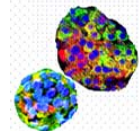


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Title:	Cell counting and Basics				
Protocol #:	1.0	Submitted:	050510	Approved:	200610
Category:	Cyto	Author(s): ¹	MRU, SNS	Checked by:	AAH

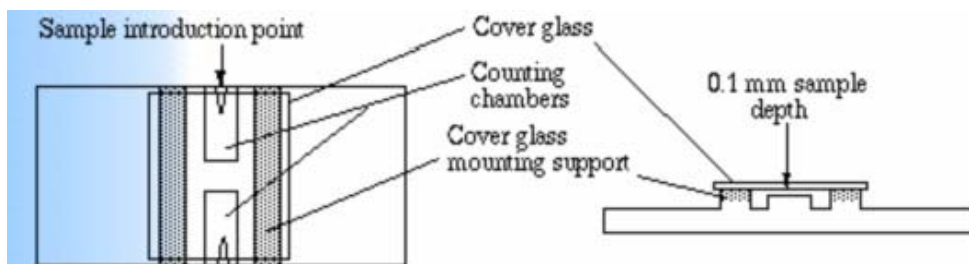
Reagents:

1. DMEM with 2 mM L-glutamine (GIBCO, cat. no. 12800-116)
2. FBS (GIBCO, cat. no. 10270-106)
3. 0.25% trypsin (GIBCO, cat. no. 27250-018)
4. Streptomycin/ Penicillin
5. L-glutamine (GIBCO, cat. no. 35050-038)
6. FBS (GIBCO, cat. no. 10270-106)
7. Trypan blue (Invitrogen)
8. Tissue culture labware (BD)

Equipment

1. Hood for cell culture with vertical laminar flow and equipped with UV light for decontamination (PC2 certified)
2. Incubator with both temperature and gas composition controls
3. Inverted microscope with phase-contrast
4. Haemocytometer
5. Haemocytometer slide

Reagent Setup



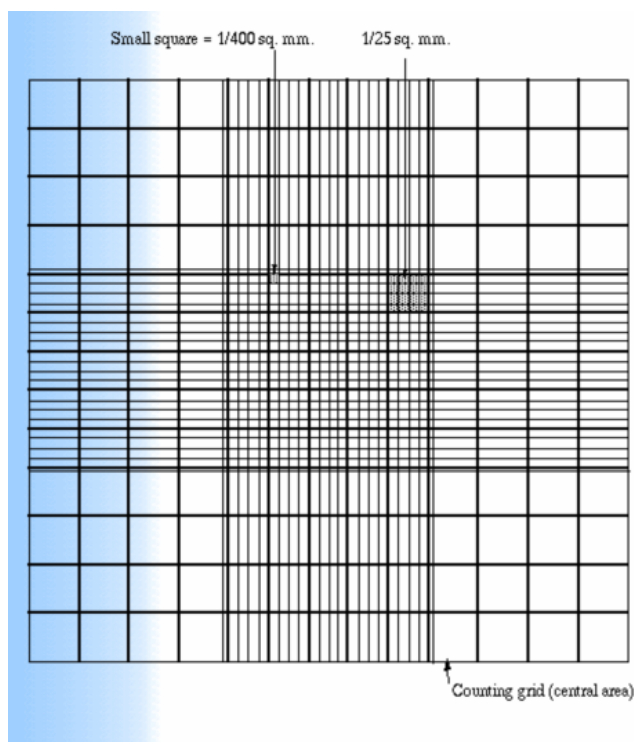
Procedure

1. To prepare the counting chamber the mirror-like polished surface is carefully cleaned. The coverslip is also cleaned. Coverslips for counting chambers are specially made and are thicker than those for conventional microscopy, since they must be heavy enough to overcome the surface tension of a drop of liquid. Do not use regular coverslips (if you break one).
2. Wet the edges of the hemocytometer with a drop of water and then place and slide the coverslip over this till it is nicely fixed on to the counting hemocytometer.

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3. The cell suspension is introduced into each of the V-shaped wells with a pipet. The area under the coverslip fills by capillary action. Enough liquid (usually $\sim 10\mu\text{l}$) should be introduced so that the mirrored surface is just covered. The counting chamber is then placed on the microscope stage and the counting grid is brought into focus at low power.

Note: It is essential to be extremely careful with higher power objectives, since the counting chamber is much thicker than a conventional slide. The chamber or an objective lens may be damaged if the user is not careful. One entire grid on standard hemacytometers with Neubauer rulings can be seen at 40x objective. However, you can use a 20X objective as well for routine measurements. The main divisions separate the grid into 9 large squares (like a tic-tac-toe grid). Each square has a surface area of one square mm, and the depth of the chamber is 0.1 mm. Thus the entire counting grid lies under a volume of 0.9 mm-cubed.



4. Cell suspensions should be dilute enough so that the cells do not overlap each other on the grid, and should be uniformly distributed. To perform the count, determine the magnification needed to recognize the desired cell type. Now systematically count the cells in selected squares so that the total count is 100 cells or greater (number of cells needed for a statistically significant count).

Note: For large cells this may mean counting the four large corner squares and the middle one. For a dense suspension of small cells you may wish to count the cells in the four $1/25$ sq. mm corners plus the middle square in the

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central square. Always decide on a specific counting pattern to avoid bias. For cells that overlap a ruling, count a cell as "in" if it overlaps the top or right ruling, and "out" if it overlaps the bottom or left ruling.

5. Here is how to determine a cell count using a standard hemocytometer. To get the final count in cells/ml,

- **Cells / ml** = total number of cells counted in each quadrant, divided by the number of quadrants counted, times the dilution factor of cells in trypan blue. Multiplied by the constant number 1×10^4 .
- **Total number of cells** = cells/ ml multiplied by the volume of cells the cell pellet was resuspended in.

For example: The cell pellet was resuspend in 5 ml media. 20ul of the cell pellet was added to 180 ul of Trypan blue.

117 cells were counted in the top grid on the haemocytometer and 139 cells in the bottom grid.

- **Cells / ml** = $(117+139) / 8 \times 10 \times 1 \times 10^4 = 640,000 = 6.4 \times 10^5$
- **Total number of cells** = $32 \times 10^5 \times 5 \text{ ml} = 1.6 \times 10^7$ total cells