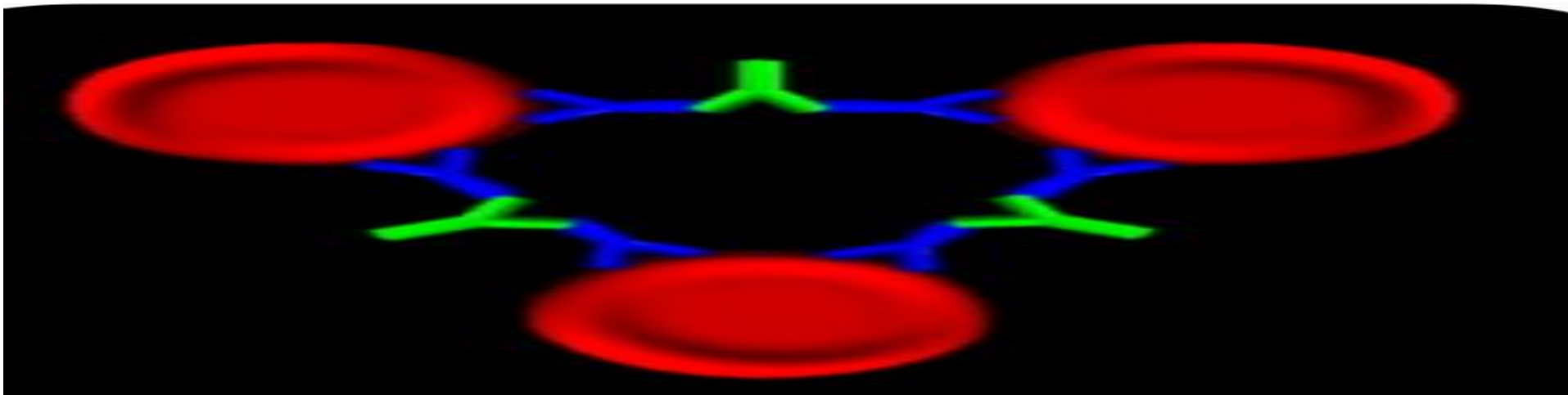
ANTI-GLOBULIN TEST (AGT)

By Mohrah Alalshaikh



Outline

- Haemagglutination process.

- Anti-globulin test:

1. Direct anti-globulin test
2. Indirect anti-globulin test

- Anti-globulin test reagent.

