RBC & WBC count

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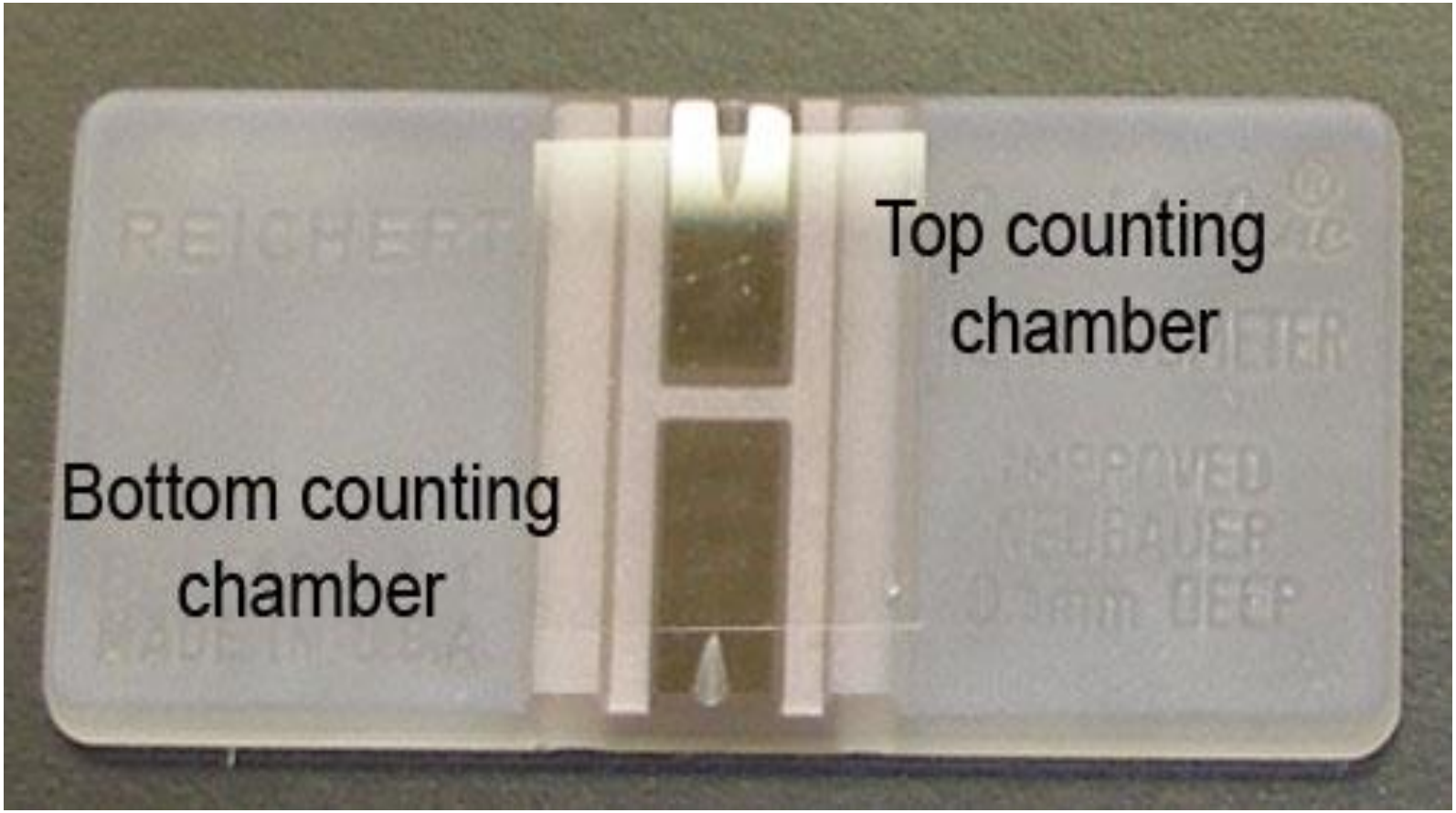
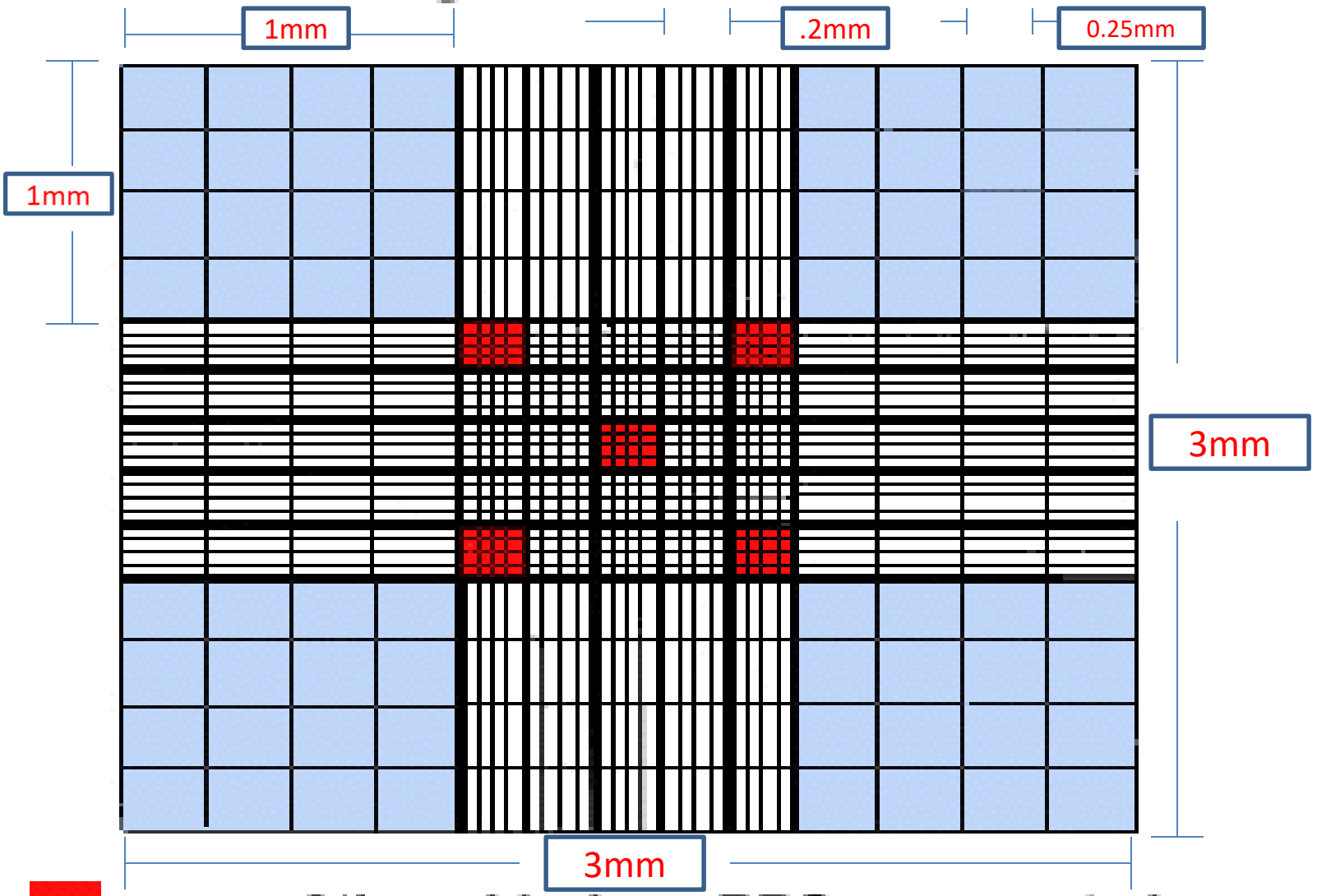
Red Blood Cell (RBC) Count

