SAWA UNIVERSITY

BLOOD TRANSFUSION

ABO BLOOD GROUPING

Sawa University

College of health and medical techniques

Department of Medical Laboratories.....

Stage: forth

جامعـــــة ســـــاوة الاهلية

كليـــة التقنيـــات الصحيـة والطبيـة

قسم تقنيات المختبرات الطبية

تدريسي المادة :د. هبة محمد

Lecture No 2 Practical

Contents:

- Introduction.
- ABO grouping tests;
  - types
  - Procedure
  - interpretation

Introduction

- Red cell membranes have antigens (glycoproteins)

on their external surfaces.

- These antigens are unique to the individual recognized as foreign if transfused into another individual promote agglutination of red cells if combine with antibody.
- more than 30 such antigen systems discovered

Presence or absence of these antigens is used to classify blood groups

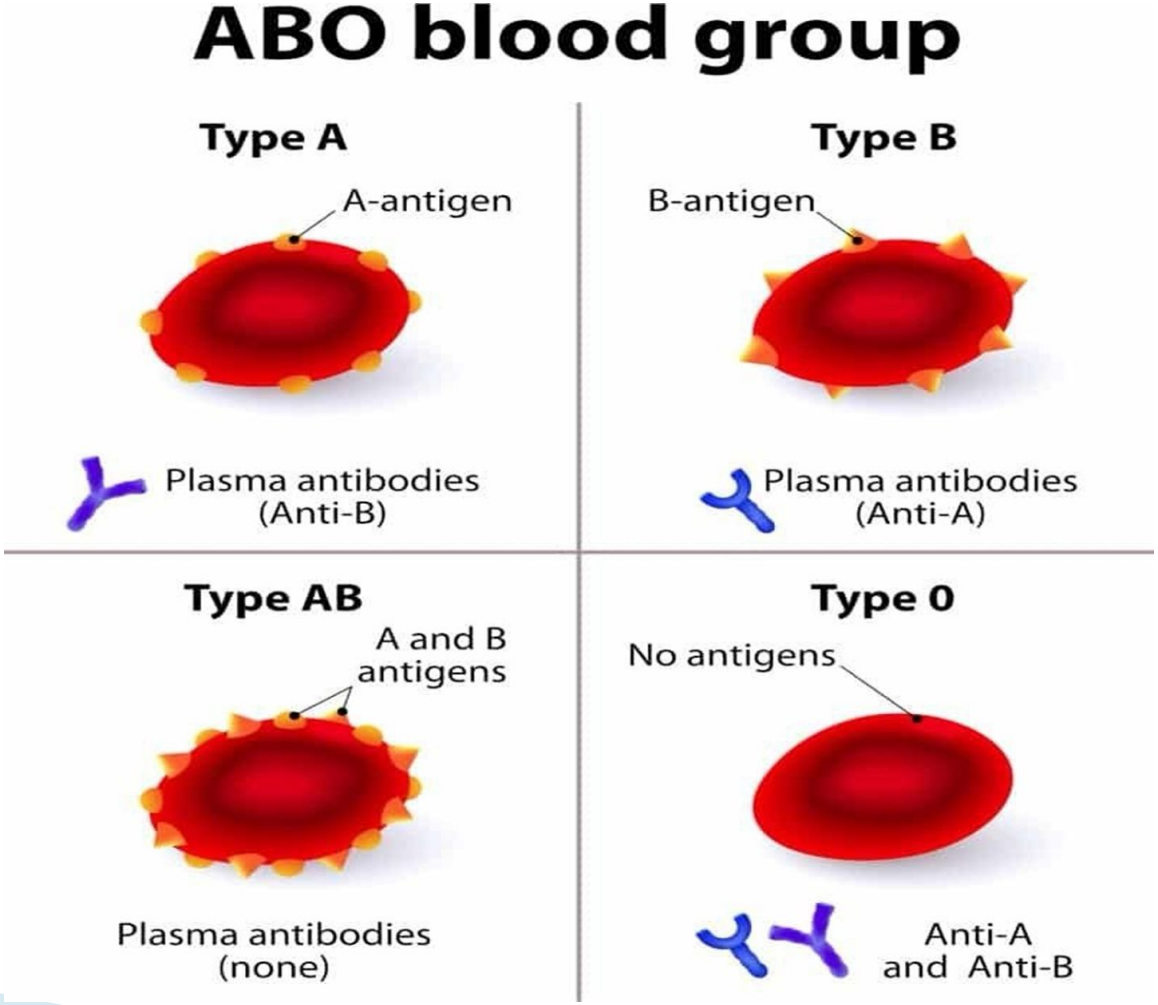
Major blood groups - ABO & Rh.

Minor blood groups - Kell , Kidd, Duffy etc.

ABO Blood Groups

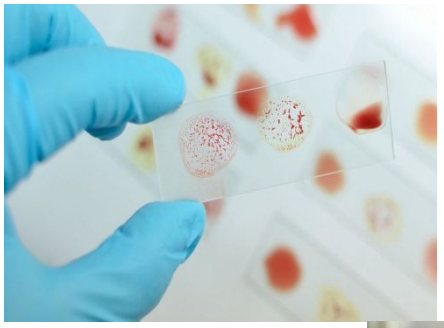
The most well known and medically important blood types are in **the ABO group**.

