7. Place dissociated cells into a centrifuge tube and allow clumps to settle for 5–6 minutes. Transfer cells into a centrifuge tube and pellet the cells at 200 × *g* for 10 minutes. Wash the cells several times to remove all fat.

1.10 CELL COUNTS WITH A HEMACYTOMETER

The hemacytometer chamber (Figure 1.2) is used to count both white and red blood cells. The large square areas indicated by the numbers 1, 2, 3, and 4 in the figure are used for counting white blood cells. The area indicated by the number 5 is used for counting red blood cells. The volume contained by each of these separate areas under a coverslip is 10^{-4} ml (in hemacytometers that are 0.1 mm deep).

Errors can be introduced in a number of ways: dilution errors, loss of cells during pipetting, uneven suspension of cells, overfilling or underfilling of the chamber, and the counting of cells before they settle. Random distribution of cells in the chamber is another source of error but can be compensated for by counting a large number of cells.

A. White Blood Cell Counts

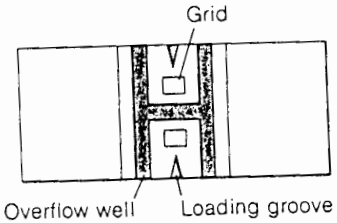
When determining the total white blood cell count, it is advantageous to first remove red blood cells by lysis in dilute acid, to prevent the erroneous counting of red blood cells as small lymphocytes. The addition of gentian violet to the acid solution facilitates counting since it stains the white blood cells. Cell populations that are grown in continuous culture, and thus do not contain red cells, are easily counted in any isotonic diluent.

MATERIALS AND REAGENTS

Cell suspension
Hemacytometer and coverslip (Scientific Products, #B3180-2)
Microscope
Pasteur pipettes and rubber bulb
Hand counters
Counting solution: 3% acetic acid in water (v/v) or 0.01% gentian violet in 3% acetic acid (Turk's solution)

PROCEDURE

1. Make appropriate dilutions of the cell suspension just prior to counting. The optimal concentration of cells for counting is 5–10 × 10^5 cells/ml (50–100 cells per large square) after dilution in the counting solution.
2. Using a Pasteur pipette with finger control, let the cell suspension flow under the coverslip until the grid area is *just* full and not overflowing into the overflow well. If the chamber is loaded too



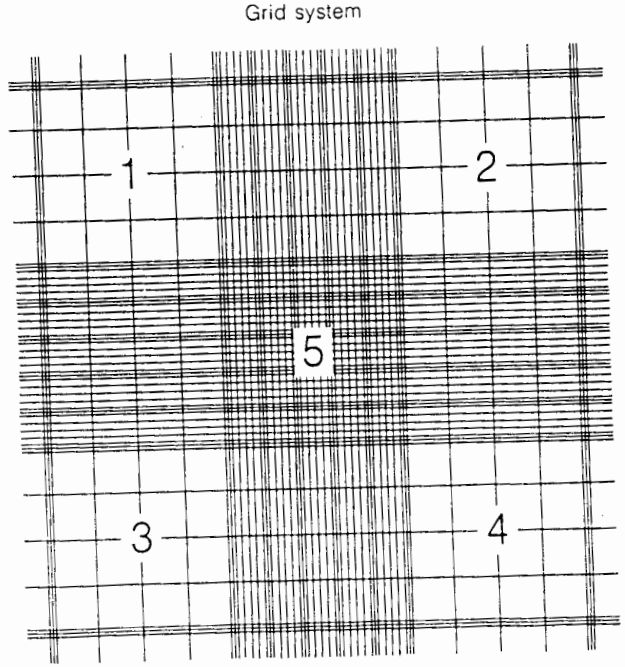


FIGURE 1.2
Hemacytometer.

heavily, clean it and begin again; do not attempt to remove excess liquid. Allow cells to settle.

3. Count all of the cells contained in each of the 4 large squares (1–4 in Figure 1.2). Some cells will be touching the outside borders. Count only those cells touching two of the outside borders (for example, the upper and left). A minimum of 200 cells should be counted. Determine the average number of cells per large square. This is the number of cells per 10^{-4} ml. Thus:

$$\text{cells/ml} = (\text{average number per large square}) \times 10^4/\text{ml} \times \frac{1}{\text{dilution}}.$$

B. Red Blood Cell Counts

MATERIALS AND REAGENTS

Similar to those for white blood cell counts except that the counting solution is a physiological medium such as BSS (Appendix A.3) or PBS (Appendix A.8).

PROCEDURE

1. Make appropriate dilutions of the red blood cells. For example, a 1 : 200 dilution of whole blood is adequate.
2. Count the number of red blood cells, using the large center square (square 5 in Figure 1.2). This large square is divided into 25 smaller squares. Count the red blood cells in 5 of these smaller squares (e.g., the four corners and the center square):

$$\text{cells/ml} = (\text{number in 5 squares}) \times 5 \times 10^4/\text{ml} \times \frac{1}{\text{dilution}}.$$

1.11 DETERMINATION OF VIABILITY BY TRYPAN BLUE EXCLUSION

The number or percentage of viable white blood cells can be determined by staining cell populations with trypan blue. Viable cells exclude the dye, while nonviable cells take up the dye, thereby fostering a visual distinction between unstained viable cells and blue-stained nonviable cells. After being stained with trypan blue, the cells must be counted within 3 minutes; after that time viable cells begin to take up the dye. Also, since trypan

blue has a great affinity for proteins (Kruse et al. 1973), elimination of serum from the cell diluent will allow a more accurate determination of cell viability.

MATERIALS AND REAGENTS

Cell suspension at 2–5 × 10^6 cells/ml
Trypan blue, 0.2% (w/v) in water
5× saline: 4.25% NaCl (w/v)

PROCEDURE

1. On the day of use, mix 4 parts of 0.2% trypan blue with 1 part of 5× saline.
2. To 1 part of the trypan blue saline solution, add 1 part of the cell suspension (1 : 2 dilution).
3. Load cells into a hemacytometer (Section 1.10) and count the number of unstained (viable) white blood cells and stained (dead) cells separately. For greater accuracy, count more than a combined total of 200 cells:

$$\text{viable cells/ml} = (\text{average number of viable cells in large square}) \times 10^4/\text{ml} \times \frac{1}{\text{dilution}};$$

$$\%\ \text{viable cells} = \frac{\text{number of viable cells}}{\text{number of viable cells} + \text{number of dead cells}} \times 100\%.$$

REFERENCE

Kruse, P. F., Jr., and M. K. Patterson, Jr., Eds. 1973. *Tissue Culture: Methods and Applications*. Academic Press, New York.

1.12 DETERMINATION OF VIABILITY BY EOSIN Y EXCLUSION

The advantage of using eosin Y as a vital stain is that the time elapsed before examining the cells is less critical than for trypan blue exclusion; The percentage of viable cells remains constant from 1–10 minutes after staining with eosin Y. However, some find red-(eosin Y)-stained cells more difficult to recognize than blue-(trypan blue)-stained cells.