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SECTION 6

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Blood group systems

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Introduction

The red cell membrane carries a great variety of surface proteins, as well as proteins that cross the lipid layer of the cell membrane itself. It is these surface proteins and glycoproteins that carry the blood group antigens and their specificity is mostly determined by the sequence of oligosaccharides (e.g. ABO) or the sequence of amino acids (e.g. Kell, Duffy, Kidd, MNS). These antigens are assigned to blood group systems or collections based on their relationship to each other as determined by serological or genetic studies. As of June 2019, the Red Cell Immunogenetics and Blood Group Terminology working party of the International Society of Blood Transfusion (ISBT) recorded that 38 blood group system genes have been identified and all known polymorphisms (alleles) sequenced. This section will primarily cover the ten major blood groups systems (ABO, Rh, MNS, P1PK, Kell, Duffy, Kidd, Lewis, Lutheran, and I) and provide some information on some of the other blood group systems. Also included is basic information about Human Leucocyte Antigens (HLA) and platelet antigens (HPA). Some references will be made to basic molecular structures, but detailed molecular structures and recent advances in DNA technology are not within the scope of this publication.

Since blood group antigens are genetically determined, the frequency distribution of the antigens often varies in different populations. It is important to know what antigen frequencies apply in your local population. Testing should relate to the antigen frequency in the population, and this may present a challenge when using red cell reagents produced in other parts of the world.

Where feasible, two references have been used for the percentages of different groups in the major blood group systems; those appearing in the first edition of this publication, and those from the Blood Group Antigen Facts Book (full reference needed here). Where possible we have identified the geographical location from which the information is derived, and the local ethnic groups are identified as “Black”, “Caucasian” and “Asian”.

Learning objectives

- By the end of the section, the student should have a good understanding of the following:
  - Blood group terminology
  - Cluster of differentiation
  - Functions of blood groups
  - ABO and H blood group systems
    - ABO grouping
    - Inheritance of ABO bloods groups
    - ABO blood group frequencies
    - Production of ABO antigens
    - H-deficient phenotypes and O_h phenotype
  - Subgroups within the ABO system
  - ABO system antibodies
  - Clinical significance of ABO system
    - Clinical significance in transfusion
    - Clinical significance in haemolytic disease of the fetus and newborn
  - Lectins (plant agglutinins)
  - ABH secretors
  - Unique features of the ABO system
  - Rh blood group system
  - Rh genetics and inheritance
    - Molecular studies
  - Rh terminology
  - Rh frequencies
  - Rh typing
  - Rh antigens
  - Clinical significance of Rh system
    - Clinical significance in transfusion
    - Clinical significance in haemolytic disease of the fetus and newborn
  - Unique features of the Rh system
  - Other major blood group systems
  - Other blood group systems
    - MNS
    - P1PK
    - Kell
    - Duffy
    - Kidd
    - Lewis
    - Lutheran
  - I
  - Additional blood group systems/collections/antibodies reacting with high and low prevalence antigens
  - Polyagglutination
  - Human Leucocyte Antigen (HLA)
    - Disease association

  - Transplantation
  - Transfusion
  - Pregnancy
  - Parentage testing
- Human platelet antigens (HPA)
  - Fetomaternal alloimmune thrombocytopaenia

Blood group terminology

As of June 2019, 38 different blood group systems are known, ten of which are considered in this publication to be major blood group systems.

In addition, there are various blood group antigens that have been allocated to collections (the 200 series), low incidence antigens (the 700 series) and high incidence antigens (the 900 series). The ISBT Working Party on Red Cell Immunogenetics and Blood Group Terminology develops and maintains guidelines for blood antigen and alleles nomenclature and assigns newly recognized antigens and alleles to the appropriate system. Currently known system and alleles are shown on the working party section of the ISBT website. Tables 1 and 2 list blood group systems and their main antigens.

There are strict criteria for:

- The allocation of a blood group antigen to a new or existing blood group system. The antigen must be shown to be an inherited characteristic, it must be defined by a human antibody, the gene encoding it must have been identified and sequenced, and the chromosomal location must be known.
- The establishment of a blood group collection, which requires two or more antigens that are related serologically, biochemically or genetically, but do not fulfil the requirements for a blood group system.
- Inclusion into the 700 series (an inherited antigen with an incidence of less than 1% in most populations tested and distinct from other systems and collections).
- Inclusion into the 900 series (an inherited antigen with an incidence of >90% in most populations tested and distinct from other high frequency antigens).

Various terminologies have been used to describe the different blood group systems and their antigens and respective antibodies ever since the ABO blood group system was first described in 1900 by Karl Landsteiner. In 1980 an ISBT committee was tasked to devise a genetically based numerical terminology for red cell antigens. This is an ongoing process and new information regarding the antigens and candidate new antigens are reviewed by the committee on a regular basis.

The numerical terminology was primarily designed to facilitate computer input. The alternative terminologies are commonly used, both in everyday communication, in laboratories and in publications. In this section the ISBT terminology for the blood group system will be shown in brackets, preceded by "ISBT" for clarity. This terminology consists of one or more letters, a space and three digits e.g. ABO 001. We will use the more 'user-friendly' alternative names.

Note: The term group or type can be used interchangeably when discussing blood groups or types. Further notes on Rh terminology will be found in the Rh section.

The number of antigens within a blood group system, collection, and series varies tremendously from 1 in the I (ISBT I 027) system to 55 in the Rh (ISBT RH 004) and 49 in the MNS (ISBT MNS 002) system.

Table 1 Ten major blood group systems

ISBT no.	Name of blood group system	Major antigens	Chromosome location no.
001	ABO	A, B, A_1	9
002	MNS	M, N, S, s, U	4
003	P1PK	P1, P^k	22
004	Rh	D, C, E, c, e	1
005	Lutheran	Lu^a, Lu^b	19
006	Kell	K, k, Kp^a, Kp^b, Js^a, Js^b	7
007	Lewis	Le^a, Le^b	19
008	Duffy	Fy^a, Fy^b, Fy3	1
009	Kidd	Jk^a, Jk^b, Jk3	18
027	I	I	6

Cluster of differentiation

The cluster of differentiation (abbreviated to CD) is a protocol for the identification of cell surface molecules that provide targets for the phenotyping of cells. CD molecules often act as receptors for various other molecules, and some play a role in cell signalling. Some examples are CD8 found on cytotoxic T-cells and NK cells, and CD4 found on T-helper cells. In order to distinguish these cells from one another, they may be referred to as CD8 cells and CD4 cells. Some CD molecules carry blood group antigens, such as CD235a which carries the MN antigens of the MNS blood group system (ISBT MNS 002) and CD235b which carries the Ss antigens of the MNS blood group system. At the time of this publication, more than 370 unique CD clusters and sub-clusters have been identified.

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Functions of blood groups

The structures of the different blood group carrier molecules and their antigens have been studied extensively, and a wealth of information has become available, particularly since the development of molecular genetic techniques and the data from the human genome project.

Table 2 Other blood group systems

ISBT no.	Name of blood group system	Main antigens	Chromosome location no.
010	Diego	Di^a, Di^b, Wr^a, Wr^b	17
011	Yt	Yt^a, Yt^b	7
012	Xg	Xg^a	X
013	Scianna	Sc1, Sc2	1
014	Dombrock	Do^a, Do^b, Gy^a, Hy, Jo^a	12
015	Colton	Co^a, Co^b, Co3	7
016	Landsteiner-Wiener	LW^a, LW^{ab}	19
017	Chido/Rodgers	Ch/Rg	6
018	H	H	19
019	Kx	Kx	X
020	Gerbich	Ge2, Ge3, Ge4	2
021	Cromer	Cr^a	1
022	Knops	Kn^a, Kn^b	1
023	Indian	In^a, In^b	11
024	Ok	Ok^a	19
025	Ralph	MER2	11
026	John Milton Hagan	JMH	15
028	Globoside	P	3
029	Gill	GIL	9
030	RH associated glycoprotein	RHAG	6
031	Forssman	FORS	9
032	Jr	Jr^a	4
033	Lan	Lan	2
034	Vel	Vel	1
035	CD59	CD59.1	11
036	Aug	Aug1	6
037	Kanno	Kanno	20
038	Sid	Sid	17

However, only a little is known about the function of the blood groups.

The red cell is a complex structure, and the red cell membrane contains many surface proteins that are anchored to the membrane, cross the lipid bilayer one or more times or are adsorbed onto the surface of the red cells. Many of the proteins expressed on the surface of the red cells are polymorphic and carry the different blood groups. Figure 1 shows the red cell membrane with representative blood groups.

The functions of some of the red cell membrane proteins have been identified, such as the carrier molecule of the Jr^a antigen of the JR blood group system (ISBT JR 032) that was identified as ABCG2, a breast cancer resistance protein (BCRP) that makes cancer cells more resistant to anti-cancer drug therapy. It has been designated as CD338.

Studies on the null phenotypes that occur in most blood group systems have contributed to knowledge of their function. For example, the Rh protein, which assists in the transport of carbon dioxide across the cell membrane, also has a structural role in maintaining the flexibility and flattened shape of the red cell. Absence of Rh antigens (i.e. the Rh null phenotype) is associated with to structural changes to the red cell membrane that can produce haemolytic anaemia. The ABO, H, I, P1PK blood groups are carbohydrate structures on the red cell membrane glycolipids and glycoproteins and less is known about their function. Table 3 provides a list of the functions of the blood groups.

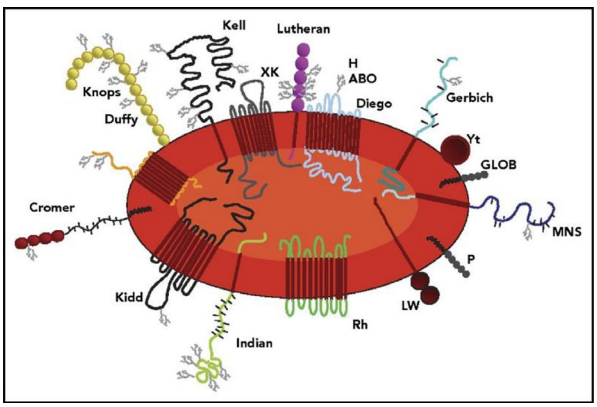

Fig. 1 RBC membrane with representative blood group antigens.

The format for describing the blood group systems in this publication is as follows:

- Antigen frequencies are given as approximate percentages, simply to make them easier to remember. This will sometimes result in the total being slightly more or less than 100%. The figures for Blacks apply to published data, or to surveys performed in southern Africa or published in the Blood Group Antigens Fact Book.
- When an antibody has an optimum reaction temperature of 37°C using the indirect antiglobulin technique (IAT), it is presumed to be an IgG antibody. These are generally clinically significant, being capable of causing *in vivo* destruction of antigen positive red cells.
- When an antibody is described as a saline agglutinin, reacting optimally at 4°C, it is presumed to be an IgM antibody. These are generally of no clinical significance (with the exception of ABO antibodies) unless they show reactivity at temperatures above 30°C or are capable of activating complement.

