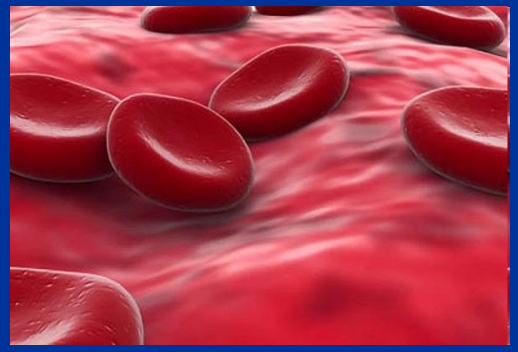
DEPARTMENT OF GENERAL SURGERY

TRANSFUSIOLOGY



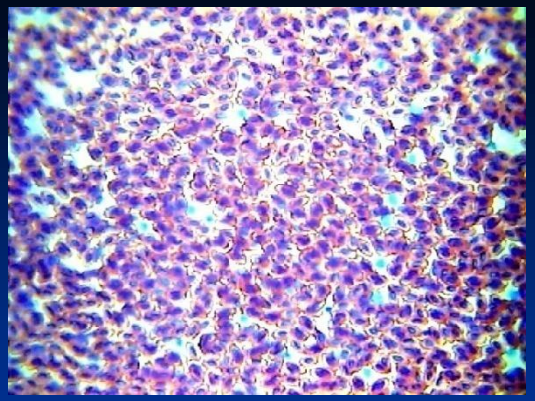
Lecture for general surgery
Chorna I.A.

Poltava

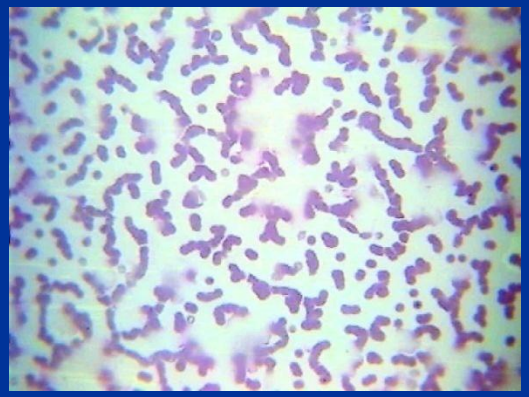
LECTURE PLAN

- What is blood
- Science about a blood.
- Blood in History
- Concept about blood types
- Methods of definition of blood types

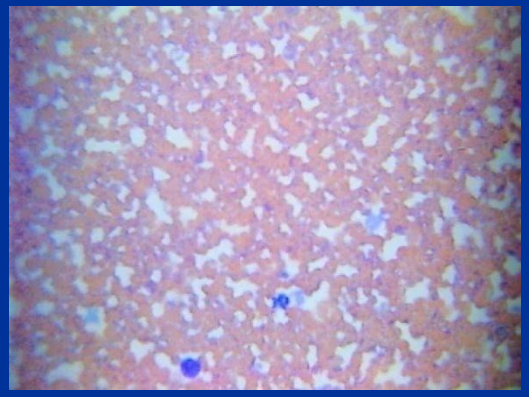
Microscopic Views



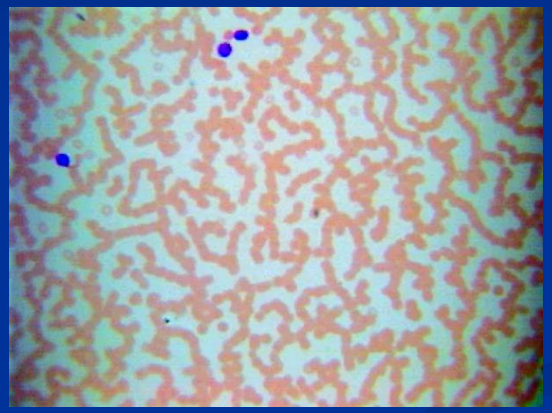
Bird Blood



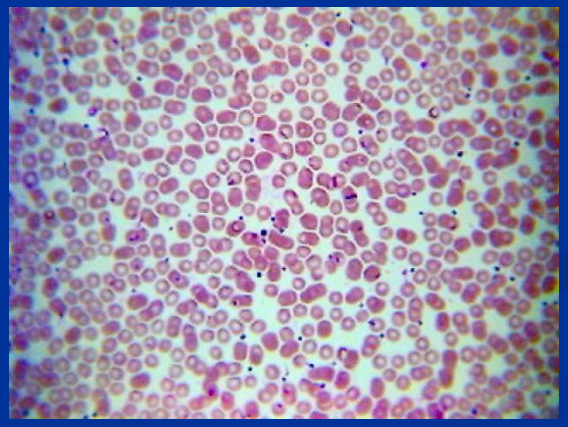
Cat Blood



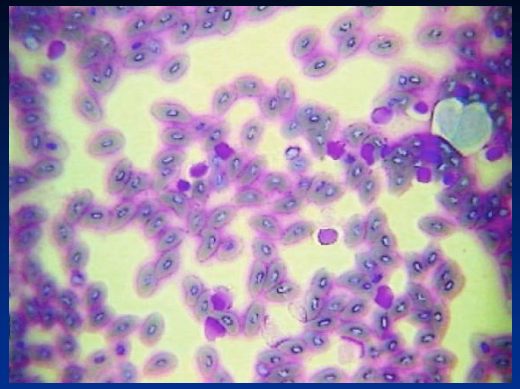
Dog Blood



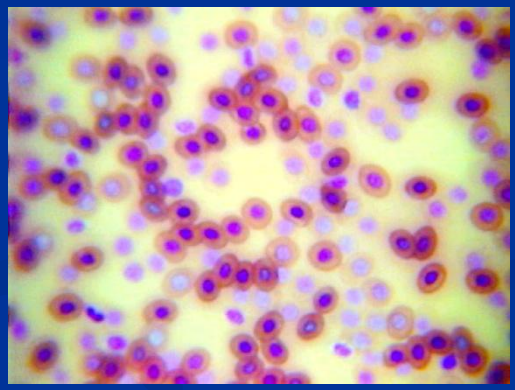
Horse Blood



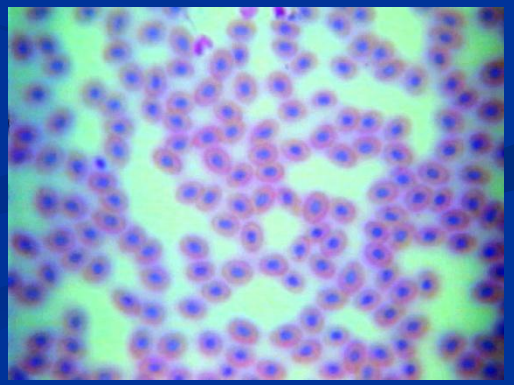
Human Blood



Fish Blood



Frog Blood



Snake Blood

What is blood?

- A highly specialised circulating tissue which has several types of cells suspended in a liquid medium called plasma.
- Origins from Greek '*haima*'
- Blood is a life sustaining fluid
- Blood is an amazing fluid!
- Keeps us warm
- Provides nutrients for cells, tissues and organs
- Removes waste products from various sites

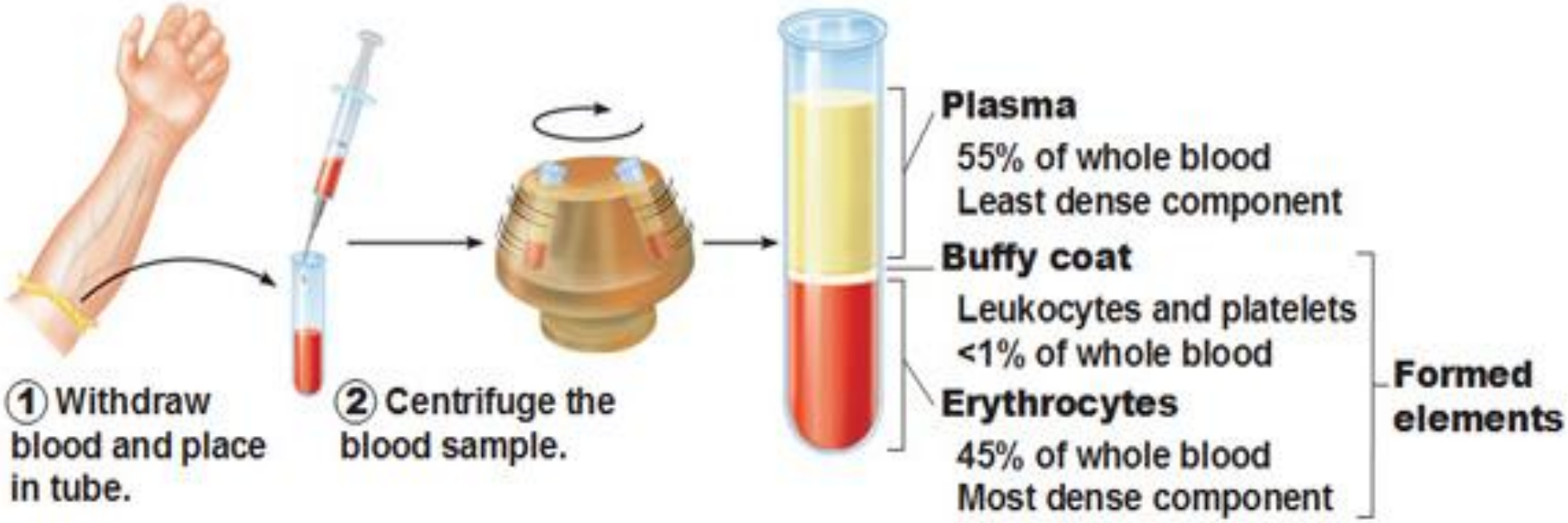
Blood is technically a specialized connective tissue. It is made up of blood cells and plasma.

