ORSZÁGOS VÉRELLÁTÓ SZOLGÁLAT

ABO grouping

Practical lecture for IV year medical students

Zita Csernus MD

National Blood Transfusion Service
Blood Transfusion Centre Pécs

www.ovsz.hu

http://aok.pte.hu/index.php?page=egyseg&egy_id=1910&menu=okt_anyag&nyelv=eng?

Basic of the blood transfusion is the best compatibility

Blood group antigens of transfused blood are not own (exept monozygotic twins)

Immune response

Blood group antibodies

Antigen – antibody reaction

agglutination or haemolysis

RBC Blood Group Systems

- 34 blood groups
- 339 authentic blood group antigens

Most important antigens: **ABO**
Rh
Kell
.
.

About 25 blood group antigens are clinically important

They may cause serious transfusion complications!

ABO blood group system

Discovery: 1900 Karl Landsteiner

2 antigens (A or B) presence or absence forms the 4 blood groups

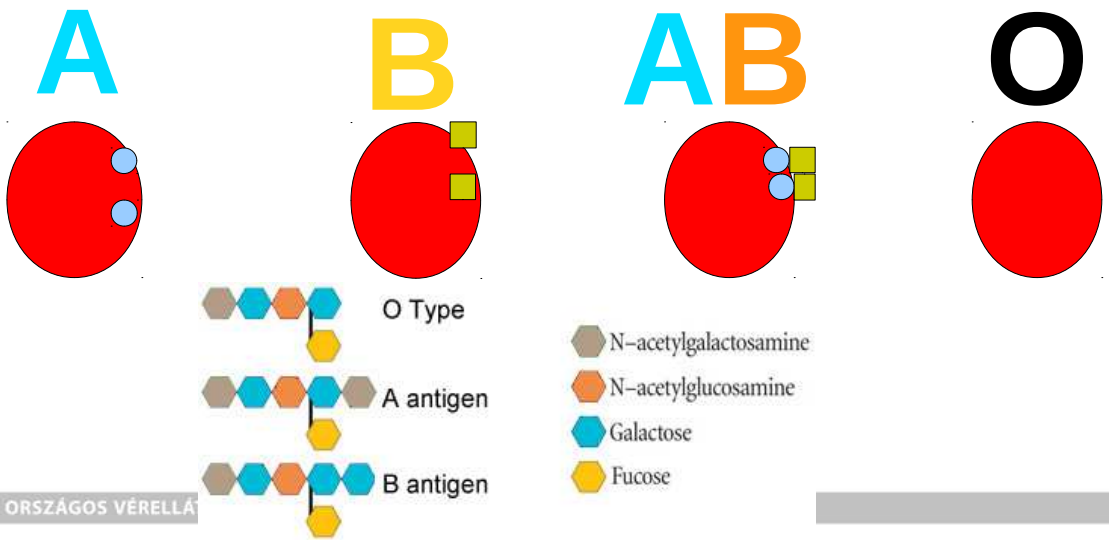


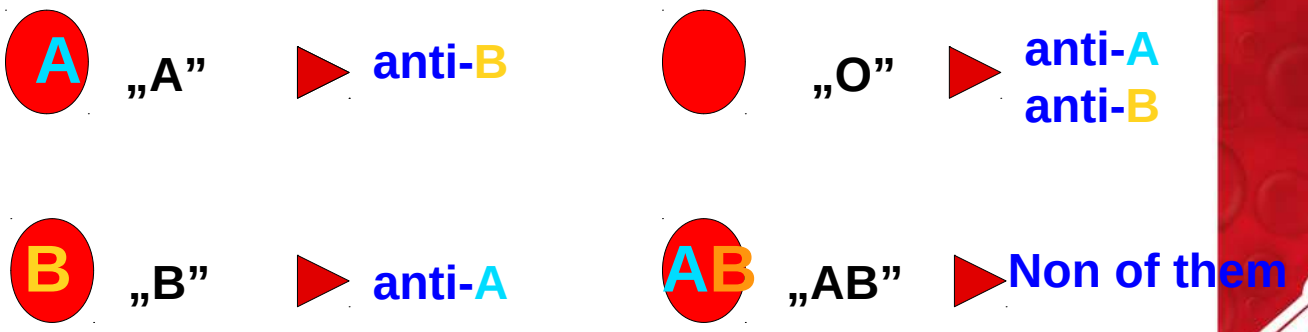
Diagram showing the carbohydrate chains that determine the ABO blood group

ABO Blood Groups

REGULAR (natutally occurring) blood group antibodies are present in the sera of all adult individuals.

Landsteiner's Law:

Two different antigens (A and B) are found on the surface of RBCs, and the "naturally occurring" antibodies against these antigens are found in the plasma of individuals who do not express them.

