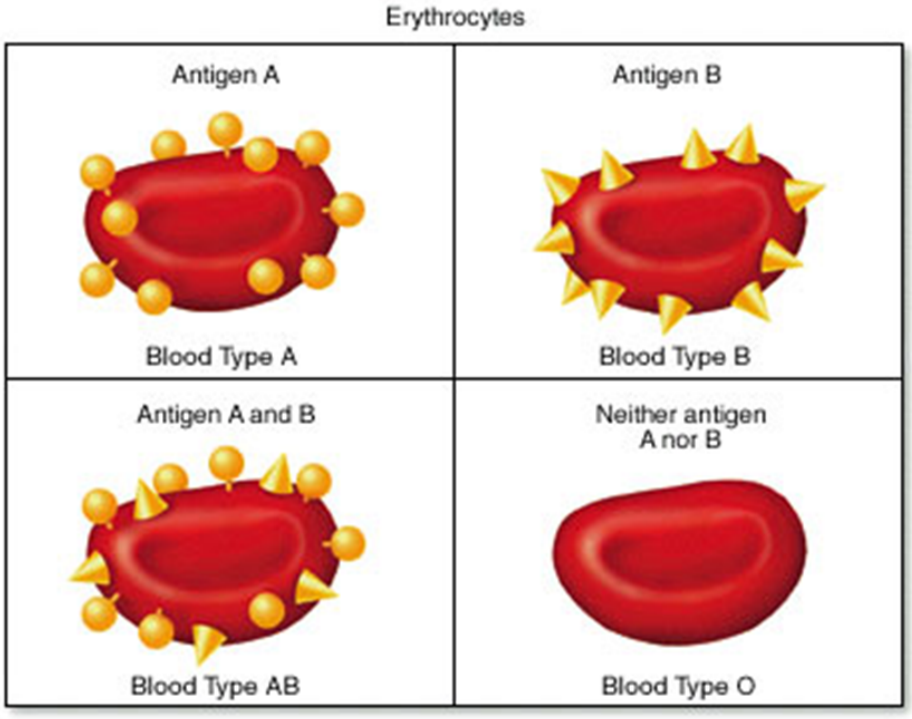
Antigens

Antigens are physical structures on red cell surfaces capable of triggering an immune response i.e. an antibody, in an individual lacking that structure.

Blood group antigens are physical structures found on the surface of red cells which are capable of triggering an immune response in the form of antibodies.

An antigen will stimulate production of an antibody in an individual who lacks that antigen. The antigen-antibody reaction is specific therefore a given antibody will react only with its corresponding antigen.

- This is commonly known as the 'lock and key' principle.
- In essence the antigen initiates antibody production and in doing so eliminates itself.

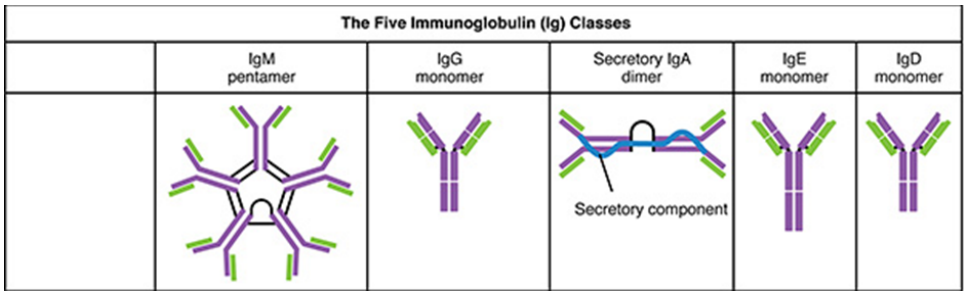


Antibodies

Antibodies are immunoglobulins produced as an immune response to a foreign antigen.

The production of an antibody is dependent on a number of factors influencing immunogenicity.

- A substance capable of triggering an immune response will typically be recognised as foreign, relatively large, protein, carbohydrate or lipid based, and introduced in sufficient amount.