The ABO blood group consists of two antigens (A & B) on the surface of the RBC & two antibodies in the plasma (anti-A & anti-B)



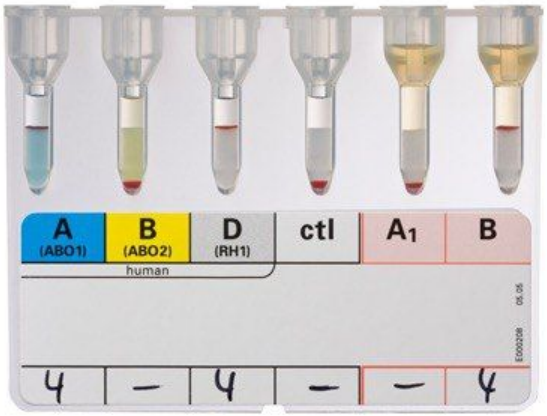
The ABO grouping can be performed by the following methods:

1. Slide method.



2. Tube method.

3. ABO gel grouping.



:Antisera used in ABO grouping

The following commercially prepared antisera are used in detection of ABO blood groups:

- **Antisera A**

The "Methylene Blue" dye present in antisera A gives it a blue color.

Antisera A carry very high titer of anti-A antibodies.

- **Antisera B**

The presence of dye "Acriflavin"

' in antisera B gives it a yellow color.

The antisera B is rich in anti-B antibodies.

Washing of red cells:

Before going for any procedure in the blood bank, the red cells have to be washed properly.

0.5 ml of red cells are mixed with normal saline filling 2/3rd of test tube.

The mixture is centrifuged at 3000 pm for 1 minute.

The supernatant is discarded.

Refill the tube with same amount of normal saline and centrifuge again.

Repeat the procedure three times and discard the supernatant every time.

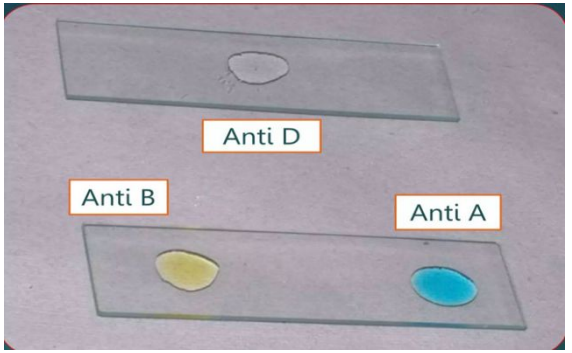
The remaining cells are washed cells.

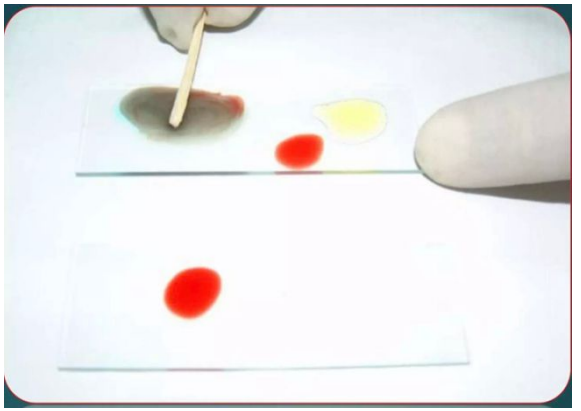
Slide method

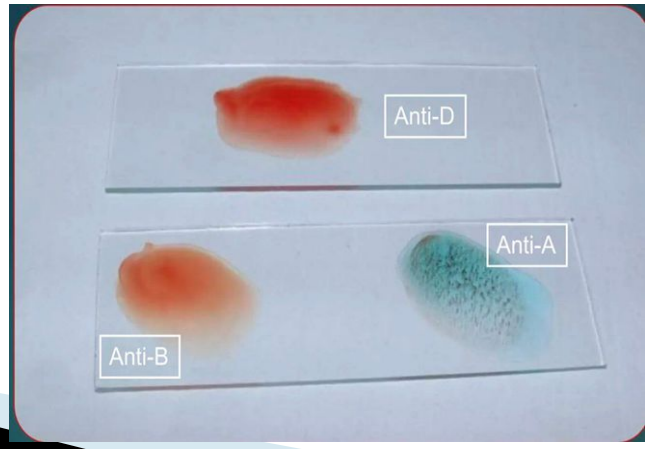
This is a simple technique, which should be employed in cases of emergency or outdoor camps. The technique is less sensitive and not capable of detecting weak antigens.

Procedure

1. Place one drop of anti-A and one drop of anti-B sera on two previously labeled slides.
2. Add one drop of blood on each slide.
3. Mix properly by a clean glass stick or the corner of another slide.
4. Rock the slides in order to mix the cells and sera
5. Leave at room temperature for 2 minutes.
6. Record the results.

