Mustansiriyah University
College of science
Biology Dept.
Zoology
4th class
Laboratory Technique LAB.
(5)

NAME :

Cell Counting

is a general name for various methods for the quantification of cells in molecular biology & medicine.

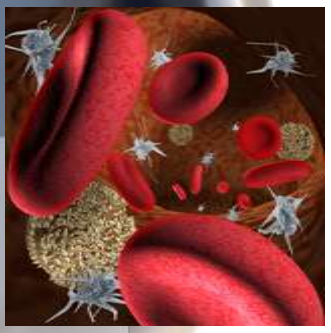
Why to count ?

1. Certain experiments require an exact number of cells to be used.
2. A lot of kits for molecular biology have the amount of reagents for a certain number of cells
3. Experiments of proliferation and survival (Usually toxicity studies).

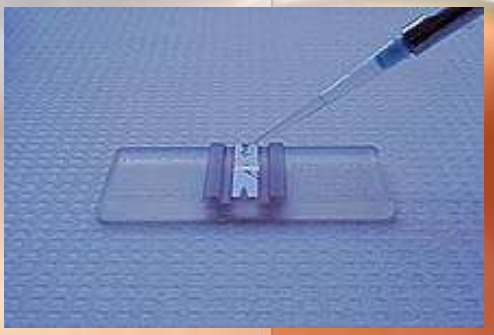
For examples:

WBC count, Sperm , RBC

Hemacytometers

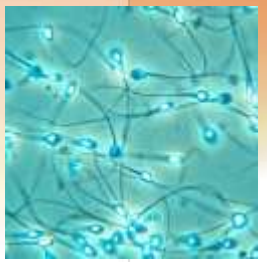


- Hemacytometers → a accuracy-made slide for performing manual cell counts with the aid of a microscope.



- Hemacytometers are used when……

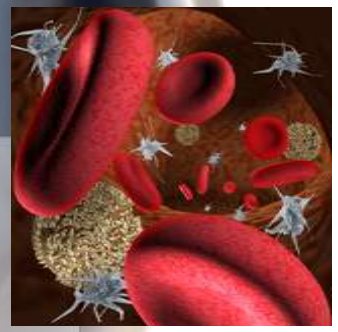
— 1. Automated cell counters and hematology analyzers are unavailable
— 2. Blood cell counts are extremely low
— 3. To get a cell count for body fluids (spinal fluid, joint fluid, semen counts, and other bodily fluids)



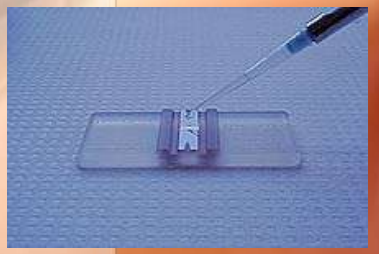
Counting Micro-organisms

- A single tiny drop of nutrient broth incubated overnight may contain 5 000 000 cells – this is a lot to count.
- $1cm^3$ may contain 10^8 cells.
- In order to estimate numbers it is necessary to dilute the sample.

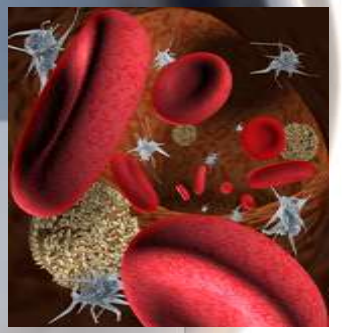
Hemacytometers

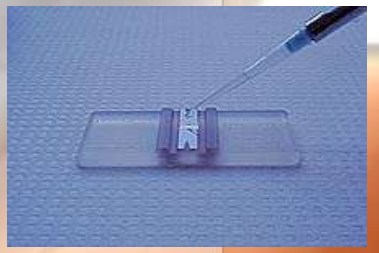


- The hemacytometer is a counting chamber that contains two microscopically ruled areas marked off by lines.
- Glass hemacytometers are made of heavy glass with two counting areas.
- The chamber areas are covered with a coverglass and placed under the microscope for visual examination.
- The chamber depth in the Neubauer-type hemacytometer is 0.1 mm.
- A glass hemacytometer is reusable if disinfected between each use.
- Disposable hemacytometers can also be purchased.
- Instead of reusing these the coverglass is plastic and can only be used once.