It also depends on the individuals immune system. Every person will respond differently to exposure to a foreign antigen and this could be due to their age, health (as they may be immunocomprimised), or immune response genes they have inherited.

- There are five classes of antibody, IgG, IgM, IgA, IgE and IgD, however within blood transfusion laboratories we are typically concerned with IgG and IgM antibodies only.

Naturally Acquired Antibodies

A person develops 'naturally acquired antibodies' against the antigens they don't have.

In blood grouping we are interested in naturally acquired blood group antibodies.

Blood Group	Antigens present	Antibodies present
A	A	Anti-B
B	B	Anti-A
AB	A and B	None
O	Neither A or B (H)	Anti-A,B

It is important to remember that antibodies will only be produced against antigens the individual does't have.

- For example an individual who is blood group A will have A antigens on their red cell surface. Through exposure to chemical structures in the environment which are identical to B antigens, they will produce Anti-B which will remain in the individuals circulation.
    - A group A individual would not be able to make naturally acquired Anti-A as they would essentially be attacking themselves.
    - The whole point of antibodies and the immune system is to protect the body from foreign invaders.
- Group B individuals would produce anti- A,
- Group AB individuals wouldn't produce any antibodies

    - You will remember me saying that group AB individuals will have separate A and B antigens on their red cell surfaces. Well in antibodies it is different. Anti- A,B is one antibody which can attach to both antigen A and antigen B.
- And Group O individuals would produce anti-A,B.

Antibodies can also be produced after exposure to foreign red cell antigens.

Rh Blood Group System

Blood group Rh is commonly used to talk about a persons D antigen status

- for example a Group O Positive individual is Group O Rh D positive.

However the Rh blood group system includes big C, little c, big E and little e.

- Again these are antigens which can be present on the red cell depending on the individuals inheritance.
- Big C is a real antigen, little c is a real antigen, big E is a real antigen and little e is a real antigen. Little d is not real and simply means lack of D, and is only written to act as a place filler
    - Again refers to antigens on a red cell
    - C is real (C+)
    - c is real (c+)
    - E is real (E+)
    - e is real (e+)
    - d is NOT real – it simply means 'lack of D' – it's a place filler.

Blood Group Testing

ABO grouping is the single most important serological test performed on pre-transfusion samples.

The uniqueness of naturally occurring antibody production in the ABO system allows two methods to be used together for blood grouping.

- This is the forward group and the reverse group.

The basis of serological testing is to add antigen and antibody and look for the presence or absence of agglutination.

- Antigens are carried on red cells and antibodies are found in patient plasma and in human derived antisera.
- The basic principle of serological testing is to test an 'unknown entity' against a 'known entity'.

Put simply:

- If we are looking for an antigen, we use an antibody to find it.
- If we are looking for an antibody, we use an antigen to find it.
    - E.g. To test if some one is group A, I want to know if they have an A antigen….use Anti-A
    - I want to know if someone has an Anti-B use cells with known B antigens on them.

This reacting plasma or antisera with red cells and checking for agglutination is the simplest test performed in the transfusion laboratory and is called **direct agglutination**.

Forward Group

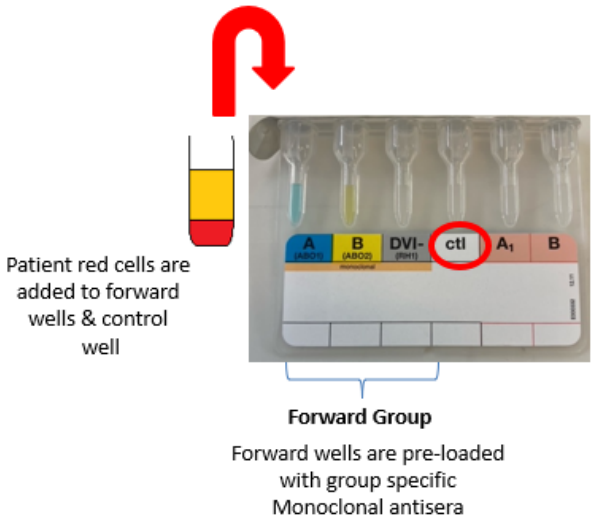
Here, column agglutination technology has been used, which is the most commonly used testing system in the UK.

