Name number Study group

Theoretical part

Determination of blood groups by slide method

Introduction

The erythrocyte cytoplasmic membrane serves as a barrier between the interior and blood under physiological conditions. Furthermore, it has a profound impact on intercellular communication and interaction. The cytoplasmic membrane is typically organised into a phospholipid bilayer with hydrophobic regions closed in on each other and hydrophilic regions interacting with the inner and outer environment. Within the phospholipid bilayer there are embedded proteins as well as lipids (mainly cholesterol) which reinforce the whole structure. A sort of plasma proteins, carbohydrates, glycolipids and glycoproteins stretches out beyond the membrane into the outer environment as antigens, based on which the wide range of blood groups is determined, such as AB0, Th, MNs, Kell, Levis, and so on. A blood group is inherited co-dominantly and remains unchanged over one's entire lifetime.

AB0 system

The AB0 system represents the most examined blood group, and is determined by the presence of the antigens A and B. These antigens are chemically glycoproteins and differ in their terminal carbohydrate chains, so-called agglutinogens. Four blood types are distinguished:

A – only agglutinogen A is present on the erythrocyte's surface
B – only agglutinogen B is present on the erythrocyte's surface
AB – agglutinogens A and B are present on the erythrocyte's surface
0 – neither agglutinogen A nor B is present on the erythrocyte's surface

Blood circulating throughout the body contains immunoglobulins IgM (anti-A and anti-B) known as agglutinins. Production of these antibodies is triggered by one's first intake of food and lasts throughout one's entire lifetime.

Group A – produces only anti-B agglutinin
Group B – produces only anti-A agglutinin
Group AB – no agglutinins are produced
Group 0 – produces both anti-A as well as anti-B agglutinin

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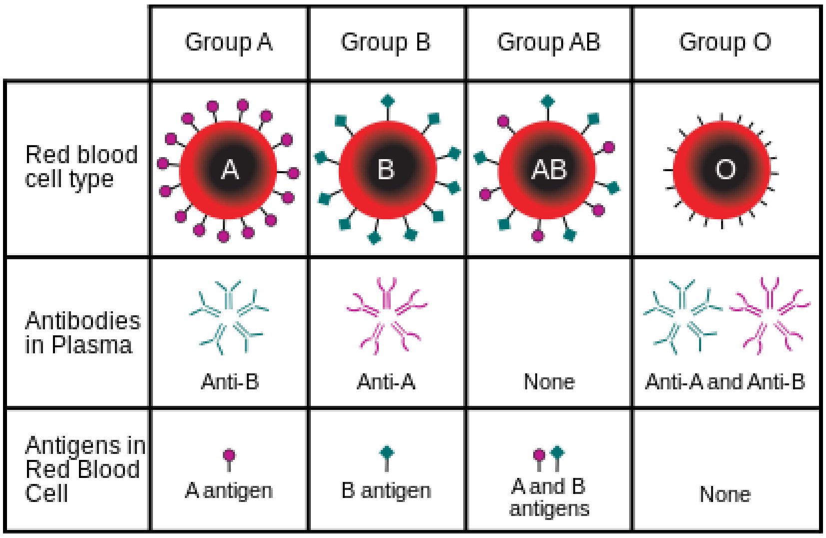
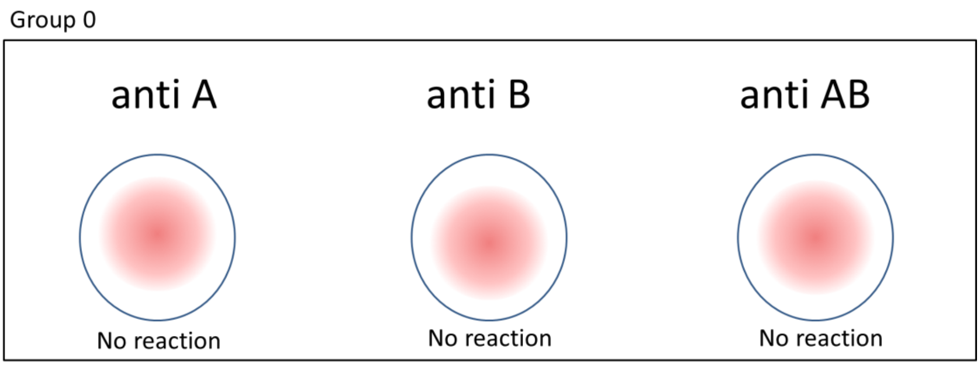
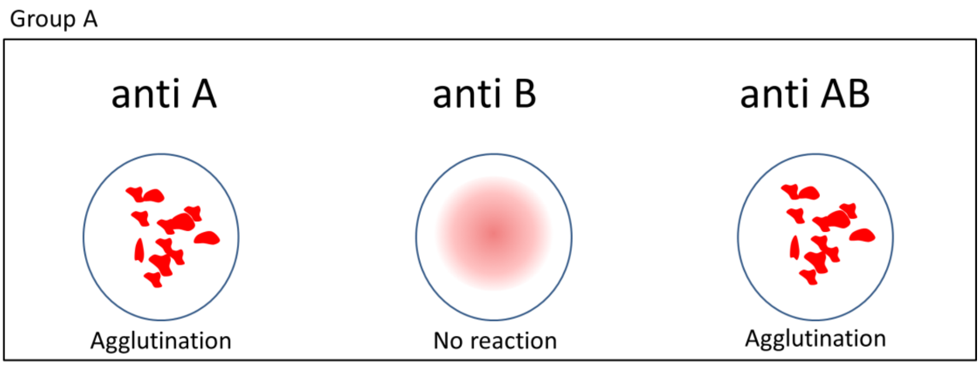