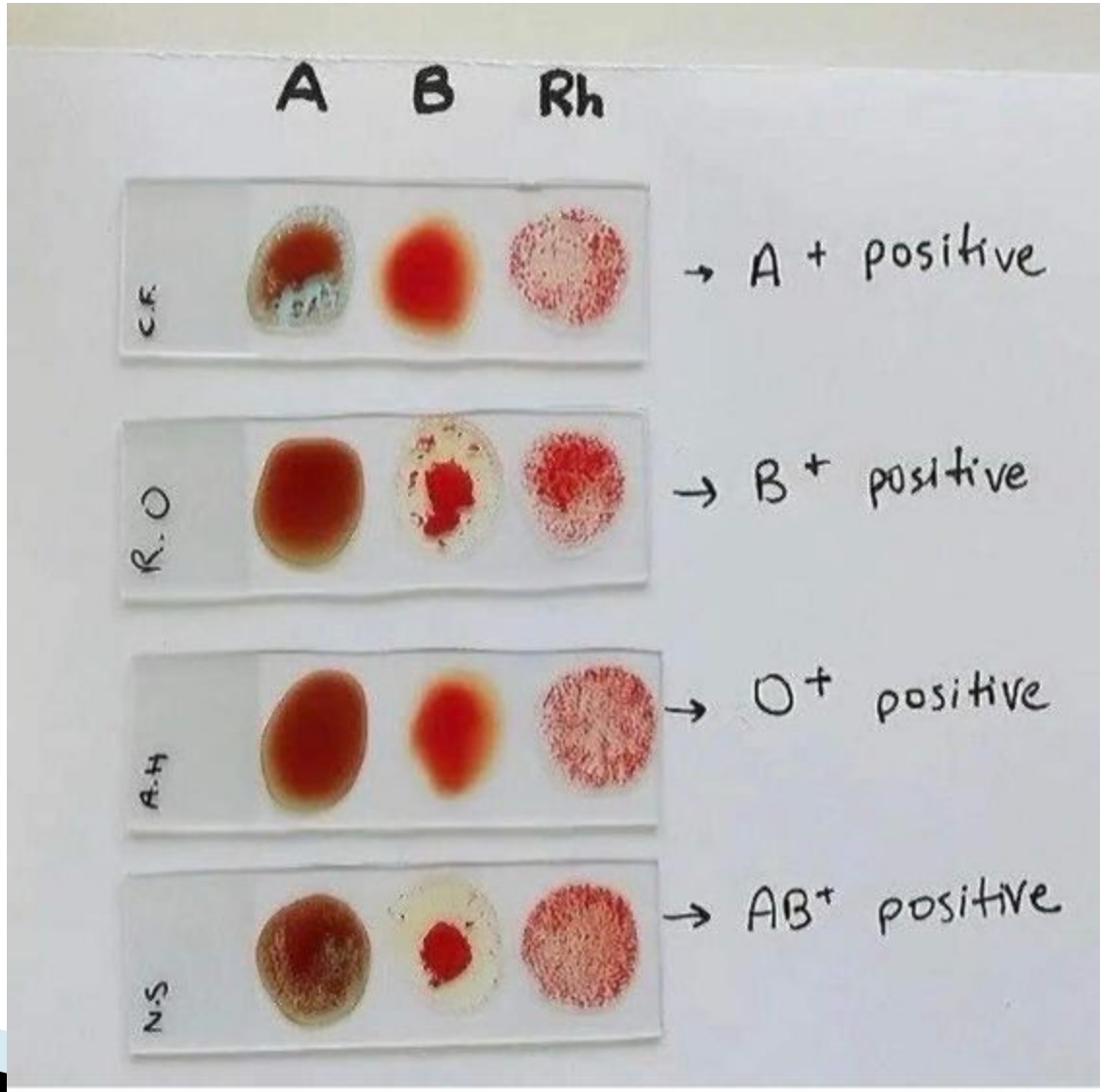






Interpretation of results

The agglutination appears like granular precipitate. Agglutination (+) in one or both antisera is interpreted as follows:



Tube method

Recommender method

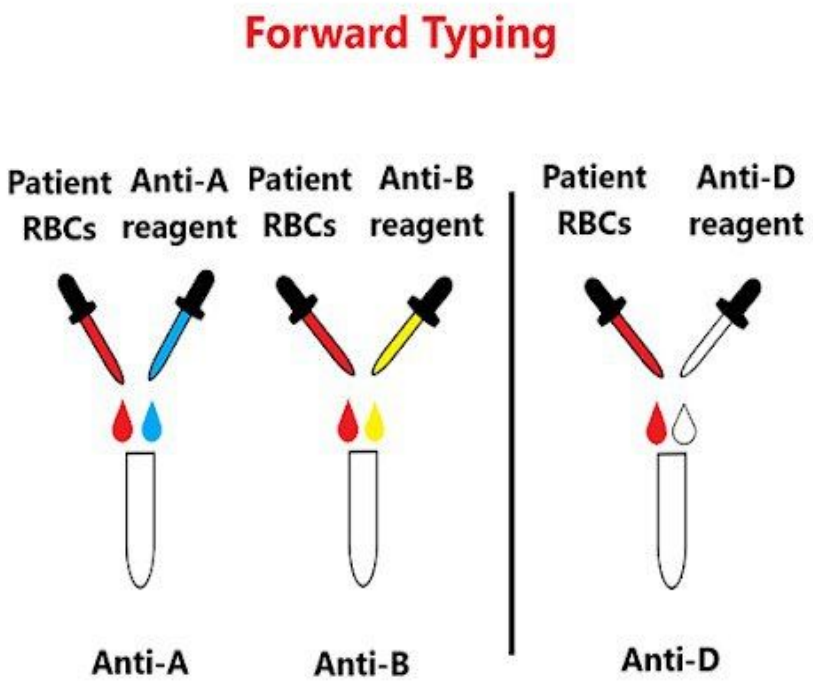
Allows longer incubation of antigen and antibody mixture.