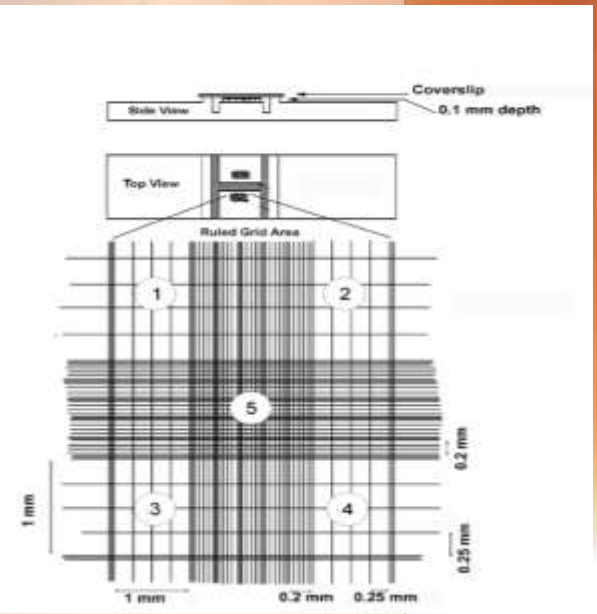


Hemacytometers

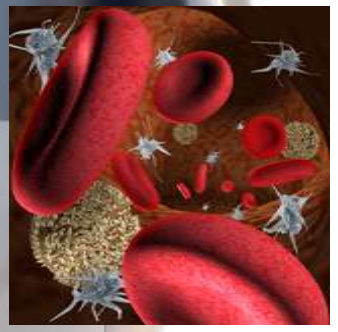


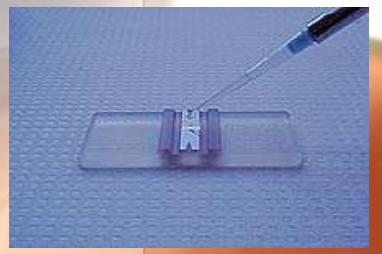


- The hemacytometer contains two identical ruled areas that each have etched lines with squares of specific dimensions.
- In the Neubauer-type hemacytometer, the total lined area on each side is made of a large square (3 X 3 mm).
- This large square is divided into nine equal squares each 1 mm^2.
- The total area of all the squares is 9 mm^2.
- When the coverglass is put in place and fluid is added, the fluid volume in the area can be calculated.