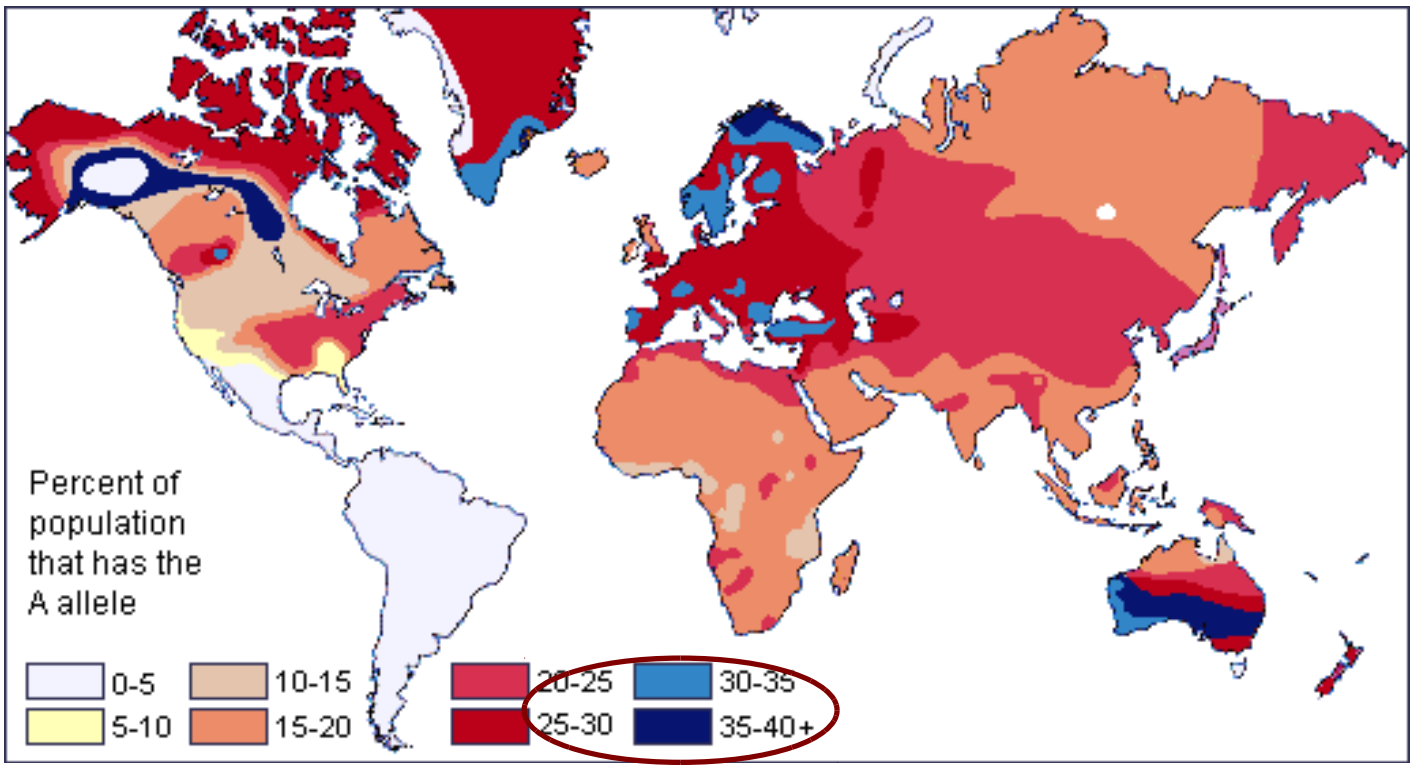


The presence of ABO antigens and antibodies (isoagglutinins) in the four blood types is summarized below:

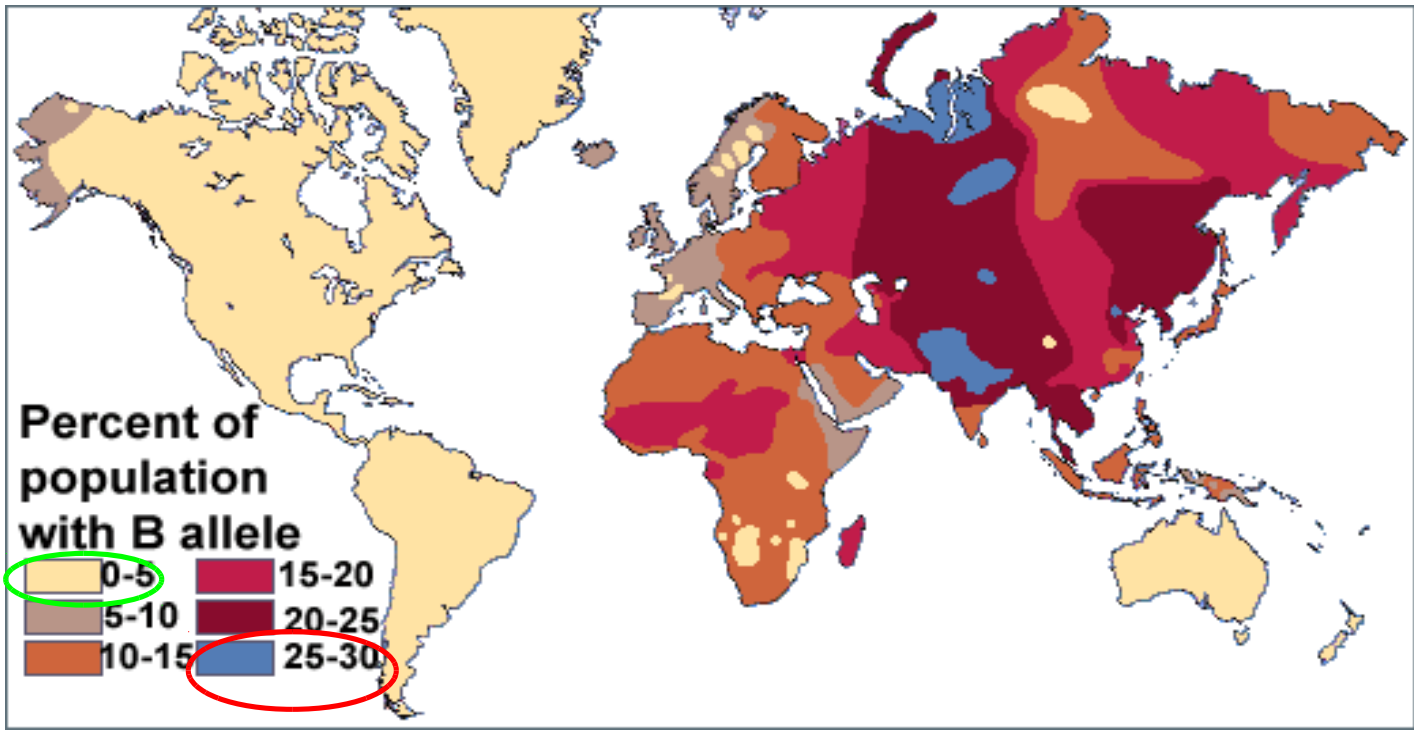
BLOOD TYPE	RBC ANTIGENS	SERUM ANTIBODIES	FREQUENCY Word	FREQUENCY Hungary
A	A	anti-B	40%	44%
B	B	anti-A	10%	16%
AB	A and B	none	5%	8%
O	none	anti-A and anti-B	45%	32%

The ABO blood group antigens also appear to have been important throughout our evolution because the **frequencies of different ABO blood types vary among different populations**, suggesting that a particular blood type conferred a **selection advantage** (e.g., resistance against an infectious disease.)