Tubes can be centrifuged to enhance reaction

Can detect weaker antigen / antibo

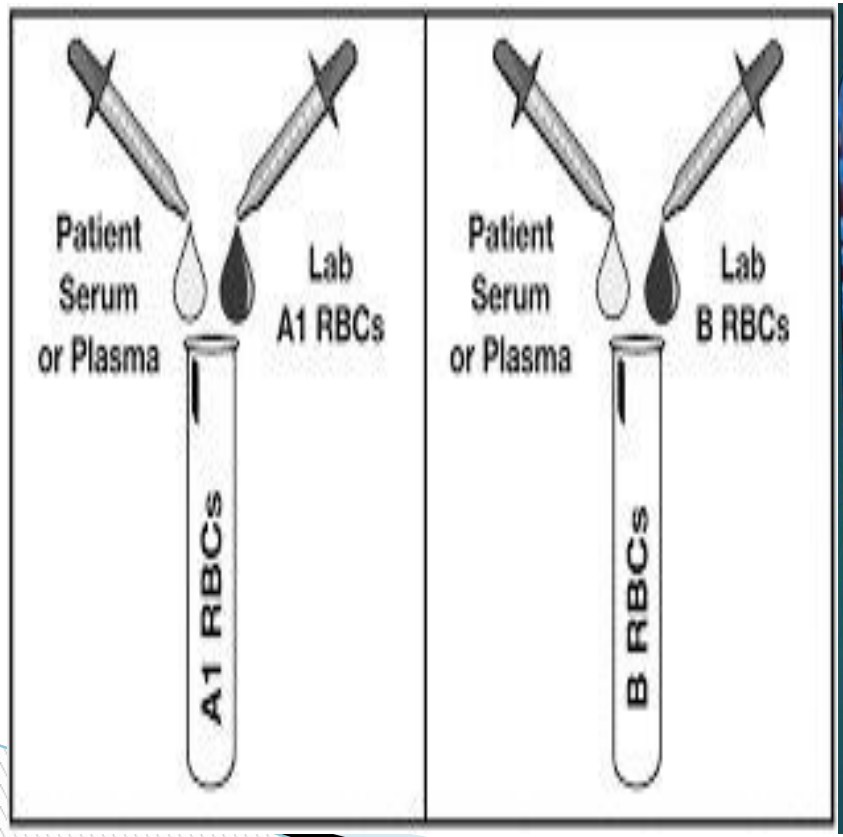
1. Cell grouping (Forward grouping

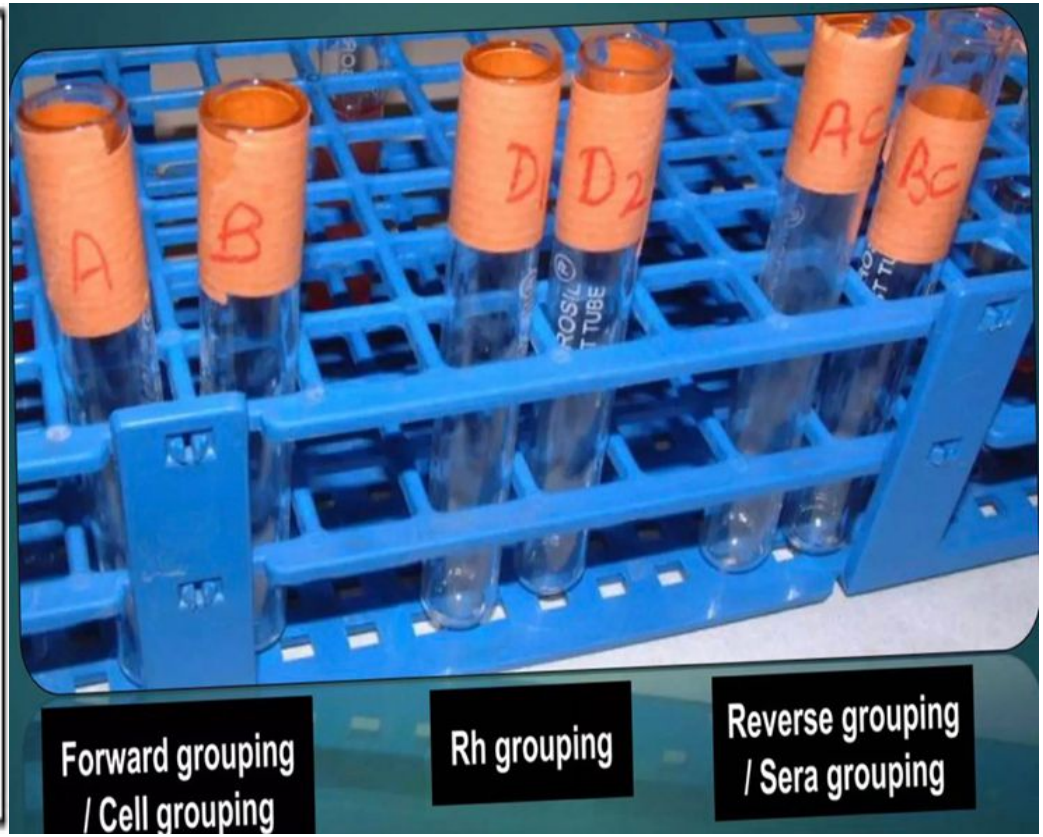
Tests the patient's red cells with known Anti-A & Anti-B to determine the antigen expressed.



