Erythrocyte Antigens
Unit 6  ABO and H  Blood Groups

A. History of ABO System

1. Discovered in 1900 by Karl Landsteiner and remains the most important of the blood group systems as far as the transfusion of blood is concerned.

2. Landsteiner tested blood samples from his colleagues by mixing each person's serum with suspensions of rbcs from the others.

3. Discovered A, B and O. Group AB was discovered in 1902 by Landsteiner's pupils von Decastello and Sturli.

4. He also demonstrated that the serum of each person contained an antibody directed against the antigen absent from the person's rbcs.

5. ABO blood group system is the only system in which the reciprocal antibodies are consistently and predictably present in the sera of normal people whose rbcs lack the corresponding antigen(s), this is known as Landsteiner's Rule.

6. ABO antigens located on RBCs, lymphocytes, platelets, tissue cells, bone marrow and solid organs.

B. Red Cell membrane structure

1. Surface of the RBC consists of a bilipid membrane in which large protein molecules are embedded.

2. The bilipid membrane is composed of a class of molecules called phospholipids which have both hydrophilic (affinity for water) and hydrophobic (antagonistic to water) properties.

3. The polar heads of the molecules are hydrophilic and the hydrocarbon tails are hydrophobic. It is known that water molecules exist outside the red cell (in the surrounding plasma) and within the cytoplasm, but are largely excluded from the bilayer, hence the orientation of the lipid molecules.
4. External surface of RBC membrane is coated with a diverse array of glycoproteins, complex carbohydrates, and lipoproteins, imparting antigenic structure to the membrane.

C. A and B Antigens

1. Inheritance follows Mendelian genetics.

2. Frequency in white population: group O 45%, group A 40%, group B 11% and group AB 4%.
   a) ABO frequencies differ in selected populations and ethnic groups.
   b) Group B higher in Black and Asian populations.

2. The A and B antigens are not fully developed in newborn infants, even though some antigens can be detected on the red cells of the embryo as early as five weeks after conception.

2. Weaker agglutination reactions are observed with fetal and newborn infants' RBCs compared to the mature RBCs of adults due to the number and strength of A and B antigen sites being less.

D. Biochemical Activities Related to the Development of A, B and H Antigens

1. ABO antigens are located on RBCs, lymphs, platelets, tissue cells, bone marrow and solid organs.

2. Inheritance of A and B genes usually results in the expression of A and B gene products (antigens) on RBCs, but H, A and B antigens are NOT the direct products of the H, A and B genes.

3. Each gene codes for the production of a specific transferase enzyme which catalyzes the transfer of a monosaccharide molecule from a donor substrate to a predeterminded precursor substance.

4. The H gene codes for the production of fucosyl transferase that catalyzes the addition of L-fucose, the immunodominant structure of H antigen, to two slightly different structures, known as the type 1 and type 2 precursor chains. The H gene and its allele h are inherited independently of the allelic A, B and O genes.

5. Once the H gene-specified transferase has acted and the L-fucose has been added to the two chains, the A and B gene-specified products can act to add sugars to the chains that now carry H.

6. The A gene codes for production of a galactosaminyl transferase that effects the addition of N-acetylagalactosamine to the preformed H-bearing chains.

7. The B gene codes for production of a galactosyl transferase that effects the addition of D-galactose to the same H-bearing structure.
8. Thus, the immunodominant structure of the H antigen is L-fucose, of the A antigen N-acetylgalactosamine and of the B antigen, D-galactose.

9. In the absence of L-fucose, the immunodominant structure of H, the A and B immunodominant sugars cannot be added. In other words, if an individual does not inherit a functional H gene, the A and B immunodominant sugars cannot be added to the structures that normally carry those determinants. This is the basis of the Bombay or Oh phenotype.

a. When an individual inherits two h genes, h being a rare allele of H, the A and B immunodominant structures are not added. The h gene is an amorph with no detectable product.

b. In spite of the dependence of A and B antigen assembly on the presence of H at the biochemical level, the Hh genes are not part of the ABO system. Independent segregation of genes at the Hh and ABO loci has been demonstrated in several families.

Genetic pathway of expression of ABH substances on erythrocytes.
c. In individuals who inherit two h genes, A and B gene function is not blocked. In other words, the A and B gene-specified transferase enzymes are still produced (dependent, of course, on the inheritance of an A or B or both genes) but because of lack of H (L-fucose) on the type 1 or type 2 precursor chains, cannot add acetylgalactosamine or galactose (respectively) to those chains.

E. The H System

1. The genes of the H blood group system are H (frequency of 99.9%) and h (<0.1% frequency).

2. H leads to the production of the H antigen that serves as the precursor molecule on which A and B antigens are built.

3. The lectin Ulex europaeus is used to detect the presence of the H antigen on the RBC.

4. The amount of H antigen present varies in quantity according to the blood group:

   \[
   O > A^2 > B > A^1B > A^1 > A^1B
   \]

   MEMORIZE!