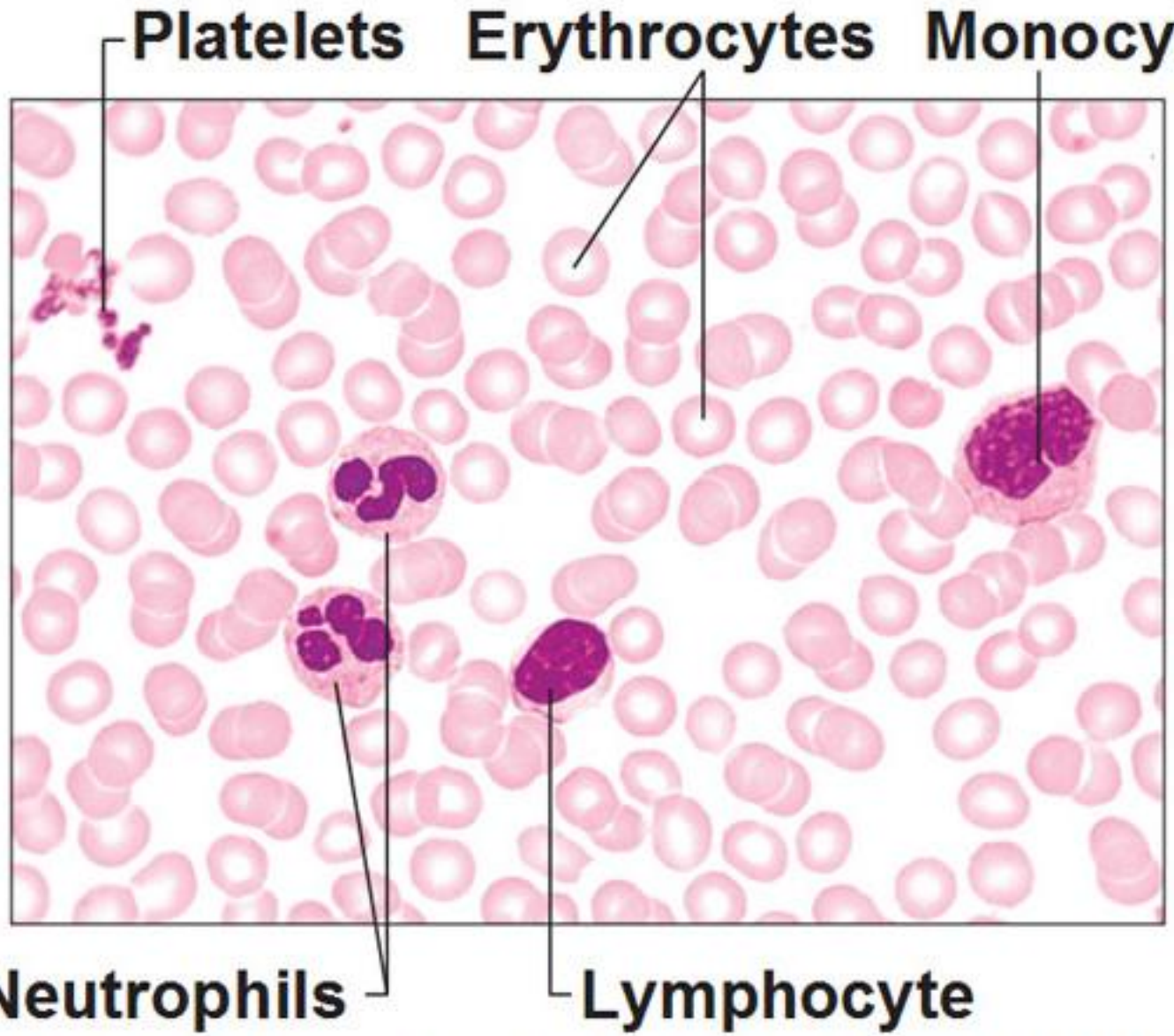
Major Components of Whole Blood

Hematocrit = %-age of blood volume that is RBCs

Males = 47% +/- 5%

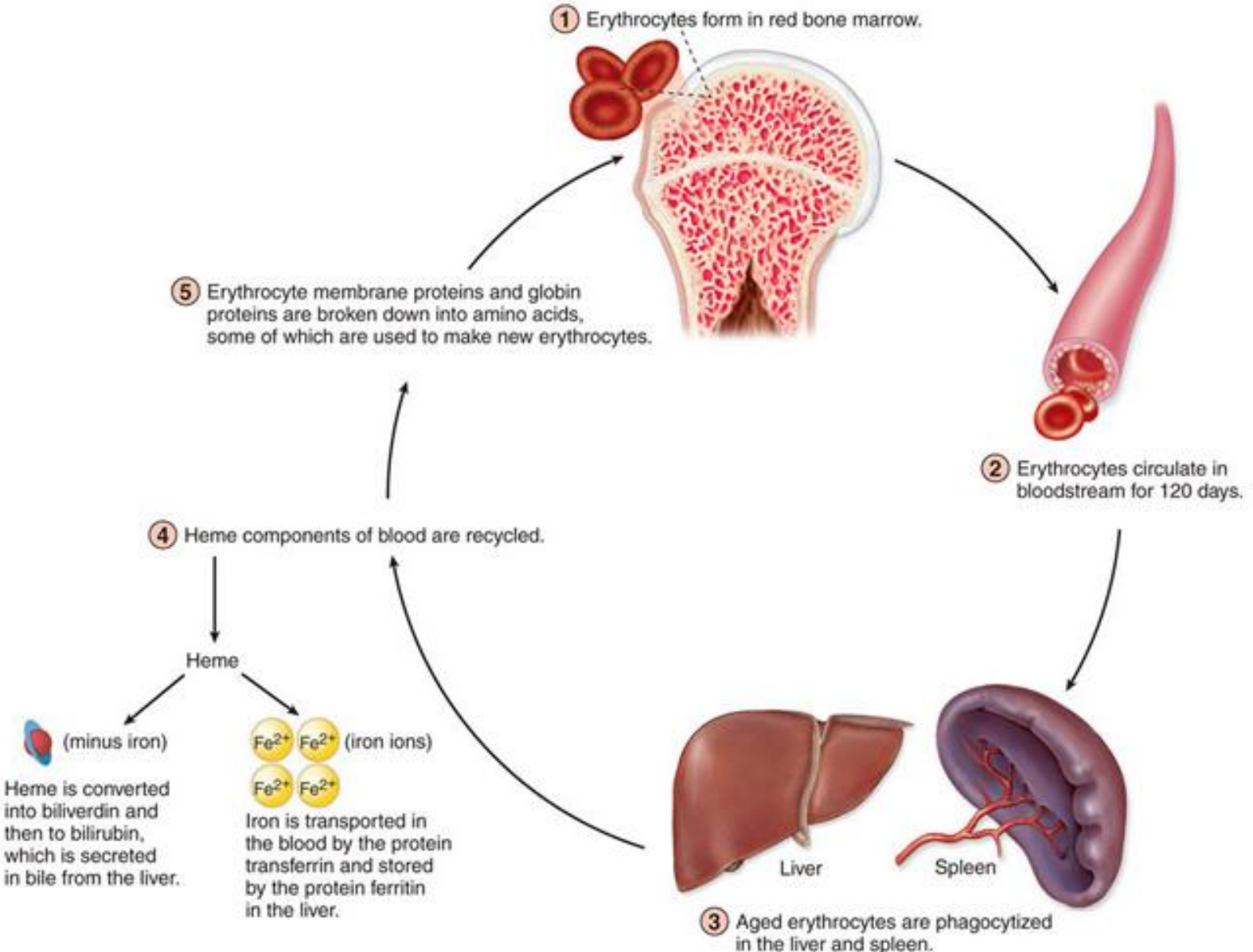
Females = 42% +/- 5%





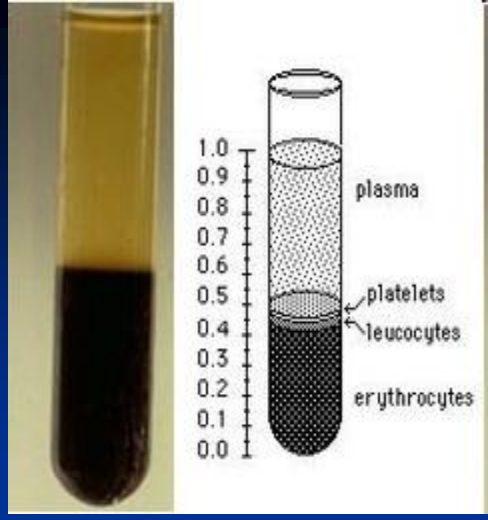
Photomicrograph of a human blood smear, Wright's stain (715×)

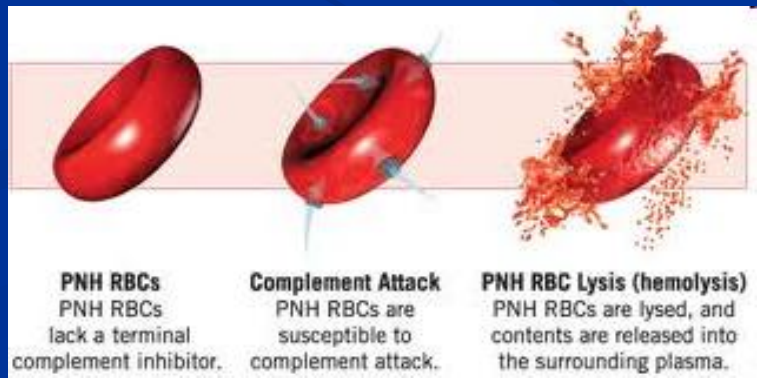
Recycling the Components of Aged or Damaged Erythrocytes



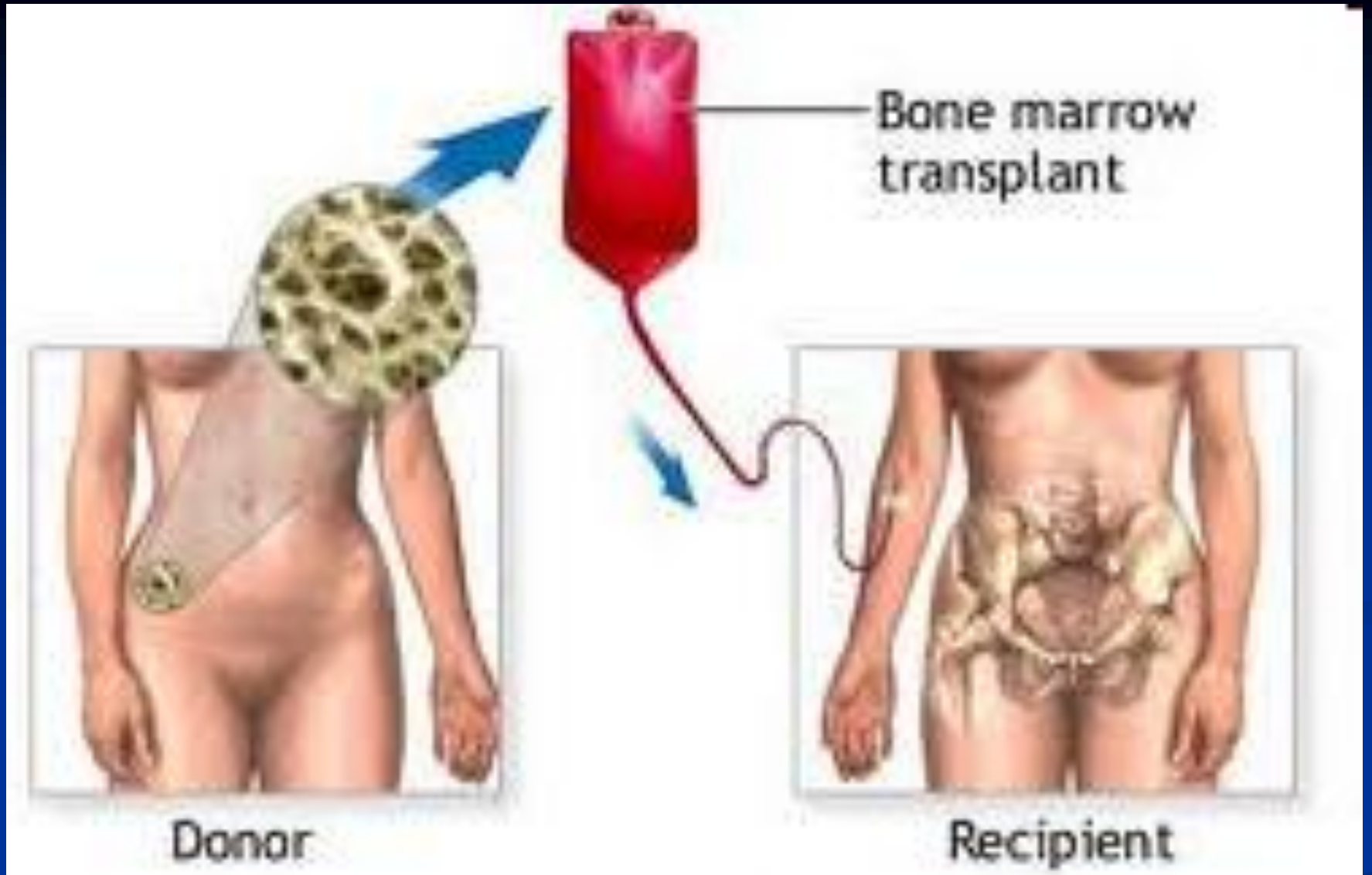
- **Serum** is the clear, straw yellow fluid that separates from the RBC's AFTER coagulation
- **Plasma** is the fluid that separates from unclotted RBC's (It is a slightly turbid/cloudy yellow color similar to plasma
- **Hemolysis** is the appearance of free hemoglobin in serum/plasma due to RBC destruction

Hemolyzed serum/plasma is UNSUITABLE for laboratory analysis

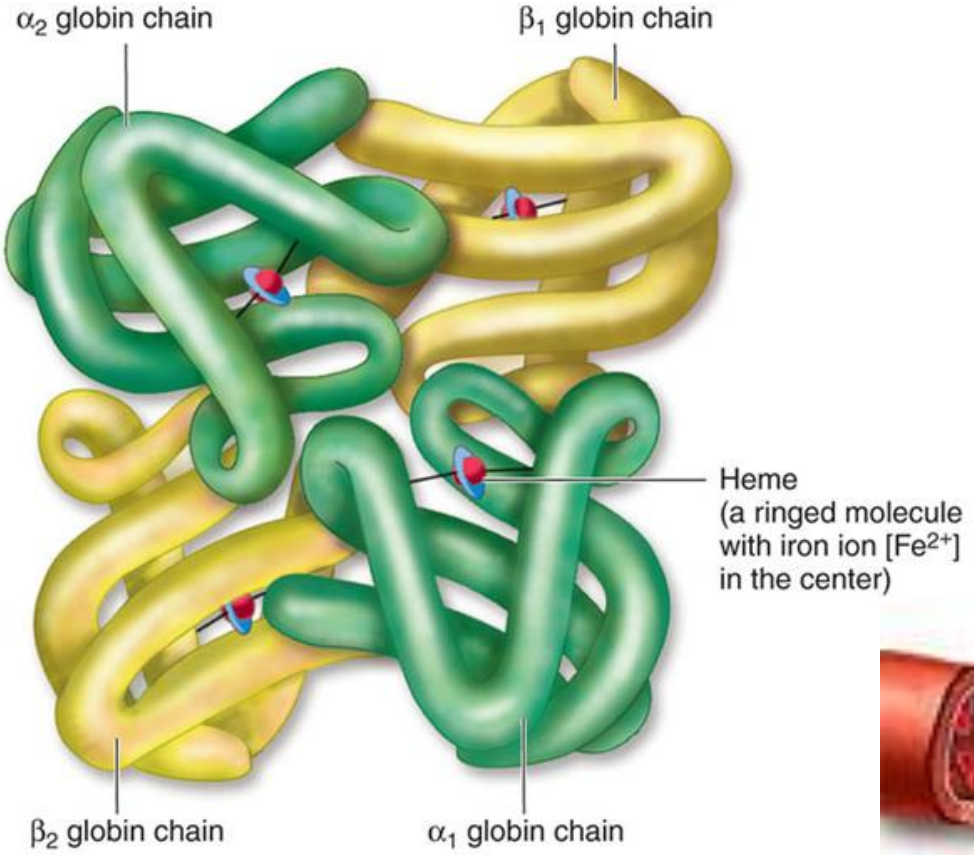


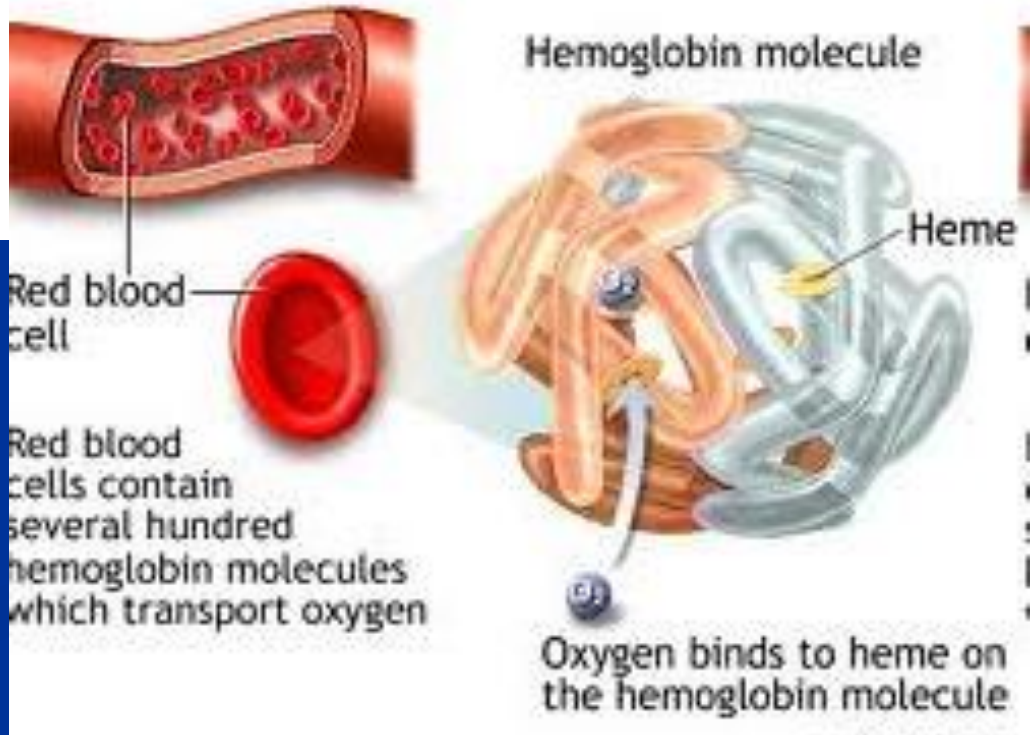


- **TRANSFUSIOLOGY** – transfusio - transfusion, mixing, logos – a science – the section of a medical science on management of organism functions by purposeful action on morphological structure of a blood with the help of transfusion of an integral blood, its components, and also blood substitutes.
- **bone marrow transplantation** - The bone marrow in the breast bone, skull, hips, ribs and spine contains stem cells that produce the body's blood cells which include white blood cells (leukocytes), which fight infection; red blood cells (erythrocytes), which carry oxygen to and remove waste products from organs and tissues; and platelets, which enable the blood to clot


