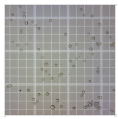

Aug 12, 2019

Manual Cell Counting

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1 Works for me dx.doi.org/10.17504/protocols.io.5zng75e

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ABSTRACT

Manual counting of cells using a Hemocytometer.

GUIDELINES

Follow biosafety level 2 guidelines handling human tissue.

MATERIALS

NAME	CATALOG #	VENDOR
P20 micropipet and filter tips		
Parafilm, 4X125'	PF002.SIZE.1	Bio Basic Inc.
Neubauer Improved (NI) Hemocytometer	22-600- 100	Life Technologies
Standard square cover slips 18×18 mm		
Trypan Blue Solution 0.4% Sterile-filtered	T8154	Sigma Aldrich

MATERIALS TEXT

Reagents

Trypan Blue

Materials

p20 Micropipette
p20 micropipette tips
Hemocytometer
Hemocytometer coverslips
Parafilm

Equipment

Microscope

SAFETY WARNINGS

Abide by biosafety level 2 standard safety procedures as human materials are used.

BEFORE STARTING

Turn on biosafety cabinet 10-15 min before start and clean all working surfaces with 70% ethanol. Make sure all materials in the cabinet are sterile and utilize standard aseptic technique.

In order to count cells, cells must be single cells suspended in solution. This can be achieved by various methods including the use of Trypsin and Accutase.

Preparation for Counting Cells

1 Remove **10 µl** of the solution containing the single cells using a p20 micropipette and add to a 1.5 ml microcentrifuge tube or onto a piece of parafilm.

2 Centrifuge the Trypan Blue and remove **10 µl** from the top of the Trypan Blue as not to get any debris using a p20 micropipette.

3 Add the **10 µl** of Trypan Blue to the 1.5 ml microcentrifuge tube or the parafilm conataining the cells and gently mix by pipetting up and down.

4 Wipe the hemocytometer and a matching coverslip with 70% ethanol and place in the biosafety cabinet.

5 Place the coverslip onto the center of the hemocytometer.

Figure Legend: Hemocytometer with coverslip.

6 Remove **10 µl** of solution from the 1.5 ml microcentrifuge tube or the parafilm using a p20 micropipette.

7 Place the tip of the pipette at the bottom edge of the coverslip and slowly pipette the solution into the hemocytometer. The bottom chamber should be filled.

8 Remove another **10 µl** of solution and add to the top chamber by placing the tip of the pipette at the top edge of the coverslip and slowly pipetting the solution into the top chamber.

9 Place the filled hemocytometer on a microscope and focus the microscope such that the cells and the grid lines in each chamber are clearly visible.

Counting the Cells

10 Pick one of the 9 boxes in the top grid (the grid in the top chamber) to begin counting. Note: Cells stained blue are dead cells and should only be counted to determine the viability, not the cell count.

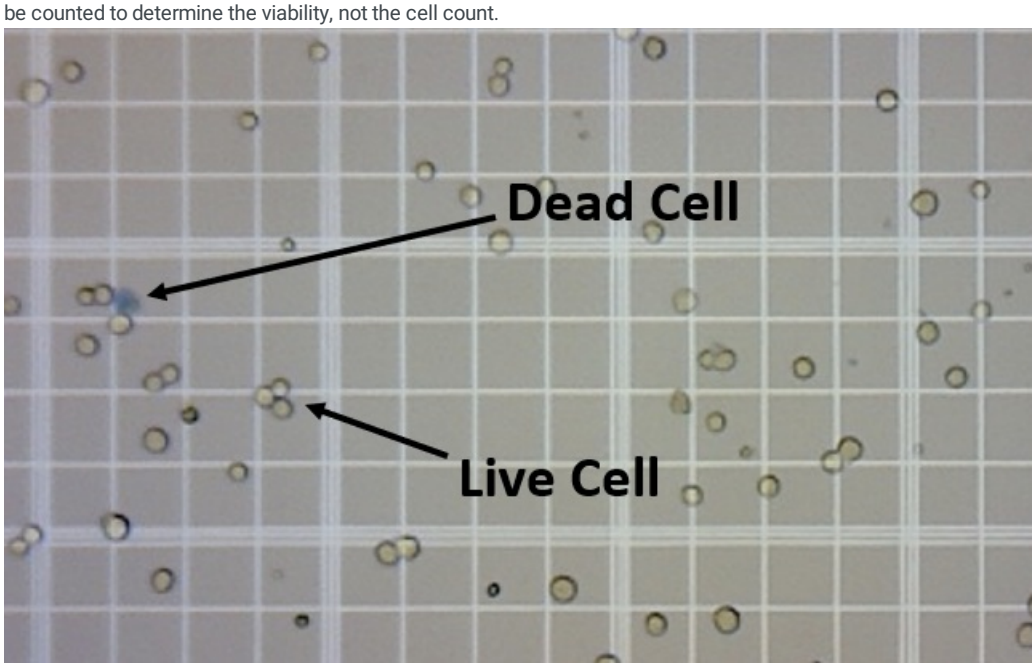

Figure Legend: Live vs Dead cells.