2. Serum grouping (Reverse grouping)

Test the patient's serum with known A & B cells to determine the presence of antibody.





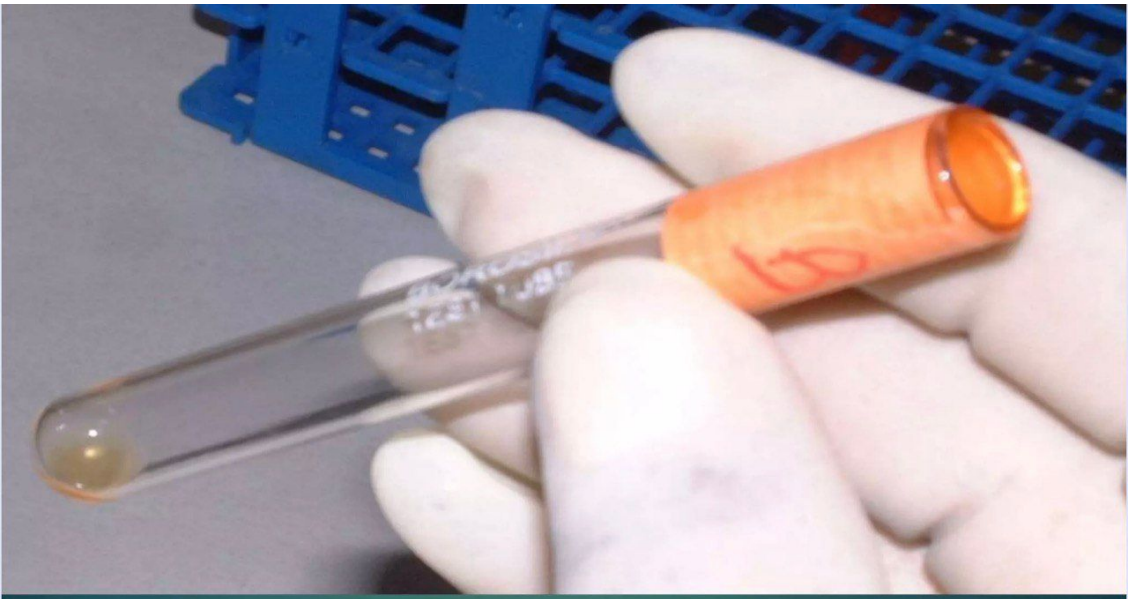
Procedure

Cell grouping (forward grouping):

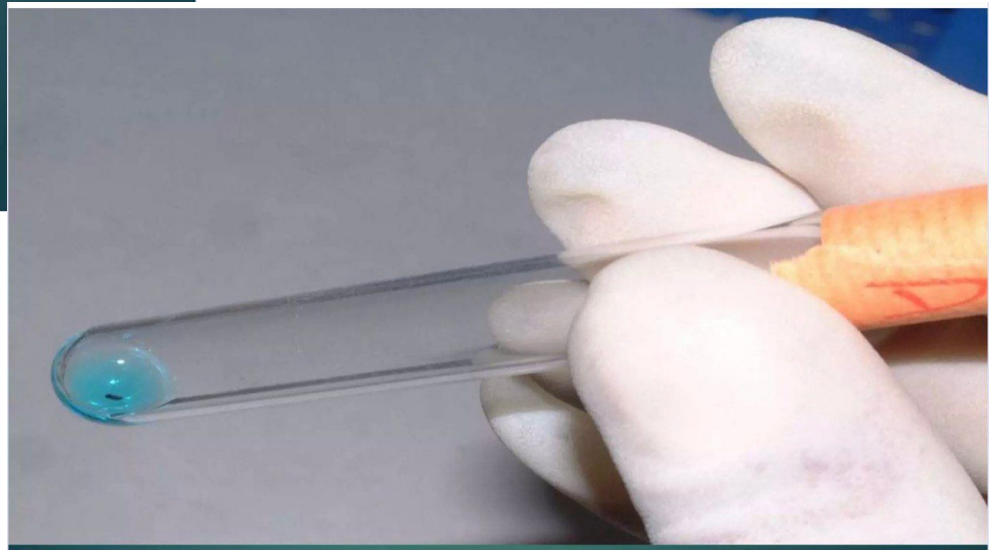
- Set up three rows of clean test tubes and label them.
- Add 2 vol of anti- A & anti-B in the tubes labeled as A & B, respectively.
- Add one vol of 2-5% suspension of test red cells in each tube
- Mix the contents of each tube by gentle shaking and leave at room temp for 5 min.
- Centrifuge the tubes at 1000 pm for 1 min.
- Observe the supernatant fluid for agglutination against a well lighted background
- Gently disperse the cell button and check for agglutination against a well lighted background
- Record results immediately

Plasma grouping (reverse grouping)

- Tests the patient's serum/ plasma with known A & B red cells to determine antibody produced.
- Acts as a confirmation of the forward group method.
- Label three clean test tubes as A cell, B cell & O cell
- Add 2 vol of test serum in each tube.
- Add one vol of 2-5% suspension of reagent red cells in respective tubes.
- Mix the contents of each tube by gentle shaking and leave at room temp (20-24°C) for 5 mins.
- Centrifuge & record the results similarly as for Cell grouping.



