[To: hematology]

Manual White Blood Cell count

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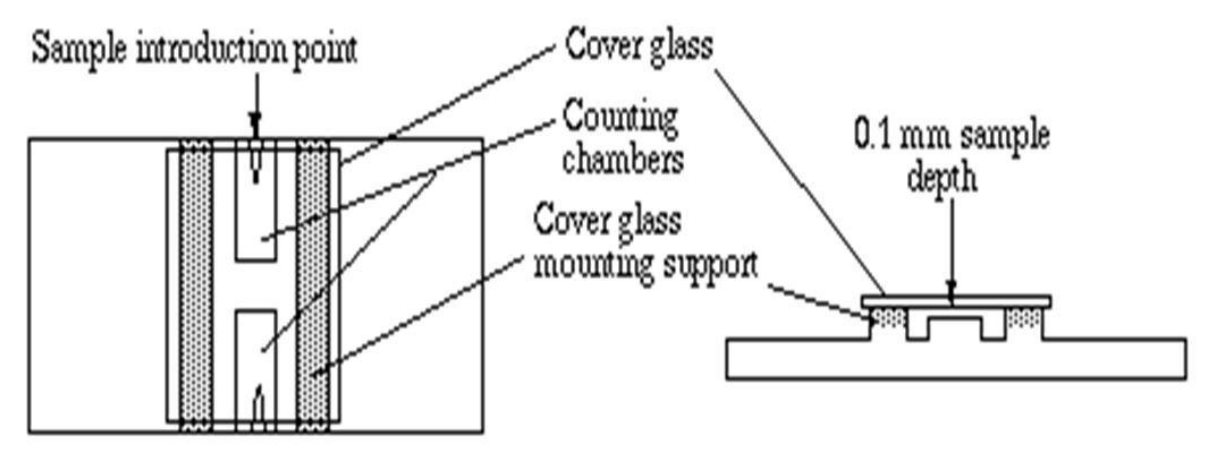
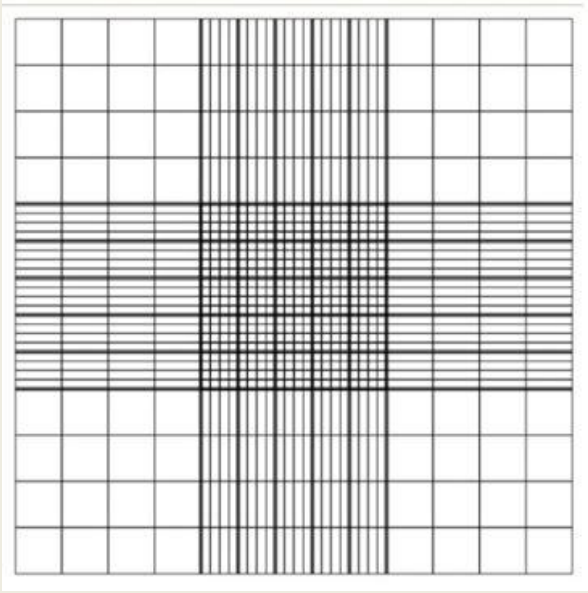
The use of a hemocytometer