- RBCs have few organelles and no nuclei, they are shaped as biconcave disks and their main function is to transport hemoglobin which in turn transports oxygen.
- The average number of RBCs in healthy men is 5.2 M/mm^3 (±300,000) and in healthy women 4.7 M/mm^3 (±300,000)
- The aim of the lab is to determine the count of RBCs in a blood sample using a hemocytometer.

- Hemocytometer is a special microscopic slide that has specific grids engraved on it's counting chamber and is designed to hold a specific volume of fluid.



areas of the grid where WBC are counted



areas of the grid where RBC are counted

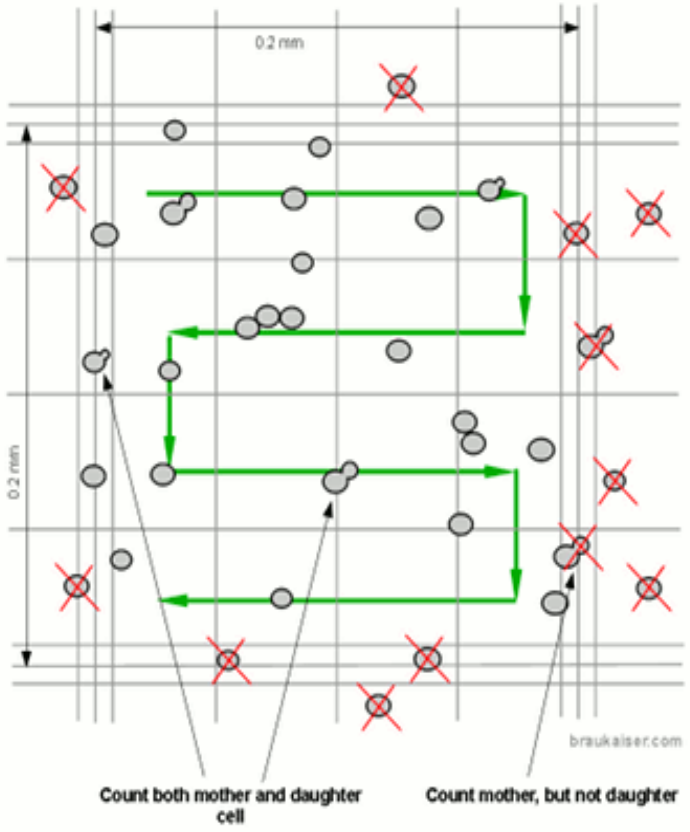
The procedure

1. Clean the hemocytometer well
2. Place a coverslip over the counting area. Now the distance between the bottom of the coverslip and the surface of the counting area is **0.1 mm**
3. Dilute the blood sample by adding 1 unit of blood to 199 units of an isotonic solvent and thoroughly mix the mixture
4. Draw a sample using a pipette and gently touch the junction of the coverslip and hemocytometer and slowly put the sample. Let it stand for 3 min.
5. Use the 10X lens to identify the center square , then use 40X lens to focus on the smaller squares (RBCs appear circular in shape)

6. Count the number of cells in the five small squares and obtain an average number.

- Start counting from the left to the right and proceed in a zig-zag.

- Regarding cells that touch boundaries, count the cells that touch two of the boundaries and ignore the Cells touching the other two boundaries



The calculation

- If we counted an average of 95 cells in the five squares what is the number of RBCs in the sample?

- RBCs/mm^3 = average number of counted cells **X** dilution factor (DF) **X** volume correction factor(VCF)
- DF = $\frac{Final\ volume}{Volume\ of\ blood} = 200$
- The volume of fluid in one small square is (0.2 **X** 0.2 **X** 0.1 = 0.004 mm^3)
- VCF = Desired Volume/ Counted Volume = 1/.004 = 250
- RBCs/mm^3 = 95 **X** 200 **X** 250 = 4,750, 000 cells /mm^3

Knowing that the blood sample was taken from a female, the number of RBCs is normal

- **Causes of high RBC count (Polycythemia)**

1. Living at high altitudes
2. Cardiac or pulmonary diseases
3. Erythropoietin secreting tumors
4. Dehydration
5. Polycythemia Vera

- **Causes of low RBC count (Anemia)**

1. Internal or external bleeding
2. Nutritional deficiencies
3. Bone marrow failure
4. Hemolysis of RBCs
5. Chronic Renal failure

WBC count

- White Blood Cells are the mobile units of the body's protective system
- Specifically transported to areas of severe infection or inflammation to provide a rapid and potent defense for the body
- Normal WBC count is 4000 - 11,000 cells/µL

- The principle of the procedure is similar to the RBC count procedure with some minor differences.