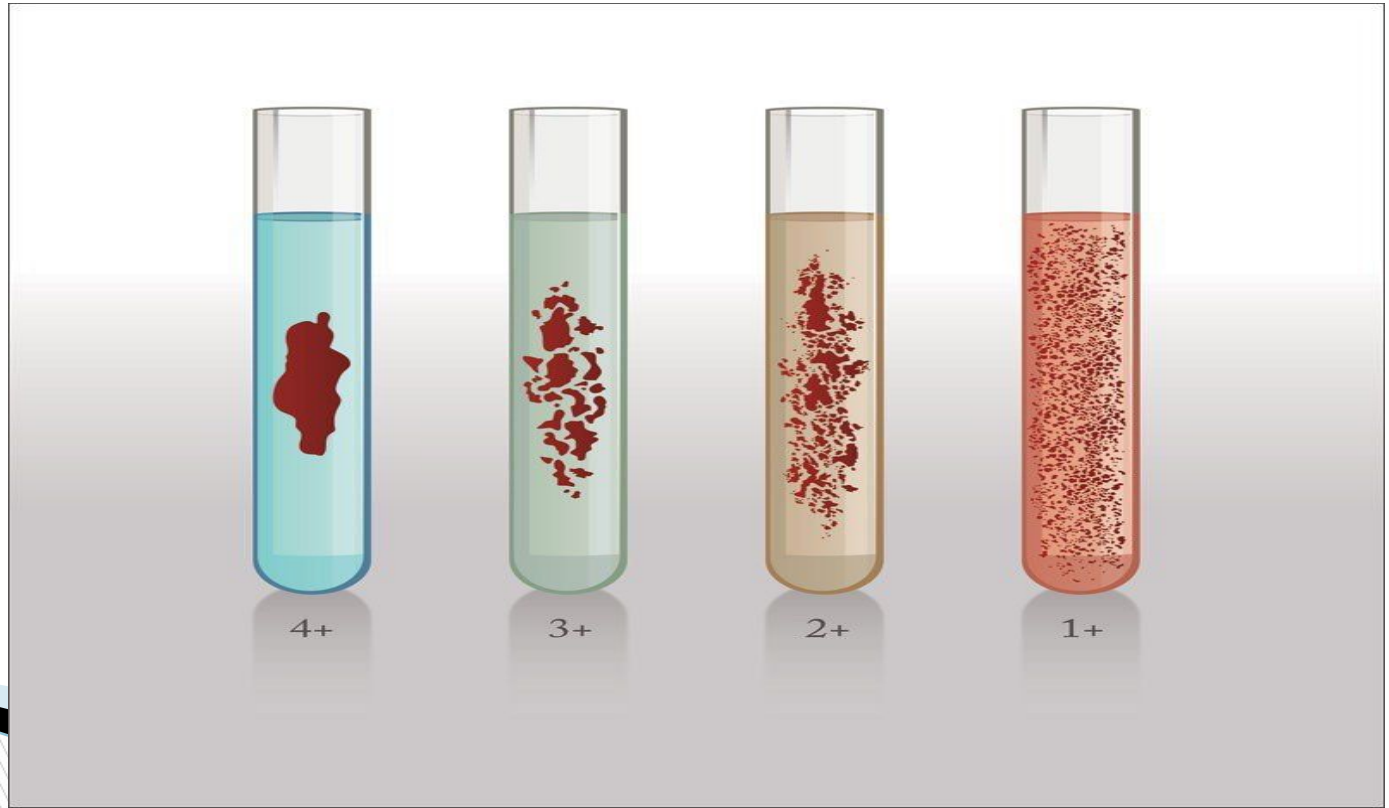
No agglutination in Anti B



Showing 4+ agglutination in Anti A

Type of agglutination observe

- 4+ cell button remains in one clump macroscopically visible.
- 3+ cell button dislodges into several large clumps.
- 2+ cell button dislodges into many small clumps.
- 1+ cell button dislodges into finely granular clumps.
- O Negative result all cells free and evenly distributed.

