Use of a Hemacytometer

P.J. Hansen[1]

[1]*Dept of Animal Sciences, University of Florida*

A hemacytometer (also spelled hemocytometer) is an etched glass chamber with raised sides that will hold a quartz coverslip exactly 0.1 mm above the chamber floor. The counting chamber is etched in a total surface area of 9 mm^2 (see Figure 1).

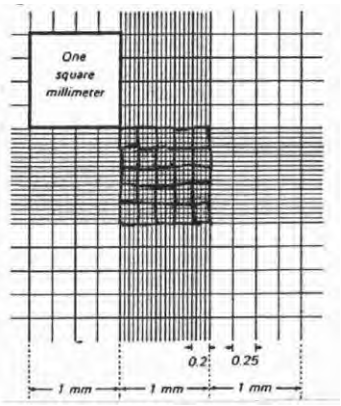

Figure 1. Dimensions of a hemacytometer.

Calculation of concentration is based on the volume underneath the cover slip. One large square (see W in Figure 2) has a volume of 0.0001 ml (length x width x height; i.e., 0.1 cm x 0.1 cm x 0.01 cm).

In both methods, the hemacytometer is filled by capillary action - place the pipette filled with a well-suspended mix of cells at the notch at the edge of the hemacytometer and then slowly expel some contents so that the fluidis drawn into the chamber by capillary action.

Staining of cells often facilitates visualization and counting. Either mix cells with an equal volume of trypan blue [0.4% (w/v) tyrypan blue in PBS] to determine live/dead count (dead cells are blue) or kill cells with 10% formalin and then stain with trypan blue or other stain (to improve visualization of all cells.

Here are two simple methods for counting cells based on the surface area of the hemacytometer used to determine cell count. Other counting schemes are accetable also. The choice of methods depends upon the cell concentration - the accuracy of the procedure depends upon the number of cells counted. When cell concentration is low, one should count more grids.

Method A

Count the number of cells in the 4 outer squares (see the left panel of Figure 2).

The cell concentration is calculated as follows:

Cell concentration per milliliter = Total cell count in 4 squares x 2500 x dilution factor

Example: If one counted 450 cells after diluting an aliquot of the cell suspension 1:10, the original cell concentration = 450 x 2500 x 10 = 11,250,000/ml

Method B

Estimate cell concentration by counting 5 squares in the large middle square (see the right panel in Figure 2).

The cell concentration is calculated as follows:

Cell concentration per milliliter = Total cell count in 5 squares x 50,000 x dilution factor

Example: If one counted 45 cells after diluting an aliquot of the cell suspension 1:10, the original cell concentration = 45 x 50,000 x 10 = 22,500,000/ml

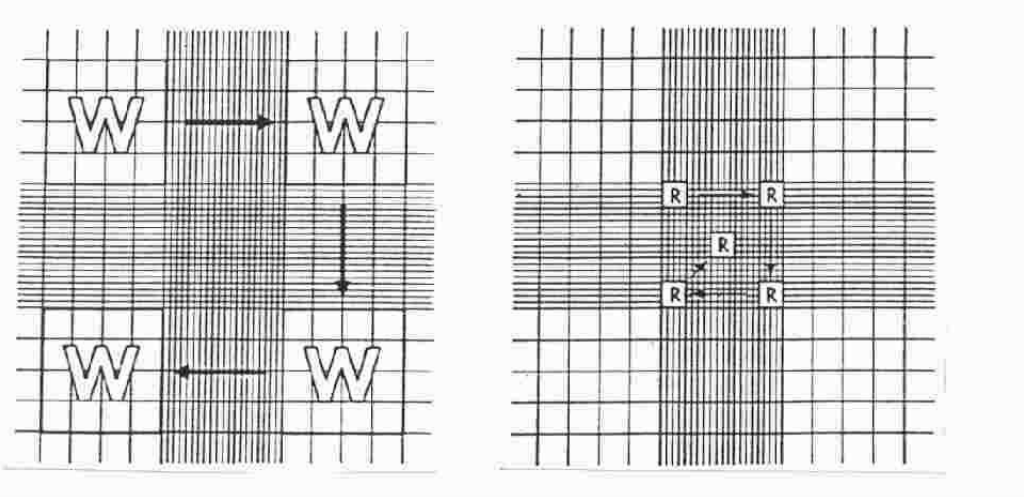

Figure 2. Counting procedure for Methods A (left panel) and B (right panel).

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