- The test has been performed using automation.
- For the majority of UK blood transfusion laboratories, computer controlled automation is used as it is the best way to reduce human error.

The forward group uses monoclonal anti-A and anti-B grouping reagents which are preloaded into the forward wells.

- Red cells are added and through agglutination or no agglutination we can identify if any corresponding antigens are present on the red cells.

The forward group is identifying what antigens are present on the red cell surface.



Patient red cells are also added to a blank control well.

The purpose of this is to ensure any agglutination is due to genuine antigen-antibody reactions.

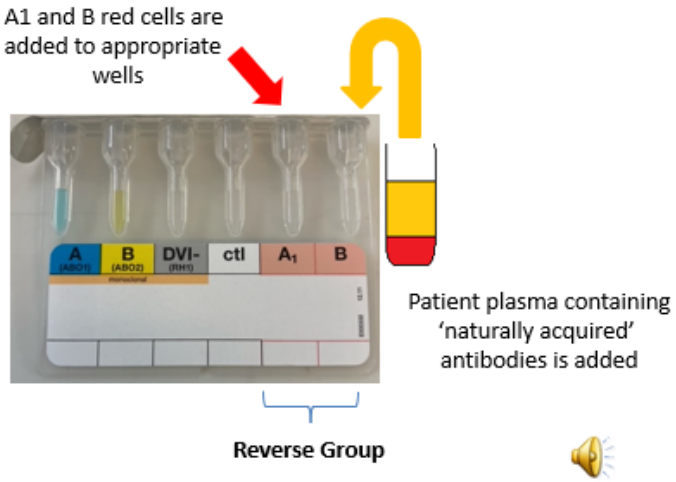
- A positive reaction in the control well would indicate a problem with the test as a persons red cells should not be able to react with themselves.
- A positive reaction in the control well means the entire test has failed and sample results should not be recorded.

Reverse Group

For the reverse group, A1 and B reagent red cells are added to the reverse wells. Patient plasma is then added.

- And again through agglutination or no agglutination we can identify if any corresponding antibodies are present in the patients plasma.

The reverse group gives an added level of confidence in results and enables the identification of rarer and more unusual blood groups.



In patient testing, a forward group only is required for all known historical patients providing there is a secure and fully automated grouping system with electronic data transfer in place.

- However all new or unknown patients must be tested twice for forward and reverse group using two separate samples.
- The second sample is to confirm the group of the first sample and the rational for doing this is to avoid wrong blood in tube events.

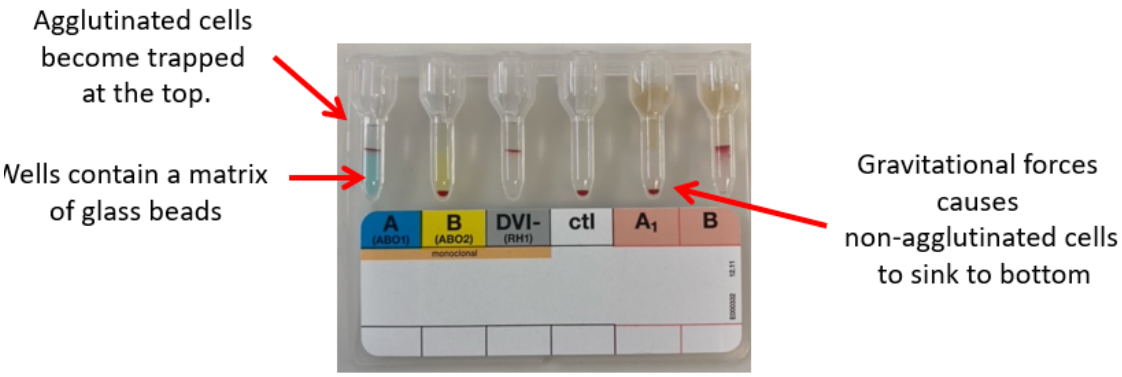
ABO Grouping Result

Blood grouping is routinely performed at room temperature which is between 18 and 22°C.

