

Counting Cells

Measuring the functionality of a medium, determining population doubling times, or tracking the growth of cells in a culture, all require a means of quantifying cell populations. The most common method of determining cell number involves a hemocytometer, an instrument for estimating the number of cells in a measured volume under the microscope. Combined with a vital stain, such as Trypan Blue, the viability of the measured volume can also be determined.

The Improved Neubauer Hemocytometer is a thick glass slide with two counting chambers, each 0.1 mm deep. Each chamber is divided into nine large squares delineated by triple black lines. The center square is further subdivided into 25 squares.

These 25 squares are again subdivided into 16 squares. This etched surface covers a total surface area of 9 mm². Hemocytometer cover slips must be used to ensure even surfaces and coverage of both counting chambers.

Trypan Blue is the most common stain used to distinguish viable cells from non-viable cells; only non-viable cells absorb the dye and appear blue and may also appear asymmetrical. Conversely, live, healthy cells appear round and refractile without absorbing the blue-colored dye. The use of this stain, however, is time-sensitive. Viable cells absorb Trypan Blue over time, and can affect counting and viability results. Make dilutions just prior to counting to avoid false data.

Procedure

1. Prepare a uniform cell suspension of the culture to be counted.
2. Dilute a small sample of the cell suspension 1:5 in 0.4% (w/v) Trypan Blue (25-900-CI). Other dilutions may be performed based on cell density of the suspension.
3. Center a cover glass over the hemocytometer chambers. Fill one chamber with the cell dilution using a Pasteur pipette. The solution will pass under the cover glass by capillary action. Do not overfill. If the solution spreads into the two lateral grooves adjoining the grid table, clean the hemocytometer and repeat the application. If there are any bubbles in the solution covering the grid table, clean the hemocytometer and repeat the application.
4. Place the hemocytometer on the stage of an inverted microscope and adjust focus using 100X magnification.
5. Use a hand-held counter to record cell counts in each of the four corner and central squares. Five squares (four corner and one center) are counted for a total of five squares. See figure 1.
6. Determine the number of cells per milliliter and total number of cells using the following calculations:
7. The percentage of viable cells can also be calculated using the following formula:

$$\text{cells/mL} = \frac{\text{\# of cells counted} \times 10^4 \times \text{dilution factor}}{\text{\# squares counted}}$$

$$\text{total cells} = \text{cells/mL} \times \text{vol. of original cell suspension}$$

$$\% \text{ viability} = \frac{\text{\# viable cells counted}}{\text{total \# cells counted}} \times 100$$

Important: Count squares touching the middle line of the triple line on the top and left of the squares. Do not count cells touching the middle line of the triple lines on the bottom or right side of the square.

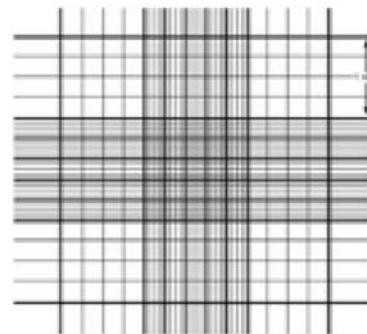


Figure 1.
Improved Neubauer Hemocytometer Counting Grid