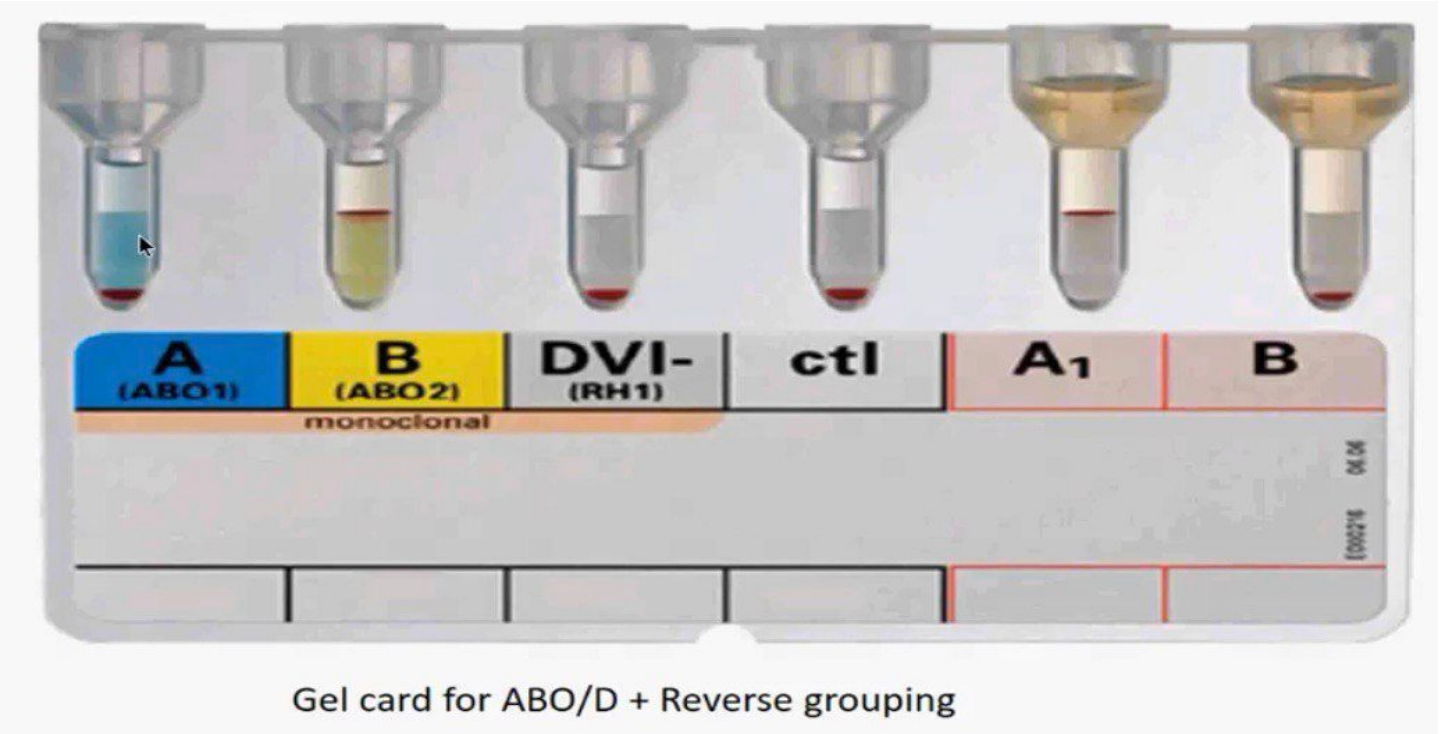


ABO Forward Grouping			Reverse Blood Group		
Patient RBC with anti A	Patient RBC with anti B	INTERPRETATION Of Blood Group	Patient serum our plasma with Reagent A1 cell	Patient serum our plasma with Reagent B cell	INTERPRETATION Of Blood Group
0	0	O	4+	4+	O
4+	0	A	0	3+	A
0	4+	B	3+	0	B
4+	4+	AB	0	0	AB

ABO gel grouping

The gel technique by its ID card system has made the blood grouping a very convenient procedure.

Forward and reverse ABO grouping and Rh grouping are done with a single card in a very short time.



Gel card for ABO/D + Reverse grouping

Reagents

The microtubes contain the following reagents:

Microtube 1 - anti-A

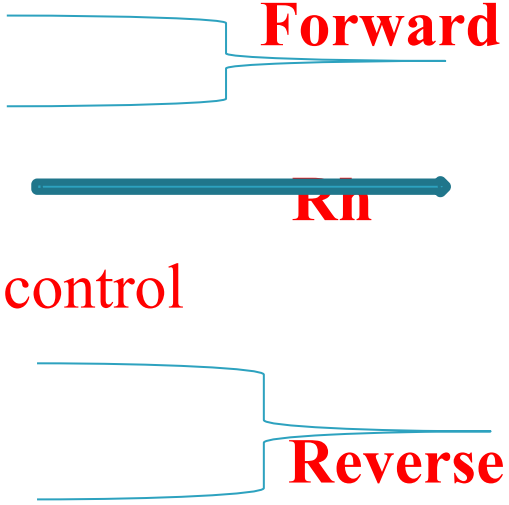
Microtube 2 - anti-B

Microtube 3 - anti-D

Microtube 4 - negative Rh control

Microtube 5 - A1 cells

Microtube 6 - B cells.



Procedure

1. Write the patient's name.
2. Remove the aluminium foil from the top of the microtubes.
3. Pipette 50 ML of patient's red cell suspension to the micro tubes 1-4 (A, B, D and control).
4. Add 50 uL of patient or donor plasma(A1 &B).
5. Centrifuge the ID card for 10 minutes in the ID-centrifuge.
6. Read and record the results.