The ABO and the Rh blood group systems are the most clinically significant blood group systems.

Table 1 shows the major blood group systems. Note that H antigen is in a separate system, H (ISBT H 018), and is not part of the ABO system (ISBT ABO 001) Table 2 provides information on blood group systems other than the ten major systems.

Table 3 Functions of blood groups

Blood group system	Red cell function
Rh, Kidd, Kx, Diego, Colton, Gill, Jr, Lan, Aug, Kanno, RhAg	Membrane transporters
Kell, Yt, Dombrock	Membrane bound enzymes
MNS, Gerbich, Vel	Structural proteins
Duffy	Chemokine receptor
Lutheran, Landsteiner-Wiener, Xg, Indian, Scianna, Ralph, John Milton Hagan, Ok	Cell adhesion molecules
Cromer, Knops, CD59, Chido/Rodgers	Complement regulation
ABO, H, I, P1, P Sid, Forssman, Lewis	Carbohydrate structures which contribute to the glycocalyx

ABO and H blood group systems (ISBT ABO 001 and H 018)

Although the ABO and H are two different blood group systems genetically, they will be described together as they are closely related, both at the biochemical and phenotype level.

The ABO system is the most important blood group system in transfusion therapy and was the first blood group system to be described. This great contribution to medicine was made by Karl Landsteiner in Vienna, Austria, in 1900 when he observed that 'the serum of healthy humans not only has an agglutinating effect on animal blood corpuscles, but also on human blood corpuscles from different individuals'. The following year, in 1901, Landsteiner was able to recognise two antigens on the red cells by separating and mixing the cells and sera of several individuals. He called the antigens A and B. Those individuals with the A antigen on their red cells were called Group A; those with the B antigen, Group B. Many individuals lack the A and the B antigens and were termed Group C, which was later termed Group O (for the German "ohne" meaning "without" or "null"). The least common group, called AB, was found by two of Landsteiner's students in 1902. Group AB individuals express both the A and the B antigens on their red cells. Landsteiner found that the serum of an individual always contained antibodies to the antigen which was not expressed on that individual's red cells. Thus, Group A individuals will have anti-B antibodies in their serum and Group B individuals will have anti-A antibodies in their serum. These facts became known as Landsteiner's Rule which states, '(In the ABO system) the antibody to the antigen lacking on the red cells is always present in the serum or plasma.'

The regular presence of anti-A and/or anti-B antibodies means that it is critical for patient safety and good transfusion practice that ABO groups are performed, recorded and interpreted correctly prior to transfusion. ABO incompatibilities are responsible for the majority of serious and/or fatal transfusion reactions and are usually caused by technical, clerical or administrative errors.

In 1930 Karl Landsteiner received the Nobel Prize in Physiology or Medicine for his work on blood types.

ABO grouping

As mentioned above, the ABO system is unique in that whenever the A or B antigens are not present on the red cells, the corresponding antibody is present in the plasma. Anti-A and anti-B isoagglutinins (also known as isohaemagglutinins) are often referred to as being 'naturally occurring'.

ABO grouping can therefore be performed by:

- Typing the red cells for the presence or absence of the A and/or B antigens. This is known as forward grouping.
- Testing the serum/plasma for the presence or absence of anti-A and/or anti-B. This is known as reverse grouping.
- The forward and reverse grouping results should correlate; refer to Landsteiner's rule.
- The general population can then be divided into four ABO groups as shown in Table 4, based on the forward and reverse grouping.

It should be noted that the anti-A,B produced by a group O individual is different from anti-A + anti-B, which is a mixture of anti-A from one source and anti-B from another source. Anti-A,B detected in group O individuals is an antibody that will react with group A and group B cells. More information on ABO typing can be found in *Section 10: Donation testing*.

Inheritance of the ABO blood groups

The ABO genes are located on chromosome number 9 (9q34.1-q34.2). The inheritance in the ABO system is controlled by various alleles, four of which are common: A^1, A^2, B and O and a series of rare alleles, for example A^3, A^x and A^m with a total of 286 alleles and 537 variants

Table 4 ABO groups

ABO Group	Antigens on red cells	Antibodies in serum/plasma
A	A	Anti-B
B	B	Anti-A
O	None	Anti-A, B
AB	A and B	None

being reported by March 2019. The *O* allele (which does not produce an antigenic product) is recessive to the *A* and *B* alleles, which are co-dominant. The ABO phenotype is shown by the grouping laboratory with ABO testing of a blood specimen, but the genotype of the individual is not obvious from these results. For example, the phenotype A_1 can result from one of several genotypes such as A^1A^1, A^1A^2, A^1A^3, A^1A^x, and A^1A^m or A^1O.

Although each individual has two *ABO* genes, serological tests do not reveal the *O* allele in the A and B phenotypes, nor can an allele producing a weak form of A be recognised if an allele higher in the scale of A antigen production is simultaneously present. The genotype can, however, be determined by DNA analysis of the gene or may be determined by family studies. Table 5 shows ABO blood group phenotypes with possible genotypes (simplified), including some of the rare alleles.

ABO blood group frequencies

The frequency of the ABO blood group genes varies between different populations. Note the variation shown in Table 6 as an example of ABO blood group distribution.

Production of ABO antigens

The ABO red cell antigens expressed on the red cells are dependent on the presence of both the *H* (or *FUT1* gene) as described below, and the *ABO* genes. The loci for the *ABO* and *FUT1* genes are not linked (although they are functionally related) and they are therefore allocated to two separate blood group systems. The *FUT1*, *A* and *B*

Table 5 ABO group/phenotype and possible genotypes (simplified)

Blood group/phenotype	Possible genotypes
A_1	A^1A^1, A^1A^2, A^1A^3, A^1A^x, A^1A^m, A^1O
A_2	A^2A^2, A^2A^3, A^2A^x, A^2A^m, A^2O
B	*BB*, *BO*
AB	A^1B, A^2B, A^3B
O	*OO*

Table 6 Example of ABO blood group distribution in percentage (The Blood Group Antigen Facts Book, used with permission)

Group	Caucasian	Black	Asian
A	43	27	27
B	9	20	25
O	44	49	43
AB	4	4	5

genes do not code directly for red cell antigens, but for enzymes known as transferases. The H-transferase (fucosyltransferase 1, hence *FUT1* as the proper name for the gene) adds the sugar L-fucose to a precursor substrate, which is a carbohydrate chain already expressed on the red cell membrane. Once this has been performed, the 3-α-N-acetyl-galactosaminyltransferse (the enzyme produced by the *A* gene and for simplicity called A-transferase) and 3-α-galactosyltransferase (the enzyme produced by the *B* gene and for simplicity called B-transferase) can act. The A-transferase adds another sugar residue called N-acetyl-D-galactosamine, which results in the expression of A antigen on the red cells. Similarly, the B-transferase adds the sugar residue D-galactose and the cells then also express the B antigen. These red cells, as a result of the actions of the H-transferase, the A-transferase and the B-transferase, type as group AB.

Therefore, the group A antigen is expressed when the H- and A-transferases are the two enzymes present; the group B antigen is expressed when the H- and B-transferases are the enzymes present, and in the case of group O only the H-transferase is present. Figure 2 shows a simplified diagram to indicate the structural differences in the molecules that result in ABH antigen expression.

The expression of A, B or AB antigens results in a relative "masking" of the H antigen. Thus, A_1, B or A_1B cells

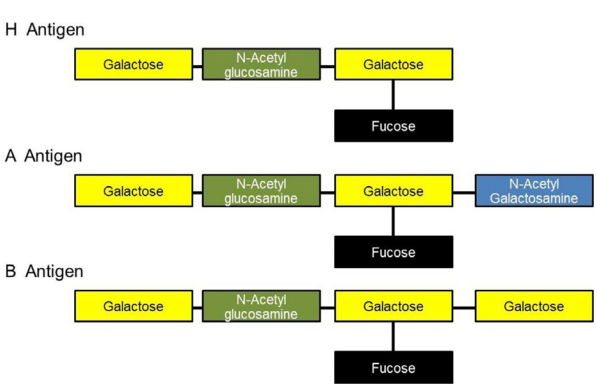

Fig. 2 Simplified diagram to indicate structural differences in ABH antigen composition.

express only small quantities of H antigen and Group O cells express the most.

The A^2 allele is less effective than the A^1 allele in masking the H determinant. A_2 cells therefore express considerably more H antigen and less A antigen than do A_1 cells. A_1 individuals express approximately 1 000 000 A antigens per red cell whereas A_2 individuals express only around 250 000 A antigens per red cell. The *O* allele in the homozygous state leads to the expression of H specificity alone, resulting in group O individuals having abundant H antigen.

The amount of H antigen that is detectable on red cells of different ABO groups, from left to right in decreasing order is as follows: most H antigen: O → Weak A → A_2 → A_2B → B → A_1 → A_1B → least H antigen.

The A, B and H antigens are detectable long before birth, although are expressed less strongly on the red cells of children than those of adults. The ABH antigen strength usually peaks at between two and four years of age and then remains relatively constant in most individuals. It may not be possible to distinguish serologically between group A_1 and A_2 groups at birth as the antigens may not yet be fully expressed.

H-deficient phenotypes

Although the ABO and H are two different blood group systems genetically (ABO Blood Group System: Number 001 and H Blood Group System: Number 018), they are closely related at the biochemical and phenotype level. The H-deficient phenotypes are very rare and include a total deficiency in H antigen (the O_h phenotype, often called the "Bombay" phenotype) or a partial deficiency ("para-Bombay" phenotype).

The O_h phenotype (Bombay phenotype)

The O_h phenotype, in which the cells lack the H antigen, arises when the individual has not inherited the very common *FUT1* gene. As there is no *FUT1* gene present, the H-transferase enzyme is absent. The precursor substance on the red cell remains unchanged and no molecules of L-fucose are present on the precursor substrate in the red cell membrane. The individual may have inherited the *A* and/or *B* genes, which code normally for the appropriate transferases. However, without the single terminal carbohydrate L-fucose at the α-1,2- position of the substrate protein, these transferases are non-reactive. The O_h phenotype, therefore, results when the individual has inherited homozygosity for the rare null allele *h*. The null *h* gene does not code for H-transferase. Individuals who have inherited one or two *FUT1* produce normal amounts of H-transferase.

O_h individuals are extremely rare. Those who were originally shown to carry the trait were individuals born in India, whose ancestors originated in Bombay (now Mumbai), hence the "Bombay blood group". There have been rare cases of O_h phenotype individual throughout the world, some with no apparent southeast Asian heritage, such as Italians living in Europe.