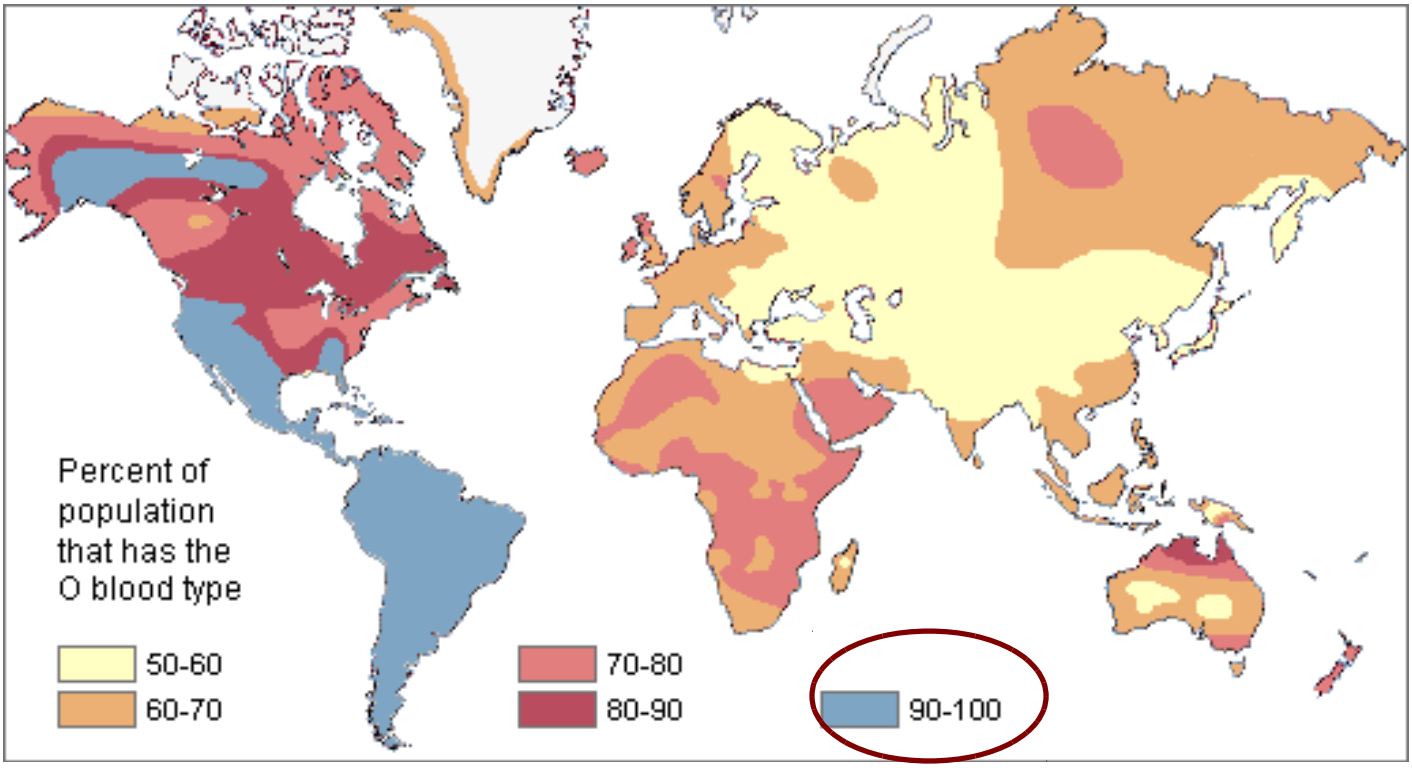
Map of A blood group allele distribution



Map of B blood group allele distribution



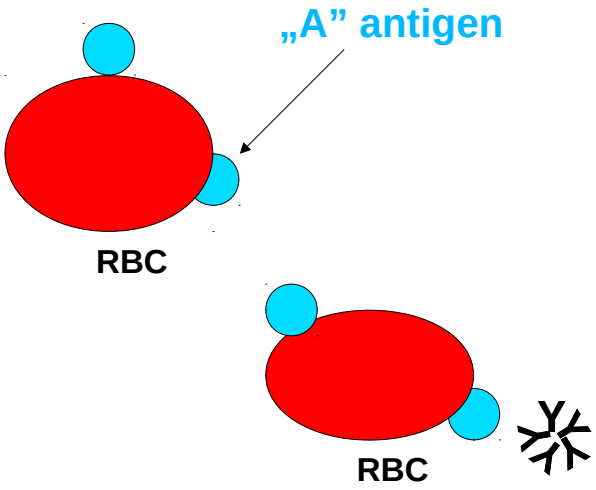
Map of O blood group allele distribution



ABO blood groups and deseases

Blood group	High risk
O	Bleedings Autoimmune diseases Virus infection Cholera Helicobacter pylori (Leb)
A	Bacterial infections Cancer Coagulation diseases
A and B	Arterial and venous thrombosis Coronary disease Malaria
RhD negative	Severe HDN
	Low risk
O	Higher malaria resistance Reduced trombosis susceptibility

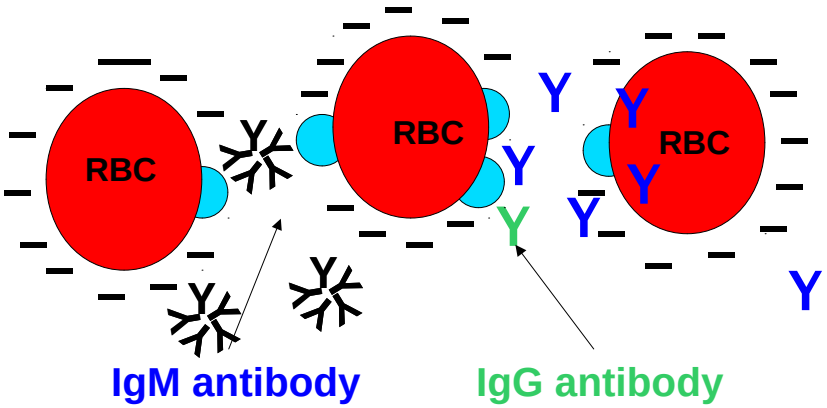
Basics of immunohaematology:



antigen – antibody interaction

Agglutination is the most common procedure used in immunohematology

AGGLUTINATION is a visible reaction



Antigen is RBC– antibody is an immunoglobulin protein mainly IgG or IgM

Antigen-antibody reactions in blood group serology are usually detected by haemagglutination