- Reaction visualisation

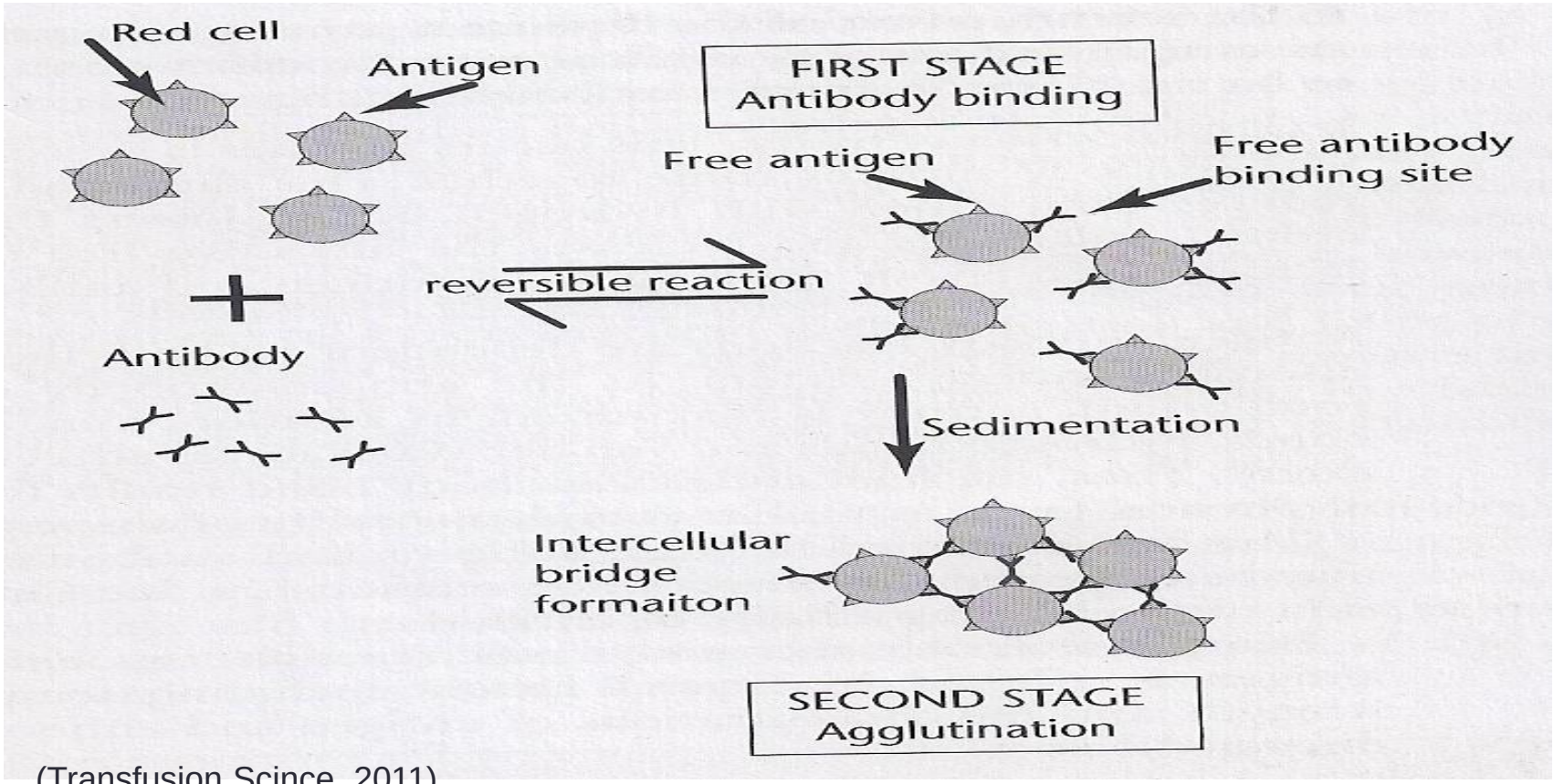
Haemagglutionation

- Generally, visualisation of Ag-Ab reactions in the laboratory is achieved by either haemagglutination or haemolysis of red cell.
- Red cell Ag-Ab reactions are used to:

1. Determine blood groups.
2. Perform antibody screening
3. For compatibility testing.

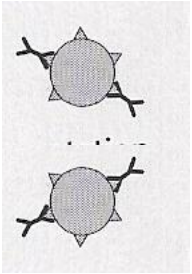
Haemagglutionation

Haemagglutination occurs in two stages. **In first stage**, red cell Abs attach to their corresponding Ags. We can call this stage 'binding' or '**sensitisation**'. **In second stage**, intracellular (between cells) bridges are formed because the free binding site of the Abs attach to a free Ag on the near RBC. This stage called **agglutination.**

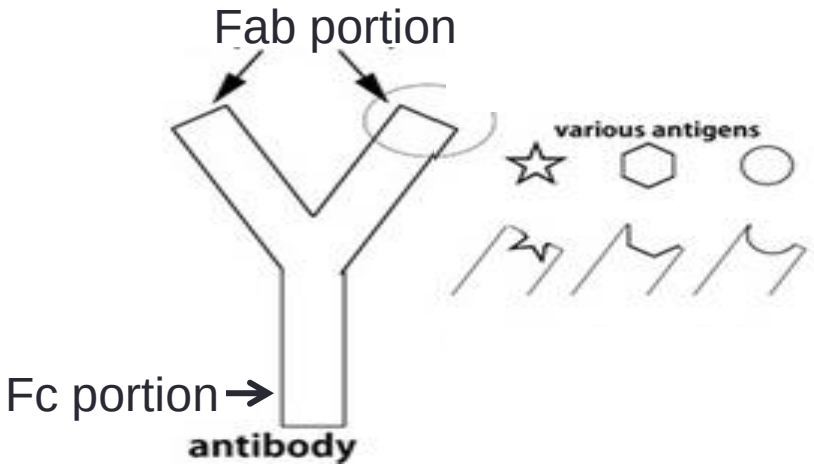


(Transfusion Scince, 2011).

