Title : **Blood – Classes**

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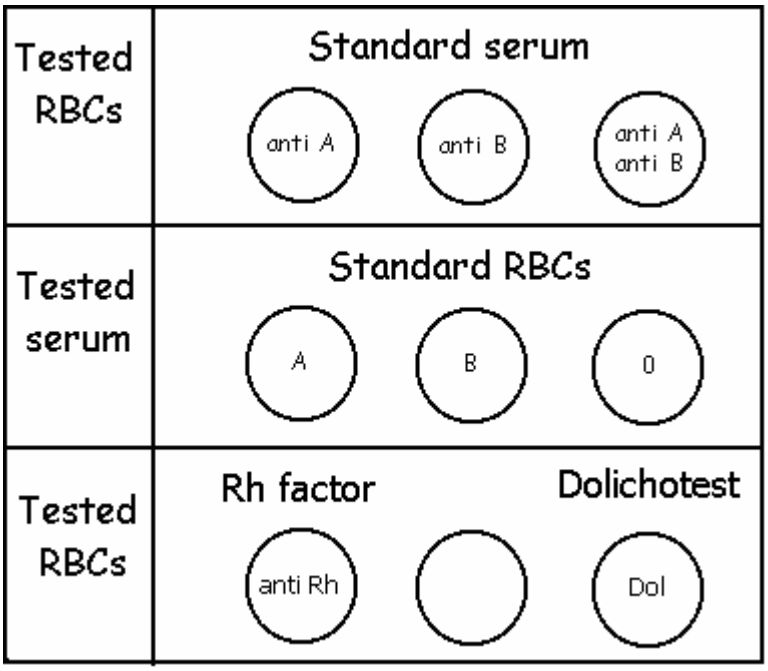
All students are expected to wear labcoats for the classes

AB0 grouping – procedure

1. In order to evaluate AB0 group first we must prepare tested RBC suspension and tested serum:

a. separate the serum from the clot

b. prepare 10% RBC suspension in 0.9% NaCl (1 drop of blood plus 9 drops of NaCl)



2. Mark three sites of a plastic slate: Anti-A, Anti-B, Anti-A and Anti-B for a standard antibodies

3. Below mark three sites of a slide: A, B, 0 for standard RBCs

4. Add a drop of Anti-A standard serum to the site labeled Anti-A, and a drop of Anti-B standard serum to the site marked Anti-B; and a drop of Anti-A and Anti-B standard serum to the site marked Anti-A and Anti-B.

5. Add one drop of standard RBCs to the appropriate sites (A, B, 0).

6. Add one drop of **tested serum** to the sites with **standard RBCs**, and one drop of **tested RBCs** to the sites with **standard serum**. Gently mix well.

7. To distinguish between group A1 and A2 use Dolichotest (an extract from Dolichus Biflorus). Dolichotest agglutinates only RBCs of A1 group.
Add one drop of Dolichotest to one drop of tested erythrocytes

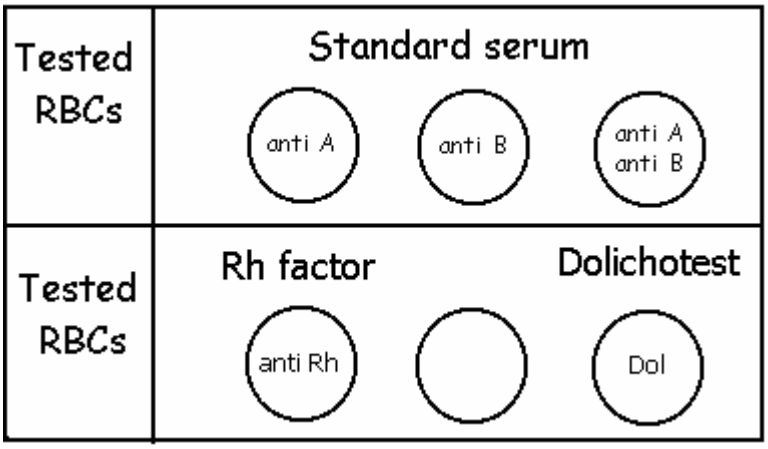
B. Rh grouping procedure

1. In order to evaluate Rh factor we use the same tested RBC suspension we've prepared before.
2. Add one drop of monoclonal antibodies anti D to one drop of tested RBCs
3. Gentlu mix well

Read the ABO and Rh group directly from the slate.

C. Examination of own blood in ABO and Rh system

1.Prepare new plastic slate with a standard suspensions as on picture bellow



2. Wash carefully your hand
3. Prepare 10% suspension of your own whole blood in 0.9% NaCl (1 drop of blood plus 9 drops of NaCl)
a. clean the tip of the finger with an alcohol .
b. prick the finger with an automatic lancet.

D. Clotting time

1. In order for blood to clot, the enzyme thrombin must be generated from the plasma precursor prothrombin. Thrombin then converts soluble fibrinogen into insoluble fibrin. Generation of thrombin involves the sequential activation of a number of other plasma clothing factors, this process is also assisted by platelets and Ca^{++}.

2. The time taken for blood to clot mainly reflects the time required for the generation of thrombin in this manner. If the plasma concentration of prothrombin or of some of the other factors is low (or if the factor is absent, or functionally inactive), **clotting time will be prolonged.**

3. The expected range for clotting time is **4-10 mins.**

4. This is the time taken from whole blood drawn from a vein to clot in vitro. The surface of the glass tube initiates the clothing process. This test sensitive to the factors involved in the intrinsic pathway.

Clotting time – procedure:

1. Clean the tip of the finger with an alcohol.

2. Prick the finger tip with an automatic lancet.

3. Note the time when blood first appears on the skin
4. Touch the tube to the drop of blood. Through capillary action the tubes are filling up with blood. Tubes cannot be heparinized.

5. Break gently 1cm of the tube at the end of 2 min, and every 30 sec these after.

6. When fibrin is formed between the two broken pieces of tube the coagulation or clotting time is noted.

E. Bleeding time

1. This test measures the time taken for blood vessel constriction and platelet plug formation to occur. No clot is allowed to form, so that the arrest of bleeding depends exclusively on blood vessel constriction and platelet action

2. The bleeding stops within 1 to 9 minutes. This may vary from lab to lab, depending on how the test is measured

3. Using the ear **lobe method**, a normal bleeding time is between **1 and 4 minutes**

Bleeding time – procedure:

1. Clean the earlobe with an alcohol.
2. Prick the earlobe with an automatic lancet.

3. Note the time when blood first appears on the skin
4. After half a minute (30 sec) place the edge of the filter paper on the top of the drop of blood.
5. Perform the operation at half minute (30 sec) interval.
6. The end point or bleeding time is the first half minute when no blood is seen on the filter paper.