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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 before 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^2 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 mm^2 should be between 15 and 50. If the number of cells per 1 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^2 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^2	Divided by 4 = cells per mm^2	Multiply by dilution factor (= volume of trypan blue)	= cells/10^{-4} ml	X 10^4 = 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 10^5 cells/ml	2.0 (1.84) x 10^6 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

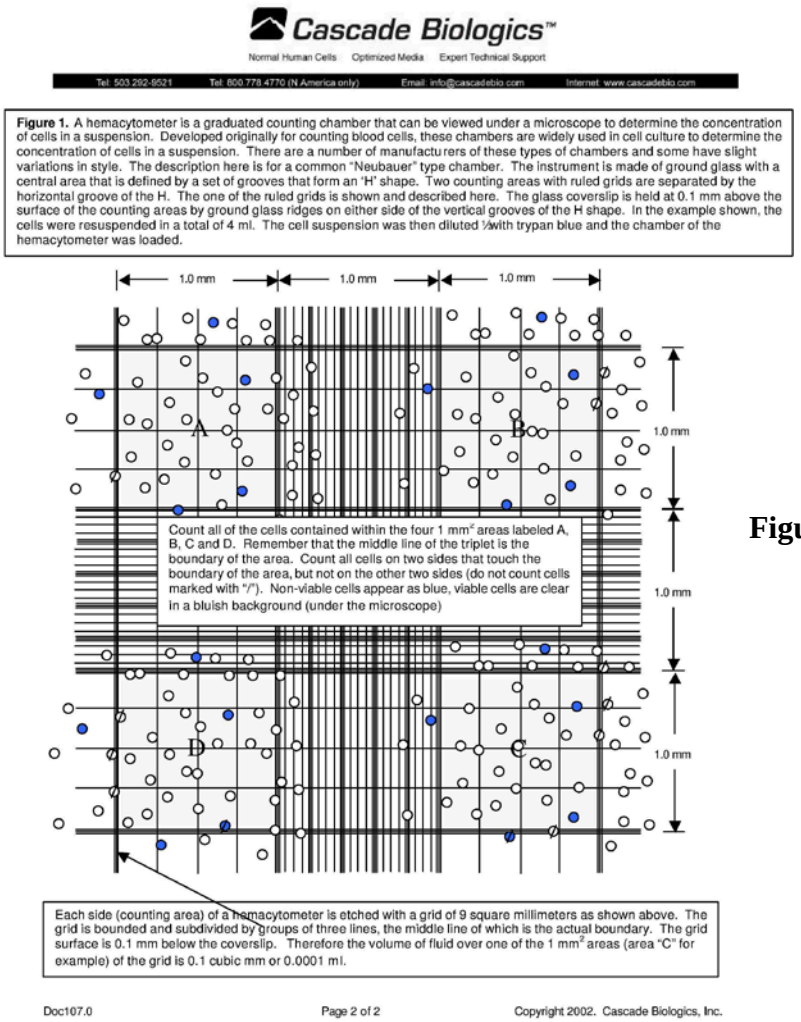

Figure 1: Hemacytometer

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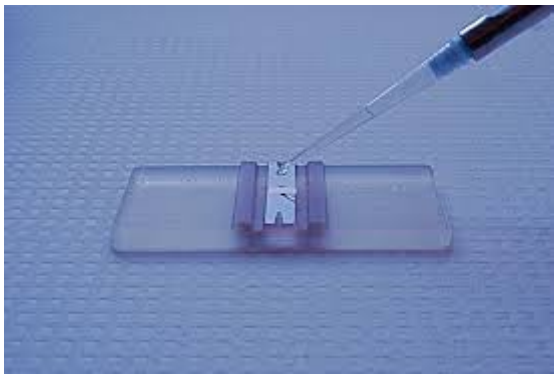

Figure 2: Loading chamber/coverslip photo

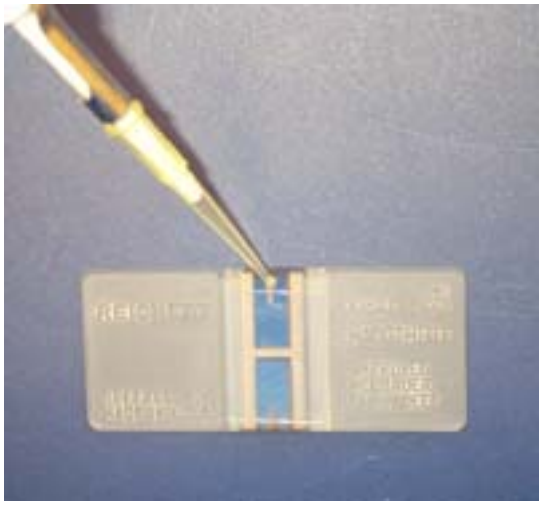

Figure 3: Loading chamber/coverslip photo 2