Division of
Blood Transfusion Services

Ministry of Health and Family Welfare
ABO and Rh Blood Grouping & Typing
Teaching Aim

• Understanding inheritance, synthesis, various antigens and antibodies and their clinical significance in ABO & Rh blood group systems

• Understanding practical aspects of ABO & Rh blood grouping
Human Blood Groups

• Red cell membranes have antigens (protein / glycoprotein) 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

• Most well known & clinically important blood group system.

• Discovered by Karl Landsteiner in 1900

• It was the first to be identified and is the most significant for transfusion practice

• It is the ONLY system that the reciprocal antibodies are consistently and predictably present in the sera of people who have had no exposure to human red cells

• ABO blood group consist of
  o two antigens (A & B) on the surface of the RBCs
  o two antibodies in the plasma (anti-A & anti-B)
### Reciprocal relationship between ABO antigens and antibodies

<table>
<thead>
<tr>
<th>Antigens on RBCs</th>
<th>Antibody in plasma / serum</th>
<th>Blood group</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Anti-B</td>
<td>A</td>
</tr>
<tr>
<td>B</td>
<td>Anti-A</td>
<td>B</td>
</tr>
<tr>
<td>AB</td>
<td>None</td>
<td>AB</td>
</tr>
<tr>
<td>None</td>
<td>Anti-A, Anti-B</td>
<td>O</td>
</tr>
</tbody>
</table>
Development at birth

- All the ABH antigens develop as early as day 37 of fetal life but do not increase very much in strength during gestational period.

- Red cell of newborn carry 25-50% of number of antigenic sites found on adult RBC.

- Although cord red cells can be ABO grouped, the reactions may be a bit weaker than expected.

- A or B antigen expression fully developed at 2-4 yrs of age and remain constant throughout life.
Expression of ABO Antigens

- Although the ABO blood group antigens are regarded as RBC antigens, they are actually expressed on a wide variety of human tissues and are present on most epithelial and endothelial cells.

- ABH antigens are not only found in humans, but also in various organisms such as bacteria, plants, and animals.

- Present both on red blood cells and in secretions only in humans and some of the apes (chimpanzee, gorilla).

- In all other mammalian species these substances are found only in secretions.
Anti-A and anti-B antibodies

- Not present in the newborn, appear in the first years of life (4-6 months usually), reach adult level at 5-10 years of age, decreases in elderly

- Naturally occurring as they do not need any antigenic stimulus

- However, some food & environmental antigens (bacterial, viral or plant antigens) are similar enough to A and B glycoprotein antigens and may stimulate antibody development
Anti-A and anti-B antibodies (contd…)

- Immunocompetent person react to these antigens by producing antibodies to those absent from their own system
- Usually IgM, which are not able to pass through the placenta to the fetal blood circulation
  - Anti-A titer from group O > Anti-A titer from group B
  - Anti-A titer from group B > Anti-B titer from group A
# ABO antigens & corresponding antibodies

<table>
<thead>
<tr>
<th>Red blood cell type</th>
<th>Group A</th>
<th>Group B</th>
<th>Group AB</th>
<th>Group O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antigens present</td>
<td>A antigen</td>
<td>B antigen</td>
<td>A and B antigens</td>
<td>None</td>
</tr>
<tr>
<td>Antibodies present</td>
<td>Anti-B</td>
<td>Anti-A</td>
<td>None</td>
<td>Anti-A and Anti-B</td>
</tr>
</tbody>
</table>

The table above summarizes the ABO blood group system, which is based on the presence of specific antigens on red blood cells and the corresponding antibodies in the plasma. The ABO blood group system is determined by the presence of A, B, or both antigens on the red blood cells, which react with antibodies in the plasma to determine the group type.
'Landsteiner's law : the plasma contains natural antibodies to A or B, if these antigens are absent from the red cells of that person
Inheritance of ABO Blood Groups

- Follows Mendelian principles
- Blood group antigens are “codominant”- if the gene is inherited, it will be expressed.
- There are three allelic genes - A, B & O
- Some aberrant genotypes do occur but they are very rare.
- Understanding of basic inheritance important.
Inheritance of ABO Blood Groups

- Two genes inherited, one from each parent.
- Individual who is A or B may be homozygous or heterozygous for the antigen.
  - Heterozygous: AO or BO
  - Homozygous: AA or BB
- Phenotype is the actual expression of the genotype, ie, group A
- Genotype are the actual inherited genes which can only be determined by family studies, ie, AO.
ABO and H Antigen Genetics

- Genes at three separate loci control the occurrence and location of ABO antigens
- Presence or absence of the ABH antigens on the red cell membrane is controlled by the H gene
- Presence or absence of the ABH antigens in secretions is indirectly controlled by the Se gene
  - H gene – H and h alleles (h is an amorph)
  - Se gene – Se and se alleles (se is an amorph)
  - ABO genes – A, B and O alleles
H Antigen

- The H gene codes for an enzyme (fucosyltransferase) that adds the sugar fucose to the terminal sugar of a precursor substance.

