# SOP: Trypan Blue Assay for Cell Viability Determination

**Approvals:**

Preparer: Jason McMillan — Date: 02JAN14
Reviewer: Hetal Doshi — Date: 20DEC18
Reviewer: Dr. Maggie Bryans — Date: 20DEC18

1. **Purpose:**
   1.1. Use of the Trypan Blue Assay.
2. **Scope:**
   2.1. Applies to determining viable cell count of mammalian and insect cells.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described 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. http://plaza.ufl.edu/johnaris/Protocols/MiscMethods/HemoCytometer.pdf
   4.2. microscope SOP
5. **Definitions:**
   5.1. Hemacytometer: a specialized microscope slide with etched glass in grid formation
6. **Precautions:**
   6.1. Trypan Blue Solution is a teratogen. It may cause birth defects. It may cause cancer. Wear gloves, eye protection and a lab coat.
7. **Materials:**
   7.1. 0.4% Trypan Blue Solution
   7.2. microfuge tubes
   7.3. P-20 micropipette and tips
   7.4. cell sample in solution
   7.5. hemacytometer
   7.6. hemacytometer coverslip
   7.7. microscope
   7.8. lab towels
   7.9. lab tissues such as Kimwipes
   7.10. deionized water
   7.11. push button counter
8. **Procedure:**
   8.1. **Mix Trypan Blue Solution with cell sample solution**
      8.1.1. Mix culture sample well to resuspend cells.
      8.1.2. Remove 50µL of culture sample and dispense into a microfuge tube.
      8.1.3. Add 50µLof 0.4% Trypan Blue Solution to the same tube.
      8.1.4. Mix the above solution by gently aspirating and dispensing the solution with the micropipette. Proceed to the next step immediately.

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8.2. **Transfer sample to hemacytometer**

8.2.1. Center the coverslip on top of the hemacytometer. The metal notches on a hemacyotometer should be partially exposed.

8.2.2. Hold the micropipette straight up and dispense 15µL of the cell/Trypan Blue solution into a metal notch of the hemacytometer. The tip of the pipette should be very close to the metal surface. The solution will spread through capillary action.

8.2.3. Repeat step8.2.2 for the second grid.

8.3. **Observe cells under the microscope**

8.3.1. Turn on the microscope per SOP.

8.3.2. Place the hemacytometer on the microscope stage.

8.3.3. Focus on the hemacytometer grid using 100X magnification (10X objective lens). Live cells are clear. Dead cells are blue.

8.4. **Count cells**

8.4.1. The grid is divided into four main quadrants (Figure 2). Beginning with quadrant 1 and moving through to quadrant 4, depress the correct button on the push button counter for every cell in each square.

8.4.1.1. Make sure the counter is set to 0. Count total live cells first. After live cell count is completed, count total dead cells.

8.4.1.2. Count in a serpentine fashion: work left to right across the top row of the quadrant. Move down to the second row and count the cells in each square moving right to left. Change to opposite direction each time a row is completed.

8.4.1.3. Count cells touching the top and left borders of a main quadrant, but not the bottom and right borders. **Do not count cells outside of the main quadrants**.

8.4.1.4. Record the number of live and dead cells each time a quadrant is completed.

8.4.2. Repeat counting procedure per section 8.4.1. for the second grid.

8.4.3. Average the cell counts of the two grids to obtain the live cell count for the viable cell concentration calculations.

8.5. **Clean the hemacytometer**

8.5.1. Remove the coverslip.

8.5.2. Blot dry the coverslip and hemacytometer on a lab towel.

8.5.3. Rinse the coverslip and hemacytometer with 70% EtOH by holding each one over a lab towel and using a squirt bottle containing 70% EtOH. Repeat rinsing with milliQ $H_2O$ and use squirt bottle labelled milliQ $H_2O$. **Note:** Handle the hemacytometer and coverslip gently. The coverslip is not disposable. Do not discard it.

8.5.4. Dry the coverslip and hemacytometer with a lab tissue.

8.6. **Calculate viable cell concentration.**

8.6.1. Formula to determine live cell count: **C = (N/V) x D**

**C** = live cell count in cells per milliliter

**N** = total number of live cells obtained from averaging the number of live cells

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counted in the two grids
**V** = volume of counting area
**Note:** The total volume of the four quadrants is 0.0004mL. (Each quadrant is 0.0001mL.)
**D** = dilution factor. For this procedure the dilution factor is 2.

8.7. Calculate percent viability
8.7.1. Formula for percent viability: **% viability = (live cell count/total cell count) *100**

**9. Attachments:**
9.1. Figure 1: Diagram of hemacytometer and cover glass
9.2. Figure 2: Diagram of hemacytometer quadrants

**10. History:**

| Name | Date | Amendment |
|---|---|---|
| Jason McMillan | 02JAN14 | Initial Release |
| Hetal Doshi | 20DEC18 | Change the sample and trypan blue volume to 50µl and sample loading volume to 15µl |

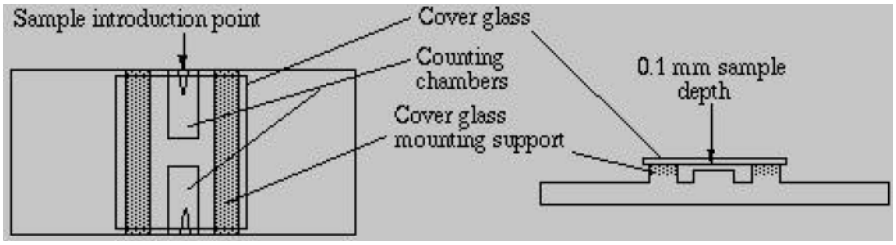


**Figure 1: Diagram of hemacytometer and cover glass**
Image: http://plaza.ufl.edu/johnaris/Protocols/MiscMethods/HemoCytometer.pdf

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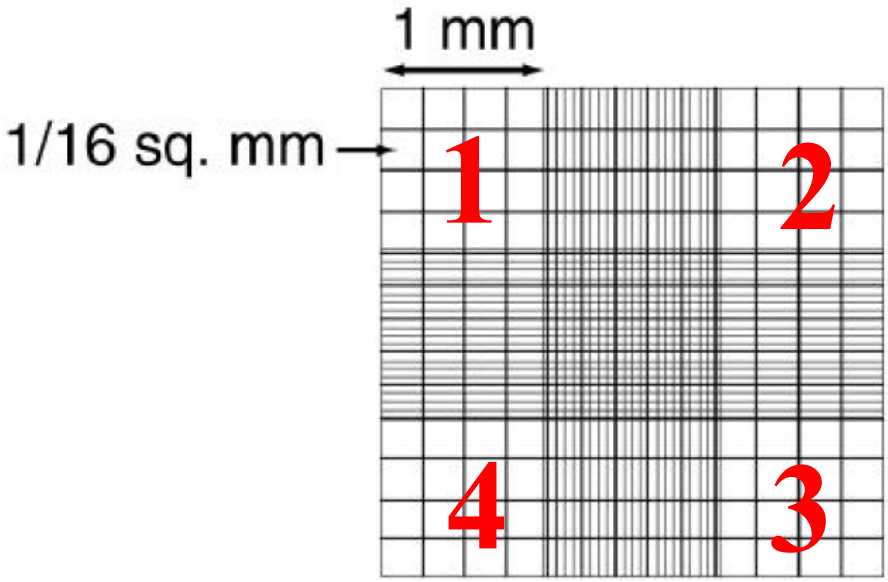


**Figure 2: Diagram of hemacytometer quadrants**