Factors affecting the antigen-antibody reaction

Factors acting on the equilibrium constant

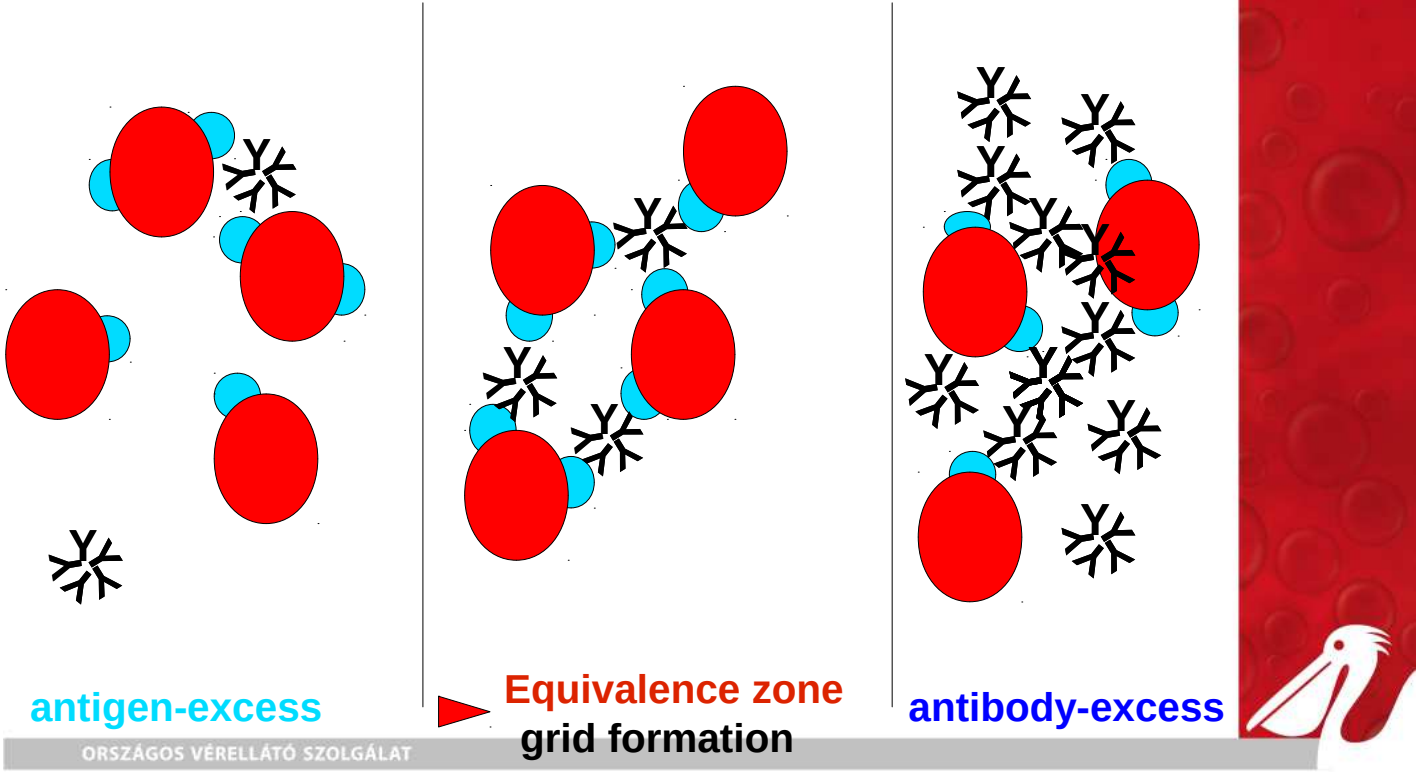
- **Temperature** - room temperature (20-25 °C)
- **pH**
- **Ionic strength** - physiological saline
- **Enzyme treatment of red cells** (papain, bromelin)

Other factors

- **Concentrations of antigen and antibody**
  - 10% RBC suspension
- **Zygosity** (number of antigen sites per cell)
- **Duration of incubation**
  - 10 minutes with human sera
  - 5 minutes with reagents sera

Haemagglutination

It is important to ensure adequate antigen – antibody ratio



No agglutination | **Visible agglutination exists** | **No agglutination**

Limitations of the Procedure:

Sources of errors

Antigen – antibody tests met a lot of requirements. Factors affecting the antigen-antibody reactions shoud be considered to establish the suitable reaction. If the reaction conditions are not followed, false negative or false positive results can occur, which can lead to incorrect blood group determination.

Reaction conditions for ABO test

- Medium of reaction (ionic strength) – **Phys. salin**
- Antigen - antibody ratio (**10% suspension**)
- Reaction temperature (**+2O °C – room temperature**)
- Reaction time (**forward: 5 minutes, reverse: 10 minutes**)

Main causes of false positive reactions

- Rouleaux formation
  marginal drying
- Little drops
- Late evaluation
- Contamination

Main causes of false negative reactions

- Early evaluation
- Inadequate
  antigen-antibody ratio
- Expired reagents and test cells

The sympexis may be differentiate from real agglutination with dropping of phys. saline.

The sympexis dissolved but no agglutination.

Sympexis = rouleaux formation of RBCs

Physicochemical changes not real agglutination

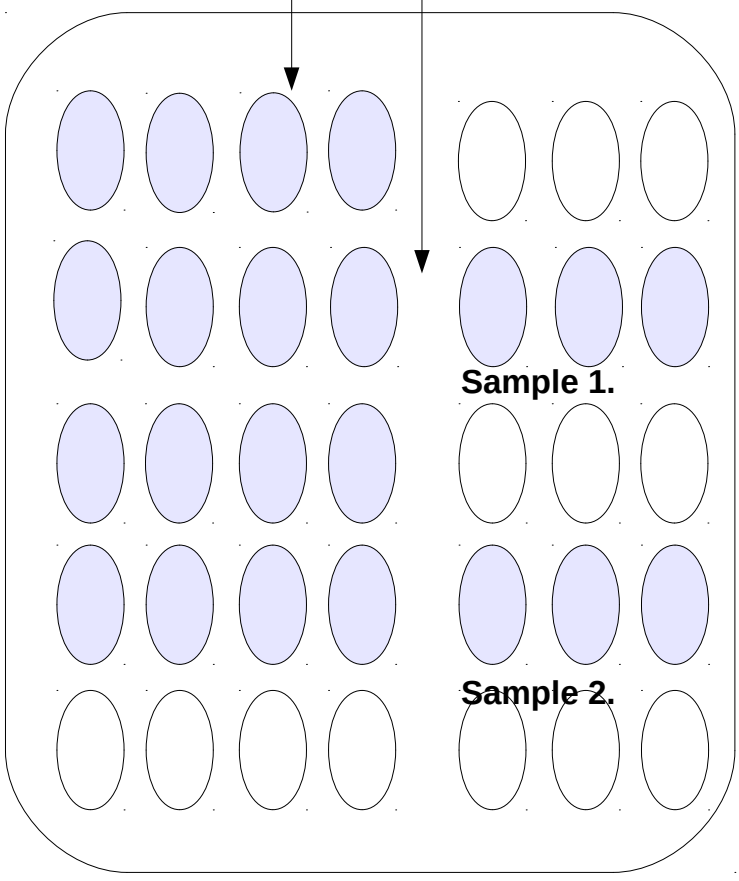
The RBC's here have stacked together in long chains. This is known as "rouleaux formation" and it happens with increased serum proteins, particularly fibrinogen and globulins. Such long chains of RBC's sediment more readily. This is the mechanism for the sedimentation rate, which increases non-specifically with inflammation and increased "acute phase" serum proteins.