Their red cells are not agglutinated by anti-A, -B, -A,B or -H. O_h individuals usually have powerful anti-H and -A,B antibodies in their serum/plasma. To avoid serious transfusion reaction, recipients can therefore only be transfused with group O_h blood. Table 7 shows the difference between group O and group O_h blood.

Subgroups within the ABO system

Subgroups of A

About 10 years after the description of the ABO groups, the first subgroup of A was described. It was observed that not all group A bloods gave identical results when tested with anti-A from Group B individuals. It was realised, furthermore, that the common A antigen occurred in two forms: A_1 and A_2. Later studies on transferase enzymes of A_1 and A_2 individuals showed that fewer antigenic sites are produced in group A_2 individuals as the enzyme is less effective in converting the precursor H substance into A antigen. However, with the use of monoclonal anti-A blood grouping reagents, little if any, difference between the reactions of A_1 and A_2 cells can be detected in the laboratory. In one survey in Southern Africa, about 99·9% of all group A bloods from Caucasians and about 96% of group A bloods from Blacks, were either A_1 or A_2, with A_1 being more frequent than A_2 in both populations. A higher incidence of A_1 was detected in the Black population.

The anti-A found in the serum/plasma of group B individuals consists of two separate antibody specificities, anti-A and anti-A_1, the latter being specific for the A_1 type. Group A or AB individuals who do not express the A_1 antigen may form an irregular, normally "cold-reacting", anti-A_1 antibody in their serum/plasma. The lectin *Dolichos biflorus* (from which an anti-A_1 reagent can be prepared and standardised) or monoclonal anti-A_1 reagents, are usually used to type red cells for the A_1 antigen.

Table 7 Differences in blood groups O and Oh

Forward grouping	Reverse grouping						
Group	Anti-A	Anti-B	Anti-A,B	Anti-H	A cells	B cells	O cells
O	0	0	0	4	4	4	0
O_h	0	0	0	0	4	4	4

Further subgroups of group A and subgroups of group B

A number of other subgroups of group A and B have been described. The subgroups are caused by genetic variations that result in a variety of weakened expressions of the antigens. The subgroups cannot be detected when the gene for the weak antigen is inherited together with a normal *A* or *B* gene. The subgroups may be detected in the laboratory when weak or unexpected negative results are obtained with the forward grouping and/or anomalous results with the reverse grouping. For subgroups A_{el} or B_{el}, the presence of A or B antigens can only be demonstrated by an adsorption and elution technique with the corresponding antibodies.

Weak A

The term weak A covers a large range of reactivity, some bloods giving clear (although weak) results and other bloods giving such weak reactions that detection may prove difficult.

The weak A types include A_3, A_m, A_x, A_{bantu}, A_{el}, A_{finn} and $A_{end.}$ Weak A type A_3 gives a characteristic mixed field agglutination pattern when tested with polycolonal anti-A and anti-A,B grouping agents. However, stronger agglutination is detected when using monoclonal blood grouping reagents. Table 8 compares reactions between groups and subtypes. Anti-A_1 may or may not be produced, although it is often produced by A_x individuals. Note that type A_3 shows mixed field agglutination with anti-A and anti-A,B and that type A_x reacts macroscopically with monoclonal anti-A,B

Weak B

Subgroups of group B are suspected when the expression of the B antigen is weak or cannot be easily detected. Subgroups of B are very rare and are found mainly in populations where the frequency of group B is high as in African and Far Eastern populations. The subgroup cannot be detected if inherited with a normal *B* allele. The weak B subgroup may be inherited with an *A* allele giving rise to a normal A, weak B phenotype, AB_{weak}.

Acquired-B

Acquired-B is caused by the action of enzymes that deacetylate the group A_1 antigen N-acetyl-D-galactosamine to D-galactosamine which is similar to the structure of the group B antigen sugar residue (D-galactose). Some anti-B reagents, especially monoclonal reagents that contain clone ES4, react with the acquired-B phenotype and a group A individual could be incorrectly grouped as group AB. It is important to select anti-B grouping reagents carefully to ensure that they do not react with the acquired-B phenotype. The condition is rare but may be associated with gastrointestinal bacterial disease or caused by bacterial contamination of a blood sample. The individual's red cells often become polyagglutinable.

ABO system antibodies

Healthy adults who do not express a given ABO antigen on their red cells usually have the corresponding antibody in their serum/plasma as a result of stimulation from the environment, such as exposure to certain bacteria or food that may express A-, B- or H-like substances. Additional exposure to the antigen can result in more potent antibody formation.

This immune response may be induced by:

- Presence of ABO incompatible fetal red cells in the maternal circulation during pregnancy and/or at delivery.
- Injection of A or B substances that may be found in vaccines, either in the culture medium or on the micro-organisms themselves.
- The accidental transfusion or injection of ABO incompatible red cells.

Isoagglutinins that are weak or missing in adults may occur in weak subgroups of A or B, either hypogammaglobulinaemia or agammaglobulinaemia (patients with no, or low levels of serum globulins), twin chimerism, old

Table 8 Comparison of reactions: group A and subtypes

Monoclonal reagents	Reactions of serum/plasma with reagent red cells							
Red cell phenotype	Anti-A	Anti-B	Anti-A, B	Lectin anti-A_1	A_1	A_2	B	O
A_1	4	0	4	4	0	0	4	0
A_2	4	0	4	0	+ or 0	0	4	0
A_3	1 mf	0	1 mf	0	+ or 0	0	4	0
A_x	Micro+/1	0	1*	0	+ or 0	0	4	0

*A_x red cells can, and often do, give quite strong reactions with anti-A,B.

age or treatment with immunosuppressive drugs, or as the results of a bone marrow/stem cell transplantation.

Isoagglutinins in infants

Isoagglutinins are not normally detected in newborn infants but develop after three to six months of life due to exposure to A-like and B-like antigens in the environment. If ABO antibodies are detected in neonatal blood samples, they are usually agglutinating IgG antibodies of maternal origin. Table 9 shows the normal grouping results of a group B newborn and an infant of six months of age.

Anti-A_1

Individuals of phenotypes A_2, A_2B and weaker subgroups of A may have anti-A_1 in their plasma. This antibody will react with group A_1 cells. Anti-A_1 is usually a "cold-reacting antibody", which is not of clinical significance. As it seldom reacts above 25°C, it is unlikely to cause transfusion reactions or haemolytic disease of the fetus and newborn (HDFN). It may, however, mask a clinically significant antibody.

Anti-A_1 occurs naturally in the plasma of about 2% of A_2 individuals and 26% of A_2B individuals. The antibody occurs more frequently as the strength of the A antigen decreases, therefore weak A (or weak AB) individuals are more likely to have anti-A_1 in their serum/plasma than A_2 (or A_2B) individuals.

Anti-H (other than O_h anti-H, -A, -B)

As individuals of group A_1, A_1B and B have very little H antigen expressed on their red cells, they sometimes develop anti-H in their plasma. This antibody can be recognised by its strong reaction with O red cells, a weaker reaction with A_2 cells and usually a failure to react with A_1 or B red cells. Anti-H of this nature, which is formed by individuals who are not H-deficient, is usually a benign autoantibody.

Group O serum

Group O serum is not a simple mixture of anti-A and anti-B. It cannot be separated by selective adsorption using either group A or group B cells and is a cross-reacting antibody generally known as anti-A,B. Various theories have been suggested to explain this cross-reactivity (including Wiener's C theory) and it appears that the anti-A,B produced by group O individuals detects a structure common to both A and B antigens.

Clinical significance of the ABO system

Clinical significance in transfusion

Of all the blood group systems, the ABO is the most important in transfusion because the isoagglutinins are normally present in the absence of the corresponding antigen. Strong reactions take place when incompatible bloods are mixed with each other, not only *in vitro,* but also *in vivo*. Even an initial transfusion of group A blood into a group O or group B patient may be disastrous, because the naturally occurring anti-A in the blood of the recipient would react immediately with the incoming group A cells, activating complement and causing haemolysis of the donor cells. This would lead to an acute haemolytic transfusion reaction which may be fatal.

Universal blood donor

Group O individuals of are sometimes termed universal blood donors, as their blood can usually be safely infused into recipients of other ABO groups (heterologous group transfusion) because:

- They do not have A or B antigens on their red cells to react with antibodies within the circulation of the recipient.
- Their naturally occurring anti-A,B antibodies are not usually harmful to the red cells of the recipient if whole blood is transfused, provided the isoagglutinins are 'low titre' i.e. have a low level of ABO haemolysins.

However, whole blood from 'high titre' group O donors, which contains immune anti-A and/or -B, may only be transfused into group O recipients (homologous group transfusion). This is because these 'dangerous' universal donors have potent isoagglutinins with haemolysing characteristics in their plasma, which may cause severe haemolytic reactions when infused into recipients with A and/or B antigens on their red cells. The risk of transfusing harmful

Table 9 Newborn and infant ABO grouping results

Forward grouping	Reverse grouping	Interpretation					
Age of infant	Anti-A	Anti-B	Anti-A,B	A cells	B cells	O cells	Group
Newborn	0	4	4	0	0	0	B
Infant of 6 months	0	4	4	3	0	0	B

anti-A and anti-B in blood group O whole blood can be reduced by the transfusion of group O red cell concentrates, from which most of the plasma has been removed.

In practice, however, it is better to transfuse a patient with blood of the same ABO group (ABO identical) and to conserve stocks of group O blood for group O patients and for emergency use.

Clinical significance in haemolytic disease of the fetus and newborn (HDFN)

Some individuals produce potent, high titre anti-A and/or anti-B, consisting of a mixture of IgM and IgG antibodies, with haemolysing characteristics in the presence of complement. This immune anti-A and/or -B in pregnant women can cause ABO HDFN with varying degrees of severity, although the fetus is rarely affected *in utero*. ABO HDFN typically develops within a few days of birth. See *Section 7: Haemolytic diseases*, for more information.

Lectins (plant agglutinins)

Certain plant extracts (usually seeds) agglutinate human and animal red cells. Two names have been suggested for these plant agglutinins: phytagglutinins and lectins, the latter term used for those which show red cell antigen specificity. Note that these substances are not antibodies. Lectins are sugar-binding proteins or glycoproteins of non-immunological origin. Some lectins are described in succeeding discussions:

Lectin anti-A_1

The most useful lectin, anti-A_1, is extracted from the seeds of *Dolichos biflorus*: the extract strongly agglutinates A_1 and A_1B cells; it reacts less strongly with A_2 cells and very weakly with A_2B cells. The extract can therefore be standardised by dilution as a specific anti-A_1 reagent. It also reacts with uncommon red cells that express the polyagglutinin antigens Tn or Cad.

Lectin anti-H

Lectin anti-H can be extracted from the seeds of *Ulex europaeus* or the common European gorse. *U. europaeus* is invaluable for the classification of group O secretor/non-secretor saliva (or group O secretor status) and confirming an O_h phenotype.