5. H-like antigens are found in nature.

6. Occasionally group A, A^1B or (less commonly) B persons have so little unconverted H antigen on their RBCs that they may produce anti-H.

   a. It is relatively weak.
   b. It virtually always reacts at RT.
   c. It is considered clinically insignificant in these blood groups.

7. In contrast persons of the rare O_h (hh) phenotype (have no A, B or H antigens on their RBCs) form a potent clinically significant anti H which reacts well over a wide thermal range and with all RBCs except those of other O_h people.

F. O_h Phenotype (Bombay)

1. This phenotype occurs when two hh genes are inherited at the Hh locus.

   a. These individuals possess normal A or B genes but are unable to express them because they lack the gene necessary for production of H antigen, the required precursor for A and B.

   b. These individuals will transmit the normal A or B gene to offspring.

2. The term "Bombay" used for O_h phenotype because examples of such RBCs were first discovered in Bombay, India.

3. Symbol O_h denotes the phenotype because results obtained in ABO grouping mimic those of group O persons.

   a. The RBCs are not agglutinated by anti-A, -B or -A,B.
   b. The serum agglutinates the reverse A and B cells.
4. Generally not recognized until the serum is tested against group O cells in the antibody screen and agglutinates all O cells tested.

   a. Because these individuals lack A, B and H antigens, they form potent anti-A, -B, -A,B and anti-H, which is the most clinically significant.

   b. These individuals can only be transfused with Bombay blood which occurs in <0.01% of the population.

5. Confirmatory testing for Oₜₜ

   a. Test patient with anti-H lectin *Ulex europaeus*. Normal group O cells are strongly agglutinated while Oh cells are not agglutinated.

   b. The patient's serum will agglutinate all blood types (A, B, AB and O).

   c. The patient's serum will fail to agglutinate Oh cells.

G. The Secretor genes

1. The A, B and H antigens are not confined to red cells but may be present in body fluids also.

2. The secretion of A, B and H substances in saliva and other body fluids is controlled by a pair of alleles, Se and se, called the secretor genes.

3. Secretion of A, B and H soluble substances is accomplished even when only one locus carries Se.

4. There can be no Se when se is present on both chromosomes. *The gene se is an amorph.*

5. The secretor genes are not linked to the ABO locus, they are inherited independently.

![Diagram of H, AB, and Lewis antigens](image-url)
6. Persons who have A, B and/or H substances in saliva are called **secretors** and the following will be present in saliva and all body fluids except cerebrospinal fluid:

<table>
<thead>
<tr>
<th>Blood Group</th>
<th>Substances in Saliva</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>A and H</td>
</tr>
<tr>
<td>B</td>
<td>B and H</td>
</tr>
<tr>
<td>AB</td>
<td>A, B and H</td>
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<td>0H</td>
<td>H</td>
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7. Provided the person is a secretor (whether Se/Se or Se/se), saliva tests can be helpful in defining a subgroup or resolving the genetic makeup of individual who appears to have an unusual blood group.

8. **About 80 percent of Caucasians are secretors, 20% are non-secretors.**

9. Subgroups of A (A¹ and A²)

   1. Subgroups of A are phenotypes that differ from others of the same ABO group with respect to the amount of A antigen carried on RBCs, and, in secretors, present in the saliva.

   2. They are usually recognized by the fact that the variant gene produces a weaker than normal red cell antigen - in some cases because of a difference in the transferase produced.

   3. Different levels of expression of A (or B) on RBCs are classified into **subgroups**.

   4. **About 80 percent of group A individuals are A¹, the remaining 20 percent are A².**

   5. It has been shown that the transferase produced by the A² gene differs from that produced by A¹ in that it is less efficient in converting H chains to A; however, the terminal sugar of A² is the same as the terminal sugar of A¹.

   6. The difference between the A¹ and A² phenotype is believed to be both quantitative as well as qualitative.

      a. A¹ cells are believed to have more A antigen and less H antigen while it is believed that A² cells have more H and less A antigen.

      b. The quantitative difference cannot be detected serologically.

      c. There must also be a qualitative difference in the antigen structure since A² individuals can produce an anti-A¹ antibody.

7. **Lectins** are a group of naturally occurring materials (usually from plant sources) that react specifically with blood group antigens.

8. The serologic distinction between A¹ and A² is based on results obtained in tests with reagent anti-A¹ prepare from *Dolichos biflorus* seeds. This lectin will cause agglutination of A¹ cells, but A² cells will not be agglutinated.