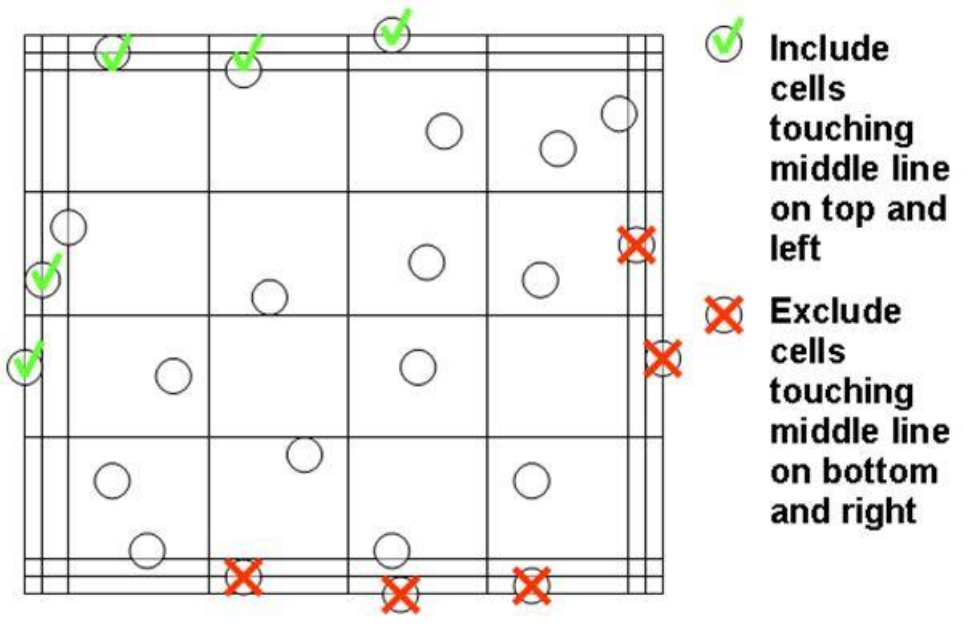
A hemocytometer is a counting chamber used to determine the number of blood cells per unit volume of a suspension. It can be used to count RBCs, WBCs or platelets. Staining of cells facilitates visualization and counting. Cell counts require a properly collected and anti-coagulated blood sample that is fresh and well mixed. If the sample has been refrigerated, it should be warmed to room temperature and remixed. The blood is then diluted using either glass blood dilution pipettes or the Whi-pette™ system.
The hemocytometer contains two raised bridges which hold the coverslip and two counting areas that are completely surrounded by a moat. Coverslips for counting chambers are specially made and are thicker than those for conventional microscopy. They should be kept with the hemocytometer and not used for other purposes.



The sample counting area contains a ruled grid, most commonly Neubauer ruling. Neubauer ruling consists of 9 large squares, each measuring 1 mm2. The depth of the chamber (distance between the grid area and the coverslip) is 0.1mm. The squares are further subdivided into smaller squares. The center square is divided into 400 small squares arranges as 25 groups of 16 each. One entire grid Neubauer rulings can be seen at 40x (4x objective).



Neubauer ruling



The use of a WBC-count Whi-pette™

Materials:

- Pre-filled amber tubes (included in Whi-pette® Kit)
- 10 microliter pipette and tips (included in Whi-pette® Kit)
- Anti-coagulated (EDTA) whole blood sample
- Microscope
- Hemacytometer

Procedure

1. Draw 10 µl of blood using a fresh pipette tip attached to the microliter pipette. Wipe any excess blood from the outside of the pipette tip with a lint free wipe. Don't forget to depress the pipette half-way down when drawing the sample and all the way down when dispensing the sample.

2. Dispense the sample into an amber tube and flush the pipette several times using the solution in the tube. Be careful not to spill any of the solution.

3. Cap the tube and mix thoroughly.

4. Allow to set 5 minutes to permit the stain to penetrate the cells. During this time set up the hemacytometer and position the cover slip.

5. Use the flushed pipette tip to instill just enough sample to fill both sides of the counting chamber. Take care that the counting area is completely filled and not allowed to overflow into the moat. Should this occur the hemacytometer should be cleaned and recharged before proceeding.

6. Let the sample to sit in the counting chamber for 5-10 minutes before counting (allow cells to settle).

7. Using 100X magnification, count the cells in the 9 large squares on both sides of the chamber. The count should proceed in an orderly fashion, starting at one end of the square, going across to the other side, then down one microscope field and back across until all cells within the square are counted.
Cells that are touching the line between two squares are counted with that square if they are touching either the top or the left line. Do not count cells touching either the bottom or right lines.

8. The totals from each side should be within 10% of each other.

9. Take the total of both sides of your counting chamber and enter the numbers into the equation below:

(Side A + B/2) x 110
Example: (78 + 82/2) x 110 = 8,800 µl