2.0 (1.84) x 10 <sup>6</sup> cells

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#### 8.5. Cleaning the hemacytometer

- 8.5.1. Clean hemacytometer immediately after use (as soon as possible). Use protective clothing gloves, and eyewear. Trypan Blue is a mutagen (see manufacturer's MSDS).
- 8.5.2. Clean instrument with 10% dilute bleach solution followed by 70% isopropanol followed by deionized water. Air Dry
- 8.5.3. Dispose of trypan blue-contaminated articles in biohazard waste.
- 8.5.4. SAVE THE COVERSLIPS. They are specially made for the hemacytometer.

## 9. Attachments:

- 9.1. Figure 1: Microscope view of slide/ How to count cells
- 9.2. Figure 2: Loading chamber/coverslip photo
- 9.3. Figure 3: Loading chamber/coverslip photo 2

Cascade Biologics™ man Cells Optimized Media Expert Techn Figure 1. A hemacytometer is a graduated counting chamber that can be viewed under a microscope to determine the concentration of cells in a suspension. Developed originally for counting blood cells, these chambers are widely used in cell culture to determine the concentration of cells in a suspension. There are a number of manufacturers of these types of chambers and some have slight variations in style. The description here is for a common "Neubauer" type chamber. The instrument is made of ground glass with a central area that is defined by a set of grooves that form an 'H' shape. Two counting areas with nucle grids are separated by the horizontal groove of the H. The one of the ruled grids is shown and described here. The glass coverslip is held at 0.1 mm above the surface of the counting areas by ground glass ridges on either side of the vertical grooves of the H shape. In the example shown, the cells were resuspended in a total of 4 ml. The cell suspension was then diluted /with trypan blue and the chamber of the hemacytometer was loaded. hemacytometer was loaded. — 1.0 mm — ->|+ \_\_\_\_\_ 1.0 mm 1.0 mm ≱⊲ 0 lo 0 0 0 С 0 0 Q 0 0 o 0 0 C 1.0 mm 0 0 O 0 0 0 C 0 0 0 0 0 0 Count all of the cells contained within the four 1 mm<sup>2</sup> areas labeled A, B, C and D. Remember that the middle line of the triplet is the boundary of the area. Count all cells on two sides that touch the boundary of the area, but not on the other two sides (do not count cells marked with "/"). Non-viable cells appear as blue, viable cells are clear 1.0 mm in a bluish background (under the microscope) ob 0 00 0 0 0 0 0 0 0 0 0 C 1.0 mm 00 0\_0 ø 0 0 0 0 0 С \_o 🛨 C Each side (counting area) of a hemacytometer is etched with a grid of 9 square millimeters as shown above. The grid is bounded and subdivided by groups of three lines, the middle line of which is the actual boundary. The grid surface is 0.1 mm below the coversilp. Therefore the volume of fluid over one of the 1 mm areas (area "C" for example) of the grid is 0.1 cubic mm or 0.0001 ml.

Figure 1: Hemacytometer

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Figure 2: Loading chamber/coverslip photo



Figure 3: Loading chamber/coverslip photo 2