EDUCATIONAL COMMENTARY – CEREBROSPINAL FLUID CELL COUNTS

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Learning Outcomes
Upon completion of this exercise, the participant will be able to:

- Explain how to perform cerebrospinal fluid cell counts with a hemacytometer.
- Calculate the number of cells per microliter from the raw cell count.
- Discuss techniques used to concentrate cerebrospinal fluid (CSF) specimens for a differential cell count.

Cerebrospinal fluid (CSF) is collected in aliquots of 1 mL to 4 mL in 3 (or sometimes 4) sterile tubes which are labeled 1, 2, 3, and 4 in the order they are collected. The last tube collected (tube 3 or 4) is used for the cell count because it is less likely to contain red blood cells (RBCs) introduced by the lumbar puncture.

Both white blood cells (WBCs) and RBCs begin to degrade as soon as 1 hour after collection, and as many as 40% of any neutrophils present can disintegrate within 2-3 hours. For this reason, the cell count should be performed STAT. If this is not possible, the specimen can be refrigerated for up to 4 hours.

Although cell counts can be performed on both WBCs and RBCs, usually only WBCs are counted. This is because the number and types of WBCs present are valuable clues to the presence and nature of an infection. Red blood cell counts, on the other hand, are of limited diagnostic value; these are usually performed only when it is necessary to correct for the WBC count or protein introduced into the specimen during a traumatic tap.

The Hemacytometer
CSF cell counts can be performed on automated equipment if the laboratory has documented linearity, background, and correlation studies. However, automated methods often lack precision in the low counts typically found in CSF specimens. For this reason, cell counts are usually performed with a hemacytometer, which is a precisely manufactured thick glass slide specially designed for manual cell counts on body fluids.

The hemacytometer has 2 raised platforms, each etched with an improved Neubauer counting chamber. The ruled area on each counting chamber is 9 mm², and this area is divided into 9 large (1 mm²) squares (Figure 1). The 4 large corner squares are subdivided into 16 equally-sized squares. The large center square is subdivided into 25 equally-sized squares, and each of these 25 squares is further subdivided into 16 squares.
A calibrated coverglass precisely controls the depth of the field, so that each large (1 mm²) square encloses a volume of 0.1 µL. The calibrated coverglass must be used when performing cell counts—any other coverglass will cause inaccurate results.

Performing the Cell Count
The standard formula for calculating the number of cells per microliter is:

\[
\text{cells/µL} = \frac{\text{number of cells counted} \times \text{dilution factor}}{\text{number of squares counted} \times \text{volume of 1 square}}
\]

Counting only the cells in the 4 large corner squares and the large center square (see Figure 1) of both counting chambers (10 squares) eliminates the need to correct for volume because 10 squares x 0.1 µL/square = 1 µL. Hence, the number of cells per microliter is simply the number of cells counted times the dilution factor. For example, assume that you dilute a specimen 1:2, load it into both counting chambers of the hemacytometer, and count a total of 25 cells in 10 large squares. The standard formula shows that the specimen contains 50 cells/µL (25 cells multiplied by the dilution factor of 2):

\[
\frac{25 \text{ cells} \times 2}{10 \times 0.1 \text{ µL}} = \frac{50 \text{ cells}}{1 \text{ µL}} = 50 \text{ cells/µL}
\]

If the specimen is undiluted, the calculation is even simpler because the dilution factor is 1. In this case, the number of cells per microliter equals the number of cells counted in 10 large squares.

Figure 1. Location of the large squares on the improved Neubauer counting chamber.

By counting cells in the 5 shaded squares in both counting chambers (for a total of 10 squares), the standard formula is simplified so that cells/µL = number of cells counted x dilution factor.

Differential Cell Count
The types of cells present in a CSF specimen provide valuable clues to the etiology of disease, and for this reason a differential cell count should always be performed along with the cell count. However, a differential performed on the unstained hemacytometer preparation is unacceptable for 3 reasons:

1. Precise identification of unstained cells is difficult.
2. Only a few cells are available for identification.
3. There is no permanent slide.
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To avoid these limitations, the differential count should be performed on a Wright’s stained smear prepared from a concentrated specimen. Specimens can be concentrated by cytocentrifugation, filtration, or sedimentation. Of these methods, most laboratories prefer cytocentrifugation because it is rapid, requires minimal training, uses small samples, and provides good cell recovery with little distortion of cellular elements. Filtration and sedimentation also yield quality results, but they are costly and time-consuming and they require more skill. Ordinary centrifugation is not recommended because, although it is rapid and does not require special equipment, it produces high rates of cellular distortion and fragmentation.

Suggested Reading