11 Any cells that are on the bottom and right edge lines should be counted while any cells overlapping the top and left edge should not be counted.

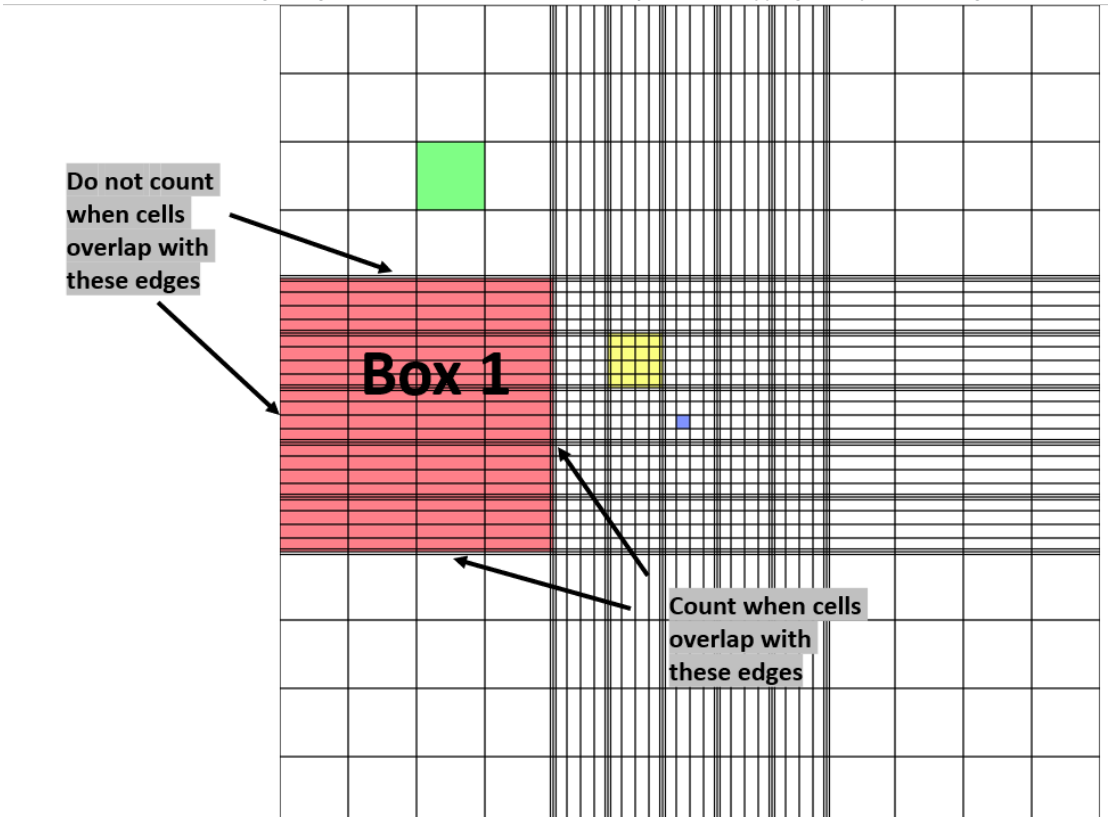

Figure Legend: Cell counting grid.

12 Using a tally counter, keep track of the number of cells counted in one box.

13 Note the total cells in one box and reset the counter.

14 Repeat steps 10 - 13 four more times, choosing a different box in the same grid.

15 Move on to the bottome grid (the grid in the bottom chamber) and repeat the process of counting by counting the cells in 5 boxes and making a note of the counts.

Calculating the Number of Cells

16 Find the average number of cells per box by adding up the total number of cells and dividing by the number of boxes that where counted. Note: In this protocol, 10 boxes where counted total, 5 in the top grid and 5 in the bottom grid, so the total number of cells should be divided by 10.

17 Multiply the final number from step 16 by 2 and then by 10,000. The result is the total number cells in the original solution (before the addition of Trypan Blue) per mL.

18 Total number of cells = (number of cells per mL) * (mL of original solution containing the cells)

19 Cell viability = 100 * (Live cells)/(Live cells +Dead cells)