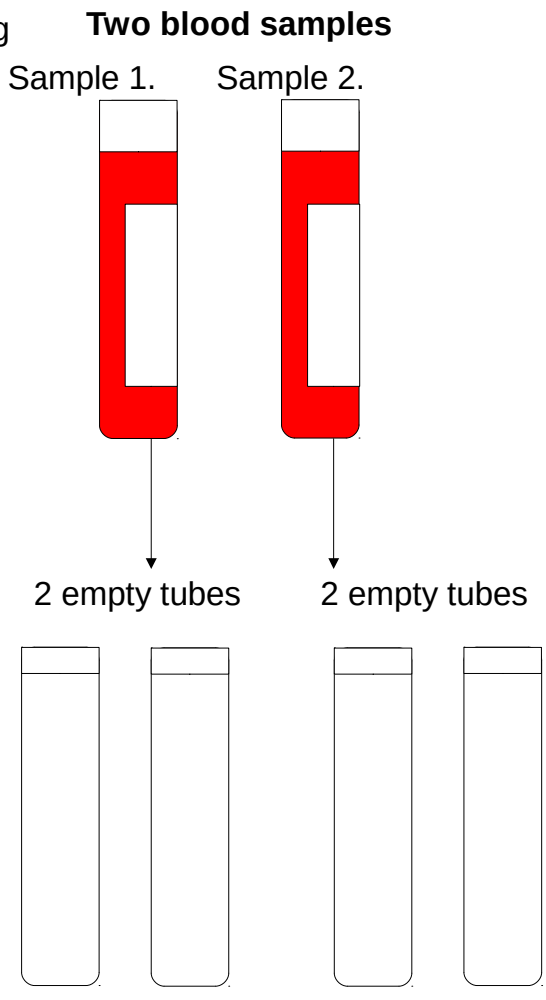
Causes: infections
multiple myeloma, chirrosis
(an increase in the ratio of immunoglobulins to albumin)
inflammatory and connective tissue disorders
cancer
diabetes mellitus
an increase in the ratio of RBCs to plasma volume
(anemia, hypovolemia)
macromolecules, contrast medium

Acute phase proteins, particularly fibrinogen, interact with sialic acid on the surface of RBCs to facilitate the formation of rouleaux. Rouleaux formation is retarded by albumin proteins, in vitro by physiological salin.

TASKS

1. forward typing
2. forward and reversed typing

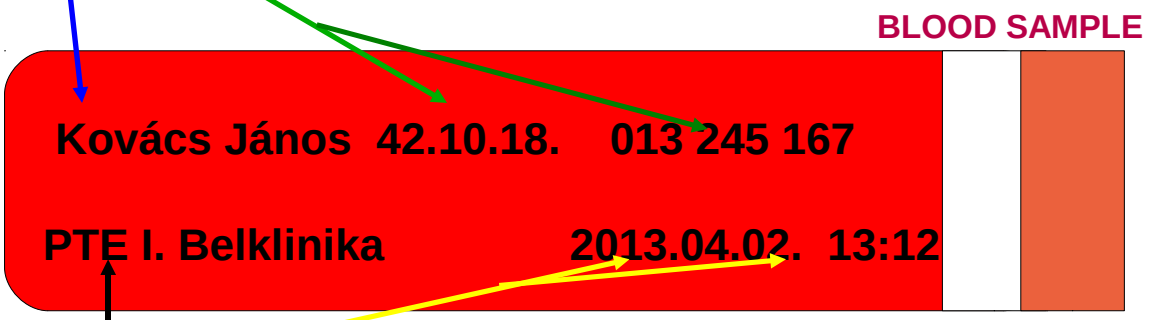




BLOOD SAMPLE LABELING

Details must include:

- Patient's registered family name and given name (unabbreviated)
- Date of birth or social security number (TAJ) (both preferred)

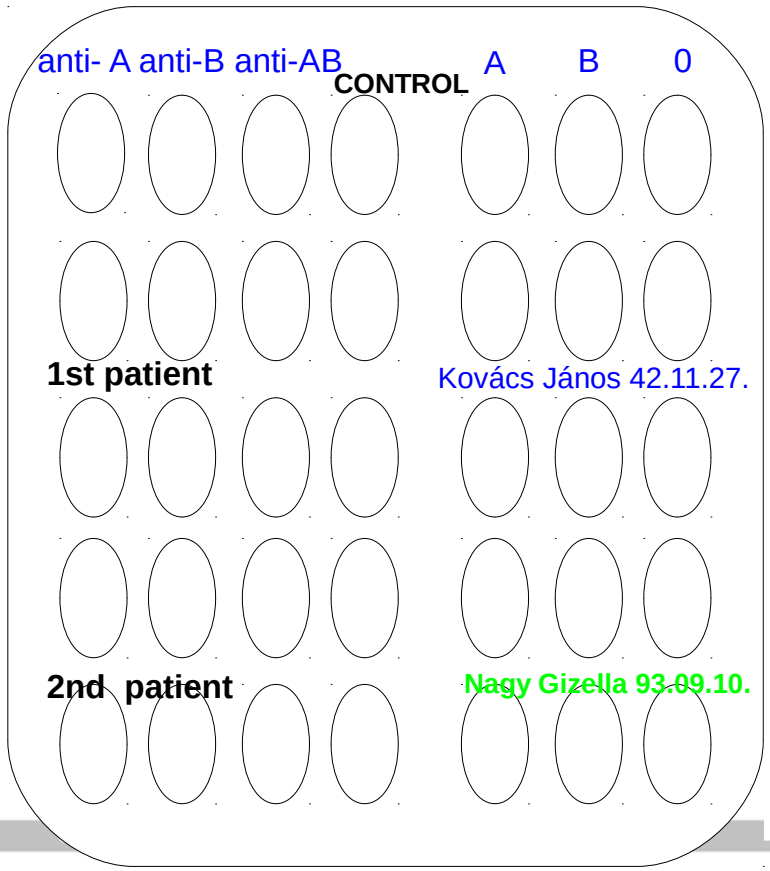


- Date and time of collection
- Name and code of institute requiring

(Signature or initials of the collector)

