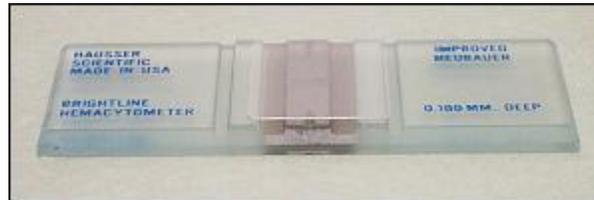


Counting Cells with a Hemacytometer

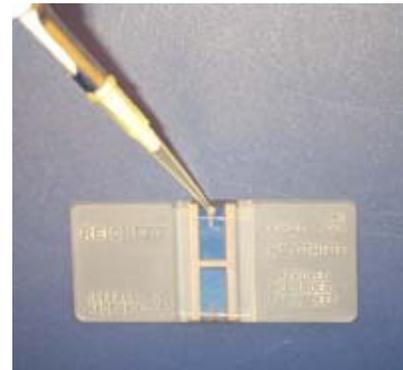
Hemocytometers were developed for counting blood cells, but can also be used to count spermatozoa. A hemocytometer has two chambers and each chamber has a microscopic grid etched on the glass surface. The chambers are overlaid with a glass coverslip that rests on pillars exactly 0.1 mm above the chamber floor. Thus, the volume of fluid above each square of the grid is known with precision.



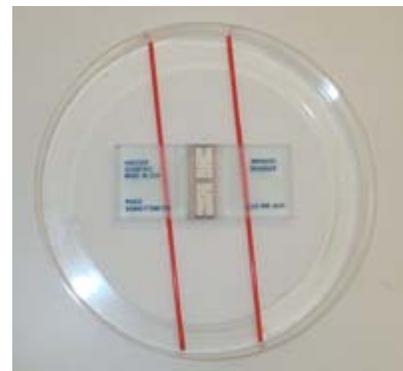
Procedure

The semen must be killed to prevent movement and diluted before loading into the hemocytometer. This can be done by diluting the semen into a buffer containing a small quantity of formaldehyde. The dilution factor must be recorded to allow calculating the concentration. When there are 20-25 cells per large square (see below) the sample is at the proper dilution.

Loading the Hemacytometer: The tip of the pipette is placed in the V-shaped groove on the hemocytometer to load the sample into the chamber (about 15 microliters.) Capillary action will draw the fluid into the chamber. It is important not to overload the chamber, as doing so will give an inaccurate count. The same is true if the cover slip is moved after the sample is loaded.

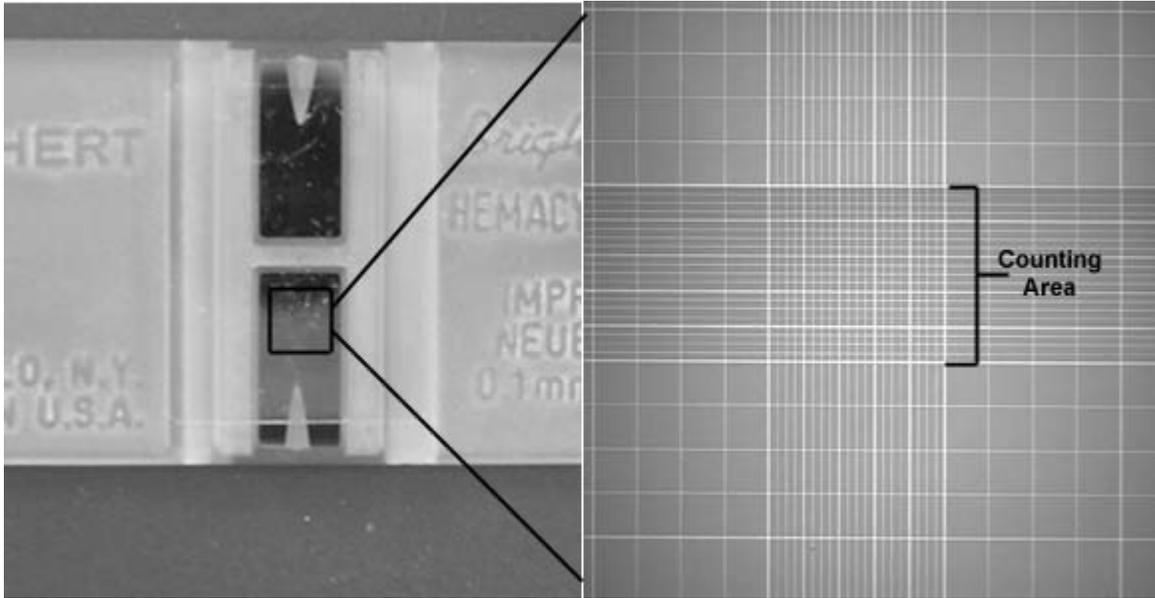


The sample is allowed to settle for 2 or 3 minutes so that the cells stop drifting around the chamber and most will be in the same plane of focus. It is important not to allow the sample to settle too long or it will dry out, concentrating the cells over the grid. To avoid drying, the hemocytometer can be placed on straws within a petri dish containing a moistened filter paper.