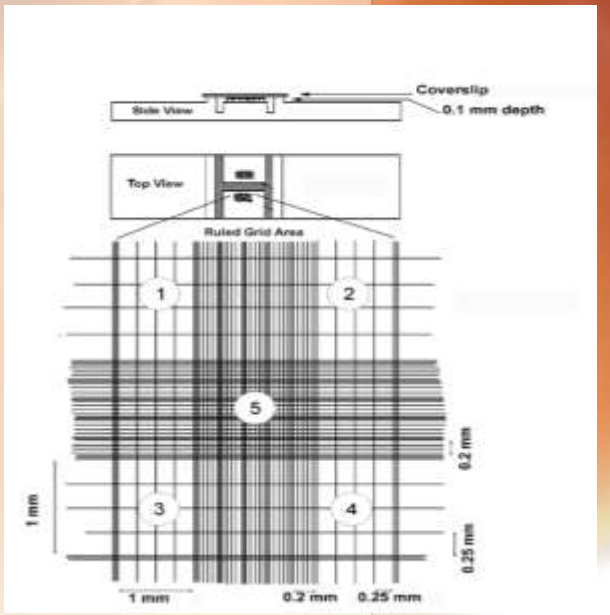


WBC Count

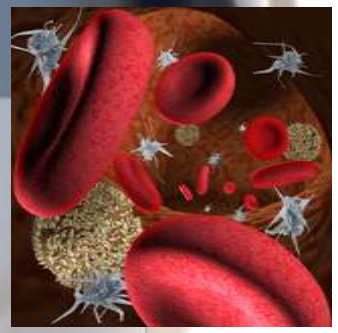




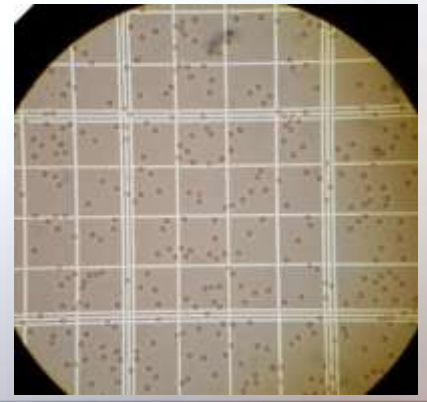
- The area used for counting WBCs is determined by how the sample of blood or fluid is diluted.
- Usually if there are a few cells the entire chamber is counted to determine the most accurate number of WBCs.
- If there are more than a few cells the 4 outer squares numbered 1-4 on the diagram are counted.
- Different diluting kits are available to help in the dilution ratio and saline can be used.

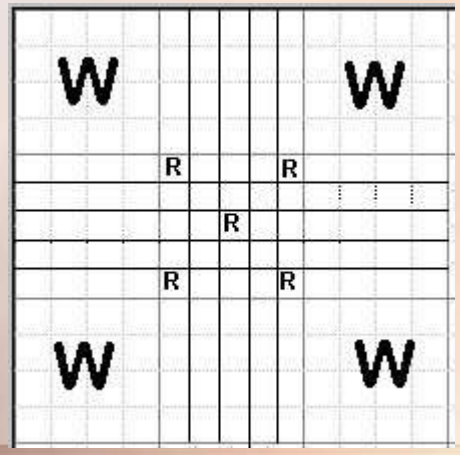


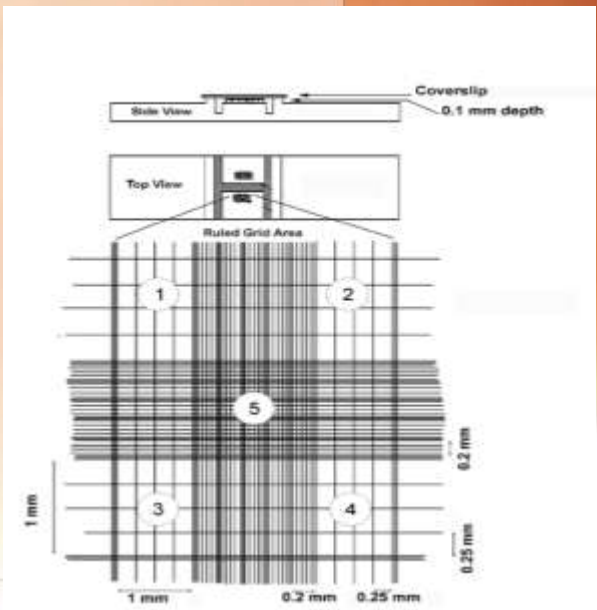
Performing a Manual WBC Count



- First determine if the liquid needs to be diluted.
- If it does follow the instructions for each diluting kit.
- Load the hemacytometer.
- Leave the hemacytometer for a few minutes to allow the cells to settle.
- Use the microscope (10 X) to locate the WBC counting area.
- You may have to lower the light on the microscope to visualize the WBCS.