- Cards are spun using a centrifuge before being read for agglutination.
- Each well contains a matrix of glass beads.

Agglutinated cells are unable to pass through this matrix and become trapped at the top- indicating a positive reaction.

- Agglutination indicates a positive reaction.



Blood Group A Rh D Positive

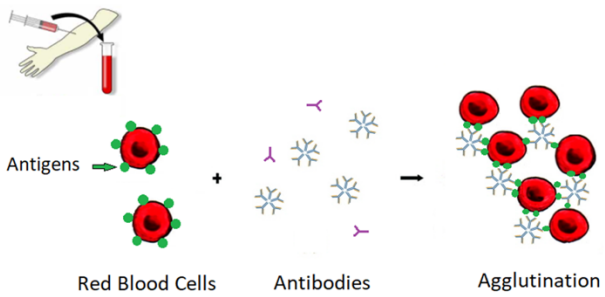
Gravitational forces in centrifugation causes non agglutinated cells to sink to the bottom indicating a negative reaction.

Abajo = Gravitational forces in centrifugation causes non agglutinated cells to sink to the bottom = negative reaction

Medio = agglutinated cells become trapped at the top = Agglutination indicates a positive reaction.

Agglutination

When red blood cells with antigens on their surface mix with the corresponding antibody, agglutination occurs which is the cross linking of cells to form a clump.



Examples of non-matching forward group

Occasionally we get an unexpected result where the reverse group does not match the forward group.

Additional antigens detected

Post transfusion of non identical ABO red cells
Post bone marrow transplant
Weak expression of A or B antigen

Missing antigens

Malignant diseases

Red cells from foetus or newborn infant

This can be due to additional antigens or missing antigens in the forward group or additional antibodies or missing antibodies in the reverse group.

- Unexpected positive reactions in the forward group are rare, however mixed field reactions can be seen in patients who have for example recently been transfused, in post bone marrow transplant patients and in individuals with weak expression of A or B antigen.
- Unexpected negative reactions in the forward group or very weak reactions can be a result of malignant diseases or red cells from a fetus or newborn (however it should be noted that most of the time ABO antigens are easily detectable from birth.

Examples of non matching reverse group

- Unexpected positive reactions in the reverse group is the most common cause of problems in blood grouping.

Blood grouping is performed not at body temperature, but at room temperature which is between 18-22°C.

- As a result any cold reacting antibody present in patients plasma can also be detected.
- Remember A1 and B red cells used for reverse grouping also carry other blood group antigens which antibodies in plasma can react against.
- Anti-A1, anti-M and anti-P1 are the most common cold reacting alloantibodies which cause issues in grouping.

Patients with cold autoimmune haemolytic anaemia can also be difficult to group.

- Anti-I is the most common cold reacting autoantibody and with majority of individuals carrying the I antigen, it is likely to react with the A1 and B cells.

Patients may have also been recently transfused with non ABO group specific plasma components, or treated with intravenous immunoglobulin which is a human derived product made from pooled plasma.

Additional antibodies

- Cold reacting alloantibodies (most commonly anti-A1, anti-M and anti-P1) and cold reacting autoantibodies (most commonly anti-I).
- Transfusion of plasma components
- Intravenous Immunoglobulin treatment (IVIg)

Missing antibodies

- Immunosuppressed
- Age (elderly or infant)

- Bone marrow transplant
- Transfusion of plasma components e.g. AB plasma

- Unexpected negative reactions in the reverse group can occur if the individual is immunosupressed, because of the age- remember infants won't have fully formed antibodies from birth. Elderly can have weakened immune systems when they are greater than 80 years old.

  Again, missing antibodies may be due to incompatible bone marrow transplant or because of transfusion of AB plasma components. AB plasma can be transfused to all recipients because an individual who is group AB will have no antibodies in their plasma. If given to a group A person this will be reflected as a negative or weak reaction in the reverse group.