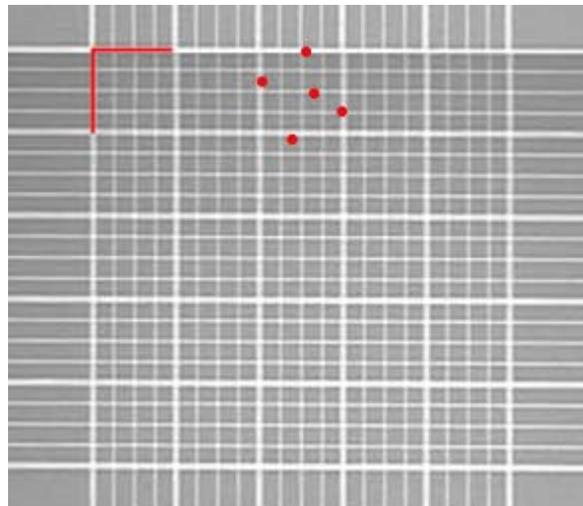


Counting: The full grid on a hemocytometer contains nine squares, each of which is 1 mm square (see figure below). The central counting area of the hemocytometer (as it will be called here) contains 25 large squares and each large square has 16 smaller squares. When counting, count only those cells on the lines of two sides of the large square to avoid counting

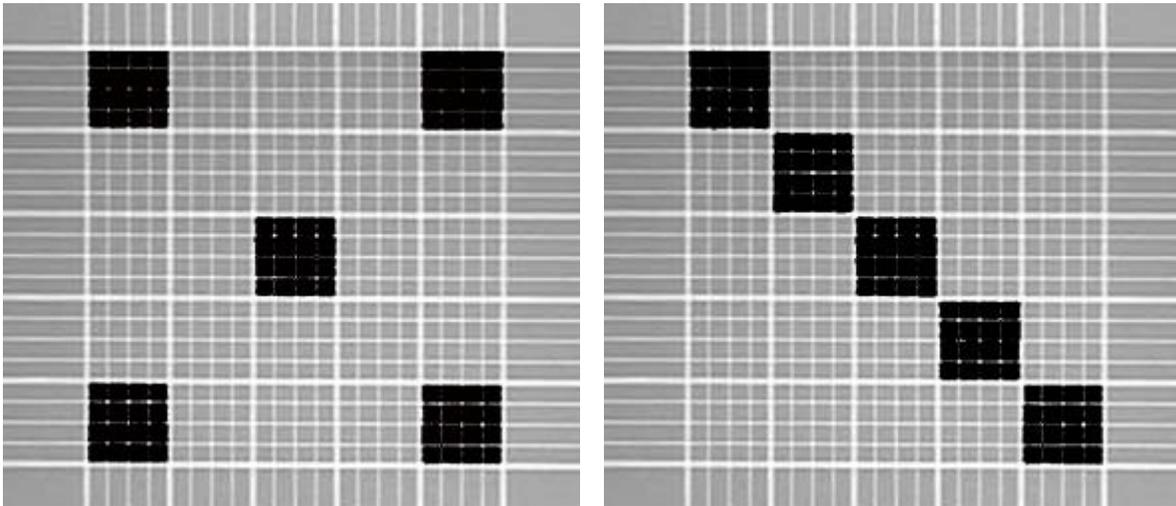
cells twice.



The example at right shows red lines where cells on the line would be counted. If red dots represent cells, one would count 3 cells in the top middle large square.



All 25 large squares can be counted, or a counting pattern using fewer squares can be used like the ones below. It is important to distribute the counting areas in a non-biased manner since cells can be more concentrated on one side of the chamber. If you count over only 5 of the 25 large squares, then multiply that value by 5 to obtain the number of cells per central counting area.



At least two chambers should be counted, including at least 100 cells within each central counting area of each chamber. For higher precision, additional sperm can be counted and the average used to calculate cell concentration.

Calculating Concentration

Each of the nine squares on the grid, including the central counting area of 25 large squares, has an area of 1 square mm, and the coverglass rests 0.1 mm above the floor of the chamber. Thus, the volume over the central counting area is 0.1 mm^3 or 0.1 microliter. You can thus multiply the average number of sperm over each central counting area by 10,000 to obtain the number of sperm per ml of *diluted sample*.

In other words, to calculate the number of sperm per ml of original sample:

1. Calculate the mean number of sperm counted for each chamber (i.e. for each of the central counting areas of each chamber).
2. Multiply the mean obtained in (1) by 10,000 to obtain the number of cells per ml of diluted sample.
3. Multiply the count obtained in (2) by the dilution factor.

Example: Assume that you dilute the original semen sample by adding 0.1 ml of semen to 9.9 ml of diluent (1:100 dilution factor). You then count the number of sperm in 5 of the 25 large squares within the central counting area of two chambers, obtaining counts of 132 and 128 cells.

1. The mean number of sperm per chamber is thus 130×5 or 650 cells per counting area (650 cells per 0.1 microliter).
2. Multiply the 650 cells per counting area by 10,000 to obtain the number of cells per ml of diluted sample (answer = 6,500,000)
3. Multiply 6,500,000 cells per ml of diluted sample by 100 (the dilution factor) to obtain 650,000,000 per ml of original semen sample.