9. **Anti-A¹ occurs in the serum of 1-8% of A² persons and 22-35% of A²B persons.**

   a. **Anti-A¹ can cause discrepancies in ABO testing**, they will forward type as an A but reverse type like an O due to their anti-A¹ causing agglutination of the A¹ reverse cells.
b. Incompatibilities in crossmatches may also be a problem as these individuals will also cause agglutination of donor cells which are A\(^1\).

c. Anti-A\(^1\) is not considered to be clinically insignificant unless it reacts at 37 C.

10. It is not necessary to test group A individuals with the A\(^1\) lectin unless you are trying to prove that the ABO discrepancy is due to the individual being an A\(^2\) with anti-A\(^1\) or having problems with compatibility testing in which you suspect anti-A\(^1\).

I. Subgroups of A weaker than A\(^2\)

1. Occur infrequently and, in general, are characterized by decreasing numbers of A antigen sites on the red cells and a reciprocal increase in H antigen activity.

2. The genes responsible constitute less than 1% of the total pool of A genes.

3. **Classification of weak subgroups is generally based on the following:**
   
a. Degree of RBC agglutination by anti-A and anti-A\(^1\) lectin (*Dolichos biflorus*).
b. Degree of RBC agglutination by anti-A,B
c. Degree of H reactivity of the RBC, this is done using the anti-H lectin *Ulex europaeus*.
d. Presence or absence of anti-A\(^1\) in the serum.
e. Presence of A and H substances in the saliva of secretors.

4. Subgroups of A weaker than A\(^2\) (A\(^{e1}\), A\(^{e1}\), A\(^1\), A\(^3\), A\(^4\), etc) are seen only infrequently (<1%) in routine transfusion practice.

5. **NOTE:** The subgroup A\(^1\) is characterized by mixed field agglutination.

J. Subgroups of B

1. Even less common than subgroups of A.
2. Criteria for their differentiation resemble those for subgroups of A.
3. Usually detected because they fail to forward type as a B, but do reverse type as a B.
4. The subgroups of B do not make anti-B as is commonly the case in subgroups of A.
5. **NOTE:** The subgroup B\(^1\) is characterized by mixed field agglutination.

K. Antibodies to A and B

1. Landsteiner reasoned from his observations that most individuals possess antibodies directed against the antigens that are absent from their own cells.

2. These antibodies were called "naturally occurring", but this term is a misnomer although continued to be used.
   a. Anti-A and anti-B are stimulated by agents such as bacteria, pollen or other substances present in the internal or external environment that have molecular configurations similar to the A and B antigen, it is an actual immune response.
   b. Non-red cell stimulated may be better term.

3. The predictable relationship between antigens and antibodies in the ABO system permits the use of both serum and cell tests in ABO grouping.
J. Development of anti-A and anti-B

1. Production usually begins during the first few months of life.

2. Babies cannot be reversed typed for two reasons:
   a. Antibodies present in babies serum have crossed the placenta from the mother.
   b. Babies do not have detectable antibody in their serum until 3 to 6 months of age.

3. Antibody production remains constant throughout life and may decrease significantly in the elderly.

4. Complete absence of the expected ABO antibodies is exceedingly rare.

L. Anti-A,B (Group O serum)

1. Group O individuals have three ABO antibodies present in their serum: anti-A, anti-B and anti-A,B.
2. Anti-A,B may react more strongly than anti-A and anti-B with some weak A or B subgroups.
3. Many laboratories use anti-A,B to test group O donors to ensure they are not weak subgroups.
4. Anti-A,B is also used to type babies, as they may type as group O at birth due to the A and/or B antigens not being well-developed at birth.

M. Antibody Characteristics

1. React best at room temperature.
2. These antibodies will agglutinate saline suspended red cells, no additional reagents are necessary.

N. Routine Testing For ABO grouping

1. Forward typing involves testing known anti-serums (antibodies) with unknown patient cells (antigens).
2. Reverse typing involves testing the unknown patient serum (antibodies) with known reagent A and B cells (source of antigens).

O. ABO Discrepancies

1. A discrepancy exists when the interpretation of the forward type does not correlate with that of the reverse type.

2. Observations which indicate a discrepancy:
   a. Strength of agglutination weaker than expected
      1) normal positive forward type should always be 4+
      2) normal reverse positive should always be 2+ - 4+
   b. Unexpected negative reaction in reverse type
   c. Unexpected positive reaction in forward OR reverse.

3. ABO interpretations must be delayed until the discrepancy is resolved. If emergency transfusion is indicated give group O RBCs of the appropriate D type.

