Blood grouping test, RBC and WBC count and Respiratory Pigments:

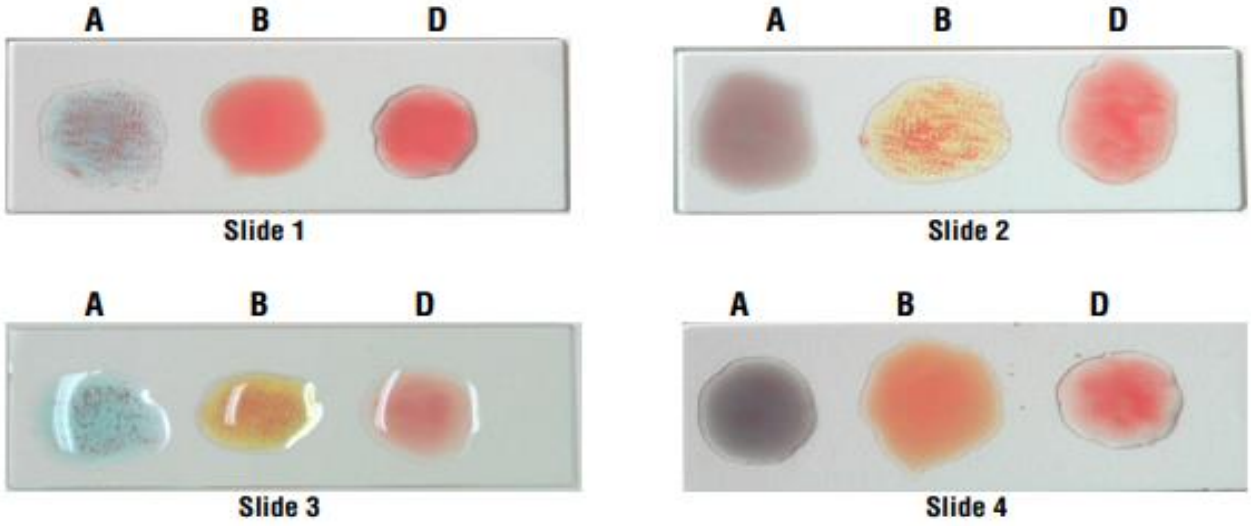
*Blood grouping test:

Material and method:

1. You mix the blood with three different reagents including either of the three different antibodies, A, B or Rh antibodies.

2. Then you take a look at what has happened. In which mixtures has agglutination occurred? The agglutination indicates that the blood has reacted with a certain antibody and therefore is not compatible with blood containing that kind of antibody. If the blood does not agglutinate, it indicates that the blood does not have the antigens binding the special antibody in the reagent.
3. If you know which antigens are in the person's blood, it's easy to figure out which blood group he or she belongs to!

- If coagulation happened in the first drop the blood group is A
- If coagulation happened in the second drop the blood group is B
- If coagulation happened in the first and second drop the blood group is AB if not the blood group is O
- If coagulation happened in the third drop the blood group is Rh^+ if not blood group is Rh^-