ABO grouping – slide test

1st step



Labeling of slide

1. Order of reagents important

anti-A
anti-B
Anti-AB

2. Negative control

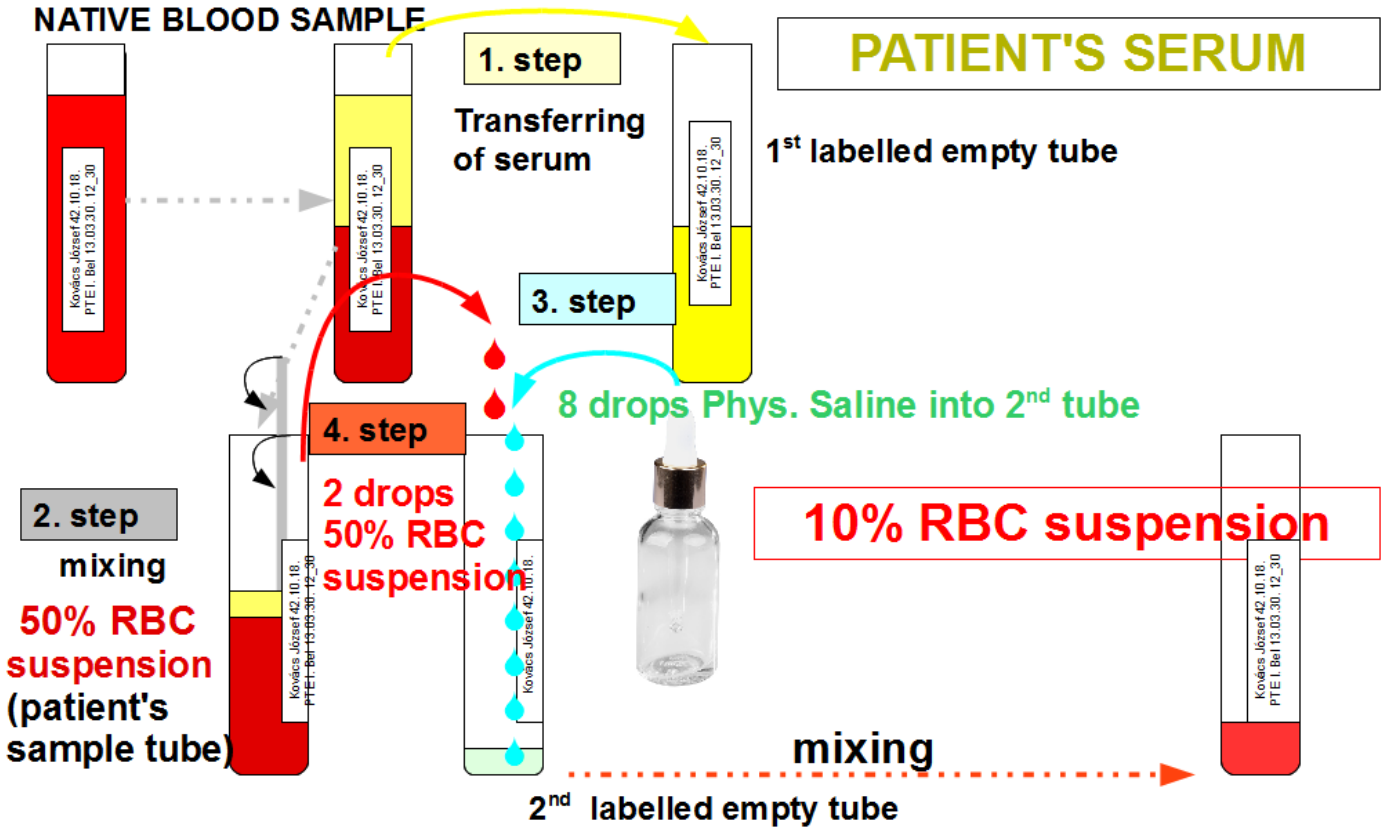
Patient's sera + patient's RBC

3. Test cell suspensions

A
B
O

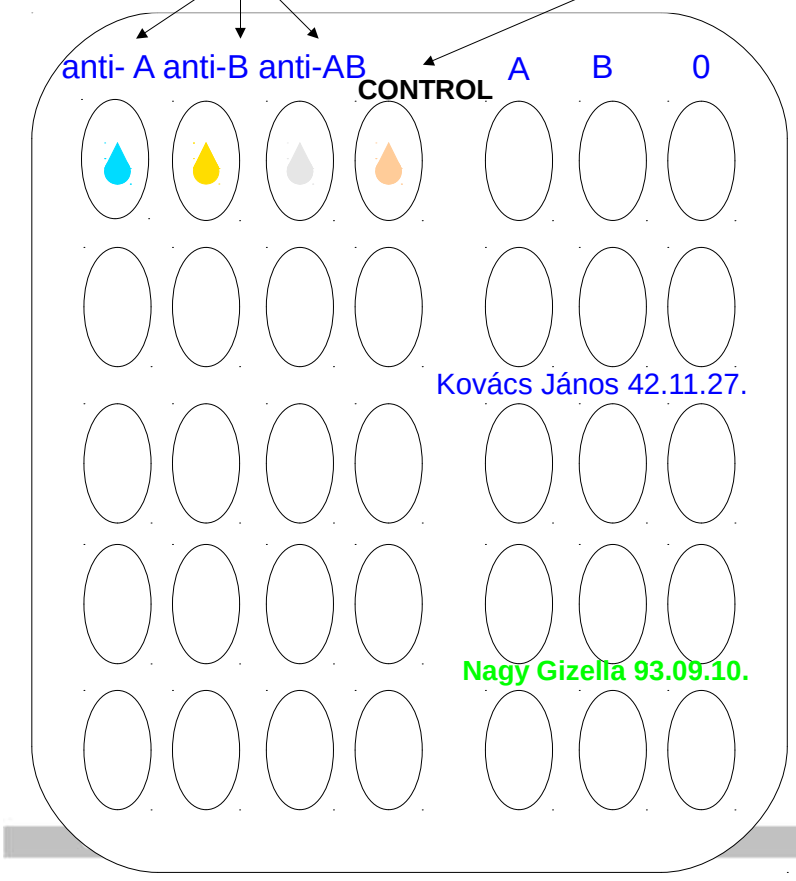
4. Patient's name and code

PREPARATION OF RBC SUSPENSION



ABO blood grouping – forward typing

2nd step dripping reagents sera and patient's serum to negative control **1 drop in each place**