ABH secretion

In addition to being expressed on the red cells, A, B and H antigens are also expressed on most other tissues as glycolipids and glycoproteins. Soluble blood group substances of the same ABO group as the red cells may also be found in the serum/plasma and are readily detectable in the saliva and other body fluids of most individuals.

The secretor status is controlled by the *SE (*or *FUT2)* gene on chromosome 19. *SE* is a dominant hemizygous gene and is responsible for the secretion of A, B and/or H. Approximately 80% of the general population secrete ABH substances (in the form of water-soluble antigens) in abundance in almost all their body fluids (not found in cerebrospinal fluid). There is no *se* allele and therefore "*se*" is used only to indicate the absence of *SE*. As there is no *SE* gene product in the absence of the *SE* gene i.e. in those individuals designated *sese*, these individuals are "non-secretors" and produce no water-soluble ABH antigens. The ABO group of a secretor may be determined by testing the saliva to determine the presence or absence of A, B and H substance. The remaining 20% of the population are termed non-secretors. Table 10 shows the soluble antigens secreted according to ABO group.

Unique features of ABO and H systems

The critical unique feature of the ABO and H blood group systems is that unlike other blood group systems, the anti-A and/or anti-B isoagglutinins are invariably present in the serum/plasma of every healthy adult when the corresponding antigen is absent from their red cells.

As the ABO and H antigens are widely distributed throughout the body, the ABO group must be considered in organ transplantation. Some organs, e.g. the heart, must be ABO compatible with the recipient. In bone marrow transplantation, ABO incompatibility is acceptable because of the lack of expression of ABO on stem cells, but precautions need to be taken such as removal of the unwanted donor red cells or plasma. Note: Anomalous red cell typing may be seen post transplantation when an ABO incompatible graft was used.

Practical application

The cornerstone of safe blood transfusion practice is to transfuse safe blood of the compatible ABO group. It is

Table 10 Soluble ABH antigens according to ABO group

Group	Soluble antigens present
A secretor	A and H
B secretor	B and H
O secretor	Abundant H
AB secretor	A, B and a little H
Non-secretor	Not readily detectable

critical that the ABO group on all samples, whether from a patient or a donor, is correct, as ABO group mistyping can have fatal consequence

Rh blood group system (ISBT RH 004)

Number of antigens: 55 (2019)

CD numbers: CD240

The discovery of the Rh groups by Karl Landsteiner and Alexander Wiener in 1940, together with the work of Philip Levine and Rufus Stetson in 1939, heralded the greatest discovery in the blood grouping field since Landsteiner described the ABO system in 1900.

In 1939 Levine and Stetson described how the mother of a stillborn fetus suffered a severe haemolytic reaction when transfused with her husband's blood. The mother, who obviously lacked some 'new' antigen, must have been immunised by her fetus that expressed this antigen, having inherited the gene encoding it from the father. When the ABO compatible husband's blood was transfused, the maternal antibody reacted with this same antigen expressed on his red cells.

In 1940 Landsteiner and Wiener, having immunised rabbits with the blood of a rhesus monkey (*Macaques mulatta*), discovered that the resulting antibodies agglutinated not only the monkey red cells but also the red cells of about 85% of the Caucasians tested.

Later work, however, showed that the red cell antigens detected by the human-derived antibody and the animal antibody were not identical and in fact belonged to two different blood group systems. The blood group system detected by the human-derived antibodies is now known as Rh (not Rhesus or rhesus) and the antigen is called D. The antigen originally described by Landsteiner and Wiener is designated LW in the Landsteiner-Wiener blood group system (ISBT LW 016). The two systems are serologically, biochemically and genetically different from one another. The locus for the Rh genes (*RHD* and *RHCE*) is on chromosome 1 (1p36.11) and is linked to the gene for elliptocytosis. The locus for the *LW* gene *(*now called *ICAM-4)* is on chromosome 19 (19p13.2).

It was soon realised that the Rh antibodies produced in humans were not as simple as they had first appeared, and that many sera contained antibodies of more than one specificity. Many related antigens were found by workers in England and in the United States of America. This led to the discovery of the five major Rh antigens: D, C, E, c, and e. The Rh blood group system has now been shown to be one of the most complex multi-allelic blood group systems with 55 antigenic specificities having been described by 2019.

Rh genetics and inheritance

Most individuals are either D positive or D negative and the expression or absence of the D antigen on the red cells results from the presence or absence of an *RHD* gene. A D positive individual may inherit two *RHD* genes, one from each parent (in which case the individual is homozygous) or only one *RHD* gene from either parent (in which case the individual is hemizygous as no allele is present on the second chromosome). The two pairs of antithetical antigens C and c, and E and e are controlled by the various *RHCE* alleles. The *RHD* and *RHCE* alleles are inherited as a gene complex or haplotype. Note that genes are allelic, but antigens are antithetical (or allelomorphic). Genes cannot be antithetical (or allelomorphic), and antigens cannot be allelic.

Two different theories were initially proposed for the genetics and inheritance of the Rh blood group system, but these have subsequently been disproved by molecular genetic studies. They are described here because they are historically interesting, and because the terminology inherent in the theories is still widely used.

Fisher Race theory (UK – theory of three pairs of linked genes)

In 1943 the British statistician Ronald Fisher, studying the results of Robert Race and co-workers in England, noticed that some reactions were antithetical (opposite), and he theorised that there were three sets of alleles involved: *C* and *c*, *D* and *d*, *E* and *e*. Fisher assumed that the three genes, if separable, must be very closely linked, for no crossing over had been observed.

The CDE nomenclature was devised and although it did not accommodate subsequent complexities in the Rh system it was easy to use.

Although this theory suggested that antibodies to all the antigens described are able to be stimulated in individuals lacking the corresponding antigen, no anti-d has ever been found.

Wiener theory (USA – theory of multiple allelic genes)

This is a theory of multiple allelic genes occurring at a single chromosomal locus (rather than at three closely linked loci). One gene complex is inherited from the mother and one from the father. It was thought that each gene complex produced an agglutinogen which had several serologic specificities (i.e. several distinct antigens). One agglutinogen could react with various antibodies because it had as part of its structure more than one

antigen. The Rh-Hr nomenclature was developed to describe the gene complexes, agglutinogens and antigens.

Molecular studies

Following studies on the Rh blood group system at the molecular level, it has been shown that there are two Rh genes, *RHD* and *RHCE*, at the *RH* locus. The *RHD* gene primarily encodes for the D antigen. At the *RHCE* gene locus, depending on the allele present, one of four alternative antigenic combinations are primarily encoded for, namely ce, Ce, cE or CE. D positive individuals inherit two Rh genes: *RHD* coupled with one of the alleles of *RHCE* from each parent. In most D negative Caucasians, *RHD* is deleted, and individuals possess the *RHCE* genes only. As a result, most D negative individuals lack the total D protein (the D antigen) on their red cell membrane, but this does not appear to have an adverse effect on the cell function. Note that the genetic mechanism in many D negative Blacks is not deletion of the *RHD* gene, but the presence of the *RHD* pseudogene (*RHD**Ψ) containing a 37 base pair duplication and a nonsense mutation. Figure 3 illustrates genomic organisation of RH genes.

The *RHD* and *RHCE* genes produce separate proteins, that are located in the red cell membrane next to each other, forming a complex of antigens. As the two gene loci are in such close proximity, many of the unusual Rh variants are the result of various genetic occurrences within the two loci, such as unequal crossing over, or mutations. Both the D protein and the RhCcEe proteins comprise 416 amino acid residues each. Whereas D– individuals generally lack the entire D protein, the difference between ce and Ce proteins are differences in four amino acid residues, three of which are transmembranous, and only one extracellular, and ce and cE proteins differ by one amino acid residue, which is extracellular.

The presence or absence of the *RHD* gene, together with one of the four possible alleles of the *RHCE* gene, results in eight possible gene combinations or complexes. The Rh genotype is therefore a combination of any of the eight possible haplotypes, shown in Table 11. One *RH* gene complex or haplotype is inherited from each parent.

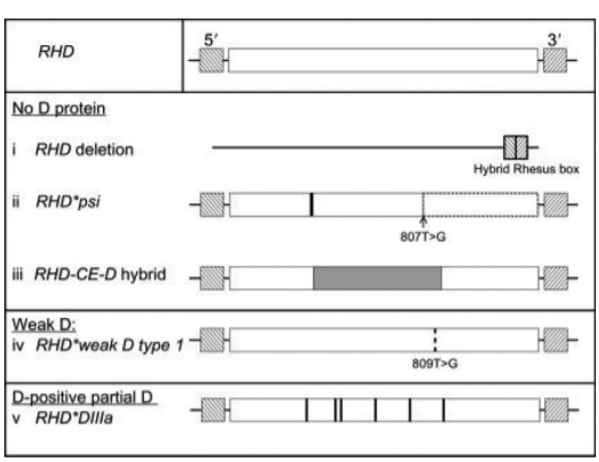

Fig. 3 Genomic organisation of RH genes in D positive and D negative haplotype and RHD* Ψ that contains 37 base pair duplication and a nonsense mutation from the Black ethnicities with the D negative phenotype.

Rh Terminology

The eight possible gene complexes, or haplotypes are shown in Table 11. The symbol "d" denotes the absence of the D antigen. There is no *d* gene or *d* gene product and d therefore represents an *RHD* deletion or an inactive *RHD* gene. The eight Rh gene complexes, or haplotypes have each been allocated a shorthand notation, the symbol R indicating the presence of an *RHD* gene and D antigen and r the absence of the *RHD* gene and the D antigen. Table 12 shows phenotyping results using Rh antisera and the frequencies of some Rh genotypes in a UK population.

Numerical terminology

In 1962 Richard E Rosenfield introduced a new terminology for the Rh system based on a numerical system. Each antigen was numbered, as was the antibody detecting it. For example, the D antigen is Rh:1 and anti-D is anti-Rh1. Blood lacking the D antigen is noted as Rh:−1 (minus one). This numerical terminology lends itself to computerisation and is now the basis for the ISBT terminology for all the blood groups.