Bone marrow transplant
Donor
Recipient
Donor bone marrow cells repopulate recipient bone marrow

Molecular Structure of Hemoglobin



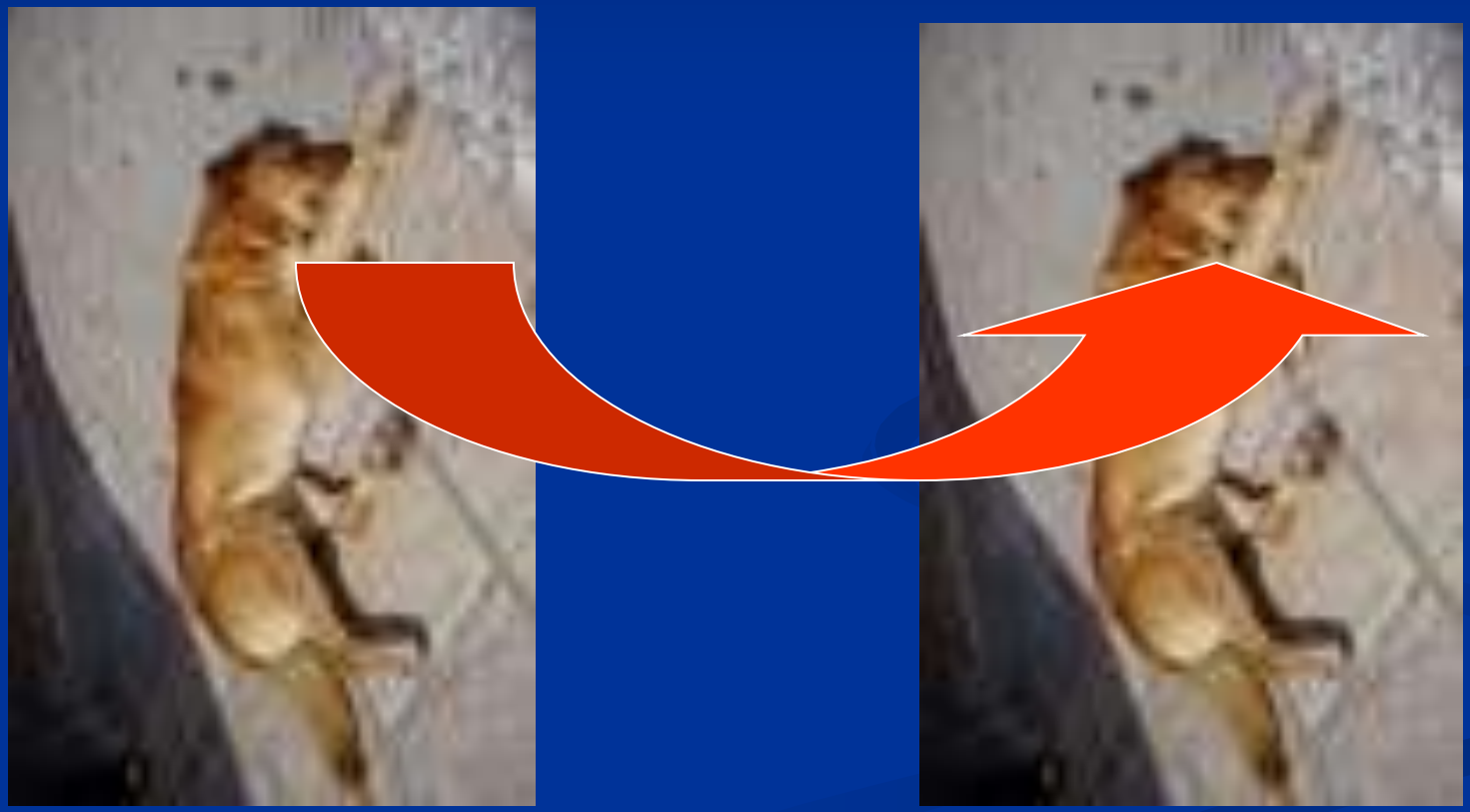


Blood in History

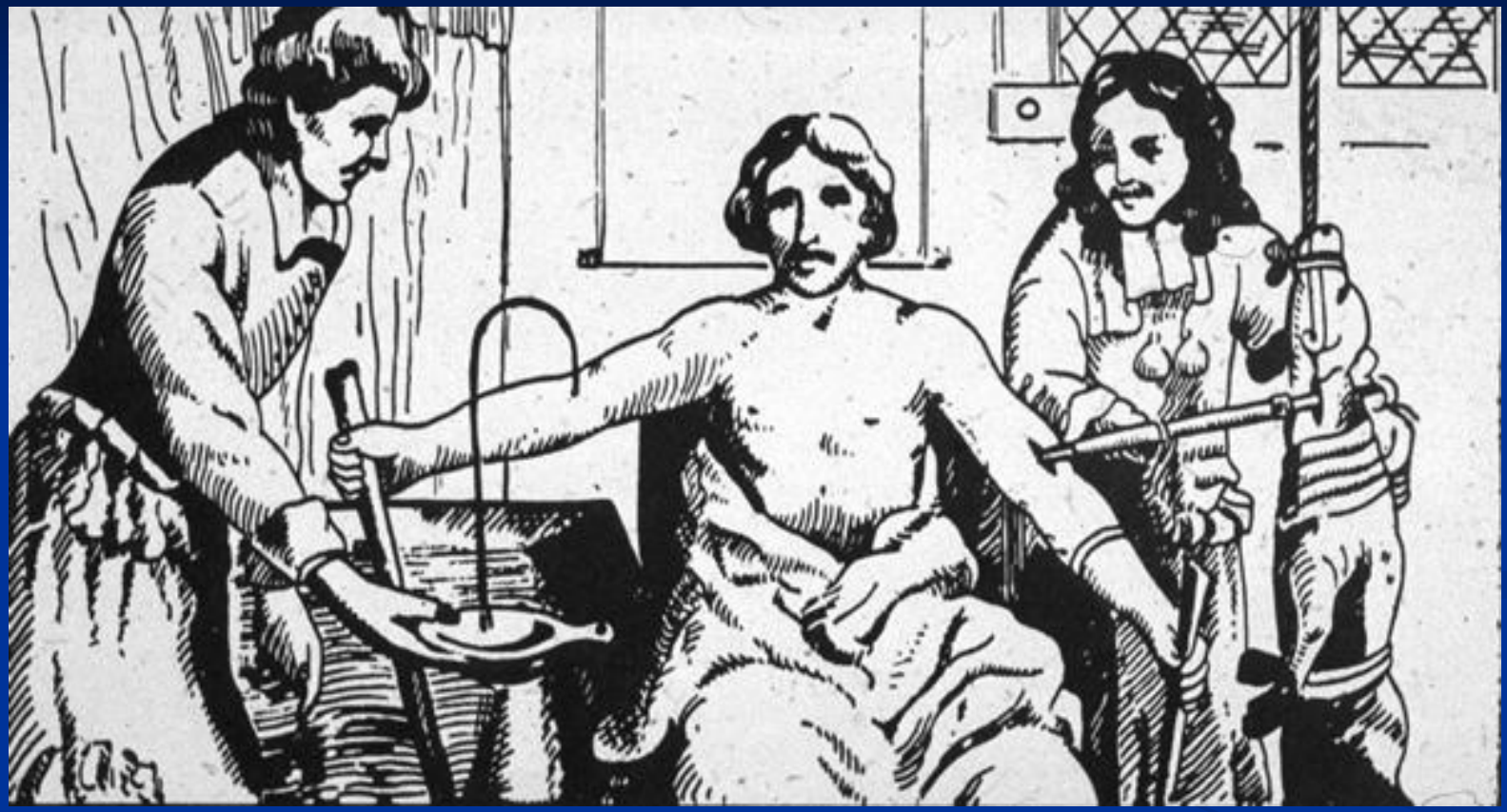
- The first documentary hemotransfusions were carried out in the XVII century from sheep and calfs - to the Human
- Hemotransfusions of the human to the human appeared on a regular basis in the beginning the XIX century — in England.

- China, 1000 BC
- The soul was contained in the blood.
- 1665: Dog to dog transfusion
- Egyptians bathed in blood for their health.
- Pliny and Celsus describe Romans drinking the blood of fallen gladiators to gain strength and vitality and to cure epilepsy.
- Taurobolium, the practice of bathing in blood as it cascaded from a sacrificial bull, was practiced by the Romans.