RBC and WBC count by using hemacytometer

Methods

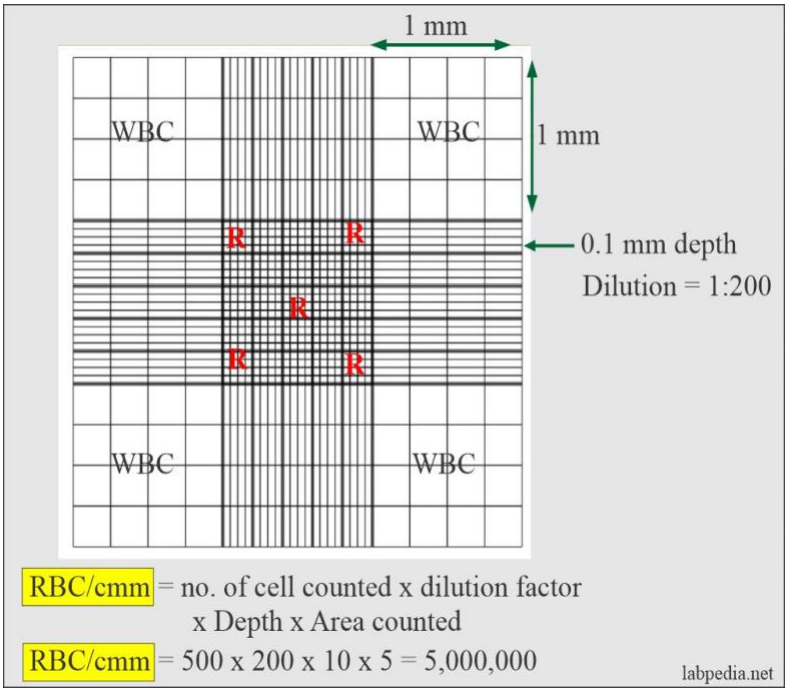
1-Manual method

2-Electronic method

Red blood cells count:

Materials Required for the Total Red Blood Cell (RBC) Count:

1- Blood sample (Capillary blood or EDTA anticoagulated tube)
2- RBC diluting fluid (preferably Hayem's fluid)
3- Cotton
4- RBC pipette
5- Hemocytometer (Neubauer's Chamber)
6- Coverslip
7- Microscope

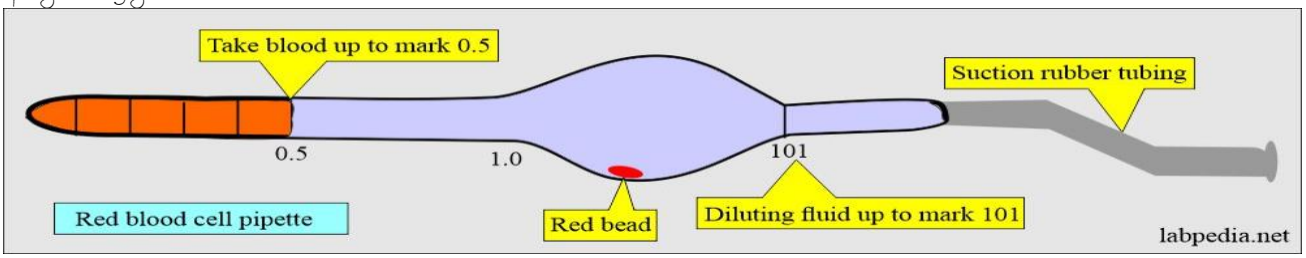


Neubauer chamber

Procedure:

- RBCs counting solution is Hayem's or Gowers isotonic saline.
- Make a dilution of 1:200 with a diluting solution.
- Fill the red bulb pipette up to 0.5 marks with the blood.
- Draw the solution to mark 101 of the RBC pipette.

- Mix the blood thoroughly in the pipette.
    - Discard the first few drops (4 to 5) and fill the Neubauer chamber.
    - Make sure that the chamber is free of air bubbles.
    - The distribution of the cells should be uniform over the ruled area.
- Allow for 2 minutes to settle the cells.
- Now count RBCs in the Neubauer chamber.
- Use 40 X to count the RBCs.
    - For RBCs, use the center square, which has 25 smaller squares.
- Count the corner 4 squares and 1 central square.
    - Count only the RBCs that fall on these squares' left and top borders.
- Repeat the count twice and divide by 2 to get the average.

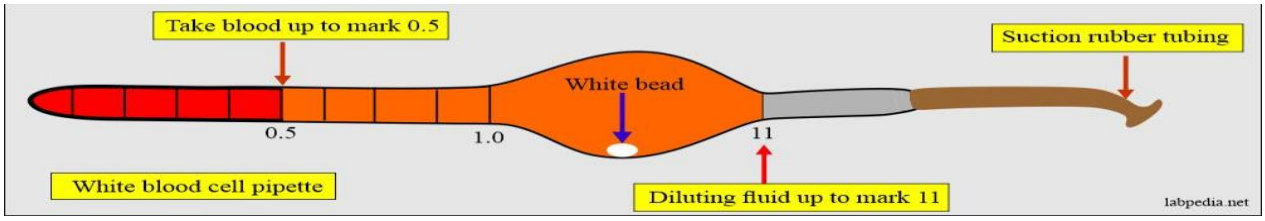


For red blood cells count this formula will be used:

50 * N * 200(dilution) = 10 000 * N **RBCs in 1mm³ blood**

White blood cells count:

In order to count the number of WBCs, the blood dilute with (Turks solution). This solution destroys the RBCs.