Rr and CDE Terminology hints

The Rh terminology can be very confusing and there are several different ways of documenting Rh. Below are

Table 11 RH genes together with gene complex (haplotype) and shorthand nomenclature

	Rh genes present			
	RHD **gene**	***RHCE*** **gene**	**Gene complex/haplotype**	**Shorthand nomenclature**
D positive	*D*	*Ce*	*DCe*	R_1
	D	*cE*	*DcE*	R_2
	D	*ce*	*Dce*	R_0
	D	*CE*	*DCE*	R_z
D negative	*d*	*Ce*	*dCe*	r′
	d	*cE*	*dcE*	r″
	d	*ce*	*dce*	r
	d	*CE*	*dCE*	r^y

Table 12 Frequencies of some Rh genotypes in a UK population

	Phenotype results using Rh antisera					
Rh genotype	Anti-D	Anti-C	Anti-E	Anti-c	Anti-e	Percentage frequency
R_1R_1	+	+	0	0	+	18
R_2R_2	+	0	+	+	0	3
R_1R_2	+	+	+	+	+	13
R_1r	+	+	0	+	+	35
R_2r	+	0	+	+	+	12
R_0R_0	+	0	0	0+	+	2
rr	0	0	0	+	+	15
$r'r$	0	+	0	+	+	0·4
$r''r$	0	0	+	+	+	0·8
$r'r'$	0	+	0	0	+	<0.1
$r''r''$	0	0	+	+	0	<0·1
$r'r''$	0	+	+	+	+	<0·1
R_zr	+	+	+	+	+	very rare
r^yr	0	+	+	+	+	very rare

several points that may be of assistance when using the shorthand notation:

- Whenever D is present, use the uppercase letter R together with the appropriate number or symbol (see second, third and fourth bullet points).
- The C antigen is associated with either $_1$ or $'$ (single prime). If C occurs with D use $_1$. When D is absent use $'$ (e.g. DCe = R_1 and dCe = r'). The 1 or $'$ indicates the C antigen and the e antigen are present.
- The E antigen is associated with either $_2$ or $''$ (double prime). If E occurs with D use $_2$. When D is absent use $''$ (e.g. DcE = R_2 and dcE = r''). The $_2$ or $''$ indicates the c antigen and the E antigen are present.
- When C and E are absent, but D is present, the notation R_0 is used. This indicates that the D antigen, c antigen and the e antigen are present.
- The phenotypes dCE and DCE are both very rare, so it seems logical to use y and z to describe them (r^y and R_z) the y and the z indicate that the c antigen and the e antigen are present.

Frequencies

The frequency of the eight possible Rh gene haplotypes varies between populations. For example, the gene combination R^0 is seen more frequently in various Black groups, particularly in sub-Saharan Africa than in Caucasian populations, whereas the haplotype *r* is more frequent in Caucasians than in Blacks. Table 12 gives the frequencies of most of the Rh genotypes in the UK population.

Rh typing

D typing

The D antigen is the most clinically significant antigen in the Rh system. Individuals are divided into D positive or D negative based on serological D typing results that detect the presence or absence of the D antigen. The frequency of the D antigen varies in different populations, e.g. in a mostly Caucasian population, 85% are D positive and 15% D negative but in Asians D negative individuals are extremely rare. On the other hand, in certain parts of the Iberian Peninsula, the percentage of D negative individuals approaches 25%.

Donors

Ideally a donor sample should be tested with two different monoclonal anti-D reagents and, if the test results concur, then the sample can be designated D positive or D negative Anti-D reagents for donor typing should be selected to detect the majority of weak and partial D types, including D^{VI}. Donations giving weak or equivocal reactions are labelled D positive to avoid the risk of transfusion to D negative recipients.

Transfusion recipients

Patients who are to receive a blood transfusion should be tested with anti-D reagents that detect all commonly encountered D types. These reagents should NOT detect partial D^{VI}. It is not necessary to detect the very weak D variants because such patients should be typed D negative and should receive D negative blood.

Prenatal testing

Routine D typing is performed in pregnancy to determine the possibility of haemolytic disease of the fetus and newborn (HDFN) based on alloimmunization to D. Testing is performed using reagents that detect all commonly encountered D types, but not partial D^{VI}. Management may vary depending on weak D subtypes (see Clinical significance of weak D and partial D below).

Rh phenotyping

The Rh phenotype can be determined by typing the red cells with specific reagents; anti-D, anti-C, anti-E, anti-c and anti-e. Positive and negative test results using these reagents denote the presence or absence of the Rh antigens and this is known as the Rh phenotype.

Table 13 shows the variation in percentage frequency of various Rh phenotypes in one study of southern African populations. The symbol d is used to denote the absence of the D antigen.

Table 13 Variation in frequency of Rh phenotypes: a study of southern African populations

Rh reagent antisera					Phenotype		Ethnicity: percentage frequency		
Anti-D	Anti-C	Anti-E	Anti-c	Anti-e			Caucasian	Black	Asian
+	+	0	+	+	DCe/dce	R_1r	33	13	30
+	+	0	0	+	DCe/DCe	R_1R_1	18	< 1	48
+	0	+	+	+	DcE/dce	R_2r	13	15	4
+	0	+	+	0	DcE/DcE	R_2R_2	2	< 1	< 1
0	0	0	+	+	dce/dce	Rr	14	2	3
+	0	0	+	+	Dce/dce	R_0r	3	66	2

It is not possible to determine the genotype of an individual from the red cell phenotype result, but the most probable genotype can be deduced using the haplotype frequency data. The phenotypes shown in Table 13 do not reflect one specific genotype. For example, cells which are phenotypically R_o may be genotypically R_OR_O or R_Or. Therefore, samples typed serologically as C-D+E- should ONLY be reported as R_O, NEVER as R_Or (or R_OR_O).

However, as the genotype incidence varies between different populations and ethnic groups, the 'probable genotype' result should be treated with reservation. It is an advantage to know the ethnicity of the individual being typed so that the appropriate frequencies can be used. The phenotype cannot determine if the individual is homozygous or hemizygous for the *RHD* gene as there is no *d* gene product. Accurate genotype determination can only be established by the use of molecular genetic techniques or by informative family studies.

Note that homozygous, heterozygous and hemizygous refer to the genes on the respective pair of chromosomes. Red cells cannot be homozygous or heterozygous/hemizygous for any blood group antigen. At best, they can have homozygous or heterozygous/hemizygous expression of an antigen.

Table 14 shows an example of a Rh phenotype and the possible genotype options, using figures derived from UK statistics. The probable genotype can then be determined.

It should also be noted that the presence of the very rare variant haplotypes, such as --- (Rh_{null}), *Dc*– and *D*– - result in all or some of the Rh antigens not being expressed on the red cells and this will affect the possible genotype calculations.

Rh antigens

The Rh antigens are encoded by the *RHD* and *RHCE* genes, each of which produces a separate protein that is inserted into the red cell membrane. The D protein crosses the red cell membrane 12 times, giving rise to six extracellular domains. Despite many studies, the exact function of the Rh proteins within the red cell membrane is unknown, but their structure suggests a transmembrane transporter function. The structure and function of the D and CcEe proteins appear similar. In cases of the very rare type Rh_{null}, the absence of the Rh proteins has shown that the red cells are abnormal morphologically and individuals often suffer from some degree of haemolytic anaemia.

D antigen

The vast majority of all populations are D positive. The D antigen is extremely immunogenic and is likely to stimulate antibody production in D negative individuals exposed to the D antigen. It is the most clinically significant of the Rh antigens and plays a significant role in HDFN (refer to *Section 7: Haemolytic diseases*).

Table 14 Rh phenotypes and possible genotype using UK-derived statistics

Rh reagent antisera						Possible option for Rh genotypes based on phenotype		Known frequency (%) of genotype (Caucasians)
Anti-D	Anti-C	Anti-E	Anti-c	Anti-e	Phenotype			
+	+	0	+	+	D+C+ c+e+	DCe/dce	R_1r	**33***
						DCe/Dce	R_1R_0	2
						Dce/dCe	R_0r'	< 0·1

*This is the probable or most likely phenotype for Caucasians.

Weak D (previously termed D^u)

Weak D describes a weaker than normal expression of the D antigen, where fewer D antigen sites are expressed on the red cell, compared to normal D positive. Studies have shown that red cells of the presumed R_1r phenotype have about 10 000 D antigen sites per cell, whereas red cells of the presumed R_2R_2 phenotypes have about 33 000 D antigens sites per cell. Weak D cells have far fewer than this number, although the number is variable (between as few as 200 and 10 000). Weak D expression primarily results from single point mutations in *RHD* that encode amino acid changes predicted to be intracellular or in the transmembrane regions of D. These affect the efficiency of insertion, and, therefore, the quantity of D protein in the membrane, reflected in the reduced number of D antigen sites on the red blood cells. See Fig. 3 for an illustration of the *RHD* genes.

The term D^u for weak D is now obsolete. It was used to describe those forms of the D antigen that reacted weakly in laboratory tests when tested with different polyclonal anti-D reagents. With the use of monoclonal anti-D reagents many weak D types now react as D positive and cannot be distinguished from normal strength D in a routine laboratory. The identification of a weak D type will depend on the anti-D reagents selected for use and the technique used. If two potent, IgM agglutinating monoclonal anti-D reagents are used routinely for patient testing, most weak D samples will type D positive. Only the weakest form of weak D will be identified as weak or may be typed as D negative and such patients will receive D negative blood. The weak D type should, however, be detected in donor samples by either proceeding to the second (IAT) phase of a monoclonal blend reagent or by using agglutinating monoclonal anti-D reagents specifically selected for the ability to detect weak D.

Partial D

In 1953 there was a report of a D positive individual with anti-D in the plasma. Since then, many examples of D positive with anti-D have been reported, although overall it is a rare occurrence.

The term partial D is used to describe the phenotype of those rare individuals, whose red cells lack one or more of the D epitopes. The D antigen is considered to be a mosaic of epitopes. If some D epitopes are missing, or are mutated, then the individual can make an antibody specific for the missing epitope/s if they are exposed to normal D positive cells and, potentially, red cells expressing one of the weak D types, or even red cells from a different form of partial D. The anti-D produced in this way reacts with all normal D positive cells, which have all the epitopes, but fails to react with their own cells and cells of the same or similar partial D types.

In their studies in the late 1950s, Alexander Wiener and Lester Unger used the Rh^A, Rh^B, Rh^C and Rh^D classification to describe the D mosaic antigen, but this terminology is now obsolete.

Patricia Tippett and Ruth Sanger used the Category Classification to describe partial D. Their original classification, using human sera, divided partial D bloods into six categories: I, II, III, IV, V and VI, with category VI expressing fewer D epitopes than the others. Note: category I is now redundant. The categories were shown to be inherited. The original framework of this study has allowed for the addition of further partial D type complexities, as more information became available. The use of epitope-specific monoclonal anti-D has enabled the partial D to be categorised further into categories II, IIIa, IIIb, IIIc, IVa, IVb, Va, VI, VII and many others (such as DAR and DOL). Panels of monoclonal anti-D reagents are now available to classify partial D types. The molecular structures of the many partial D types have been extensively studied and it has been shown that various *RHD* and *RHD-CE-D*, and *RHCE-D-CE* hybrid genes give rise to different partial D types.

Molecular *RHD* blood typing is very efficient for managing patients and donors carrying any of the various molecular types of weak D or partial D. With the advance of Next Generation Sequencing (a technique that has made genetic sequencing extremely rapid), the whole *RHD* and *RHCE* genes can be sequenced to identify the mutation.

Clinical significance of weak D and partial D

Weak D individuals do not usually produce anti-D, although isolated cases have been reported, whereas partial D type individuals may develop clinically significant anti-D.