1665- from ***DOG to DOG TRANSFUSION***

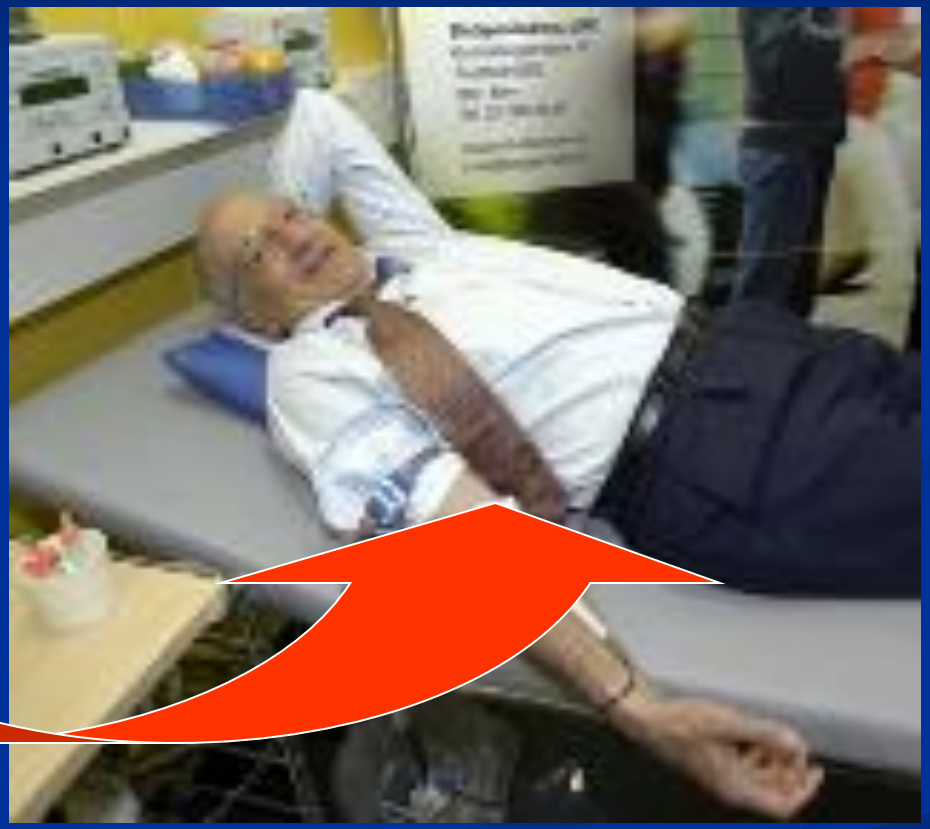


Animal to Human Transfusion

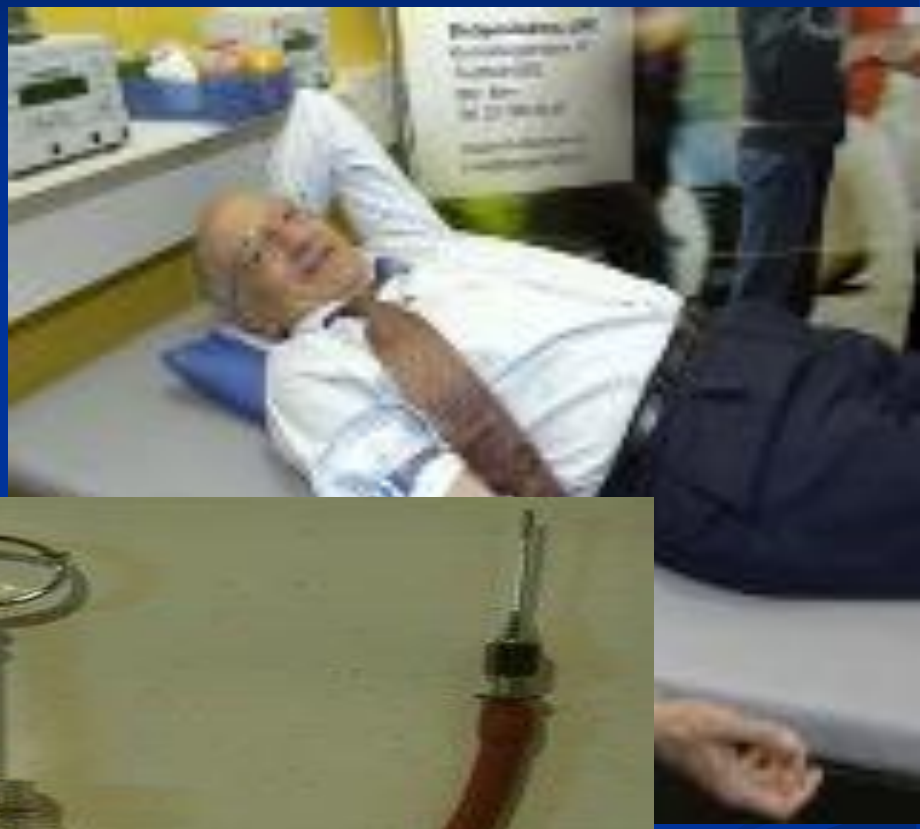


Early lamb blood transfusion

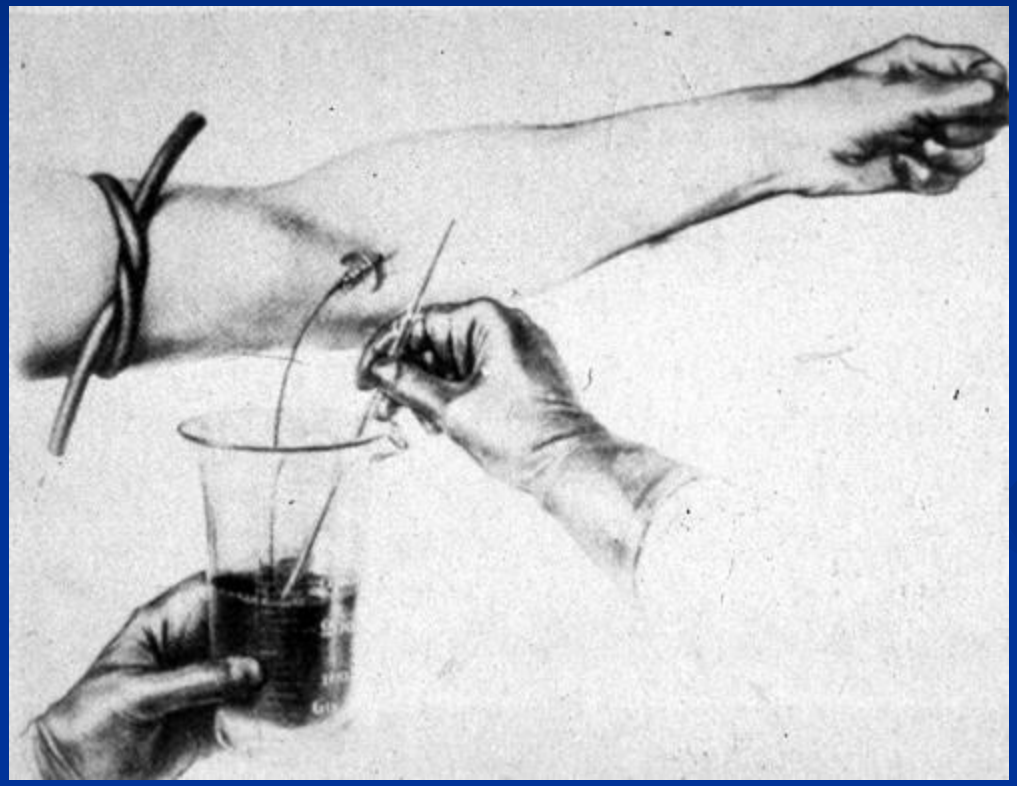
1667-*SHEEP TO HUMAN TRANSFUSION*

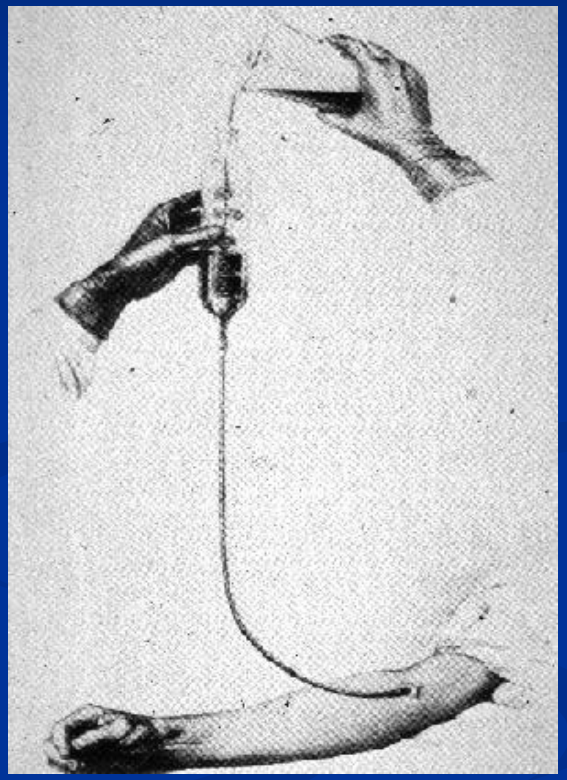


1818- ***HUMAN TO HUMAN TRANSFUSION***



Lewisohn's Method of Transfusion





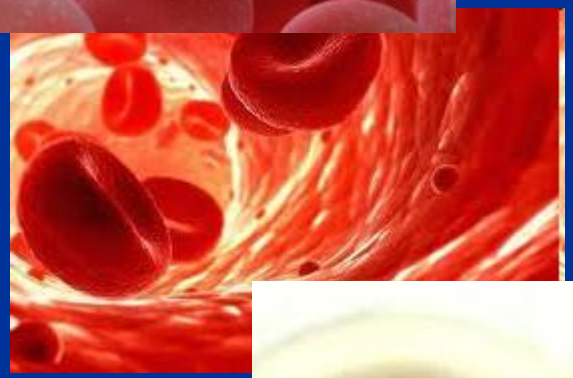
Blood is collected in a citrated flask…….and immediately transfused.

Transfusion of drags and blood components the modern medicine is surveyed as operation of transplantation of a tissue from the donor to the recipient. At this operation by a necessary condition immunologic compatibility and infectious safety of transfusion mediums is.

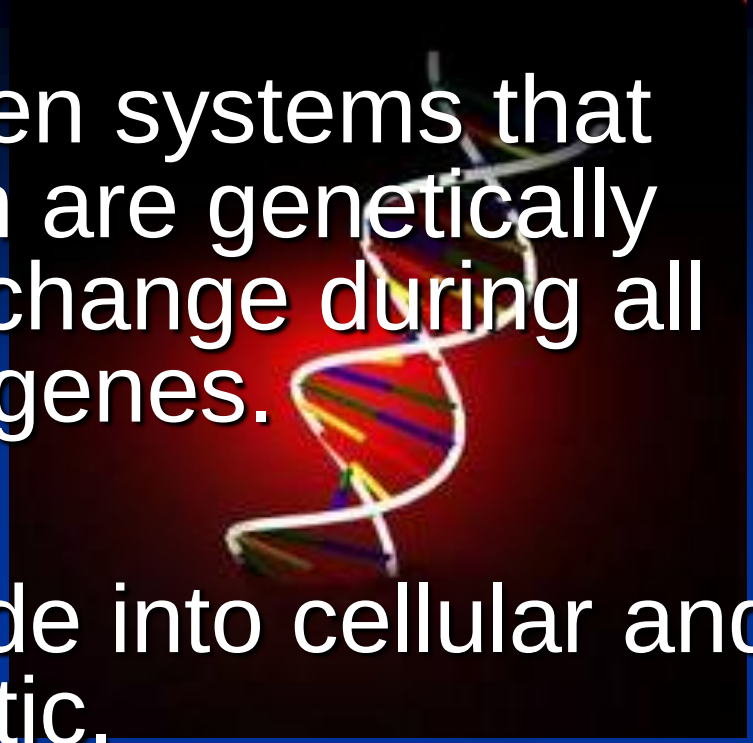
TRANSFUSION AGENTS

- - **Blood and its components** (Packed red cells, Platelets, Fresh Frozen Plasma, Frozen plasma, Cryoprecipitate, Albumin, Immunoglobulins).
- ***Transfusion of blood and its components is called a hemotransfusion***
- - **Blood substitutes** - the medical solutions intended for replacement of the lost blood or normalization of broken functions of a blood.
- **The donor** - people who give the blood (or an organ) for introduction (transplantation) by the patient.
- **Recipients** it is patients who receive donor organs and tissues.

What is blood grouping?



Blood type - it the antigen systems that discover in blood of man are genetically inherited, that does not change during all life, controlled by allelic genes.



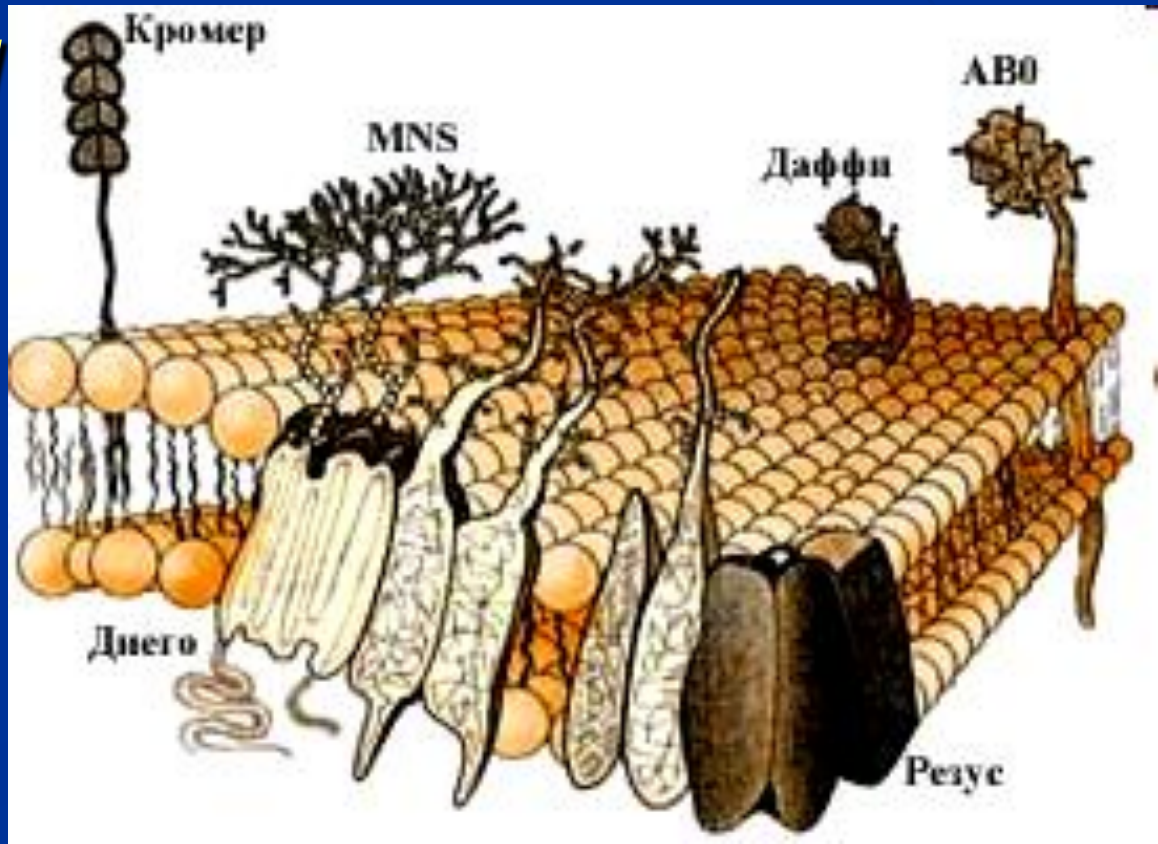
All antigens of blood divide into cellular and plasmatic.

Cellular antigens have a basic value in transfusioligy . They are divided for **erhytrocytic (red cells), leucocytic, thrombocyte**.

Over **300 antigens** of red cells, that form over 25 antigen systems, are known.

A clinical value is had: ABO, rhesus factor, Kell, Duff, MNSs, Kidd, Levis, Lutherans, Diego, Kromer.

A model of membrane of red cells is with the built-in molecules of blood of the different systems types.



Basic in Transfusiology

is the antigen systems of **AB0** and **Rh-factor**, as they are most **adjuvant**

Adjuvanticity (иммунногеность)- to induce ability of antigens products of antibodies, if they get in an organism in that such antigens are absent.

Therefore the antigen system AB0 has a basic value in compatibility of blood at transfusion.

Compatibility is combination of blood of donor and recipient for to the antigens and antibodies, that does not cause immunological co-operation (взаимодействия).