- The precursor substance (proteins and lipids) is formed on an oligosaccharide chain (the basic structure).

- The H antigen is the foundation upon which A and B antigens are built.

- A and B genes code for enzymes that add an immunodominant sugar to the H antigen.
  - Immunodominant sugars are present at the terminal ends of the chains and confer the ABO antigen specificity.
RBC precursor substance

Glucose
Galactose
N-acetylglucosamine
Galactose

Precursor Substance (stays the same)
Formation of the H antigen

H antigen

- Glucose
- Galactose
- N-acetylglucosamine

Fucose
A and B Antigen

- The “A” gene codes for an enzyme (transferase) that adds N-acetylgalactosamine to the terminal sugar of the H antigen
  - N-acetylgalactosaminytransferase

- The “B” gene codes for an enzyme that adds D-galactose to the terminal sugar of the H antigen
  - D-galactosyltransferase
Formation of the A antigen

- Galactose
- N-acetylgalactosamine
- Fucose
Formation of the B antigen

- Glucose
- Galactose
- N-acetylglucosamine
- Galactose
- D-Galactose
- Fucose
Secretor Status

- A, B, H substances are found in all body secretions (except CSF) in 80% of individuals.
- Ability to secrete these substances is determined by the presence of secretor gene (Se) in either homozygous (SeSe) or heterozygous (Sese) state.

<table>
<thead>
<tr>
<th>Blood Group</th>
<th>Substances Secreted</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>H</td>
</tr>
<tr>
<td>A</td>
<td>A &amp; H</td>
</tr>
<tr>
<td>B</td>
<td>B &amp; H</td>
</tr>
<tr>
<td>AB</td>
<td>A, B, &amp; H</td>
</tr>
<tr>
<td>Oh</td>
<td>Nil</td>
</tr>
</tbody>
</table>
Characteristics of Bombay Phenotype

- First reported by Bhende et al in Bombay in 1952.
- Frequency estimated to be about 1 in 7600 in Bombay.
- Absence of H, A & B antigens. No agglutination with anti-A, anti-B or anti-H
- Presence of anti-H, anti-A and anti-B in the serum
- No A, B or H substances present in saliva
- Incompatible with any ABO blood groups, compatible with Bombay phenotype only
- A recessive mode of inheritance (identical phenotypes in children but not in parents)
ABO Subgroups

• ABO subgroups differ in the amount of antigen present on the red blood cell membrane
  o Subgroups have less antigen

• Subgroups are the result of less effective enzymes. They are not as efficient in converting H antigens to A or B antigens (fewer antigens are present on the RBC)

• Subgroups of A are more common than subgroups of B
Subgroups of A

- Two principle subgroups of A are: $A_1$ and $A_2$
- Both react strongly with reagent anti-A
- To distinguish $A_1$ from $A_2$ red cells, the lectin Dolichos biflorus is used (anti-$A_1$)
- 80% of group A or AB individuals are $A_1$ and $A_1B$
- 20% are $A_2$ and $A_2B$
A2 phenotype

• **Clinical significance of A2 phenotype**
  
  o 8% of A2 and 25% of A2B individuals may produce anti-A\textsubscript{1} in the serum
  
  o This may result in discrepancy in blood grouping or incompatibility in cross match
  
  o However, these anti-A\textsubscript{1} antibodies are cold reacting & therefore may not cause problems routinely.