Significance in patients:

- Weak D patients very rarely form anti-D. Antenatal patients identified to be 'weak D' will usually be regarded as D positive and therefore do not require antenatal or post-natal anti-D immunoglobulin. In particular, prenatal patients with weak D types 1,2, or 3, the most common forms of weak D in Caucasian populations, can be classified as D positive.
- Patients with partial D types other than D^{VI} are most commonly identified when an apparently D positive individual makes anti-D. Routine anti-D typing reagents will react with the majority of non-D^{VI} partial D types. Partial D individuals may be stimulated to produce anti-D if transfused with D positive blood. The anti-D can cause severe transfusion reactions.

- A partial D mother will not be identified as a candidate for anti-D immunoglobulin. Should she deliver an infant with normal D she may be stimulated to develop anti-D to the missing epitope(s). Future pregnancies may be complicated by HDFN.
- The most common partial D is D^{VI}. However, reagents selected for patient typing do not detect D^{VI} therefore D^{VI} patients will be managed as D negative.

Significance in donors:

- Blood donors should be tested for weak D, and some countries perform genotyping for *RHD* and, if weak D is present, their blood is labelled as D positive.
- Most partial D blood donors will be typed as D positive.

Trans effect of *RHCE*C*

A weak D phenotype can occur as a result of the *trans* effect of *RHCE*C*. (The * indicates that the letter or symbol following is the allele present in the gene complex. Thus *RHCE*C* means that the *RHCE* gene complex contains the *C* allele.) If the haplotype encoding the D antigen is in *trans* (on the opposite chromosome) with a haplotype encoding the C antigen, but not the D antigen (e.g. *dCe*), the expression of the D antigen may be weak. This is known as the Ceppellini effect, after the principle author (the Italian geneticist Ruggero Ceppellini) on the first paper, published in 1955, to describe this effect. Family studies have shown that when the haplotype encoding the weak D is not in *trans* with C, in the other words, it is in the *cis* position, then the D antigen is expressed normally. For example, *Dce/dCe* (D and C in *trans*) may type as weak D, whereas *DCe/dce* (C and D in *cis*) will type as normal D.

Cc and Ee antigens

The Cc and Ee antigens are less immunogenic than the D antigen and may demonstrate dosage effect depending on the reagent used. A number of variants, particularly variants of the e antigen, have been described, such as the rare hr^S and hr^B found mainly in Black populations.

C^w antigen and anti-C^w

Although the C^w antigen was originally thought to be antithetical to C, it has been shown to be antithetical to high frequency Rh antigen MAR. C^w+ cells are almost always C+ although very rare examples of C^w+, C− individuals have been reported. Anti-C^w is not necessarily produced in response to a known red cell stimulus and may occur in combination with other antibodies to low prevalence antigens.

G antigen and anti-G

Red cells that are C+ or D positive are generally G+, although very rare exceptions have been reported. The Ce and CE proteins and the D proteins share an amino acid sequence that is recognised by anti-G. Many if not most anti-C+D sera may therefore contain anti-G.

Anti-G will appear to be anti-C+D in routine laboratory testing. Transfusion management in all cases is therefore straightforward with C-D- blood.

A potential complication arises in the case of an antenatal patient with apparent anti-C+D because of the possibility this may be anti-G or anti-C+G **without** anti-D. In this case, the patient remains a candidate for anti-D immunoglobulin to prevent anti-D formation. Anti-G can be isolated from anti-G+C, anti-G+D and anti-G+C+D mixtures by a sequence of absorption and elution.

Clinical significance of the Rh system

Rh antibodies are most likely to be immune IgG antibodies that have been stimulated by exposure to foreign red blood cells either through pregnancy or transfusion. Examples of 'naturally occurring' IgM anti-E and anti-C^w are however, common.

The antibodies in the Rh blood group system can cause severe transfusion reactions and are second only to the ABO and H systems in this regard.

Practical transfusion measures to avoid Rh sensitisation

Because D antigen is so highly immunogenic, transfusion of D positive blood to D negative individuals should be avoided. In some circumstances, mainly as blood stock conservation measures when there is a shortage of D negative blood, D positive blood may be transfused into D negative patients. This can only be done when consequent development of anti-D is unlikely to be a major concern. For example, in emergency situations, D positive blood may be transfused to D negative males or to D negative women who do not have childbearing potential.

Other Rh antigens are also immunogenic, though to a lesser degree than D. There is a risk that patients who lack the C, E, c or e antigens will be exposed to these antigens during transfusion. This often leads to the production of the corresponding antibodies.

While it is unnecessary for hospital laboratories to type all patients for Rh antigens other than D, there is a sound argument in favour of determining the Rh phenotype of patients likely to require repeated transfusions over multiple transfusion episodes, in order to prevent additional risks of sensitisation to Rh antigens other than D. For example, in the UK, it is standard practice to phenotype and/or genotype sickle cell disease patients for CeEe antigens as well as K and provide CcEe and K matched red cells to reduce the risk of alloimmunization.

All Rh antibodies reactive by IAT should be regarded as clinically significant and antigen-negative blood selected for transfusion into such patients. Weak, 'naturally occurring' Rh antibodies such as examples of anti-E and anti-C^w demonstrated by saline or enzyme techniques only, that fail to react by IAT, can be ignored. ABO group blood, which is D positive and untyped for these benign antigens maybe selected for crossmatch, and if compatible by IAT, may be safely given.

Clinical significance in haemolytic disease of the fetus and newborn

Rh antibodies, particularly anti-D and anti-c are capable of causing HDFN. (See *Section 7: Haemolytic diseases* for details.) Anti-D is the most common cause of severe HDFN and may be combined with other Rh antibodies, e.g. anti-D + anti-C.

Although 'enzyme only' reacting Rh antibodies may be detected (they often have anti-E specificity), the Rh antibodies that are always clinically significant and cause HDFN are IgG and are best detected using the IAT.

A fetomaternal haemorrhage (FMH), either during pregnancy or during delivery, acts in exactly the same way as a transfusion, although the volume of cells is usually much smaller. The mother may be exposed to antigens she lacks, but which the fetus expresses. The most common example of this is the D negative mother who has an FMH of D positive blood and develops anti-D, which may cause HDFN in subsequent pregnancies if the fetus is D positive.

HDFN usually increases in severity with each succeeding D positive pregnancy. The anti-D increases in titre and avidity with every FMH, followed by additional immunisation of fetal cells, so that eventually they may be of sufficient potency to cause intrauterine death of an affected fetus. Before the advent of prophylaxis (preventative treatment) with Rh immunoglobulin, anti-D was responsible for about 90% of severe HDFN cases.

Other Rh antibodies, particularly anti-c, may cause severe HDFN. For example, it is possible for a D positive (*DCe/ DCe*) mother who has been stimulated to produce anti-c, to give birth to a D positive (*DCe/dce*) infant suffering from HDFN caused by anti-c.

Other major blood group systems

MNS blood group system (ISBT MNS 002)

Number of antigens: 49 (2019).

CD numbers: CD235a, CD235b

The M and N antigens were first described by Karl Landsteiner and Philip Levine in 1927. Although the MN types and Ss types are shown separately in Table 15, the two sets of antithetical antigens form a single blood group system. The MN antigens are situated on glycophorin A (GPA, CD235a) and the Ss antigens on glycophorin B (GPB, CD235b). GPA and GPB are sialoglycoproteins (sialic acid, sugar and protein) of the red cell membrane. The gene for the antigens within the MN blood group system is located on chromosome 4 (4q32.21). There are two amino acid residue differences between the M and N antigens on glycophorin A and a single amino acid residue difference between the S and s antigens on glycophorin B. Amino acid substitutions and hybrid GPA/GPB glycophorins result in many of the large number of antigens identified within this system, e.g. M^g, Mi^a, St^a, and Dantu. The very rare null type M^k is the result of the absence of both red cell GPA and GPB.

Table 15 MNS blood group frequencies in a Southern African population (excluding U which is a high prevalence antigen)

Phenotype	Caucasian	Black
M+N–	28	26
M+N+	50	44
M–N+	22	30
S+s–	11	3
S+s+	44	28
S–s+	45	69

The S and s antigens are associated with the high prevalence antigen, U. The rare phenotype S-s-U– occurs in 0·25% of Blacks and a rare variant form of U gives rise to an S-s-U+ phenotype, as a result of a mutant form of GPB.

Antibody characteristics

- Treatment of red cells with some proteolytic enzymes, such as bromelin, papain and ficin, denatures M and N antigens, variably denatures the S and s antigens, but not the U antigen, resulting in anti-M, anti-N, anti-S and anti-s not usually being demonstrable by enzyme techniques.
- Anti-M and anti-N are usually "cold agglutinins" reacting by saline agglutination techniques. Anti-N seldom reacts above 20°C.
- "Cold-reacting" anti-M often contains a large proportion of IgG immunoglobulins.
- Reactivity of some examples of anti-M and anti-S is enhanced by lowering the pH of the serum/plasma.
- Gel column technology is very sensitive for the detection of anti-M, because the gel has a low pH.
- Occasionally, anti-M and anti-N react by IAT.
- Anti-M frequently occurs in children where it may not be immune in origin and is often reactive by IAT at 37°C.

- Although anti-S may be naturally occurring, it is usually a complement-binding type IgG antibody. Anti-S is often produced together with antibodies to low prevalence antigens.
- Anti-U is usually demonstrable by IAT and enzyme methods.
- Monoclonal anti-M, anti-N and anti-S reagents are available for red cell typing and lectin anti-N (V*icia graminea* or *Vicia unijuga, Bauhinea purpura* and *B. variegata*) is available for N typing.
- Anti-s is quite rare.

Clinical significance

Clinical significance in transfusion.

- Anti-M and anti-N, unless reacting at 37°C, are generally considered not to be of clinical significance and have rarely been the cause of transfusion reactions. IAT crossmatch compatible blood, rather than antigen-negative blood, is usually given.
- Anti-S, anti-s and anti-U are considered clinically significant and may cause moderate to severe transfusion reactions. Crossmatch compatible and antigen-negative blood should be selected for transfusion.

Clinical significance in HDFN.

- Anti-M and anti-N have occasionally caused mild HDFN but are not considered to be of obstetric significance. However, there is gathering body of evidence that anti-M may be of greater clinical significance within Japanese and Chinese populations.
- Anti-S, anti-s and anti-U may cause moderate to severe, even fatal HDFN.

P1PK blood group system (ISBT P1PK 003)

Number of antigens: 3 (2019)

The P1PK blood group system was formerly called the P system and contained three antigens: P, P_1, P^k. In 1994 the P antigen was reallocated to the Globoside system (ISBT GLOB 028). In 2011 the NOR antigen was assigned to the P system, which was renamed P1PK in 2013.

The P1 blood group was described by Karl Landsteiner and Philip Levine in 1927, after injecting rabbits with human red blood cells and screening for antibodies by testing the rabbit sera against human red blood cells from different individuals.