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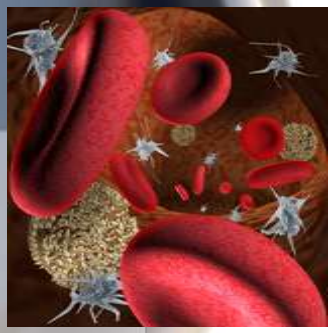
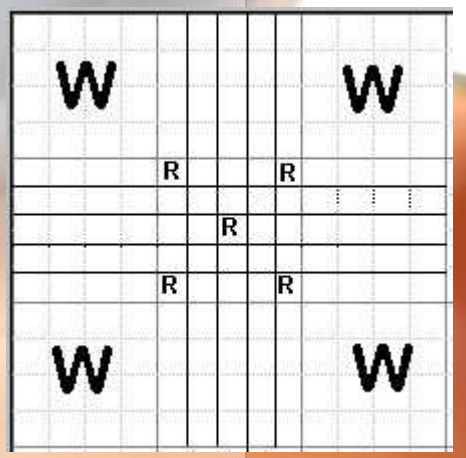


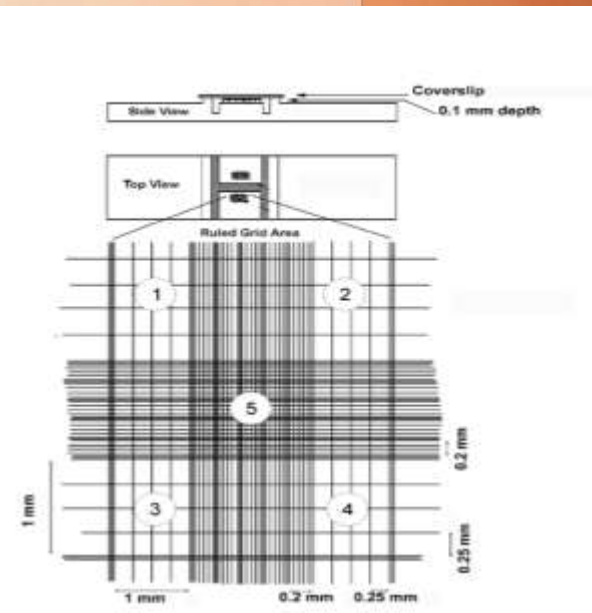
- Cells touching the upper or left border of the squares are counted.
- Cells touching the lower or right border of the square are not counted.
- Each side of the chamber should be counted and an average of the two should be taken.
- To calculate the cells if all 9 squares are counted you use the equation…..

$$\frac{\text{\# of cells X dilution}}{9 \text{ X } 0.1} = \text{\# cells / microliter}$$

- To calculate the cells in the 4 outer squares use the following equation…..

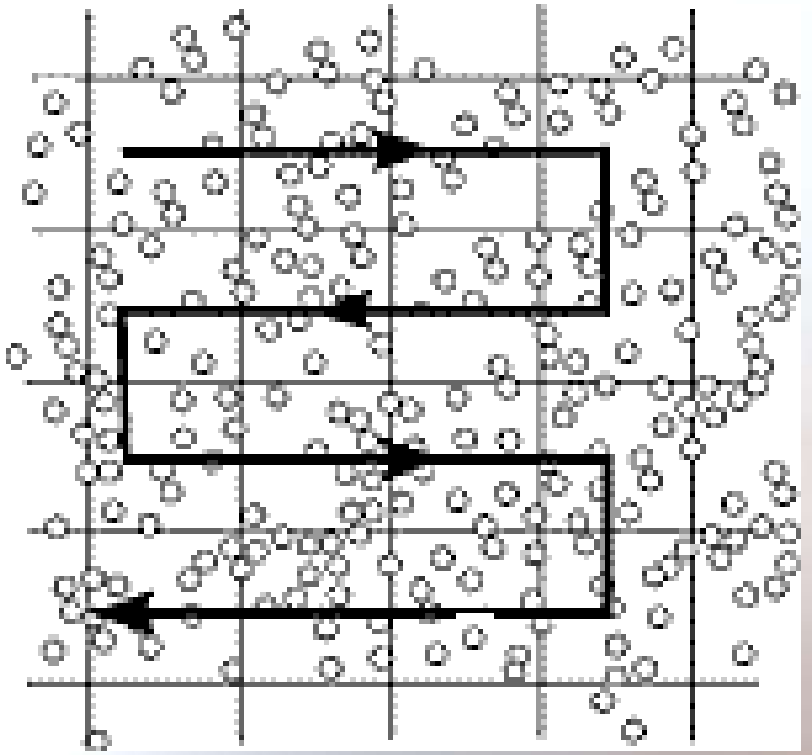
$$\frac{\text{\# of cells X dilution}}{4 \text{ X } 0.1} = \text{\# cells / microliter}$$