	Group A	Group B	Group AB	Group O
Red blood cell type	A	B	AB	O
Antibodies in Plasma	Anti-B	Anti-A	None	Anti-A and Anti-B
Antigens in Red Blood Cell	A antigen	B antigen	A and B antigens	None

The interaction of agglutinogen and agglutinin leads to agglutination, production of floating flakes accompanied by subsequent destruction of erythrocytes by the complement system.

Explanation of slide method





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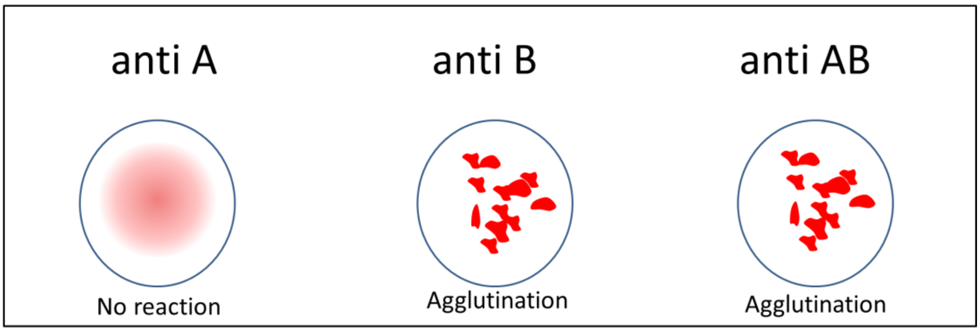
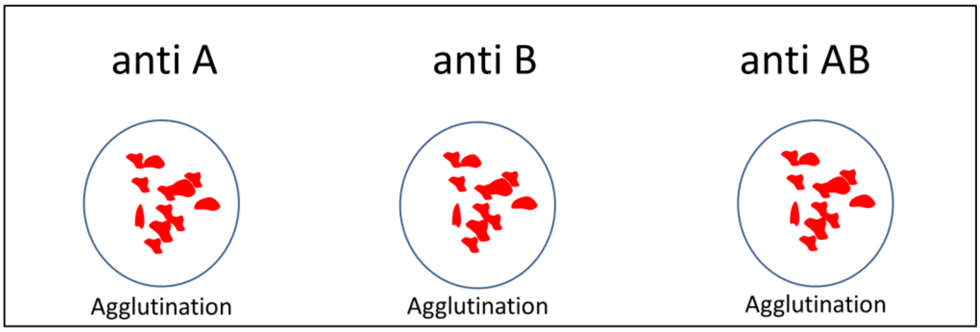
Group B



Group AB



As an example to explain the slide method, we will look at the group A. In the blue drop containing anti-A agglutinins, there is an obvious reaction of anti-A agglutinins and red blood cells holding A agglutinogens on their surface lead to the production of large erythrocyte-immunoglobulin complexes. This reaction cannot be observed in the yellow drop owing to the lack of agglutinin anti-B which does not have its agglutinogen B counterpart on the erythrocyte's surface. In the last grey drop agglutination is displayed due to the presence of both anti-A and anti-B agglutinins, but only anti-A agglutinins take part in this reaction.

Hereditability of the AB0 system

Blood groups are inherited from both parents. The AB0 blood type is determined by a single gene producing three alleles – allele A, B and i. A specific dominance relationship occurs called codominance where allele i is recessive to the dominant A and B alleles.

Phenotype – blood group	Alleles
A	AA, Ai
B	BB, Bi
AB	AB
0	Ii

Rh factor

The Rh factor represents another blood group determined by antigens C, c, D, d, E, e. Nevertheless, the Rh factor is defined by the presence or absence of the antigen D. A Rh positive subject is a carrier of antigen D on the surface of red blood cells and vice versa. Antigen D is dominantly inherited and thus a Rh negative subject has inherited the recessive allele from the both parents. Contrary to the AB0 system, antibodies against antigen D are produced just after the Rh negative subject's first encounter with antigen D – this reaction is called immunisation. Moreover, anti-Rh antibodies are produced as IgG type, and thus are capable of being transported through the placental barrier.