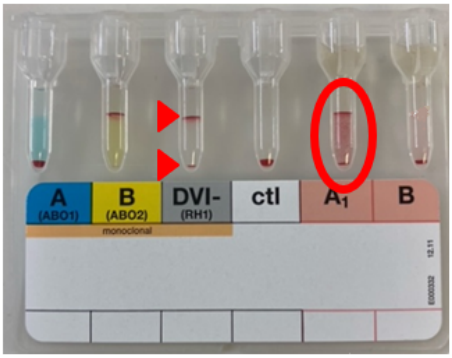
What's going on?

Here, we have an anomalous result.

You can see there is a dual population in Rh D well, and there is a weak reaction in the A1 reverse well.

So, what is going on?

1. In the first instance the test was repeated. The second result was the same indicating it is genuine.
2. On checking the patients historical group, transfusion history and clinical details, this result was explained.



✓ Repeat test
✓ Check clinical details
✓ Check history

The patient was historically grouped as B Rh D Positive and was recently transfused Group B Rh D negative blood components.

- So to give the dual population, the patients own D antigens have agglutinated with the preloaded antisera, and the transfused blood cells which lack the D antigen remain in the patients circulation giving the negative reaction.

The patients clinical details also noted they were being treated with chemotherapy. This explains the weak reaction in the reverse group as the patient will be immunosupressed.

Mismatch Resolutions

Whenever blood grouping does not give the expected reaction

1. the first step is to repeat the test.
    - This is to confirm the reaction is genuine.
2. The patients history should then be checked looking at their historical group, diagnosis and transfusion history.
3. If the anomalous result is not explained, a repeat sample should then be requested to ensure the blood wasn't taken from the wrong patient. If the result is genuine then further investigations are required.

For example, in the case of an additional antibody, the test can be repeated at 37°C to rule out the presence of a cold reacting antibody. Additional reagents if available can also be used to try resolve any discrepancies.

What is important to remember is that a blood group should not be assigned until the anomaly is resolved.

✓Repeat test
✓Check history
✓Ask for repeat sample
✓Warm sample to 37°C (if additional antibodies)
✓Use additional reagents
- ➢Forward group- anti-A1, anti-A,B, anti-H, AB serum
- ➢Reverse group- A2 cells and O cell

A blood group should NOT be assigned until the anomaly is resolved

Technical Errors

A technical error is actually more likely the cause of an unexpected result rather than the weird and wonderful.

This is not to say that the reaction isn't genuine but it is important to rule out a technical error first.

Common technical errors include wrong sample selected and tested, mislabelled samples, transposition of samples where the forward and reverse group are from two different patients, transposition of reagents where A1 and B cells have been put into the wrong well, failure to follow the standard operating procedure, expired reagents being used or results transcribed incorrectly.

- Technical errors is more likely the cause of unexpected grouping result than the 'weird and wonderful'
- Common technical errors
  -wrong sample
  -mislabelled tubes

-transposition of samples or reagents
-failure to follow Standard Operating Procedure (SOP)
-use of expired reagents
-transcription errors

Compatible Blood Components

Blood components for transfusion should be selected to match the ABO group of the recipient wherever possible.

This is the easiest way to avoid any complications. If ABO identical components aren't available there are compatible alternatives. It is paramount transfused components are compatible to avoid acute transfusion reaction.

Recipient ABO group	Antigens on recipient red cells	Antibodies in recipient circulation	Compatible Red cells	Compatible Plasma
O	None	Anti-A Anti-B Anti-A,B	O	O A,B,AB
A	A	Anti-B	A O	A AB
B	B	Anti-A	B O	B AB
AB	A and B	None	AB A,B,O	AB

The implications for transfusion are different depending on if you are transfusing red cell components of plasma components. This is because group A, B and AB red cells carry large amounts of A or B antigens and O, A and B plasma will contain large amounts of A,B and AB antibodies.

The table here gives you the antigens and antibodies for each ABO group as well as the red cell and plasma components which are compatible.