Left side of ABO test
- clinical blood grouping

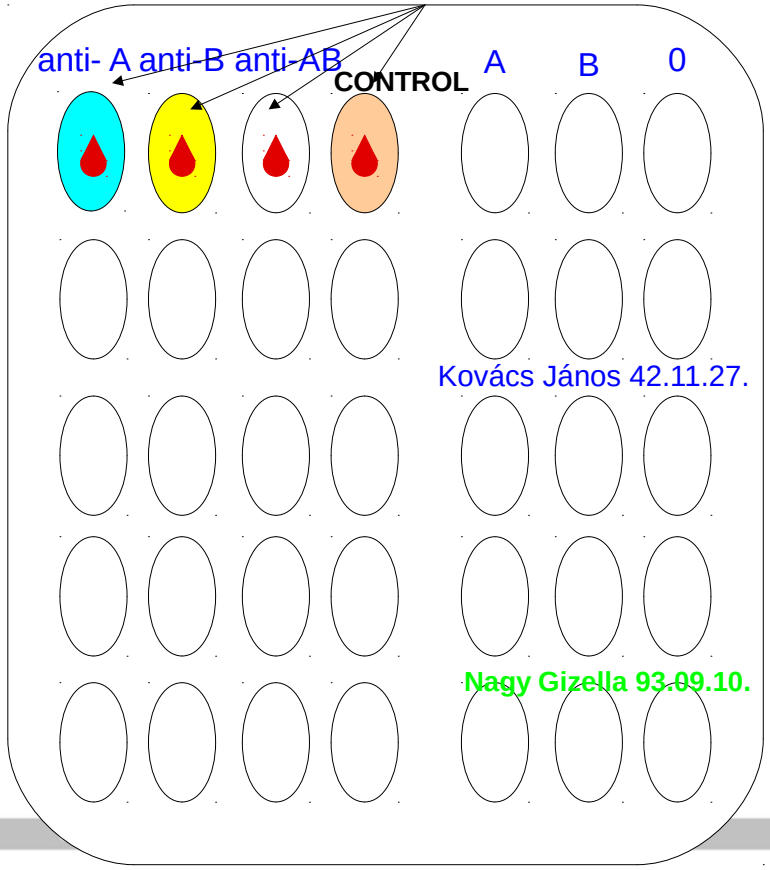
High titer monoclonal reagents

anti-A
anti-B
Anti-AB

Patient's serum

ABO blood grouping – forward typing

3rd step dripping of paient's RBC suspension 1 drop to each place



Left side of ABO test
- clinical blood grouping

Dripping 1 drop 10% RBC suspesion

Mixing and wiping

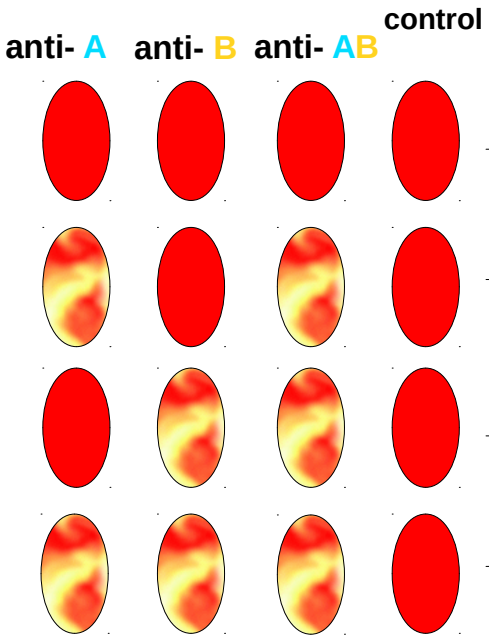
Reaction time: 5 minutes

Tilting slowly

Interprete reactions

ABO forward grouping –interpretation

Clinical blood typing or left side



Blood type

No agglutination: „0”

Agglutination with antisera:
anti-A and anti-AB: „A”

anti-B and anti-AB: „B”

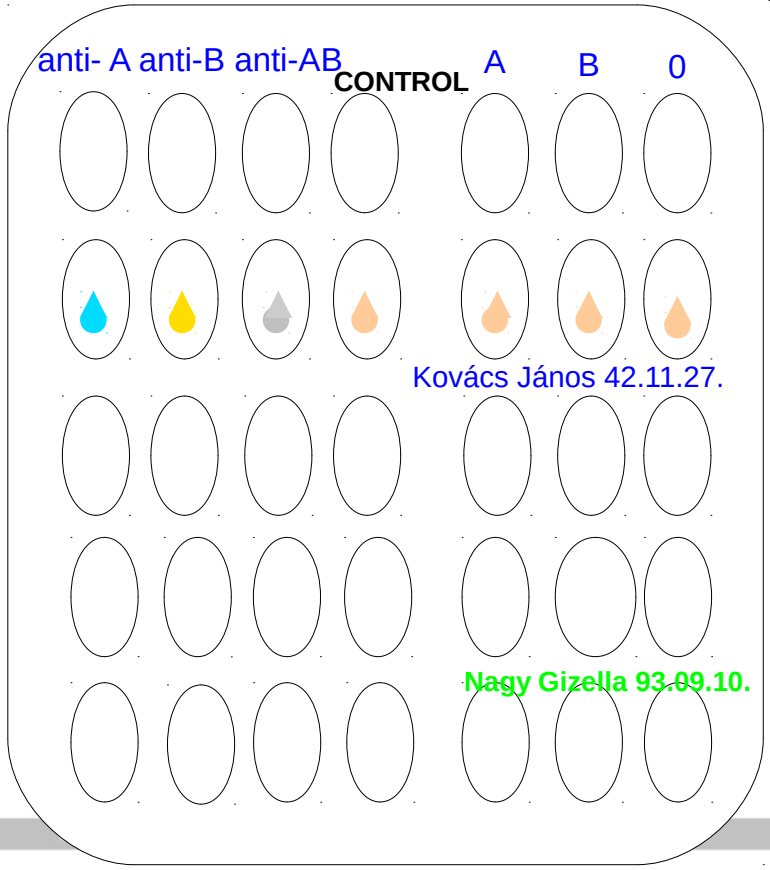
anti-A, anti-B and anti-AB: „AB”

The control must be negative.
If any agglutination occurs the test is not valid.

ABO forward and reverse typing

1st step

Laboratory typing or double sided typing



1 drop of

Forward side

High titer monoclonal reagents

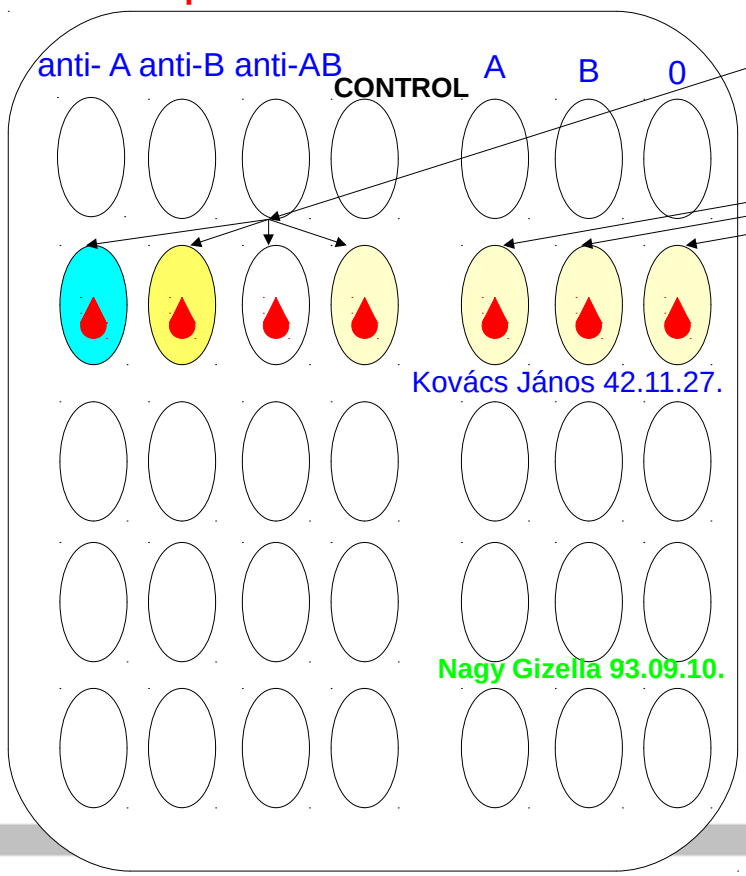
- **anti-A**
- **anti-B**
- **anti-AB**

Negative control –
Patient's **RBC** susp. + **own serum**

Reverse side –
Patient's serum
to each test cell reaction area

ABO forward and reverse typing

2nd step



dripping of **paient's RBC**
1 drop to **forward typing** areas
respectively

Dripping of **A, B, O test cells**
in signed reaction areas
1 drop respectively
Reverse typing