Haemolytic disease of newborns (Erythroblastosis fetalis)

This disorder occurs in Rh negative mothers immunised against the Rh factor (i.e. there are anti-Rh factor antibodies circulating in her blood) and carrying a Rh positive baby. The mother's immunisation might be result of either a previous pregnancy when the mother's and a Rh positive baby's blood were mixed or by administration of an incompatible blood type. The subsequently created anti-D antibodies can be transported through the placental barrier and lead to destruction of red blood cells in the newborn. An elegant method of preventing the production of anti-D antibodies has been introduced: after each delivery of a Rh positive baby, anti-D immunoglobulin is administered to the Rh negative mother in order to destroy all Rh positive erythrocytes leaked during the labour.

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Protocol

Determination of blood groups by slide method

Methods

Equipment:

Standard sera of group A (anti-B, i.e. beta), group B (anti-A, i.e. alpha) and group 0 (anti-A and anti-B, i.e. alpha and beta), slides, dropper

Procedure:

1. Drop each serum on the slide. Be careful not to mix them!
2. Using a dropper with a fine tip, take a drop of blood from the blood sample.
3. The corner of another slide is put into the blood drop and a small amount of blood is transferred to one of the sera and mixed by the same corner. In the same way, but using always another corner of the slide, transfer blood to the remaining test sera and mix the samples carefully.
4. Agglutination may be speeded up (2–10 min) by carefully rocking the slide to all four sides. The agglutination appears as visible flakes floating in the transparent serum. The result may be tested by microscopic observation: in the case of a positive reaction, the rod cells will form larger clusters.

Note:

Do not confuse the terms agglutination and coagulation, as these designate totally different physical and/or chemical events. Unfortunately, sometimes coagulation does appear in the test drop, usually when too much blood is added and mixed only slightly or not at all. To prevent this, the ratio of blood to test serum should be around 1:10.

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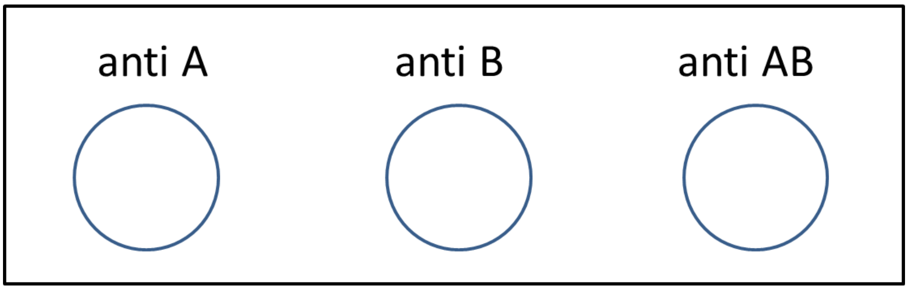
Results

Into the scheme below draw the reaction observed in your conducted experiment.

Blood 1



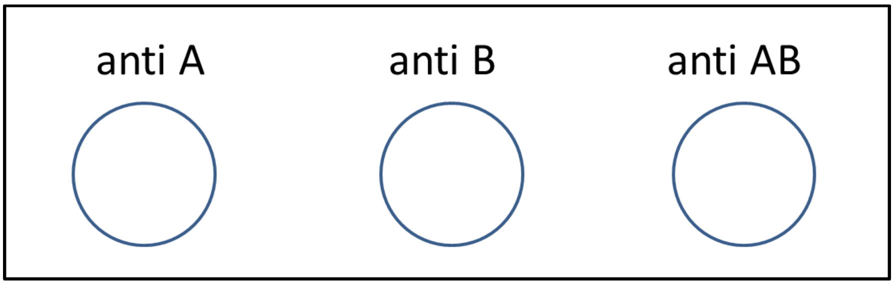
Blood group determined in your experiment:
This particular blood group is found in ………..% of population.
Based on Mendel's laws suggest all the possible combinations of the parent's blood groups.

…………….. ……………. ……………..……………. ……………..

Suggest combinations of parent's blood groups unable to produce your patient's blood group.

…………….. ……………. ……………..……………. ……………..

Blood 2



Blood group determined in your experiment:
This particular blood group is found in ………..% of population.
Based on Mendel's laws suggest all the possible combinations of the parent's blood groups.

…………….. ……………. ……………..……………. ……………..

Suggest combinations of parent's blood groups unable to produce your patient's blood group.

…………….. ……………. ……………..……………. ……………..

Conclusion

Propose possible blood groups of acceptors compatible for receiving your patient's blood products – blood plasma and packed RBCs.

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