4. Errors divided into two categories: Technical and Sample
   a) May result in false positive or negative reactions.
   b) Sample errors divided into red cell problems (forward) and antibody/serum (reverse)
5. Technical errors causing *false negative reactions* may be caused by:
   a. Failure to add serum or antiserum to a test.
   b. Failure to identify hemolysis as a positive reaction.
   c. Not using the appropriate serum (or reagent) to cell ratio.
   d. Improper centrifugation.
   e. Incubation of tests at temperatures above 20-25 C.
   f. Use of inactive reagents.
   g. Failure to interpret or record test results correctly.

6. Technical errors causing *false positive reactions* may be caused by:
   a. Over-centrifugation
   b. Use of contaminated reagent antibodies, RBCs or saline.
   c. Use of dirty glassware.
   d. Incorrect interpretation or recording of test results.

7. Problems associated with testing red blood cells (forward type).
   a. Samples obtained from a recently transfused patient or a bone marrow transplant patient.
   b. Persons who are an ABO subgroup or who have weakened antigens due to diseases such as leukemia.
   c. High levels of abnormal proteins or Wharton's jelly (cordbloods) which may cause nonspecific aggregation.
   d. High concentrations of A or B blood group substances in serum may on rare occasions inhibit activities of blood group reagents to such an extent to give a false negative with unwashed cells.
   e. Sera of some individuals contain antibodies to dyes used to color anti-A or anti-B causing a false positive when unwashed cells are used.
   f. Individuals with potent cold autoagglutinins may coat their own RBCs so heavily that they spontaneously agglutinate.

8. Problems associated with the serum testing (reverse type).
   a. *Very weak or negative reactions* from patients with immunodeficiency due to disease, therapy, depressed immunoglobulin levels, elderly patients, or patients who have received large amounts of IV fluids.
   b. Small fibrin clots.
   c. Rouleaux
   d. Patients with abnormally high levels of abnormal serum proteins or who have received plasma expanders.
   e. Antibodies other than ant-A or anti-B (unexpected antibodies).
   f. Antibodies to chemical constituents of the diluents used to preserve the reverse cells.
   g. Negative or weak reactions on specimens from infants 4-6 months of age.
   h. Bone marrow transplant from ABO non-identical donor.
   i. Unexpected reactions when patient receives sufficient volumes of blood components containing plasma of an ABO group other than their own.

P. Resolving ABO Discrepancies

1. *First course of action should always be to repeat testing* on a better washed RBC sample and using serum from the original specimen.
2. If discrepancy persists:
   a. If patient appears to be group A test the RBCs with anti-A\(^1\) lectin and serum with A\(^2\) cells. If the patient is negative with the lectin they are of the subgroup A\(^2\), and if the serum is negative with the A\(^2\) cells, this shows they have anti-A\(^1\) in their serum.
      1) *Most commonly encountered discrepancy due to unexpected antibodies.*
      2) Test with additional A\(^2\) cells to confirm specificity due to anti-A\(^1\).
   b. *Incubate at RT for 30 minutes for detection of weakened antibodies or antigens.* Frequently needed for elderly patients. Can also incubate at 4 C but *must run an autocontrol.* Cold antibodies are frequently encountered in tests performed at 4 C. Most common cause of false negative result.
   c. Test the serum against group O adult, group O cord and a patient autocontrol (patient serum plus patient cells) to determine if cold reactive antibodies are interfering with testing (also fairly frequently encountered).
   d. Wash the patient and reagent RBCs several times.
   e. Obtain a new specimen.

3. Acquired B Phenotype - B (A)
   a. Patient's RBCs appear to be group AB with a weak B antigen, yet the serum contains anti-B.
   b. This phenomena appears to be associated with carcinoma of the colon or rectum, infection with gram negative organisms and intestinal obstruction.
   c. This phenomena has been associated recently with some monoclonal anti-B typing sera.

4. Mixed field agglutination, a term used to describe discrepancies associated with samples which have two distinct cell populations. May be due to:
   a. A or B patients transfused with group O blood.
   b. D positive patients transfused with D negative blood or D negative patients transfused with D Positive blood.
   c. Patients who receive bone marrow transplants of a different ABO type.
   d. The subgroup A\(^1\) and B\(^1\) classically exhibits a mixed field agglutination when tested with anti-A, this aids in its identification.
   e. The rarest form is due to chimerism due to an intrauterine exchange of erythropoietic tissue by fraternal twins.

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Q. Transfusion Practice

1. Provide serologically compatible blood.
2. Whole blood must be ABO identical - has RBCs AND plasma
3. For Red Blood Cells the donor must lack the ABO antigen to which the patient has antibodies.
   a. Group O is the universal donor.
   b. Group AB is the universal recipient.
4. Donor products such as FFP and Platelet concentrates must lack ABO antibodies to recipient RBCs.
   a. AB is the universal donor
   b. Group O is the universal recipient.