First stage of agglutination

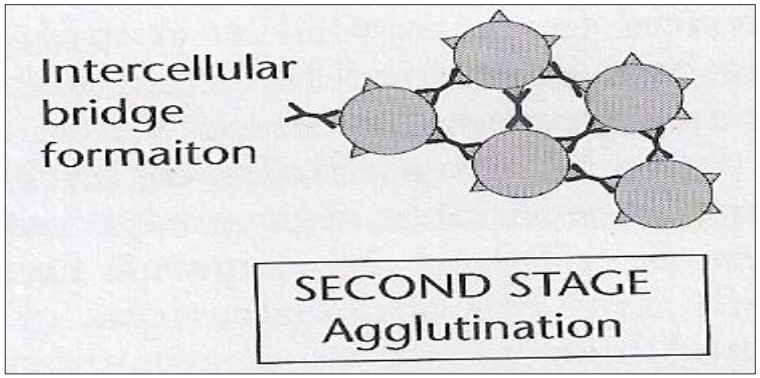


- (Fab) portion of Ab is matching in shape and charge to the corresponding Ag .
- Several forces and types of bond are involved in this process of Ab binding.
- Example of bonds: ionic bond, hydrogen bond. Example of other factors, temperature, pH, ionic strength and Ag/Ab concentration



Second stage of agglutination

- In this stage intracellular bridge is formed.

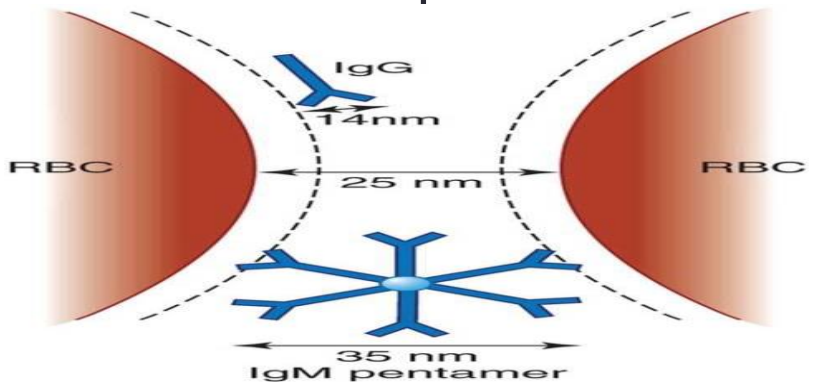


- There are some major factors affecting this stage including:

1. The force of repulsion.
2. Concentration of Ag and Ab.

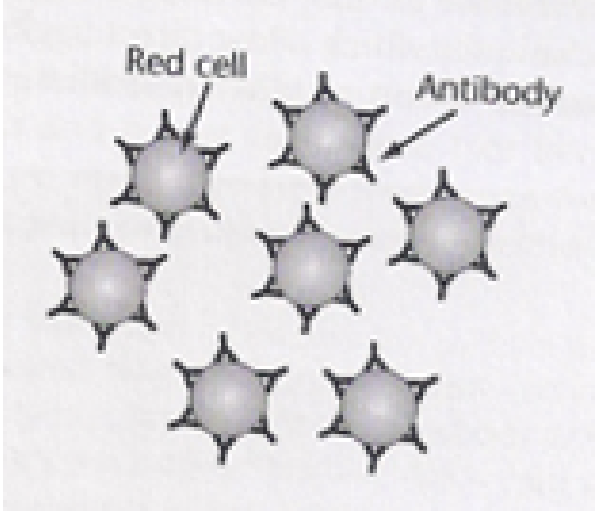
Repulsive forces

- The most important force against aggregation is the repulsive forces result from the electronegative charge of the red cell membrane arise from negatively charged amino acid (sialic acid) present on the surface of RBC.
- The repulsive forces keep the red cells apart by approximately 18-25 nm.
- A maximum distance between IgM binding site is 35 nm, so they can agglutinate RBCs directly. IgM sometimes called complete Ab .
- On the other hand, a maximum distance between IgG binding site is 14 nm, so they can not form intracellular bridge between RBC. Thus, generally IgG can sensitise RBC but NOT directly agglutinate RBC in saline. IgG also known as incomplete Ab.



Concentration of Ag and Ab

- In first stage of agglutination, the increase of Ag or Ab concentration is increase the RBC sensitisation. However, this will inhibit the agglutination in the second step.
- For this reason, the concentration between Ab and Ag should be optimised.