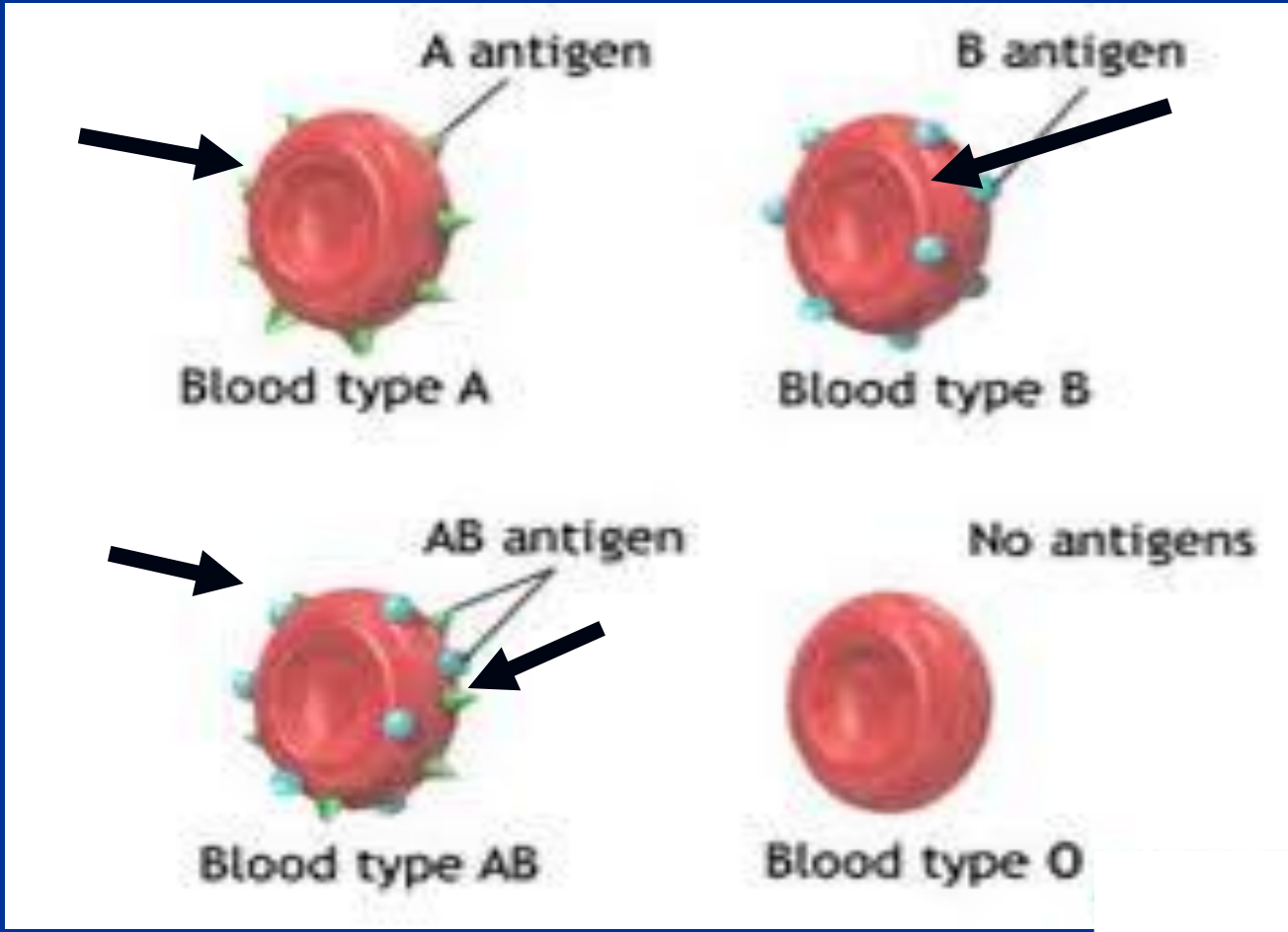
CLASSIC BLOOD TYPES by system of ABO:

Depending on a presence in the red cells of **agglutynogens A** and **B**, and in the serum of blood of corresponding to them **agglutinings** α and β, distinguish **4 blood' s groups.**

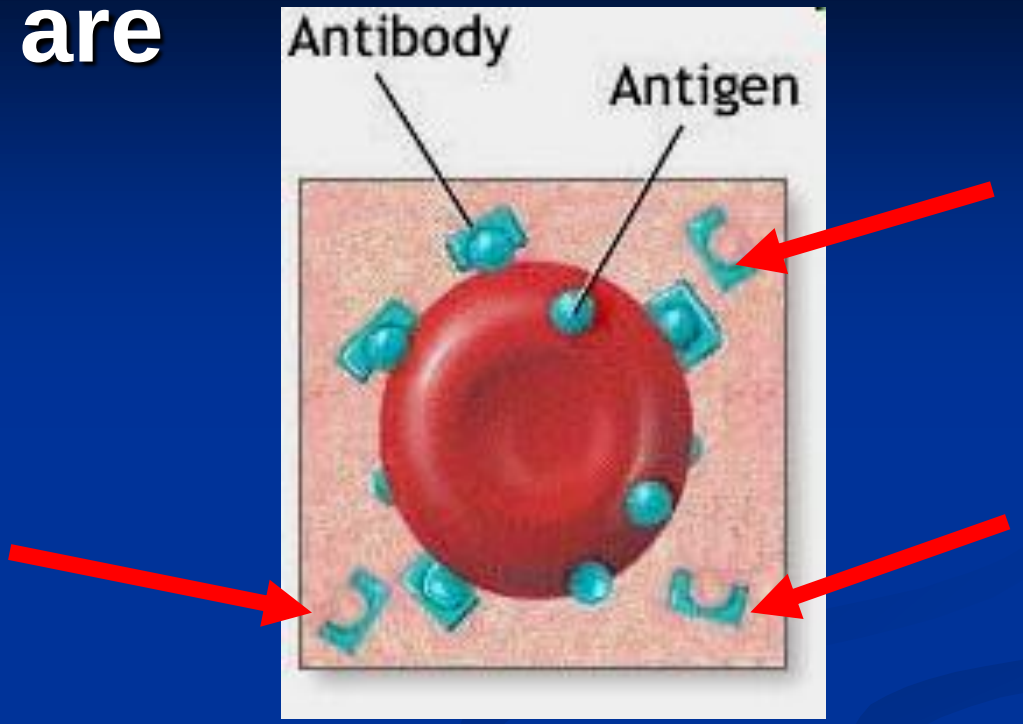
AB0 system

On the membranes of erythrocytes

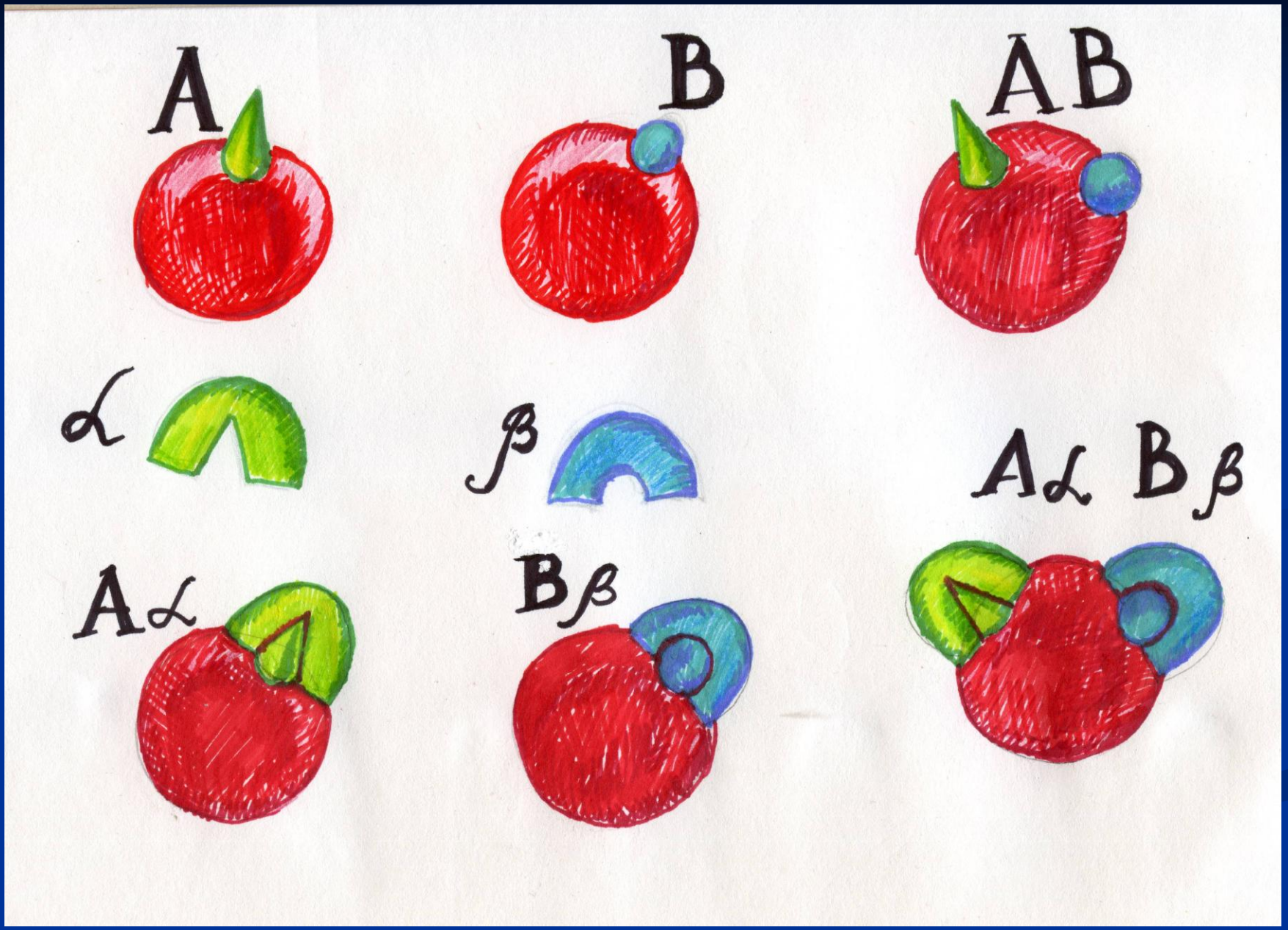
antygens – agglutinogens are



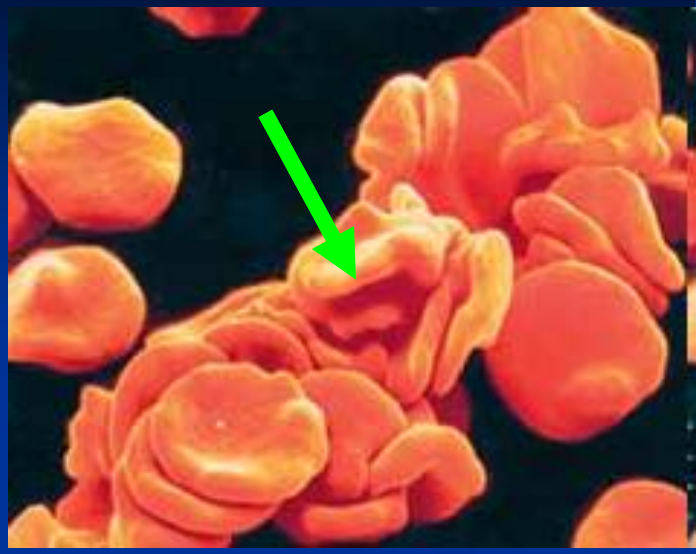
- In plasma of blood the **antybody – agglutinins** are



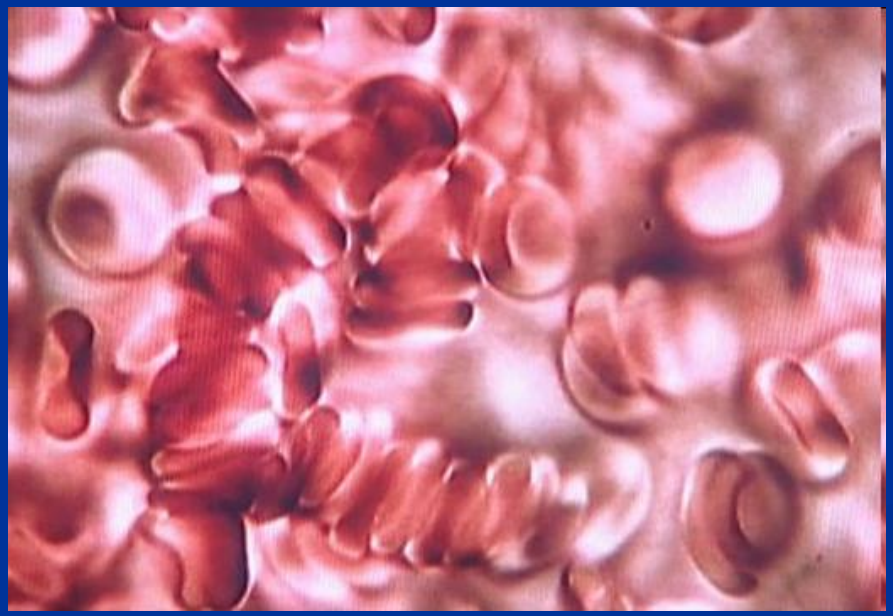
Accordingly there are of the same name antigens and antibodies in blood of human, can not be simultaneously, as at presence of such the reaction of hemagglutination occur