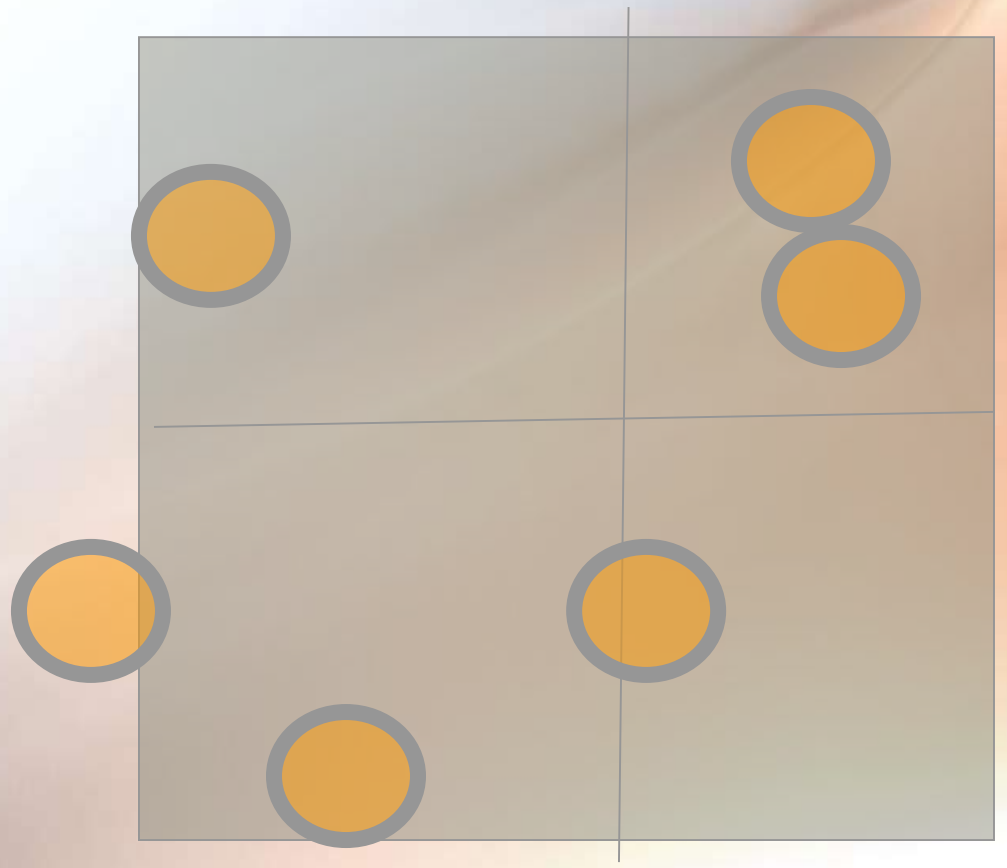




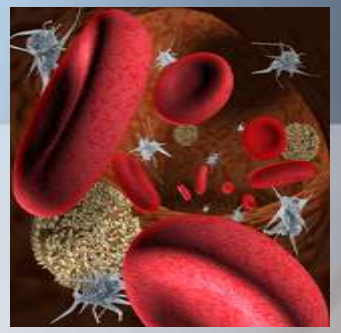
Counting Rule

- Do not count cells touching
  - Bottom line
  - Right line

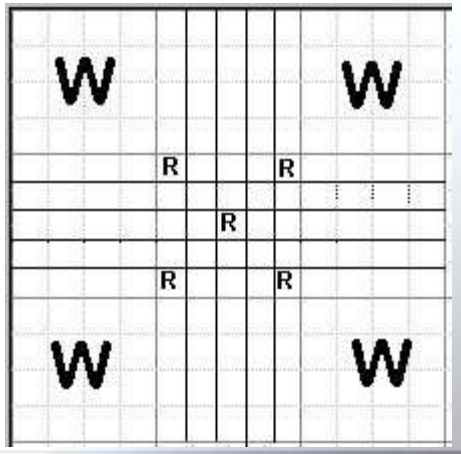


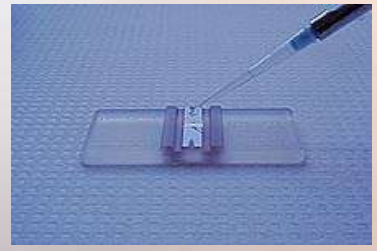


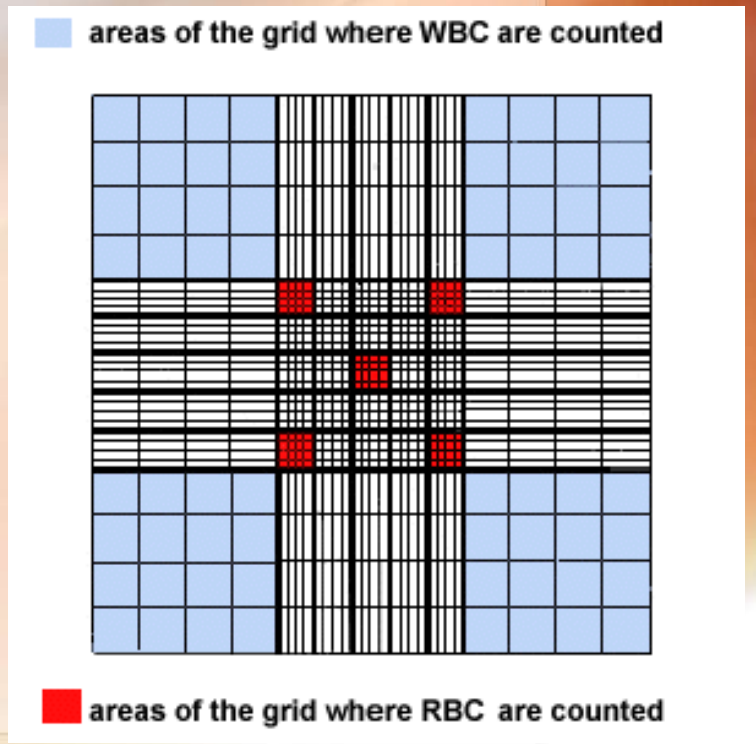
RBC Count & PLT Count



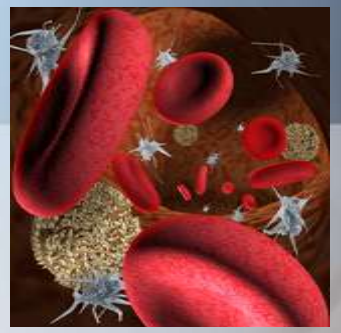
- The large center square is used for RBC and PLT counts.
- The center square is divided into 25 smaller squares, which are each subdivided into 16 squares.
- Only 5 of the 25 squares are used to count red blood cells.
- These 5 are usually the 4 outer squares and the inner most center square.
- The entire large center square is used to count platelets.