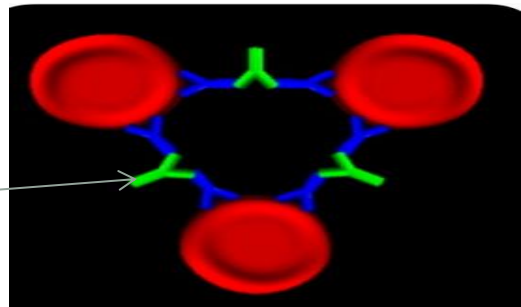


Anti-globulin test (AGT)

- It is also known as coombs test or anti-human globulin test.
- AGT is the most important method available for detecting clinically important Abs. There are two types of AGT:

1. Direct Anti-globulin Test (DAT): Used to detect Abs attached to RBCs.
2. Indirect Anti-globulin Test (IAT): Used to detect free RBC Abs in plasma.

- Anti-globulin reagent used in AGT contains anti-human globulin which is directed against the Fc portion of human Ab or/and complement (C3d).
- Both complement and Ig are globulin.



DAT

- DAT is a laboratory test that can be used to identify whether **RBCs have antibodies (IgG) attached to their surface in vivo** OR **RBCs have been coated with complement (C3d)** OR **both of them.**

- It is help in:

1-Diagnose the cause of haemolytic anaemia as caused by autoimmune disease or induced by drug.

2-Also to investigate a transfusion reaction.

3-To diagnose haemolytic disease of the fetus and newborn.

Conditions associate with sensitised RBC

A. **Autoimmune haemolytic anaemia**: in this disease the immune system of the body produce Abs directed against its own RBC Ags. **These Abs are called autoantibodies, so the autoantibodies are Abs directed to self Ags.**
B. **Drug-induced anaemia:**

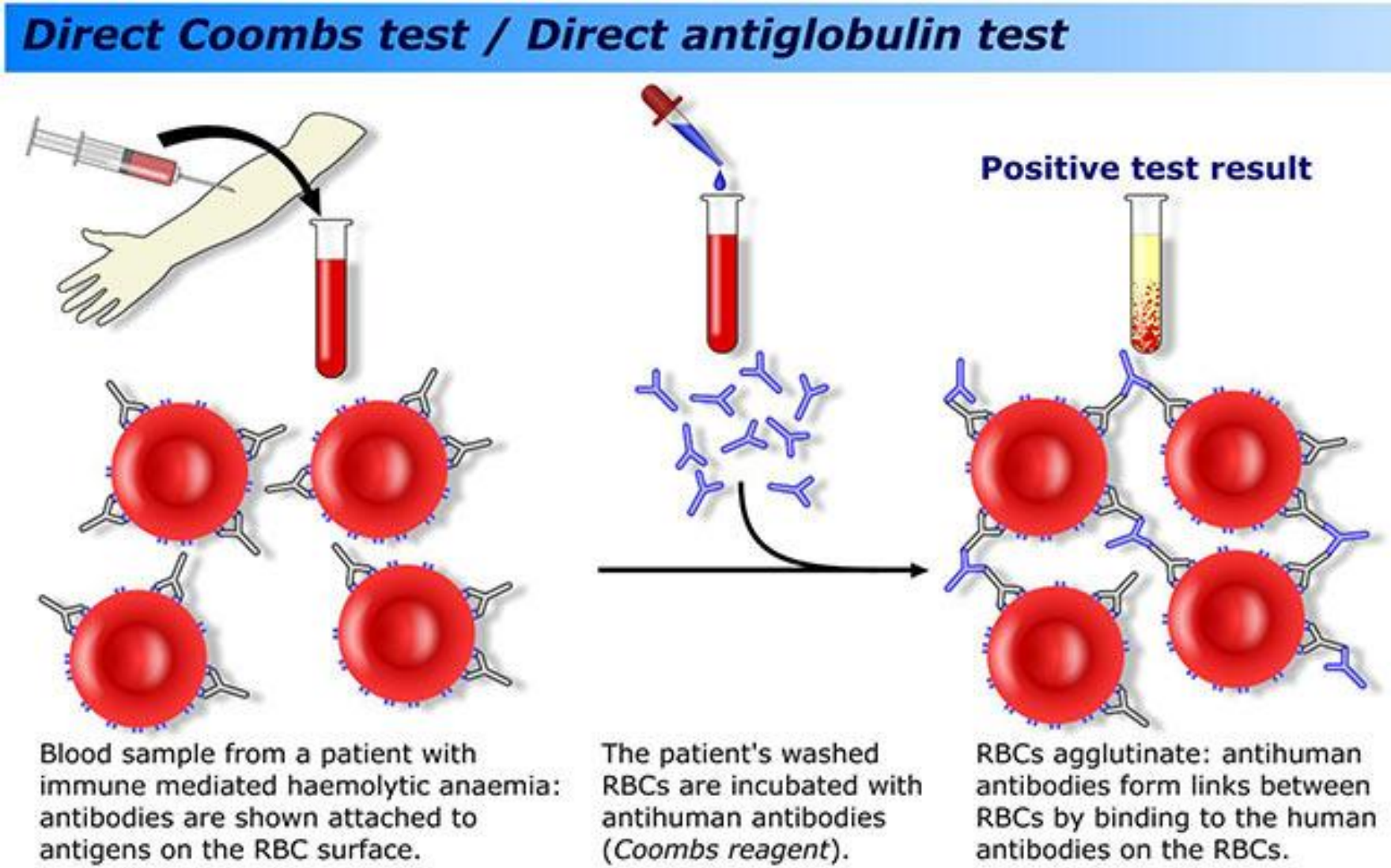
Certain drugs can induce antibody against red cell Ags. Sometimes, drugs coat the surface of RBCs, causing Abs to react with the RBCs but this happen very rare.