The antigen was named P because this was the first letter after the already assigned M, N and O.

P1 is encoded for on chromosome 22 (22q13.2) The *A4GALT* gene encodes a 4-α-galactosyltransferase enzyme, which adds an α-galactose to paragloboside on the red cell membrane to create the P1 antigen.

The P1, p^k and NOR antigens are not primary gene products. They are located on glycolipids. The terminal linkage of each antigen is synthesised by the primary gene product (4-α-galactosyltransferase). All antigens in the P1PK systems are based on lactosylceramide, which is also the immediate precursor for the p^k antigens.

P and Pk are high frequency antigens. (P is the only antigen in the Globoside blood group system ISBT GLOB 028.)

The frequency of the P_1 antigen varies in different populations as shown in Table 16.

It is important to understand the differences in characteristics and clinical significance of anti-P and P1.

Antibody characteristics

- Anti-P_1 is naturally occurring, and usually an IgM antibody. It is commonly encountered as a cold agglutinin but occasionally reacts at 37°C. Some rare examples may bind complement and react by IAT.
- Anti-P_1 that binds complement and reacts by IAT may cause a transfusion reaction.
- Anti-P is found in the serum/plasma of all P^k negative individuals and will haemolyse P_1 and P_2 cells in the presence of complement. Anti-P is also found in cases of paroxysmal cold haemoglobinuria (PCH). PCH is a haemolytic disease that occurs mainly in children following a viral infection. The sera from such patients give a positive Donath-Landsteiner test (see *Glossary* for details).

Other antibodies that are not actually part of the P1PK blood group system are noted here:

- Anti-PP_1P^k (previously called anti-Tj^a) is a rare, potent antibody found in the very rare type pp individuals. The antibody reacts at all temperatures by all methods and is frequently present as a haemolysin. It causes transfusion reactions and is a potential cause of recurrent abortions. These abortions are induced because the placenta contains high numbers of P^k and P antigens which are targeted by the IgG antibodies. It rarely causes HDFN.

Clinical significance

Clinical significance in transfusion.

- Anti-P_1 is not normally considered to be clinically significant and it is not usually necessary to select

Table 16 Frequency of P1 antigen in percentage

Phenotype	Caucasian	Black
P_1+ (or P_1)	79	94
P_1− (or P_2)	21	6

antigen-negative blood. Units found compatible by IAT at 37°C may be transfused.
- Anti-P and Anti-PP_1P^k are antibodies to high prevalence antigens and it is extremely difficult to find compatible blood for patients requiring blood transfusions.

Clinical significance in HDFN.
- Anti-P_1 has not been reported to cause HDFN.

Features of the P1PK blood group system
- P_1 antigen is weakly expressed at birth.
- P_1 substance can be found in various flatworms, and hydatid (tapeworm) cysts in sheep livers. P_1 substance from avian sources, e.g. pigeon egg albumin can be used in inhibition tests.
- The frequency and avidity of anti-P_1 are increased in P_1- individuals suffering from helminth infestations (parasitic worm, *e.g.* hookworm).

Practical application
- The P_1 antigen varies considerably from individual to individual in antigenic strength and known strong P_1+ cells should be used for antibody detection.

Kell blood group system (ISBT KEL 006)

Number of antigens: 39 (2019)

CD number: CD238

The Kell blood group system was described in 1946, and was named after the proband, Mrs Kelleher. The antibody was named anti-K (Note: *NOT* anti-Kell). It caused HDFN in this case and has done so in numerous cases since. The Kell system alleles have been identified at the *KEL* locus on chromosome 7 (7q34). The Kell system is also associated with the Kx (ISBT XK 019) and the Gerbich (ISBT GE 020) blood group systems, which adds to its already quite considerable complexity.

The most commonly encountered antigens in the laboratory are the K and k antigens (Note: *NOT* Kell and Cellano). After the ABO and Rh antigens, the K antigen is the next most immunogenic. Table 17 shows the Kell blood group frequencies.

Many different Kell antigens are produced and the frequency of the antigens, other than the high and the low prevalence antigens, varies greatly between populations. The well-known high prevalence antigens are k, Kp^b and Js^b.

Table 17 Kell blood group frequencies in percentage

Phenotype	Caucasian	Black
K+k–	0·2	0·1
K+k+	8·8	3·5
K–k+	91·0	96·5

The K_o type is the rare null type in the Kell system and lack all the Kell antigens. K_o individuals who have become immunised frequently produce anti-Ku, an antibody that reacts with all red cells except other K_o cells. This makes the provision of compatible blood for such patients extremely difficult.

Red cells of the very rare McLeod phenotype show weakened expression of the Kell antigens. The degree of depression of the Kell antigens varies from individual to individual with the McLeod phenotype. Some express the antigens almost as strongly as normal individuals, while others express the antigens so weakly that by normal serological techniques they mimic K_o red cells. The McLeod phenotype has an association with Chronic Granulomatous Disease (CGD), an inherited primary immunodeficiency disease, in which case the condition is known as the McLeod Syndrome.

Transient depression of Kell system antigens may also occur in warm auto-immune haemolytic anaemia, in microbial infections, and in cases of idiopathic thrombocytopenia purpura. Examples of auto-anti-Kp^b and anti-Ku have been reported.

Antibody characteristics
- Anti-K and anti-k are usually IgG immunoglobulins, reacting optimally by IAT and some examples also react by enzyme techniques.
- Some examples of anti-K react at temperatures below 37°C.
- Although anti-K is usually produced in response to stimulus by transfusion or pregnancy, 'naturally occurring' cases have been reported and are possibly due to bacterial infections such as *Escherichia coli*. This type of anti-K is usually transient.
- In the past, some examples of anti-K were reported to react weakly in tests using LISS or polybrene.
- If a patient develops anti-k it can be difficult to provide compatible blood because of the low frequency of k– blood. However, anti-k is a relatively rare antibody as few individuals are k–.
- The chemical dithiothreitol (DTT) denatures Kell antigens, so the detection of Kell antibodies is not possible if DTT is used to determine whether IgM or IgG antibodies are present in a sample or if a ZZAP solution that contains DTT and an enzyme such as papain or bromelain is used for auto-adsorptions.

Clinical significance

Clinical significance in transfusion. Anti-K and anti-k are clinically significant and can cause severe acute

haemolytic transfusion reactions as well as delayed transfusion reactions. K− or k− (i.e. antigen-negative) blood and crossmatch compatible blood should be provided to patients with anti-K or anti-k antibodies respectively.

The K antigen is highly immunogenic, and formation of anti-K has resulted from transfusion of K+ red cells units to K− individual. Consequently, it is now common practice in the UK and many European countries to transfuse K− females with child-bearing potential with K− red cells to avoid the formation of anti-K. In patients requiring long term transfusion therapy, units matched for K (as well as the various Rh antigens) should be provided. In the UK appropriately 1/500 donors would be expected to be k− and "wet" units are usually available from rare blood stocks.

Clinical significance in HDFN. Anti-K antibodies differ from the other blood group system antibodies that cause HDFN as they destroy early erythroid progenitor cells and suppress erythropoiesis, causing severe anaemia, and occasionally death of the fetus. High bilirubin levels are not a characteristic as the early erythroid progenitor cells are destroyed prior to haemoglobinisation. Bilirubin is therefore not released into the amniotic fluid as with Rh mediated haemolytic disease and consequently measurement of bilirubin concentration in amniotic fluid does not give an accurate indication of the severity of the disease. See *Section 7: Haemolytic diseases*, for more information.

Kp^a and Kp^b

The Kp^a and Kp^b antigens result from the presence of two allelic co-dominant genes in the Kell blood group system. The Kp^a antigen is a comparatively low prevalence antigen, being expressed on the red cells of about 2% of Caucasian individuals and is very rarely expressed on the red cells of Black individuals. The Kp^b antigen is a high prevalence antigen expressed on the red cells of more than 99·9% of all individuals. The antibodies are IgG, reacting optimally by IAT and are clinically significant both in transfusion and HDFN. The provision of Kp(b−) blood to individuals who have become immunised is difficult. Antigen-negative and IAT crossmatch compatible blood should be transfused when the intended recipient has the corresponding antibodies.

Js^a and Js^b

The Js^a antigen is expressed on the red cells of approximately 20% of Black individuals and less than 0·1% of Caucasian individuals. Anti-Js^a is commonly detected in a mixture of antibodies, rather than as a single specificity, especially in sickle cell disease patients, which increases the difficulty of finding suitable blood for transfusion.

The Js^b antigen is a high prevalence antigen found in more than 99·9% of all populations. The antibodies are IgG and react optimally by IAT. All examples of anti-Js^b have been detected in Black individuals.

Both antibodies have caused HDFN and transfusion reactions. Antigen-negative and IAT crossmatch compatible blood should be transfused. If the rare type Js(a+b−) blood is required it may be obtained through a rare donor registry.

General comment

The antibodies to the Kell system are usually of clinical significance and may cause severe or delayed transfusion reactions. The respective antigen-negative and IAT crossmatch compatible blood should be transfused. If the antibody is to a high frequency antigen in the Kell blood group system, it may be necessary to obtain the antigen-negative blood from a rare donor registry.

Duffy blood group system (ISBT FY 008)

Number of antigens: 5 (2019).

CD number: CD234

The Duffy blood group was described in 1950 by Marie Cutbush and Patrick Mollison. Anti-Fy^a was found in a haemophilia patient who had received multiple transfusions and the system was named after this patient. The Fy^a and Fy^b antigens are encoded by a pair of co-dominant allelic genes located on chromosome 1 (1q23.2). The difference between Fy^a and Fy^b is caused by a single amino acid residue variant within the protein.

The Duffy protein spans the red cell membrane seven times and the major (or α) protein contains 336 amino acid residues. There is also a minor (or β) protein containing 338 amino acid residues. The Duffy antigen, also known as the Duffy antigen receptor for chemokines (DARC), is a receptor of both the sub-families of chemokines (C-X-C and C-C).

The frequency of the Duffy blood group system antigens varies greatly between different populations. The silent recessive *FY* gene is very rare in Caucasians but occurs frequently in Blacks, particularly those living in malarial areas. Red cells lacking Duffy antigens are refractory to invasion by malaria parasites (such as *Plasmodium vivax* and *Plasmodium knowlesi*). This suggests an adaptive response to the disease or selective pressure due to the parasite; consequently, the proportion of individuals in certain African populations who do not express the DARC protein in their erythrocytes is high. Homozygous expression of the *FY* gene results in the Fy(a−b−) phenotype. Most examples of Fy(a-b-) in African Blacks are as a result of a homozygous mutation of the *GATA-1* gene in the promoter area of the Duffy gene, which

prevents the expression of the Duffy antigens ***ONLY*** on red cells (it is erythrocyte specific). The *FYB* gene is usually present (except in individuals from Papua New Guinea, where the *FYA* gene is often present, instead of the *FYB* gene). Such individuals rarely produce an anti-Fy3, however often they are stimulated to so do.