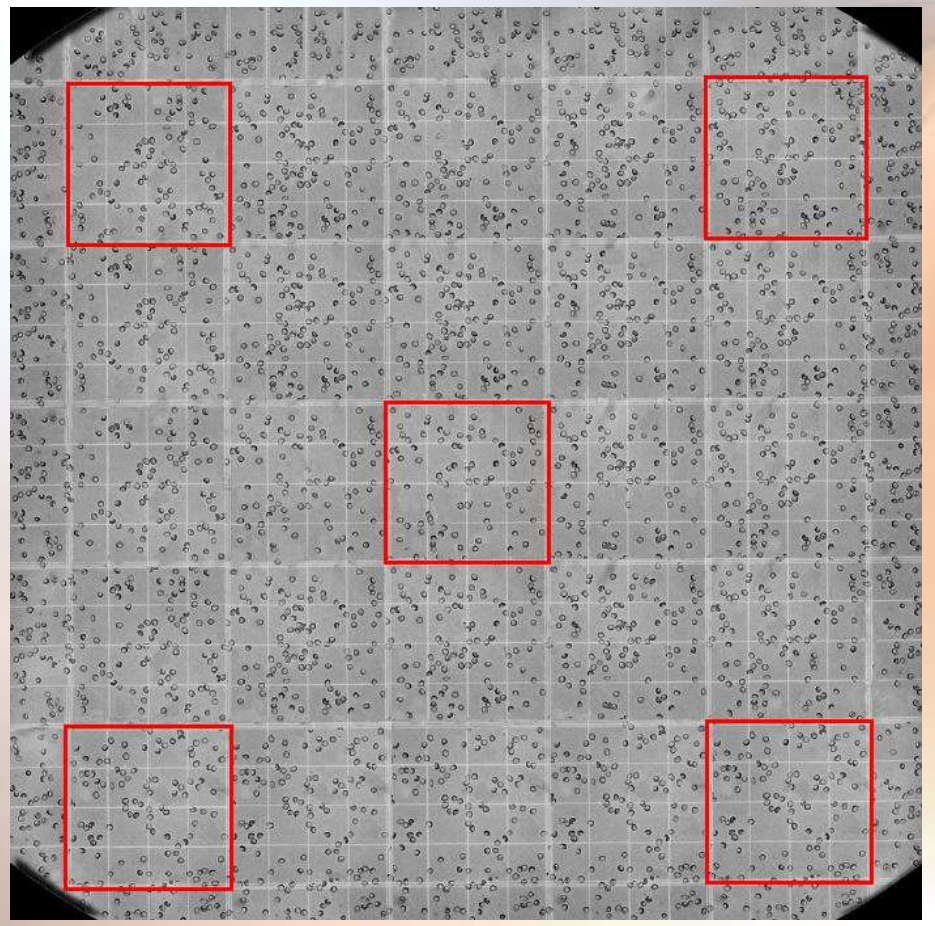




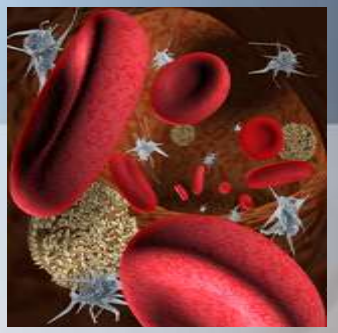
RBC Count & PLT Count

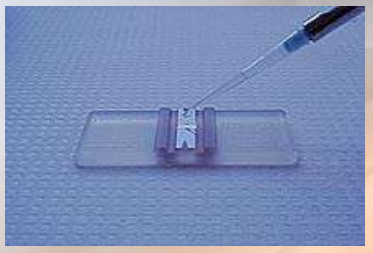


- First determine if the liquid needs to be diluted.
- If it does follow the instructions for each diluting kit.
- Load the hemacytometer.
- Leave the hemacytometer for a few minutes to allow the cells to settle.
- Use the microscope (10 X) to locate the RBC counting areas.
- The higher power (40 X) objective needs to be rotated into place to visualize the RBCs.



RBC Count & PLT Count





- RBCs are much smaller than the before mentioned WBCs.
- The cells touching either the top or left boundaries of the squares are included but the lower or right boundaries are not counted.
- The following equation is used for the RBCs…..

$$\frac{\#\ \text{cells} \times \text{dilution}}{5 \times 0.004} = \#\ \text{cells / microliter}$$