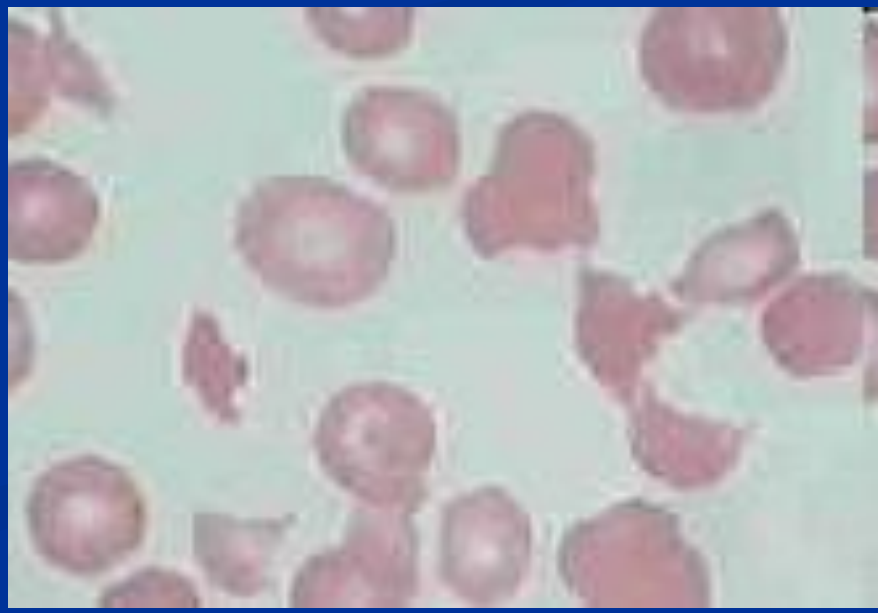

A
B
AB
α
β
Aα Bβ
Aα
Bβ

Such look has a thrombus - a clot from erythrocytes which were adhered



Hemolysed erythrocytes have such look





- Each blood type define a combination an agglutinins and agglutinogens. So, in the first group there are agglutinins α and β and agglutinogen 0; in second accordingly - A and β; in third- B and α; and in fourth AB0.
- Indication of blood type is accepted on presence of agglutinogen: 0(I); A(II); B(III); AB(IV).

O(I) – in erythrocytes agglutinogen it is not, in the serum of agglutinins α and β are

A(II) - in erythrocytes agglutinogen A is, in the serum of agglutinin β is

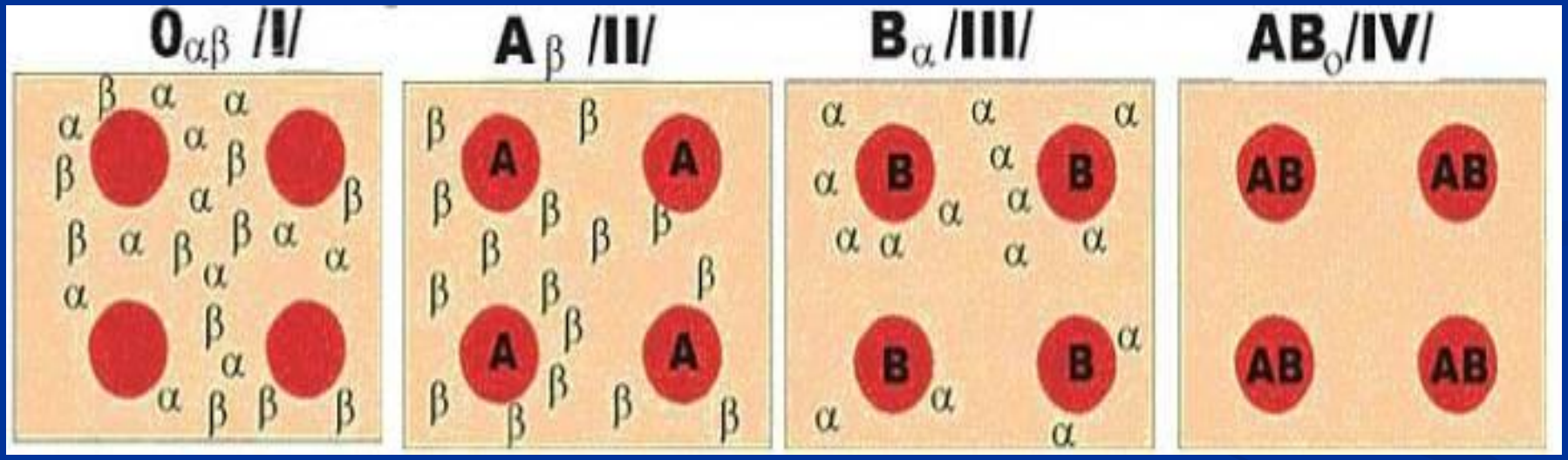
B(III) - in erythrocytes agglutinogen B is, in the serum of agglutinins α is

AB(lV) - in erythrocytes agglutinogens A and B are, in the serum the agglutinins absent.

The ABO Blood System

Blood Type (genotype)	Type A (AA, AO)	Type B (BB, BO)	Type AB (AB)	Type O (OO)
Red Blood Cell Surface Proteins (phenotype)	 A agglutinogens only	 B agglutinogens only	 A and B agglutinogens	 No agglutinogens
Plasma Antibodies (phenotype)	 b agglutinin only	 a agglutinin only	 No agglutinin	 a and b agglutinin

Serological characteristics of Blood Groups



- **The specific substance which is designated by a symbol "O" was found in the first blood type 0 (I).**
- **This factor is agglyutinogen, which is in erythrocytes of blood types about (I), A_2 (II), (IV) A_2B.**
- **For erythrocytes of all groups presence of a substance of "H" which is considered the general substance precursor** (попередник) **is characteristic.**

Some inhabitants of the Indian city of Bombay have a certain blood type, which doesn't contain agglutinogen O, A, B, H, but contains antibodies - α, β, anti-0 and anti-H.

Type "Bombay"

agglutinogen O, A, B, H - No, but contain antibodies α, β, anti-0 and anti-H.

Кров'яні химери

- коли частини еритроцитів зібрані в аглютинати, а інші залишаються вільними. Це одночасне перебування популяцій еритроцитів, що належать двом фенотипам АВО. Вони відрізняються по групі крові та іншим антигенам.

Трансфузійні химери виникають в результаті багаторазового переливання еритроцитарної маси чи еритроцитів I групи крові реципієнтам іншої групи.

Істинні химери зустрічаються у гетерозиготних близнюків, а також після пересадки аллогенного кісткового мозку.

- Визначення групи крові при кров'яних химерах утруднено, оскільки в деяких випадках половина еритроцитів, що циркулюють в кров'яному руслі, мають одну групу крові, а друга половина – іншу.
- Реципієнту, який має кров'яну химеру переливають еритроцитарну масу, яка не має антигенів, по відношенню до яких у реципієнта можуть бути антитіла.

BLOOD TYPE DETERMINATION on ABO SYSTEM:

- - by means of standard isogemagglutinated Serums
- - by means of standard isogemagglutinated Serums and standard erythrocytes (a **crossmatch** way)
- - by means of Monoclonal Blood Grouping Antibodies : (Anti-A and Anti-B).

BLOOD GROUPING DETERMINATION BY STANDARD ISOHEMAGGLUTINATED SERUMS

FOR DEFINITION IT IS NECESSARY:

- Two complete sets of standard isohemagglutinated serums 0(I), A(II), B(III) the groups of two various series
- one ampoule of serum of AB(IV) (in every ampoule with a serum put a dry clean pipette)
- a small bottle with isotonic solution of Sodium chloridum with a pipette
- cleanly washed-up dry dish(PLANE-TABLE)
- glass rods
- sterile spear-shaped needles are for the puncture of finger
- sterile gauze globules
- alcohol.

- determination make indoors with good lighting at a temperature

  15-25ºC.

- Serums make in the special serum laboratories from donor blood.
- Serums keep in a refrigerator at a temperature **4-8˚C**.

A PASSPORT of SERUM is a label on that:

- it is the marked blood type
- it is a number of series
- it is a titer
- it is a period of storage
- it is a place of making

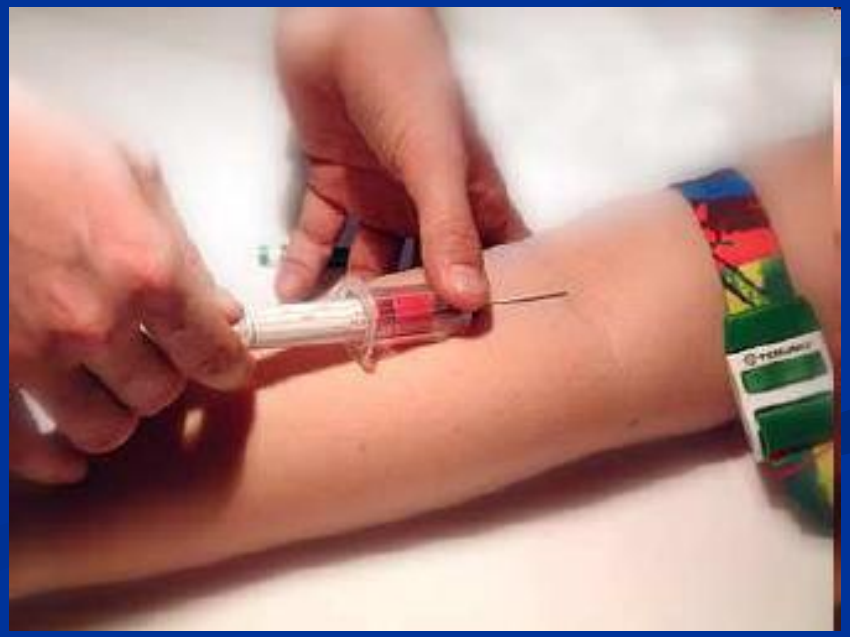
- A **SERUM MUST BE** transparent, without the signs of rotting, ampoule stored.
- A presence of flakes, sediment, dimness is the signs of uselessness of serums. Serum with the delayed period of storage can't be used.
- For convenience (comfort) the standard isohemagglutinated serums of various groups, write with the certain marking and tint:
- 0(I) - colourless(grey); strakes are not
- A (II) - blue; two strakes of blue color
- B(III) - red; three strakes of red color
- AB(IV) - bragtly- yellouw four strakes - yellow

Titer of serum

- must be not below 1: 32(for a serum B(III) not below 1: 16/32); activity is high: the first signs of agglutination must appear not later than 30 seconds.
- **Titer of serum** is that it maximum dilution at which the agglutination test can occur.

Equipment of carrying out reaction

- A reaction is conducted at a room temperature - **15-25°C**
- Blood for research is taken from a finger or from a vein



- On a dish (plate, plane-table) put a standard isohemagglutinated serums under corresponding notations of blood (I, II, III of groups in a volume 0,1 ml is one large drop an about 1 cm in a diameter) type.

- For prevention of errors at determination, put two series of serums each of groups because one of series can have low activity and not dates of clear agglutination. Thus, on a plate get 6 drops that form 2 rows for 3 drops in each in the next order on the left on the right : 0(I), A(II), B(III).

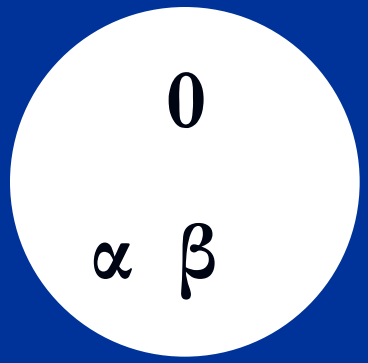
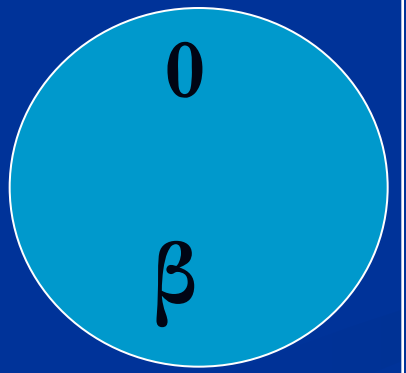
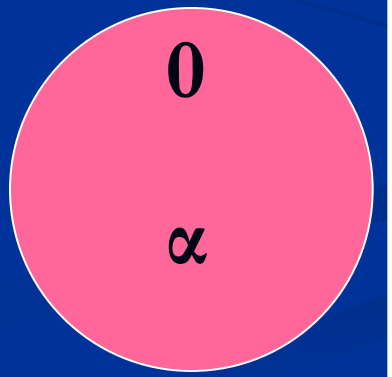
- Then investigated blood on one little drop - 0,01 ml consistently (послідовно) put by dry glass rod on a plate in 6 points, each alongside with the drop of standard serum
- an amount of the investigated blood must be approximately **in 10 times less** amount of standard serum, with that she mixed -
- hat is ratio 1: 10

That is ratio 1:10.

- after that every drop of blood and serum is mixed by means of glass rods round-edged.

