Title: Trypan Blue Assay SOP

Approvals:
Preparer:______Kari Britt ___________________________ Date ______05Aug10_______
Reviewer:_____Sonia Wallman_______________________ Date ______05Aug10_______

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 20µL of culture sample and dispense into a microfuge tube.
      8.1.3. Add 20µLof 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.
   8.2. **Transfer sample to hemacytometer**
      8.2.1. Center the coverslip on top of the hemacytometer. The metal notches should be partially exposed.

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- 8.2.2. Hold the micropipette straight up and dispense 10µL of the cell/Trypan Blue solution into a notch of the hemacytometer. The tip of the pipette should be very close to the metal surface. The solution will spread through capillary action.

8.3. **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. Left button is for live cells. Right button is for dead cells. Make sure the counter is set to 0.
  - 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.

8.5. **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 cover slip and hemacytometer with deionized (DI) water by holding each one over a lab towel and using a squirt bottle of DI water.
  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 counted in the four main quadrants
  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

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10. History:

Name	Date	Amendment
Kari Britt	26Jul05	Initial release
Deb Audino	04Apr08	College name change, format of history
Kari Britt	05Aug10	Proofreading, formatting and grammar edits throughout

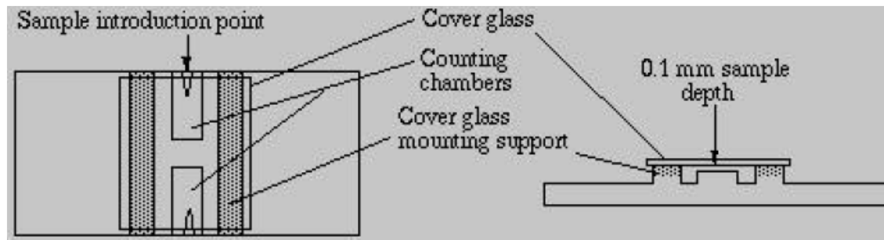

Figure 1: Diagram of hemacytometer and cover glass
Image: http://plaza.ufl.edu/johnaris/Protocols/MiscMethods/HemoCytometer.pdf

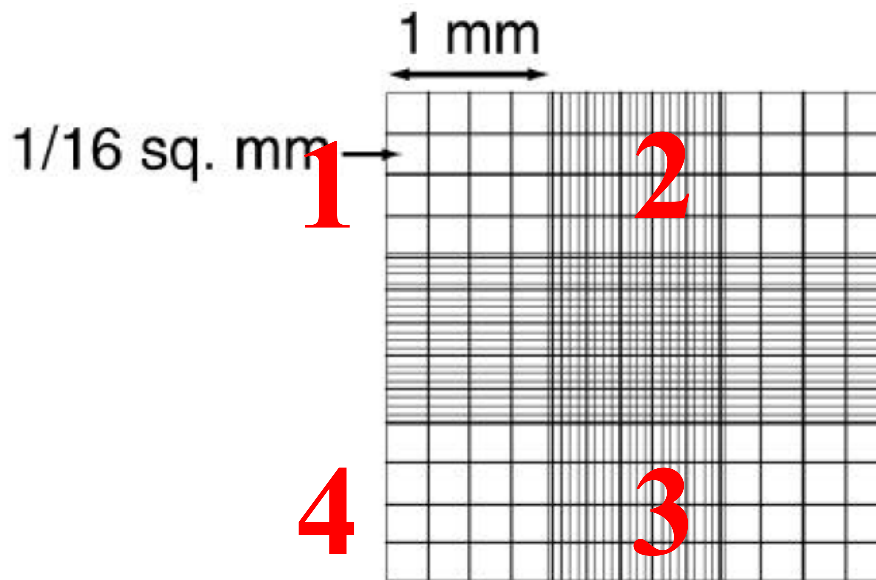

Figure 2: Diagram of hemacytometer quadrants