• **Difference between A\textsubscript{1} and A\textsubscript{2}**
  
  o It is quantitative
  
  o The A\textsubscript{2} gene doesn’t convert the H to A very well resulting in fewer A\textsubscript{2} antigen sites compared to the many A\textsubscript{1} antigen sites
B Subgroups

- B subgroups occur less than A subgroups
- B subgroups are differentiated by the type of reaction with anti-B, anti-A,B, and anti-H
- B₃, Bₓ, Bₘ, and Bₑₗ
Practical aspects of ABO grouping

• Routine ABO grouping must include both cell & serum testing as each test serves as a check on the other.
• Test should be done at room temperature or lower; testing at 37°C weakens the reactions.
• Tubes, slides should be dry and labeled properly.
• Serum should always be added before adding cells.
• Results should be recorded immediately after observation.
• Hemolysis is interpreted as positive result.
Blood Grouping

• There are 2 components to blood typing:
  o Test unknown cells with known antibodies
  o Test unknown serum/plasma with known red cells

• The patterns are compared and the blood group is determined.
Blood Sample for Blood Grouping

Blood sample

- Clearly labeled blood samples in sterile tubes (plain & EDTA)
- Test should be performed on the fresh sample for best results. In case the test can not be performed immediately, sample can be stored at 4°C & should be tested with in 48 hours
- No signs of hemolysis should be there
- If serum is not completely separated, centrifuge tube at 3000 rpm for 3 min
- Preferably use saline washed red cells and make 2-5% suspension
Red Cell Suspensions for Blood Grouping

- 2-5%: Test Tube Method
- 0.8-1%: Gel technology
- 1%: Microplate
Test Tube Method of ABO Grouping

Recommended method

• Allows longer incubation of antigen and antibody mixture without drying
• Tubes can be centrifuged to enhance reaction
• Can detect weaker antigen / antibody

Two steps in ABO grouping

• Cell grouping (Forward grouping)
  – Tests the patients red cells with known Anti-A & Anti-B to determine the antigen expressed

• Serum grouping (Reverse grouping)
  – Test the patients serum with known A & B cells to determine the presence of antibody
Lay Out of Tubes for ABO & Rh grouping

Forward grouping
Cell grouping
Rh grouping
Reverse grouping
Sera grouping
2 vol of anti-A / anti-B/ Anti-AB + 1 vol of 2-5% red cell suspension

Incubate at room temp (20-24°C) for 5 min

Centrifuge at 1000 rpm for 1 min

Check for agglutination against well lighted background
Reverse Grouping

2 vol of test serum/plasma

+ 1 vol of 5% suspension of reagent red cells in respective tubes

Shake & leave at room temp (20-24°C) for 5 min

Centrifuge at 1000 rpm for 1 min

Centrifuge & record the results similarly as for cell grouping
## Tube Agglutination Grading

### Tube Agglutination Grading Chart

<table>
<thead>
<tr>
<th>Scale</th>
<th>0-4</th>
<th>0-12</th>
<th>Macroscopically Observed Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>12</td>
<td></td>
<td>One solid agglutinate, no free red cells detected.</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td></td>
<td>One or two large agglutinates, a strong reaction.</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td></td>
<td>Medium size agglutinates, clear background.</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td></td>
<td>Small agglutinates, with a lot of free red cells.</td>
</tr>
<tr>
<td>+/-</td>
<td>3</td>
<td></td>
<td>Weak granularity in the red cell suspension.</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td></td>
<td>No agglutinates, an even red cell suspension.</td>
</tr>
</tbody>
</table>
Microplate Method

- It is ideal for testing large number of blood samples.
- More sensitive to detect weaker antigen-antibody reactions
- Results can be photographed for archival storage
- Microplate can be incubated & centrifuged
- There is significant saving in time and in the cost of disposables and reagents.
- Microplates are intended to be disposable however they can be reused after cleaning them properly making sure that all foreign protein are removed.
- Microplates can be adapted for automation
Microplate Method

Reaction in the microplate after 1 hour incubation at room temperature
Column Agglutination Technology

- One card is basically a set of 6 microtubes
- Microtubes contain either Sephadex Gel or glass microbeads impregnated with antisera
- Antigen-antibody reaction takes place in the reaction chamber of microtube
- Gel matrix or glass beads act as sieve and allow free cells (un-agglutinated) to pass through and settle at the bottom of microtube while agglutinated cells are trapped in the matrix
Grading Result
Rh Blood Group System
Rh (D) Antigen

• Of next importance is the Rh type.
  - Rh is a blood group system with many antigens, one of which is D.

• Rh refers to the presence or absence of the D antigen on the red blood cell.

• Unlike the ABO system, individuals who lack the D antigen do not naturally produce anti-D.

• Production of antibody to D requires exposure to the antigen.

• The D antigen is very immunogenic, i.e., individuals exposed to it will very likely make an antibody to it.