The procedure

1. Clean the hemocytometer well
2. Place a coverslip over the counting area. Now the distance between the bottom of the coverslip and the surface of the counting area is **0.1 mm**
3. Dilute the blood sample by adding 1 unit of blood to 19 units of solvent and thoroughly mix the mixture.
4. Draw a sample using a pipette and gently touch the junction of the coverslip and hemocytometer and slowly put the sample. Let it stand for 3 min.
5. Use the 10X lens to count the WBC in the four large corner squares .(WBCs appear as dark dots)

*The dilution fluid causes RBCs lyses and stains the nuclei of WBCs. This allows a proper count of WBCs.

The calculation

1. Blood is diluted at (1:20) so DF = 20
2. The volume of fluid in the corner square is (1 **X** 1 **X** 0.1= 0.1 mm^3) SO the VCF is 10

✓ If we counted an average of 40 cells in the 4 squares the count of WBCs is….

40 **X** 20 **X** 10 = 8000 cells/mm^3 which is a normal value

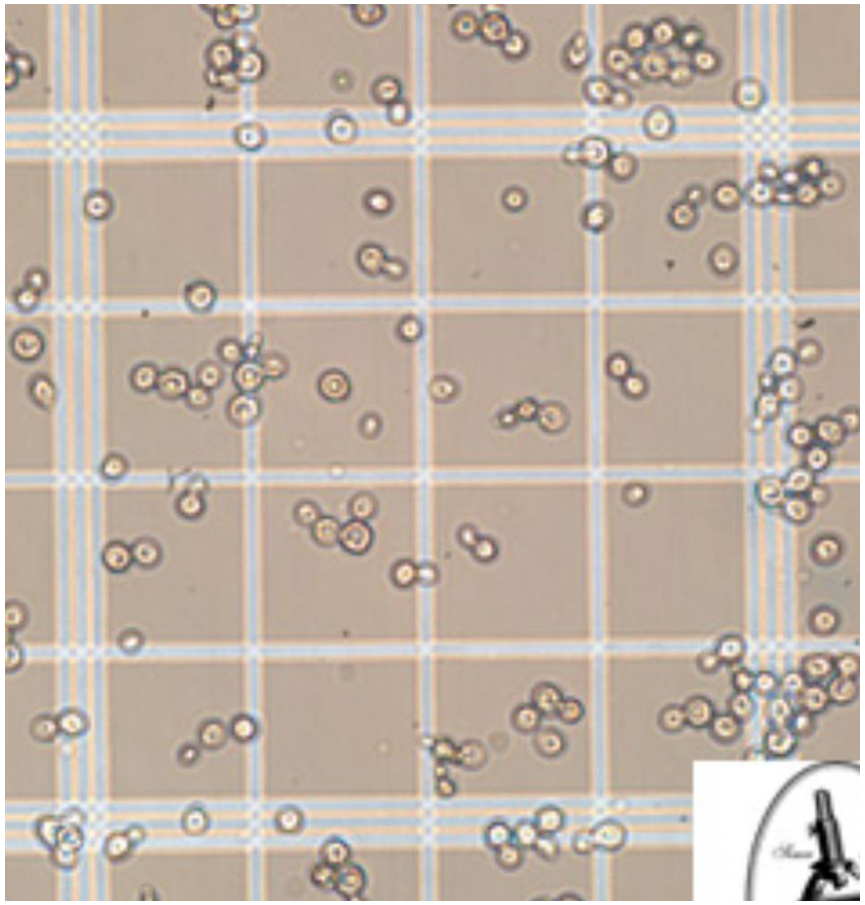
- **Causes of High WBC count (Leukocytosis)**

1. Active inflammation or infection.
2. Certain malignancies
3. Physiological processes (stress, exercise)
4. Dehydration

- **Causes of Low WBC count (Leukopenia**)

1. Bone marrow failure due to radiation or malignancy
2. Autoimmune diseases.
3. Infections like HIV & tuberculosis.

RBCs



WBCs