Mix thoroughly

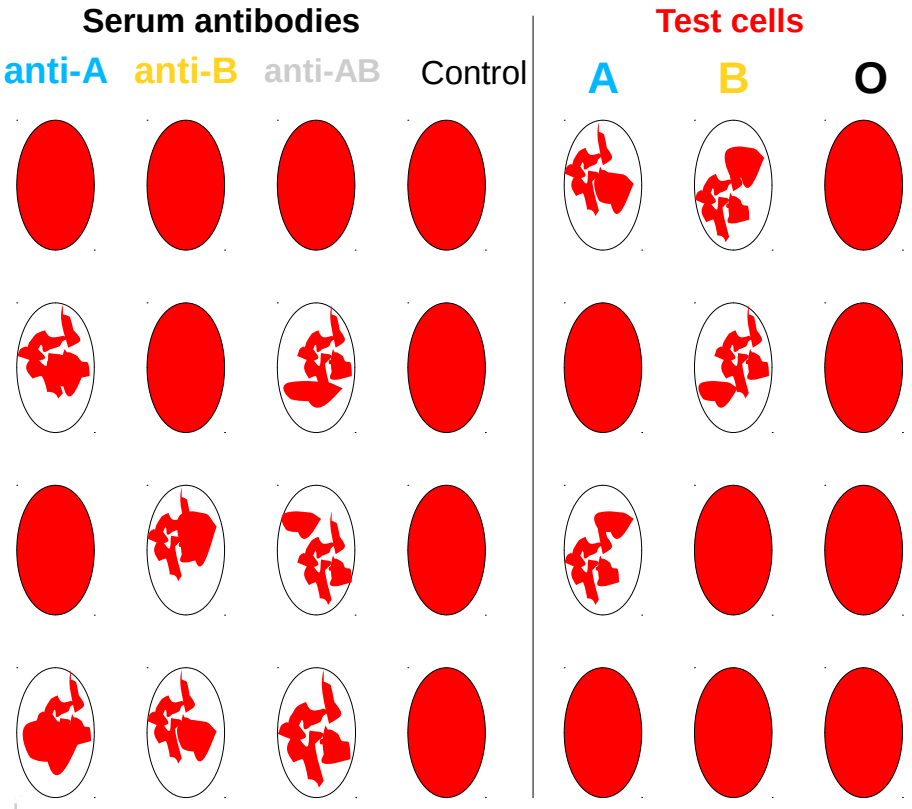
Reaction time of reverse typing: 10 minutes

Tilting slowly

Interprete reactions

ABO forward and reverse typing (laboratory test)

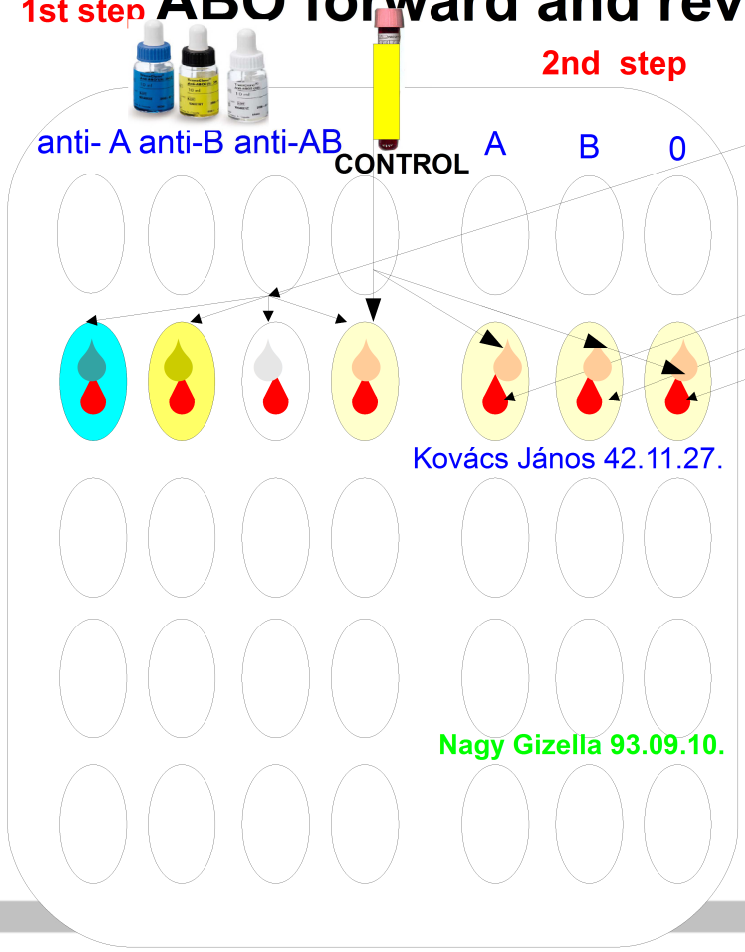
INTERPRETATION



BLOOD TYPE	ISOAGGLUTININ
„O”	anti-A anti-B
„A”	anti-B
„B”	anti-A
„AB”	none

LANDSTEINER'S RULE

1st step ABO forward and reverse typing



2nd step

dripping of **paient's RBC**
1 drop to **forward typing** areas
respectively

Dripping of **A, B, O test cells**
in signed reaction areas
1 drop respectively
Reverse typing

Mix thoroughly

Reaction time of reverse typing: 10 minutes

Tilting slowly

Interprete reactions


A
RhD positive


Serafol® ABO+D
Anti-A
Anti-B
Anti-D
Ch. - B./Lot No. 080601
Expiry date 31.12.01
Name
(Name/ Nom)
Geb.Dat.
(Date of Birth/ Date de Naissance)
Kons.- Nr.
(Unit No / No Poche)
Datum
(Date)
Blutgruppe
(Blood Group/ Groupe Sanguin)
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