Materials & instrument

1- Blood sample (Capillary blood or EDTA anticoagulated tube)
2-Turk's diluting fluid composed of:-

- Glacial acetic acid (3ml) to hemolysed RBCs.
- Aqueous gention violet (1ml) to color the nuclei of WBC.
- Distilled water up to 100 ml.

3- WBC pipette.
4- Haemocytometer (Neubauer's counting chamber) with a cover slip.

5- Microscope
6- Lancet
7- Cotton

Procedure:

- Obtain a drop of blood in the same manner as in RBC count. Draw blood up to the mark 0.5 using WBC pipette.
- Aspirate diluting fluid up to mark 11. the dilution is 1:20.
- Remove blood from outside of the pipette with clean gauze.
- Gently rotate the pipette horizontally with your hand to ensure a proper amount of mixing for 3 minutes.
- After mixing discard the first four drops of the mixture.
- Fill the counting chamber with diluted blood by holding the pipette at 45 with the slide and allow the mixture to seep under the cover slip, the filled chamber should be allowed to stand for a minute prior to counting.
- Count the WBC using the low power 10x objectives.
- Count all WBCs in four large corner squares and add the result together to obtain the total number of cells counted. In counting the cells that touch the outside lines of the large square, count only those that touch the left and lower outside margin.
- The WBCs look like black dots.
  For white blood cell count this formula will be used:

$$2.5 * N * 20(\text{dilution}) = 50 * N \quad \text{WBCs in 1mm3 blood}$$

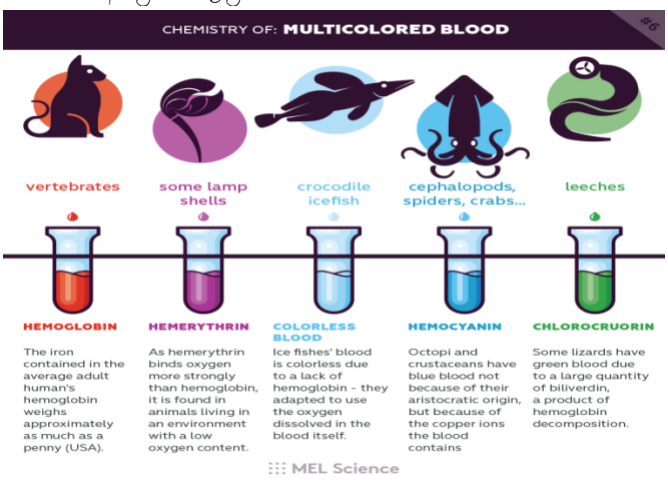
*Respiratory Pigments and Blood Color:

Animal blood refers to the fluid, which carries oxygen, nutrients and metabolic wastes throughout the animal body. Vertebrates consist of a closed circulatory system

The size of the red blood cells widely varies among animals. Red blood cells of mammals lack a nucleus and organelles.

Human blood color: Hemoglobin is the respiratory pigment of humans. Which is red in color.

Animal blood color: Hemoglobin, haemerythrin, haemocyanin, and chlorocruorin are the four types of respiratory pigments in both of vertebrates and invertebrates. Red, pink (marine worms), blue (horseshoe crabs), and green (New Guinean lizards) the colors of the blood in animals.


CHEMISTRY OF: MULTICOLORED BLOOD
#6
vertebrates
some lamp shells
crocodile icefish
cephalopods, spiders, crabs...
leeches
HEMOGLOBIN
The iron contained in the average adult human's hemoglobin weighs approximately as much as a penny (USA).
HEMERYTHRIN
As hemerythrin binds oxygen more strongly than hemoglobin, it is found in animals living in an environment with a low oxygen content.
COLORLESS BLOOD
Ice fishes' blood is colorless due to a lack of hemoglobin - they adapted to use the oxygen dissolved in the blood itself.
HEMOCYANIN
Octopi and crustaceans have blue blood not because of their aristocratic origin, but because of the copper ions the blood contains
CHLOROCRUORIN
Some lizards have green blood due to a large quantity of biliverdin, a product of hemoglobin decomposition.
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