Conditions associate with sensitised RBC con.

C. **Mother/Baby blood type incompatibility**: a DAT is performed on the blood of **a baby** who is at risk of HDN.

D. **Following blood transfusion**: always patients receive compatible ABO and RhD blood, BUT sometimes recipients immune system recognise RBC Ags from OTHER systems such as Kell or Duffy, so the body produce Abs against these Ags. **These Abs are called alloantibodies, so alloantibodies are the Abs produced against foreign Ags.** These Abs may attach to the incompatible Ags on DONOR RBCs circulating in the blood stream and sensitise them.

DAT principle



Precautions

- We have to wash red cells before add the AGT reagent because globulin in plasma may neutralise the AHG Abs, consequently No agglutination is detected leading to have false negative result.
- **Control:** The test should be controlled by red cell pre-senstised with IgG Abs, so the control cells should be agglutinated after the addition of AHG Abs

Result interpretation

- A- DAT+ (agglutination is detected): Means there are antibodies attached to the RBCs. A small percentage of normal people will be DAT+ and not have haemolytic anaemia.
- B- DAT- (NO agglutination is detected): Means most likely there is NO Ab attached to RBCs.

IAT

- Indirect antiglobulin test (IAT).
- The aim of this test is to detect FREE (**unbound)** Abs in plasma against RBC **other than** the anti-A and anti- B Abs (WHY?).
- If Abs are detected, then antibody identification test must be done to determine which Abs are present.
- Individuals may develop Abs against RBCs circulating in their blood because they have been exposed to RBCs other than their owns (foregone RBCs). For example, after blood transfusion or through pregnancy.

IAT

- Applications of IAT:

1- Usually it is used as **Ab screening test** for:

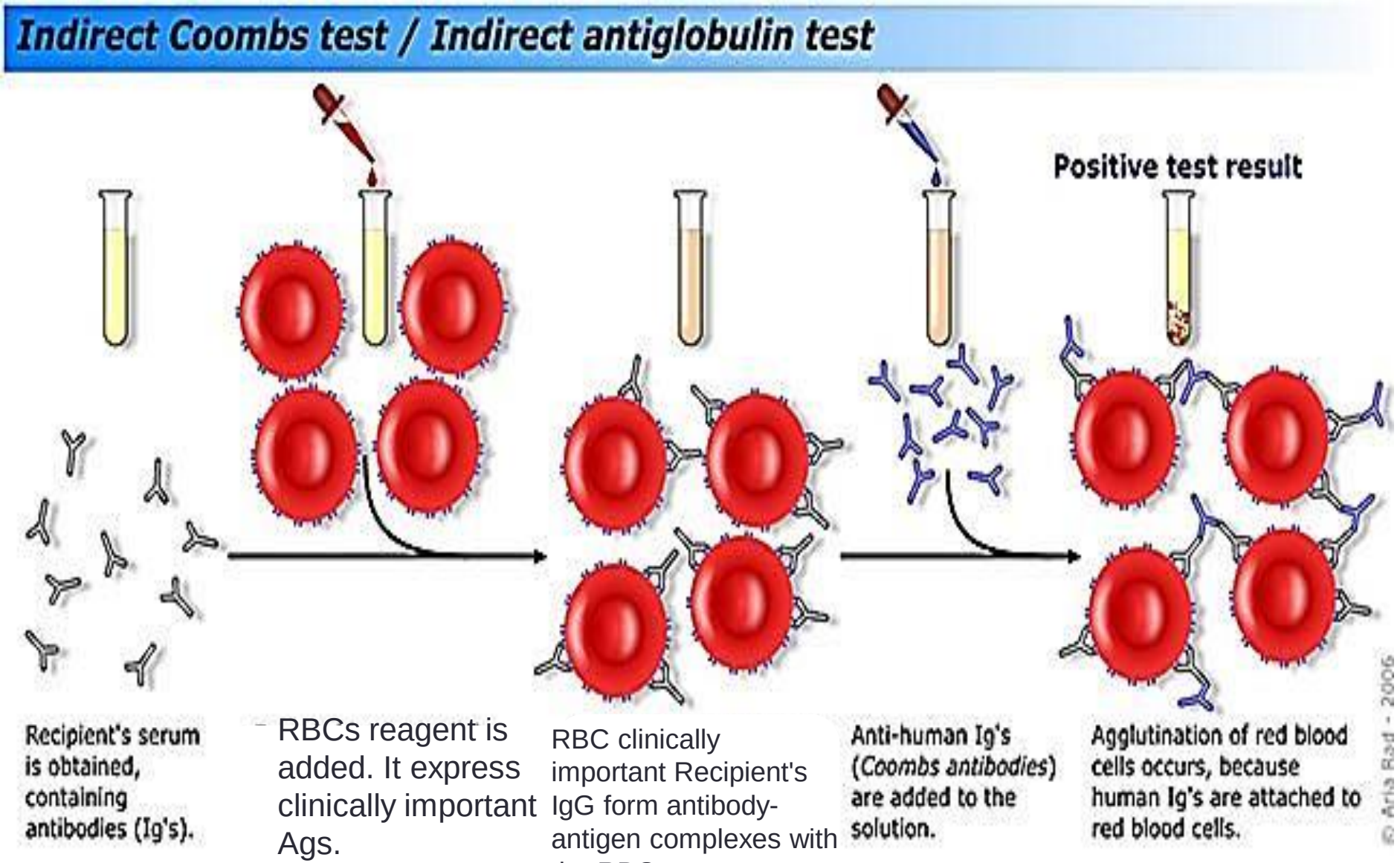
- A- **Patients when preparing for a blood transfusion**: patient who previously had blood transfusion may develop Abs against one or more Ags such anti-K or anti-Fy(a). It helps in choosing the right blood for crosshatching and transfusion.
- Antibody screen best indication of antibodies, more sensitive than cross-match against donor cells.

B- **Pregnant women**: the RBC screening (IAT) is used to screen for antibodies in the **mother blood** that may cross the placenta and attack the baby‘s red cells, causing HDN. So, DAT for babies where IAT for mothers. It is called antenatal antibody screening.

2- Part of compatibility testing (Cross-matching).

3- Determination of RBC phenotype using known antisera (eg., Kell typing).

IAT principle



Results interpretation

A- IAT+: Means there are RBC antibodies present in the serum. Following a positive antibody screening, Ab identification test should be performed to determine the type of the Abs.

B- IAT-: Means most likely there is NO Free clinically significant RBC Ab present in the patients serum.

AGT reagents

- For DAT we just use anti-globulin Ab (anti-IgG or/and anti-C3).

- For IAT: First, we use **RBC reagents**. The patient's serum / plasma is tested against a 2 or 3 cell red cell reagents. These RBC reagents are of specially selected, fully phenotyped red cells. It is called 'Reagent Screening Red Cells' (always from O group, WHY??). We use O red cells because we do not want the naturally occurring Abs, Ant- or Anti-B that normally present in the patient's serum to agglutinate the reagent cell. Second, we add the **anti-globulin Ab**.

Anti-globulin reagent

- Anti-human globulin reagent can be:

1. Polyclonal: contains anti-IgG and anti-C3d. It is prepared by inject non-human species (rabbit for example) with human serum. So the animal immune system produce anti-human globulin (anti-IgG and anti-C3d)
2. Monoclonal: just contain one specificity: anti-IgG **OR** anti-C3d.