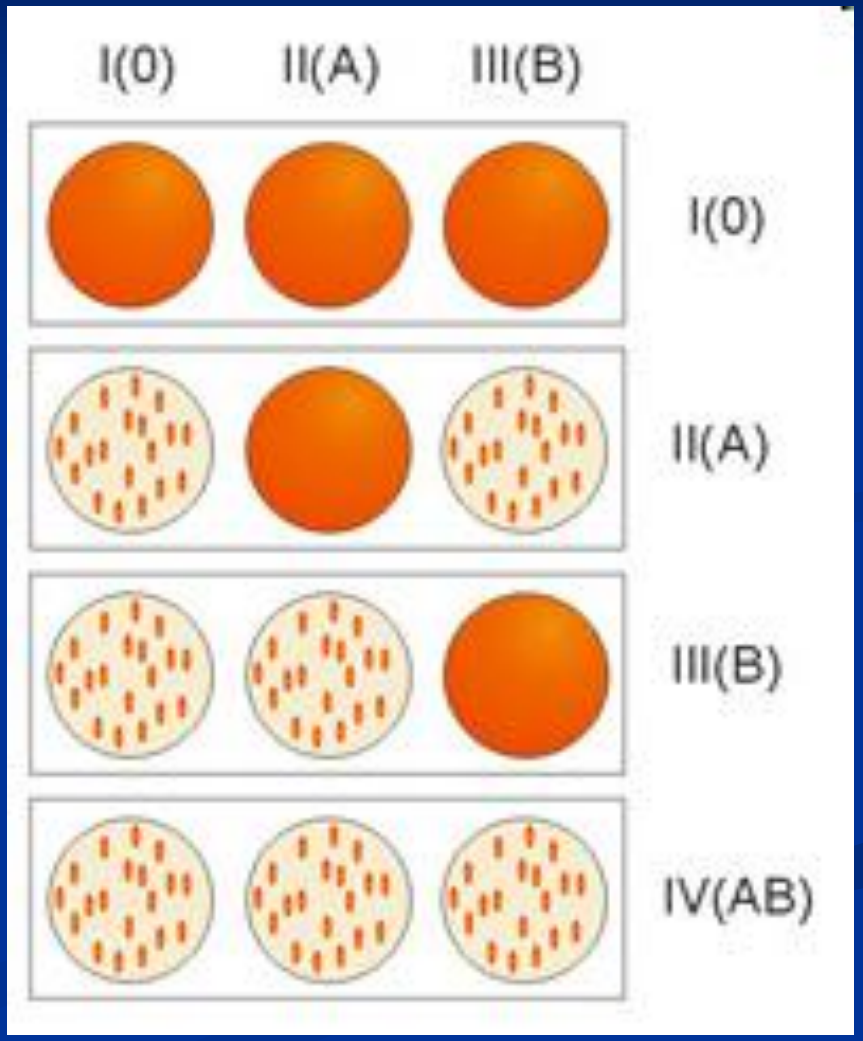
- After mixing a dish is periodically rocked.
- Agglutination begins during the first 10-30 seconds.
- Observation is necessary for carrying out to **5 minutes** through possibility of later agglutination.
- When agglutination occur, but not earlier than after 3 min, in a drop add **isotonic solution of chloride of natrium** on one drop, after that estimate a result

1. Standard serums all three groups in both series are not caused by agglutinations of red cells. It specifies on that red cells do not contain agglutinogen A and B, that that is the studied sample of blood belongs to the **group 0(I).**

Standard serums			studied erythrocytes
0 (I) αβ	A(II) β	B(III) α	
- 	- 	- 	0 Blood typing 0(I)αβ

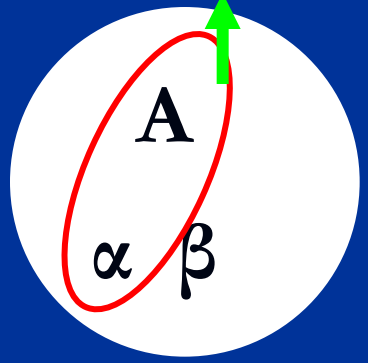
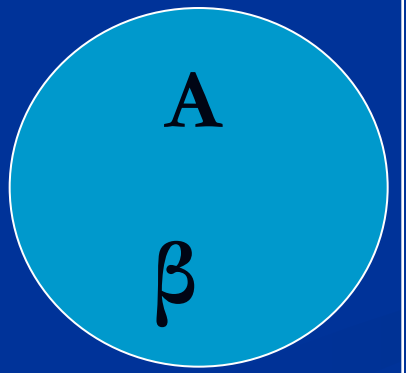
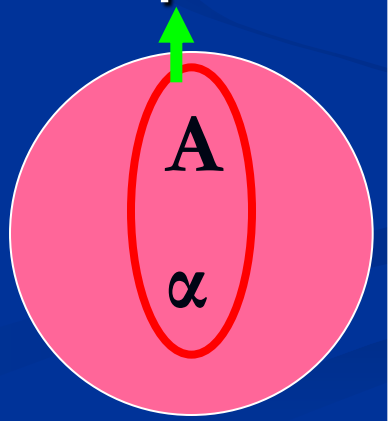
ESTIMATION OF RESALT

Blood typing
0 (I) αβ
A (II) β
B (III) α
AB0 (IV)

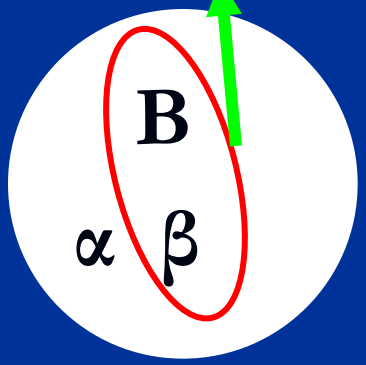
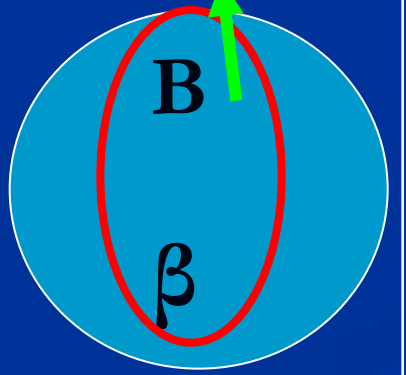
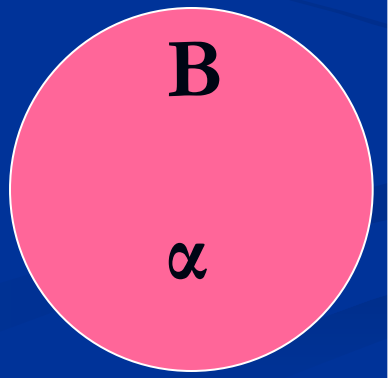


2. The reaction of isohemagglutination is negative with a serum A(II) groups of both serums and positive with serums 0(I) and B(III) groups. The red cells of the investigated sample of blood contain agglutinogen A.

Investigated blood A(II).

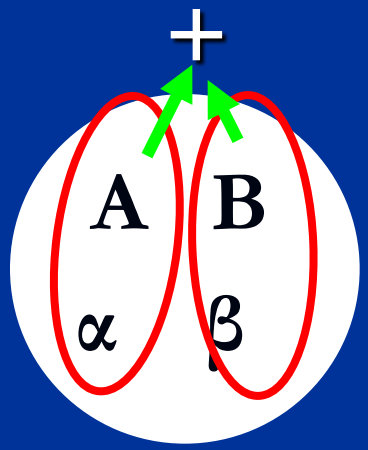
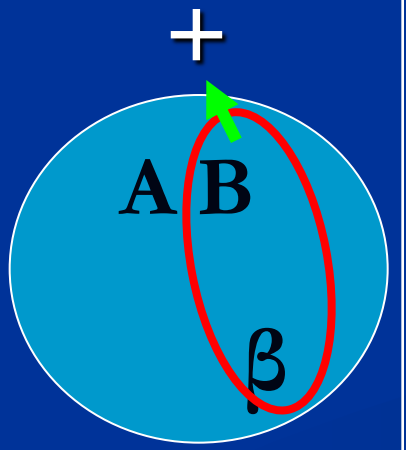
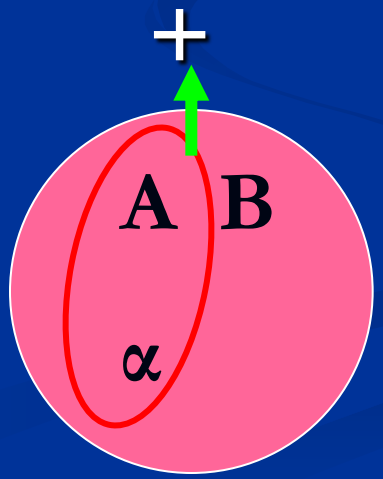
Standard serums			studied erythrocytes
0 (I) αβ	A(II) β	B(III) α	
+ 	- 	+ 	A Blood typing A(II)β

3. The reaction of isohemagglutination is negative with a serum B(III) groups of both serums and positive with serums 0(I) i A(II) groups. The red cells of the investigated sample of blood contain agglutinogen B. **Investigated blood B(III).**

Standard serums			studied erythrocytes
0 (I) αβ	A(II) β	B(III) α	
+ 	+ 	– 	B Blood typing B(II)α

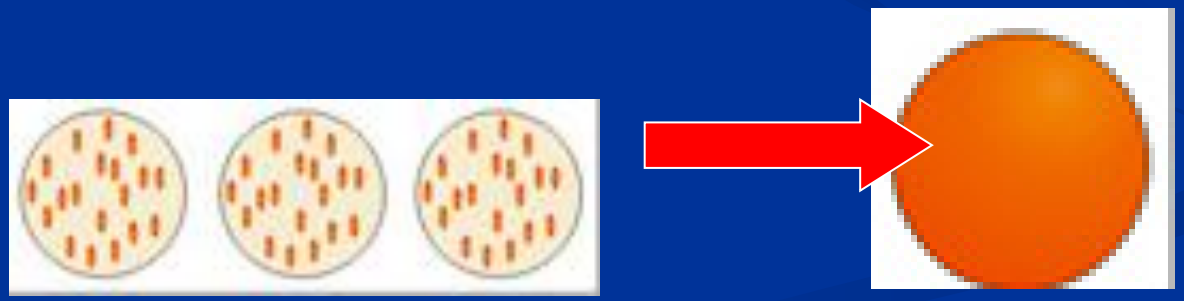
4. Standard serums all three groups give a positive reaction in both series. The investigated blood contains аглютиногени A and B.

Investigated blood AB(IV).

Standard serums			studied erythrocytes
0 (I) αβ	A(II) β	B(III) α	
			AB Blood typing AB(II)

1. **Standard serums all three groups in both series are not caused by agglutinations of red cells. It specifies on that red cells do not contain agglutinogen A and B, that that is the studied sample of blood belongs to the group 0(I).**
2. **The reaction of isohemagglutination is negative with a serum A(II) groups of both serums and positive with serums 0(I) and B(III) groups. The red cells of the investigated sample of blood contain agglutinogen A.**
   Investigated blood A(II).
3. **The reaction of isohemagglutination is negative with a serum B(III) groups of both serums and positive with serums 0(I) i A(II) groups. The red cells of the investigated sample of blood contain agglutinogen B. Investigated blood B(III).**
4. **Standard serums all three groups give a positive reaction in both series. The investigated blood contains аглютиногени A and B.**
   Investigated blood AB(IV).

- However for a final conclusion in relation to IV of blood type it is necessary to do the reaction of isohemagglutination with the standard serum of AB(IV) of group on the same methodology. The negative reaction of isohemagglutination allows finally to take the investigated blood to AB(IV) of group.



The exposure of other combinations testifies to the error of determination of blood type of patient.

Mistakes at determination of blood type :

Mistakes at determination of blood types are possible in situations when at the actual presence of agglutination it isn't determined or agglutination is determined visually at its actual absence

I. Poor quality of reagents:

- **- suitability term** (срок годности)
- **- storage conditions** (условия хранения)
- **- appearance (transparence, smell, etc.)** (внешний вид прозрачность запах)

2. Technical mistakes:

1) non-compliance with external conditions

- а) bad illuminating intensity
- б) temperature increase (it is more 25°C - the slowed-down agglutination)
- в) low temperature (less 15°C - cold panagglutination)

2) wrong carrying out the reaction:

- а) disturbance of placement of serums on a dish (PLANE-TABLE)
- б) Serum and blood ratio
- в) connection of the next drops
- г) early estimation (special antigen A_2)

3) not use of physiological solution - PSEUDO-AGGLUTINATION - pasting of erythrocytes in monetary columns with conservation of integrity of membranes

4) features of a studied blood

- а) nonspecific panagglutination (low temperature of a blood, quality)
- б) Thompson's phenomenon (in 1927) - bacteriemic pollution of a studied blood. Thomson's phenomenon is characterized by blood agglutination with Serums of all groups and Serum of own blood.
- в) autoagglytinaion - fresh bloods that is described at a series of diseases: illnesses of a blood, splenomegaly, infectious diseases, etc.
- г) other features - change of properties of erythrocytes at different pathological conditions.
  - It can be shown in the raised agglutinability of erythrocytes which is observed at patients with a hepar cirrhosis, at combustions, a sepsis. Agglutinability can be so high that erythrocytes stick together in own Serum and physical solution.
  - At leukoses depression agglutinability of erythrocytes therefore a significant amount of erythrocytes remains not involved in agglutination even when using highly active standard reagents - an artificial bloody chimera is observed.

Determination of blood typing by standard isihemagglutining serum and standatd erhytrocite (cross-math method).

- Standard erythrocytes for determination of blood types

SHORT OF METHOD

Do at the same time recognition of agglutinogens in erythrocytes of a studied blood by means of standard Serums *and* identification of agglutinins that are in Serum of this blood by means of standard erythrocytes.