Interpretation of results

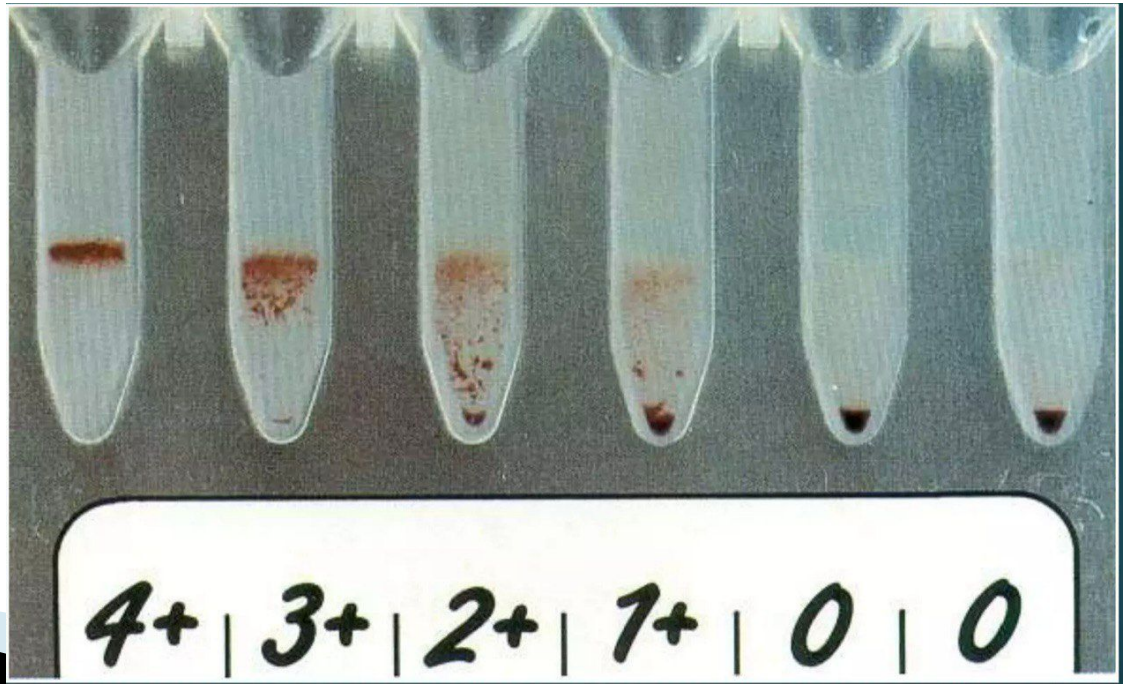
1-Negative

Compact button of red cells on the bottom of the microtube

(Strength: 0)

2-Positive

Red cells forming a single red line at the supernatant/gel interface (Strength: 4) or agglutinates dispersed through the gel column (strength: gradation 3,2,1.



Precautions

- ID cards which show air bubbles in the gel or drops in the upper part, or the covering aluminium foil is removed, the ID card must be discarded.

- Bacterial or other contamination of materials may cause false positive or false negative results.

- The negative control must not show any agglutination. If it shows agglutination, your procedure is invalid. Repeat the whole procedure.

Q, Regarding ABO grouping, antisera A contains (T or F):

1. A antigen.
2. B antibody.
3. D antigen.
4. A antibody.

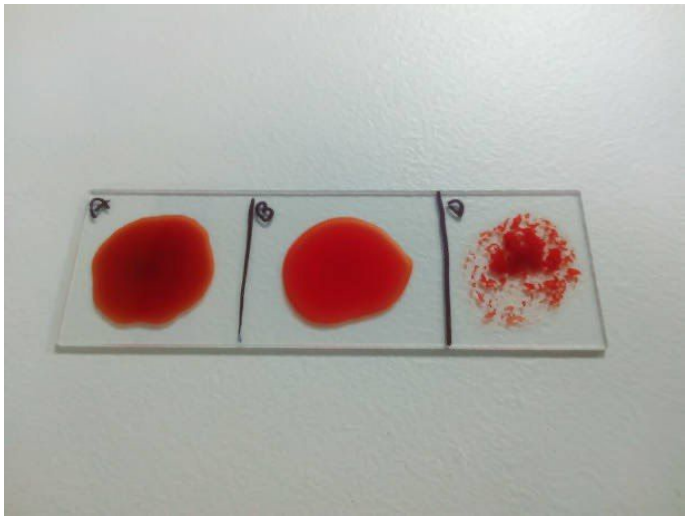
Q, Why wash red blood cells?

Q: in forward grouping, which one tested:

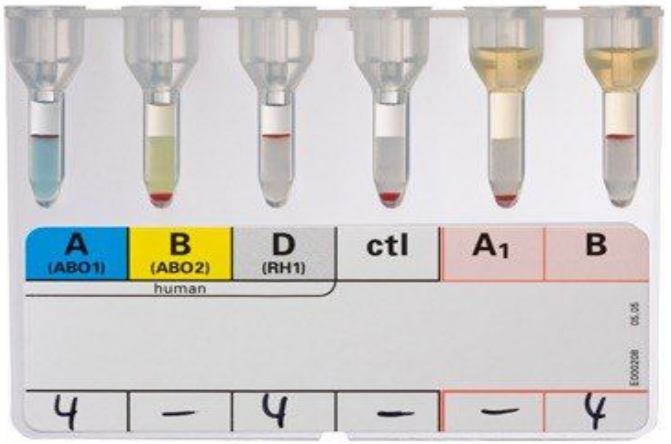
1. Patient RBC.
2. Donor antibodies.
3. Patient serum.
4. Clotting factors.

Q: interpret the results:

1.



2.



References

1. Modern Blood Banking & Transfusion Practices.
2. Practical Transfusion Medicine.