- The (Fab) portion of anti-human globulin reacts with the (Fc) portion of human IgG.

IAT screening RBC

- Usually a panel consists of two to three RBC reagents with known, full phenotype is used.

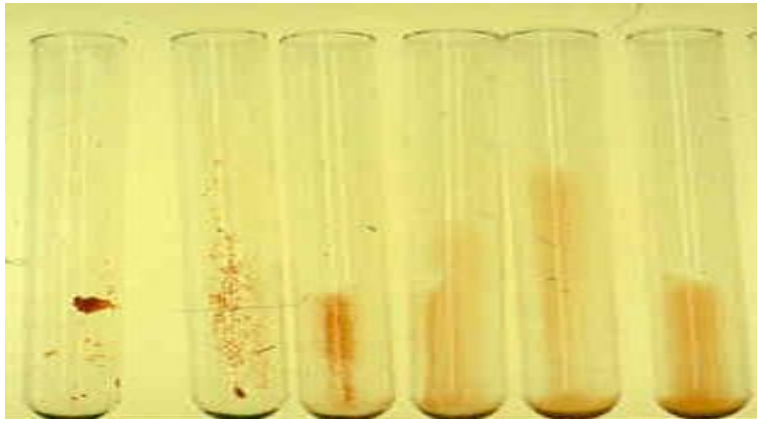
Results with Screening Cells

Rh	D	C	c	E	e	M	N	S	s	P1	K	k	Le^a	Le^b	Fy^a	Fy^b	Jk^a	Jk^b	Pt A	Pt B
O R1R1	+	+	0	0	+	0	+	0	+	3	+	+	0	+	0	+	+	0	3	0
O R2R2	+	0	+	+	0	+	0	+	0	4	0	+	+	0	+	0	0	+	3	0
O rr	0	0	+	0	+	+	+	+	+	3	0	+	+	0	+	+	+	+	0	0

Visualisation of agglutination

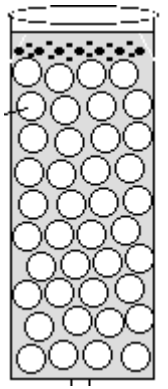
- scoring: we give score for agglutination, where 5 means very agglutinate and 1 is less agglutinate.
- Ag/Ab reactions can be detected in many ways including:

1. Reactions in test tube.
2. Reactions on slide.
3. The use of microplates.
4. Reaction in microtubes (also called Gel card) (used most common).



Gel card

- Gel card contains gel beads inside the microcolumns. So, if there is agglutination, the clumping stay at the top. But if there is no interaction between the Ab and Ag the cell will go down.
- For DAT:



-These cards contain beads + anti human IgG +anti C3d.

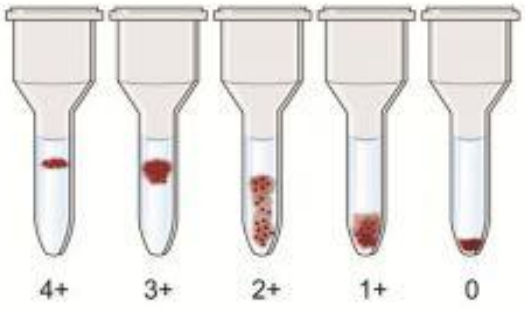
-Add patient's RBC Incubate and centrifuge.

- For IAT:

-These cards contain beads + anti human IgG +anti C3d.

-Add reagent red cells then patient's plasma onto the column

-Incubate and centrifuge.



summary

- First and second stages of haemagglutination process.
- Factors influence the agglutination: repulsive forces and Ag/Ab concentration.
- Anti-globulin test types:
- **DAT:** detect senstised RBCs.

Conditions associated DAT+: autoimmune haemolytic aneamia, HDN, drug induced anemia and after incompatible transfusion.

Principle of DAT, precautions and results interpretation.

- **IAT**: detect free RBC Abs.

Used usually for patient pre-transfusion and for pregnant women.

Principle of IAT.

- Reagent used in AGT: 1- anti-human globulin reagents: monoclonal and polyclonal specificity. 2- RBC reagent from O group expressed clinically importance Ags between them (for IAT).
- Different methods for visualising Ag/Ab reactions, the most common one for AGT is Gel card method.