Technique of carrying out reaction

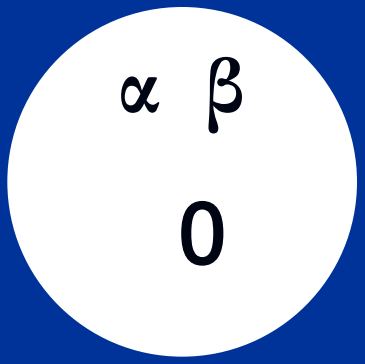
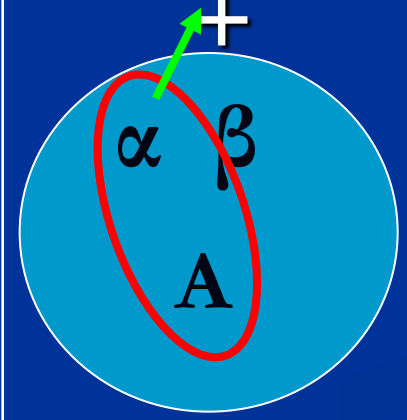
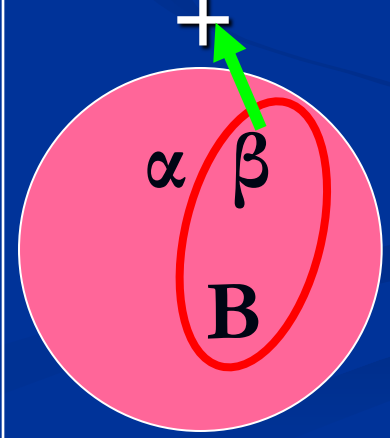
- Blood for research take in a dry test tube, centrifuge or leave alone for 20-30 minutes for serum unit.
- Determination carry out on a white plate, with a pipette in 3 places put on one **big drop of Serum** of a studied blood from a test tube (0,1ml)
- On the lower part of a plate also under the corresponding notation put on one small **drop of standard erythrocytes** in such order from left to right: 0(I), A(II), B(III).

- Reaction is carried out at room temperature 15-25°C
- Watch a reaction course not less than 5 minutes.
- Moderately agglutination emergence, but not earlier, than in 3 minutes, in those drops in which it came, adds on one drop of a **isotonic solution of chloride of natrium** then estimate result.
- At treatment of results estimate the data received at both reactions (with standard isohemagglutinated serums and standard erythrocytes).
- **Feature of treatment of results** of reaction with standard erythrocytes is that erythrocytes of group 0(I) is control because in them there are no antigens that does impossible a specific agglutination test with other Serum.

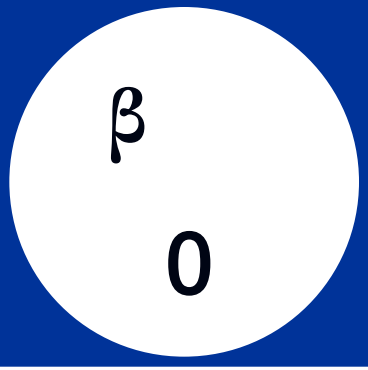
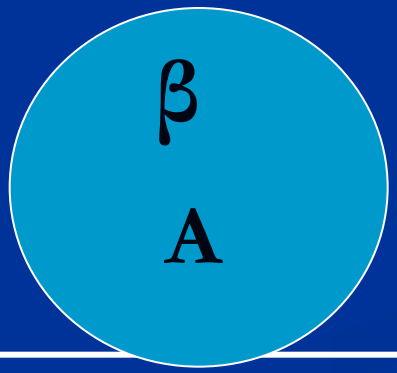
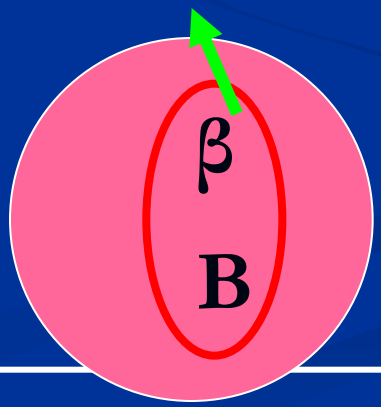
Estimation of results of determination of blood types by a cross method

Presence of agglutination at a reaction with the standard isohemagglutinated serums of the following groups :				Presence of agglutination at a reaction with the standard erythrocytes of the following groups :			Blood typing
0 (I)	A(II)	B(III)	AB(IV)	0 (I)	A(II)	B(III)	
-	-	-		-	+	+	0αβ(I)
+	-	+		-	-	+	Aβ (II)
+	+	-		-	+	-	Bα(III)
+	+	+	-	-	-	-	AB(IV)

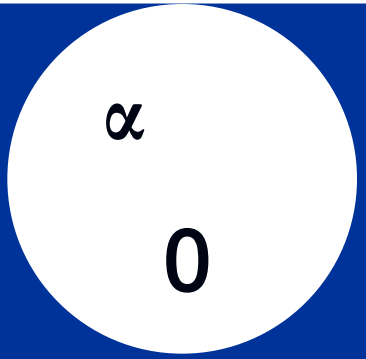
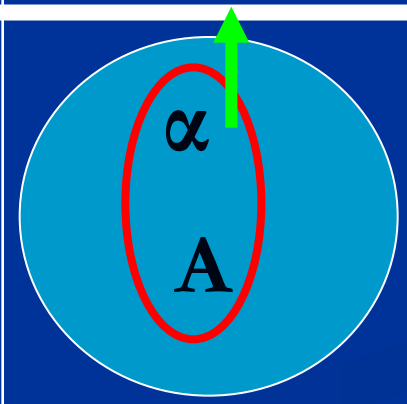
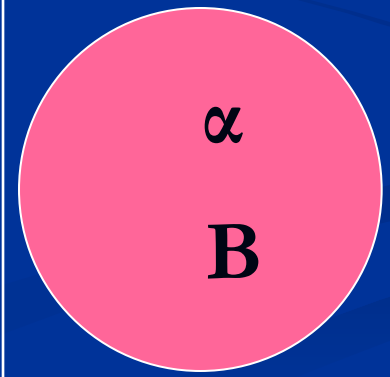
1. Standard serums II and III groups cause agglutination. It specifies that serum contains agglutinins α and β, that is the studied sample of a blood belongs to **group 0(I)αβ.**

Standard erythrocytes			Studied serum
0 (I) 0	A(II) A	B(III) B	
- 	+ 	+ 	αβ Blood typing 0(I)αβ

2. Standard serums III groups cause agglutination. It specifies that serum contains agglutinins β, that is the studied sample of a blood belongs to **group A(II)β.**

Standard erythrocytes			Studied serum
0 (I) 0	A(II) A	B(III) B	
-	-	+	β Blood typing A(II)β
			

3. Standard serums II groups cause agglutination. It specifies that serum contains agglutinins α, that is the studied sample of a blood belongs to **group B(III)α.**

standard erythrocytes			studied serum
0 (I) 0	A(II) A	B(III) B	
			β Blood typing B(III)α

4. Standard serums don't cause agglutination. It specifies that serum doesn't contain agglutinins, that is the studied sample of a blood belongs to **group AB(IV).**

standard erythrocytes			studied serum
0 (I) 0	A(II) A	B(III) B	
-	-	-	AB Blood typing AB(IV)
0	A	B	

Determination of blood of the system ABO types by means of monoclonal antibodies Anti-A and Anti-B

Monoclonal antibodies (**Seraclone**) Anti-A and Anti-B are used for determination of a blood type of the person of ABO system instead of standard isohemagglyutinated serums, by identification of antigens A and B in erythrocytes by standard antibodies which are in monoclonal antibodies.

MONOCLONAL ANTYBODY

- Monoclonal antibodies Anti-And and Anti-In are produced by two various hybridomas which are formed by a way of merge muscular an antibody - forming B-lymphocytes with cells of a mouse myeloma. The called monoclonal antibodies are presented by divorced ascitic liquid of mice carriers of a hybridoma which contains the M immunoglobulin against antigens A and B.
- Monoclonal antibodies give faster and more expressed agglutination test, than standard serums, and also full their standartisation allows to apply one series.
- Monoclonal antibodies anti-A and Anti-B are made in the form of liquid or bottle, liquid is painted in red (anti-A) and dark blue (anti-B) color.
- Store them in a refrigeration at temperature 2-8˚C, term of conservation of 2 years

EQUIPMENT of CARRYING OUT REACTION

- Determination make indoors with good lighting at a temperature **15-25ºC.**

On a porcelain plate put on one big drop of monoclonal antibodies Anti-A and Anti-B, nearby put a drop of a studied blood in 10 times of the smaller size

- Admix rods this drops.
- Plate slightly shake and watch reaction within 2-3 minutes.
- Reaction as a rule arises in the first 3-5 seconds and is characterized by formation of fine red units, and then and flakes.

ESTIMATION OF RESALTS

1. Agglutination is absent with monoclonal antibodies Anti-A and Anti-B - the blood *doesn't contain* agglutinogens **A** and **B -** a studied blood of **group O(I).**
2. Agglutination arises with monoclonal antibodies Anti-A, erythrocytes of a studied blood contain an agglutinogen **A** - a studied blood of **group A (II).**
3. Agglutination arises with monoclonal antibodies Anti-B, erythrocytes of a studied blood contain an agglutinogen **B -** a studied blood of **group B (III).**
4. Agglutination arises with monoclonal antibodies Anti-A and Anti-B. Erythrocytes contain an agglutinogens **A** and **B** - a studied blood of **AB (IV) group**.

The scheme of estimation of results with monoclonal antibodies

Presence of agglutination at a reaction with monoclonal antibodies		Blood typing
Anti-A	Anti-B	
-	-	O(I)αβ
+	-	A(II) β
-	+	B(III) α
+	+	AB(IV)

- Express-test – «Eldoncard», 73x105 мм.

Determination blood grouping for the AB0 system and Rh-factor i резус- RhD, control. The photo is pasted.

- 1 card = 1 test

Rh Factors

- Scientists sometimes study **Rhesus monkeys** to learn more about the human anatomy because there are certain similarities between the two species. While studying Rhesus monkeys, a certain blood protein was discovered. This protein is also present in the blood of some people. Other people, however, do not have the protein.
- The presence of the protein, or lack of it, is referred to as the Rh (for **Rhesus**) factor.
- If your blood does contain the protein, your blood is said to be Rh **positive** (Rh+). If your blood does not contain the protein, your blood is said to be Rh **negative** (Rh-).

A+ A-
B+ B-
AB+ AB-
O+ O-

Rh Factor

- System antigens a rhesus have ability to cause formation of alloimmune antibodies.
- Rh blood types were discovered in 1940 by Karl Landsteiner and Alexander Wiener.
- Rhesus (Rh) factor is an termostabile specific protein found on the surface of red blood cells
- On erythrocytes of the human there are 5 main antigens of rhesus system (**D**, C, c, E, e). From them the most immunogenic is **D [its complete notation** Rh-(D)]. A presence or absence of this antigen determines rhesus-belonging of blood : Human that have D- antigen, belong to the group rhesus-positive (among the persons of white race them approximately 85%, human, that does not contain D - antigen, behave to Rh-negative (them, accordingly, about 15%

Rh(+) –85% Rh(-) – 15%

Importance of the Rh system

- After the A and B antigens, the D antigen is the most important red cell antigen in blood banking
- The D antibody can cause transfusion reactions and hemolytic disease of the newborn (HDN)/*Erythroblastosis fetalis*
- Rh antigens are highly immunogenic, the D antigen is most potent
- **D ➢ c ➢ E ➢ C ➢ e**

  Highly Rarely

  Immunogenic

- Exposure to less than 1 ml of Rh positive red cells can stimulate Ab production in an Rh negative person.

Rh Blood Types		
	Antigen D	No antigen D
Erythrocytes		
Plasma	No anti-D antibodies	Anti-D antibodies (after prior exposure) 
Blood type	Rh positive Erythrocytes with type D surface antigens and plasma with no anti-D antibodies	Rh negative Erythrocytes with no type D surface antigens and plasma with anti-D antibodies, only if there has been prior exposure to Rh positive blood

BLOOD TYPE DETERMINATION on Rh - SYSTEM

- There are many methods of determination of rhesus factor.
- There is a reaction with anti-D monoclonal antibodies (**Seraclone** Anti-D) in a clinic - most often used

Determination of Rh-фактора with Seraclone Anti-D

- Determination make indoors with good lighting.
- The best results a test gives at the use of high concentration of red cells and temperature about 37°, that is to use a warmed-up plate.
- For research use whole blood, washed red cells, red cells in plasma, serum, preservative or physiological solution.

Methods performing

- a) Put the large drop (about 0,1 ml) of reagent on a plate or plane-table.
- b) Put near a little drop (about 0,03 ml) to the investigated blood (red cells).
- c) Carefully mix up a reagent with blood by a clean glass rod.
- d) In 10-20 seconds softly rock a plate. Without regard to that clear agglutination comes in the first 30 seconds, take into account the results of reaction in 3 minutes after mixing.
- e) Write down the results of reaction immediately after determination.

Estimation of result

- Appearance of agglutination testifies about a rhesus - positive blood (existence antigen-D)
- Absence of agglutination - a blood a rhesus negative (the antigen-D is absent)

Mistake at determing of Rh-factor

1. Errors of organizational and technical character

- - the wrong ratio between serum and erythrocytes (erythrocytes should be approximately in the 10th times less, than serums);
- - non-compliance with necessary temperature
- - non-compliance with time necessary for carrying out reaction;
- - the conclusion becomes from the dried drop;
- - use for determing of a Rh factor of the old, hemolised or infected blood;

2. The mistakes bound to use of substandard Serums

- - use of delayed Serums;
- - use of low-active Serums;
- - use of polluted, infected Serums.

3. The mistakes predetermined by biological features of a studied blood

- - phenomenon of a polyagglytination of erythrocytes.
- - existence of an antigen of D^U (a weak version of an antigen of D). Erythrocytes with that agglytinogen give very indistinct agglutination with standard Serums of anti-Rh. Recipients with an antigen of DU consider Rh-negative, donors - Rh-positive;
- - depression of level of Rh- agglutinogens at some diseases (illness of system of a blood, a liver, kidneys, immune system)

Thanks for your attention!