• For this reason all individuals are typed for D, if negative must receive Rh (D) negative blood.
Rh (D) Antigen (contd…)

- Rh antigens are an integral part of the red cell membrane.
- They are protein in nature with an active phospholipid component.
- Rh antigens do not exist in the soluble form and, therefore are not excreted in body fluids.
- Unlike ABO antigens, Rh antigens are present only on red blood cells. These antigens are not found on other blood cells including platelets and leukocytes.
Rh (D) Antigen (contd...) 

- A very potent antigen (50% may form antibody to exposure)
- Frequency in Indian population
  - 92-95% Rh positive
- The most important patient population to consider is females of child-bearing age.
- If immunized to Rh (D) antigen the antibody can cross the placenta and destroy Rh (D) positive fetal cells resulting in death.
- This is why Rh negative women are given injection anti-D after birth of Rh positive baby.
Rh Antibodies

- All Rh antibodies are immune in nature, developed after immunizing event

- React at 37° C and require anti globulin test to demonstrate the reaction

- Generally do not react at room temperature in saline

- Most are IgG in nature and therefore can cross the placenta

- Generally, do not fix complement and cause extravascular hemolysis

- All are important in HDN and delayed HTR
Rh typing

- Normal typing for Rh antigens only includes typing for Rh (D).
- The result of this typing determines the Rh status of the cells (Rh - positive or Rh - negative).
- Some Rh typing sera is diluted in high protein solutions and may require a negative control.
- It is recommended to use two monoclonal anti-D sera from two different manufacturers labeled as D1 and D2, especially to confirm all Rh negatives.
Monoclonal Anti-D

Three types

1. IgM anti-D monoclonal reagent
2. Blend of IgM and IgG monoclonal antibodies reagent
3. Monoclonal IgG anti-D

- IgM antibodies are highly specific and saline reacting equally at RT and 37°C but unreliable for detection of weak D
- Blended antibodies are now routinely used and can be used for detecting weak D
Tube Technique for Rh Typing

- Prepare 5% washed red cell suspension of test sample.
- Take three clean test tubes and label tubes 1 & 2 as “test” and tube 3 as “control”.
- Place 1 drop of anti-D (D1) in tube 1 and 1 drop of anti-D (D2) in tube 2.
- Place 1 drop of 22% bovine albumin / control in tube 3.
- Add 1 drop of 5% test cell suspension to each tube.
- Mix well, centrifuge at 1000 rpm for 1 min.
- Resuspend cell button & look for agglutination
- Control tube should show no agglutination
- For all RhD negative test on blood donor, D^u test recommended
Method for Rh Typing

1 drop anti-D1

+ 1 drop 5% red cells

1 drop anti-D2

+ 1 drop 5% red cells

Mix well and centrifuge at 1000 RPM x 1 min

Look for agglutination
Weak D

Inheritance of D genes which result in lowered densities of D Antigens on RBC membranes, gene codes for less D.

RBC with normal expression of D antigen

Decreased number of D antigens in Weak
Partial D

- Absence of a portion of the total material that comprises the D antigen (qualitative defect)

- If the partial D patient is transfused with D positive red cells, they may develop an anti-D alloantibody to the part of the antigen (epitope) that is missing
Method for Weak D Testing

• Add 1 drop of 2-5% suspension of D negative red cells to a test tube and add 2 drops of Anti D (blend of IgG + IgM).

• Incubate at 37°C for 30 minutes.

• Wash three times with normal saline.

• Make dry red cell button and add polyspecific AHG reagent.

• Look for agglutination.

Results:

• If there is agglutination Du Positive.

• If there is no agglutination Du Negative.
Significance of Weak D

Donors

• Weak D testing on donors required.
• Labeled as D positive
• But as recipient D negative

Patients

• Weak D testing on patients not required.
• Standard practice to transfuse with D negative
Significance of Weak D

• Weak D is much less antigenic in comparison to D, however, such red cells may be destroyed if transfused to a patient already having anti-D. Hence, weak D donor units are labeled as Rh positive.

• The weak D positive recipients are classified as Rh negative and safely transfused with Rh negative blood

• D^u positive infant can suffer from HDN if the mother possess anti-D antibodies

• Rh immunoprophylaxis is recommended for the Rh negative mother if the newborn is D^u positive.
Learning Outcome

- You should now be able to perform ABO & Rh grouping on the donor and recipients sample

- You should be able to resolve discrepancies in the blood grouping

- You should be able to perform weak D testing if required