Table 18 shows the Duffy blood group frequencies.

Table 18 Duffy blood group frequencies in percentage

Phenotype	Caucasian	Black
Fy(a+b–)	20	9
Fy(a+b+)	47	1
Fy(a–b+)	33	22
Fy(a–b–)	0	68

Antigen characteristics

- Fy^a and Fy^b antigens are denatured by enzyme treatment (i.e. bromelin, ficin and papain, but not trypsin).
- Fy3 is expressed on all red cells, apart from those of the Fy(a–b–) phenotype and is resistant to enzyme treatment.
- Fy3 negative i.e. Fy(a-b-) is rare in Caucasians but not uncommon in Blacks where it may reach 68%.

Antibody characteristics

- Anti-Fy^a and anti-Fy^b are IgG and can be stimulated by transfusion and pregnancy,and both can cause HDFN.
- Anti-Fy^a and anti-Fy^b react by antiglobulin technique and are not detected by most enzyme techniques.
- Anti-Fy^a is often detected together with other red cell antibodies, particularly Rh antibodies.
- Anti-Fy^b is not frequently encountered but is not uncommon in Chinese populations.
- Anti-Fy3 was first found in an Fy(a-b-) Caucasian Australian multiparous woman who had also been previously transfused.
- Anti-Fy3 is a rare antibody found in Fy(a-b-) populations.
- Anti-Fy3 from individuals who not from one of the Black ethnicities reacts strongly with cord blood cells, while anti-Fy3 from Black ethnicities does not react or reacts only weakly with cord cells.
- Anti-Fy3 formation is usually preceded by formation of anti-Fy^a.
- Anti-Fy3 can cause haemolytic transfusion reactions and mild HDFN.

Kidd blood group system (ISBT JK 009)

Number of antigens: 3 (2019)

The Kidd blood group system was described in 1951 by Fred Allen, Louis Diamond and Beverly Niedziela and the first antigen found was named after the initials of the sixth child (John Kidd) of the first proband to make anti-Jk^a, which resulted in haemolytic disease of newborn. The blood group system was named after the proband.

The antigens Jk^a and Jk^b of the Kidd blood group system are encoded by a pair of co-dominant alleles located on chromosome 18 (18q11-12).

The frequency of the Kidd blood group system antigens varies between different populations. The Jk^a antigen is expressed more frequently in Black individuals, than in Caucasian.

The rare Jk(a–b–) or Jk:-3 phenotype (the null type in the Kidd blood group system), has been reported in some Polynesian and Finnish populations, and less frequently in other populations. It should be recognised that the 0·9% of Polynesians with the Jk(a-b-) phenotype could be an exaggeration as, according to one source (Issitt and Anstee), kinship was not taken into account. The urea lysis test is a useful test for screening for the rare Jk:−3 phenotype, as Jk:−3 cells, unlike Jk(a+) and/or Jk(b+) cells, are resistant to lysis by 2 M urea.

Antibody characteristics

- Anti-Jk^a and anti-Jk^b are IgG antibodies that can activate complement and are stimulated by transfusion or pregnancy.
- The detection of these antibodies can be difficult, because frequently they are weakly reactive and may show dosage, i.e. antibody reacts more strongly or reacts only with homozygous red cells, i.e. those carrying a double dose of the antigen.
- The antibodies react optimally by IAT, although some weak examples react only by enzyme IAT techniques and solid phase technique.
- Some examples may be complement-dependent and require the presence of activated complement before they can be detected.
- They are relatively rare antibodies and are most often present in serum/plasma containing other blood group antibodies.
- The avidity and the titre of the antibodies often diminish rapidly, and the antibodies may not be demonstrable in subsequent samples from the patient.
- Anti-Jk3 can be detected by IAT.

Clinical significance

- Both anti-Jk^a and anti-Jk^b (including weak examples) have caused severe acute haemolytic transfusion reactions due to an anamnestic response.

- Kidd antibodies can cause delayed haemolytic transfusion reactions.
- Antigen-negative and IAT crossmatch compatible blood should be transfused to patients with these antibodies. If further transfusions are required at a later date the antibodies may be difficult to detect at the time of the crossmatch or may no longer be demonstrable by routine serological techniques. Antigen-negative blood should be transfused to prevent a delayed transfusion reaction. Good records of patients with clinically significant antibodies should be kept so that the appropriate blood can be crossmatched. Anti-Jk3 should also be considered to be clinically significant. Compatible rare type Jk:−3 blood may need to be obtained from a rare donor registry.

The antibodies rarely cause HDFN. Table 19 shows the Kidd blood group frequencies.

Lewis blood group system (ISBT LE 007)

Number of antigens: 6 (2019).

The Lewis blood groups system was first described by Arthur Mourant in 1946 and named after one of the two donors in whom anti-Le^a was first identified. Lewis antigens are not actually intrinsic to the red cell membrane but are adsorbed onto the red cells from the plasma. The presence or absence of the Le^a and Le^b antigens is determined by genes at three different loci on chromosome 19:

- *H (or FUT1)*: responsible for production of H substance (the precursor substance for the A and B antigens).
- *SE (or FUT2)*: enables the A, B and H antigens to be secreted in most body fluids.
- *LE (or FUT3)*: which is the gene for the Lewis alleles. The symbol *le* is used to show the absence of the *Le* gene.

When the *Le* gene is present, the enzyme produced reacts with one of two possible H substrates. The preferred substrate is secreted H substance. This is produced when both *H* and *SE* genes are present. The secreted H substance is then converted into the Le^b antigen. If the secreted H substance is not present as a result of the absence of an *SE* gene, (or because of the inheritance of the very rare *hh* genes, i.e. O_h type), the enzyme product of the *LE* gene acts on the precursor H substance instead, giving rise to the Le^a antigen.

If no *LE* gene is present, neither the Le^a or Le^b antigens are produced, resulting in the Le(a−b−) phenotype.

Table 20 provides a summary of the Lewis blood groups, together with their interactions with the ABH and secretor genes.

The Le^a and Le^b antigens are adsorbed onto the red cells from the plasma, but the antigenic strength is variable and may change under various circumstances, e.g. pregnant women often type as Le(a−b−). Cord cells type as Le(a−b−) as the red cell Lewis antigens only develop during the first 12–15 months after birth.

The Le(a−b−) phenotype occurs more frequently in Black individuals than in Caucasians, as shown in Table 21.

Unusual phenotype of Le(a+b+)

The Le(a+b+) phenotype is caused by mutation(s) in secretor *FUT2* which reduces the efficiency of the FUT2 enzyme, and as a result the Lewis FUT3 enzyme become relatively more efficient and is thus able to compete more

Table 19 Kidd blood group frequencies (approximate) in percentage

Phenotype	Caucasian	Black	Asian
Jk(a+b–)	26	51	23
Jk(a+b+)	50	40	27
Jk(a–b+)	23	8	49
Jk(a–b–)/Jk: –3	Very rare	Very rare	<1*

*Polynesians.

Table 21 Lewis blood group frequencies in percentage

Phenotype	Caucasian	Black
Le(a+b–)	22	23
Le(a–b+)	72	55
Le(a–b–)	6	22

Table 20 Summary of LE, A, B and H and SE gene interactions

Phenotype	Genes present	Comment (ABH)	Comment (Lewis)
Le(a+b−)	*LE sese*	ABH non-secretor	*LE* gene product acts on H precursor substance
Le(a–b+)	*LE Se*	ABH secretor	*LE* gene product acts on secreted H substance
Le(a–b–)	*lele Se*	ABH secretor	No *LE* genes are present
Le(a–b–)	*lele sese*	ABH non-secretor	No *LE* genes are present

effectively for precursor. More Le^a means less H type 1 is made with less Le^b and ABH substance, hence the association of this phenotype with the partial/weaker secretor phenotype.

Antibody characteristics

- Anti-Le^a and anti-Le^b are often IgM, naturally occurring antibodies. They are usually developed by type Le(a−b−) individuals. The antibodies generally react best at temperatures below 37°C and react by saline and enzyme techniques. Some examples of anti-Le^a and anti-Le^b are IgG and react by IAT. Strong reacting IgG antibodies may cause *in vitro* haemolysis in the presence of complement.
- In the presence of multiple antibodies in a patient's plasma, the presence of Lewis antibodies can interfere with antibody identification; the use of Lewis substance for neutralisation can be helpful in resolving the problem.
- Some anti-Le^b reacts better with group O or group A_2 Le(b+) cells than with group A_1 or group B Le (b+) cells. These are termed anti-Le^{bH} as they react with cells with the most H antigen.

Clinical significance

- The majority of Lewis antibodies do not cause transfusion reactions.
- Generally, patients with anti-Le^a and anti-Le^b which reacts at 37°C can be transfused with crossmatch compatible blood. There are, however, some rare examples of Lewis antibodies, which react strongly at 37°C by IAT and activate complement. These antibodies should be considered to be of potential clinical significance, and it may be wiser to select antigen-negative units of blood for crossmatch.
- Lewis antibodies do not cause HDFN as fetal and newborn red cells do not normally express Le^a and Le^b antigens.

Lutheran blood group system (ISBT LU 005)

Number of antigens: 27 (2019)

CD number : CD239

Anti-Lu^a was described by Sheila T. Callender and Robert Race in 1945, and should have been named Lutteran, after the first Lu(a+) donor, but the writing on the label of the blood sample was misread as Lutheran.

The Lutheran glycoproteins (CD239) are immunoglobulins that bind the extracellular glycoprotein laminin. The protein is encoded by the *LU* gene which is located on chromosome 19 (19q13.2). Lu-glycoproteins may be involved in facilitating movement of maturing erythroid cells from the erythroblastic island of the bone marrow to the peripheral circulation and may play a role in the migration of erythroid progenitors from fetal liver to the bone marrow. The *LU* locus is part of a linkage group situated on Chromosome 19, which includes the *SE*, *LE*, *H* and *LW* loci.

In addition to the two major antigens, Lu^a (a low prevalence antigen) and Lu^b (a high prevalence antigen), there are three further pairs of allelic co-dominant genes that control the antithetical Lu6 and Lu9 antigens, the antithetical Lu8 and Lu14 antigens, and the antithetical Au^a and Au^b antigens.

Nineteen other high prevalence antigens are part of the system. There is a very rare recessive type, which genuinely expresses no Lutheran antigens, and these individuals can be stimulated to produce anti-Lu3. Additionally, there is the In(Lu) dominant type, which is itself rare, but not as rare as the recessive type. This type does express Lutheran antigens very weakly (and normally types as Lu (a-b-) by routine serological techniques), but the antigens can be detected by adsorption elution. These individuals cannot produce anti-Lu3.

Table 22 shows the Lutheran blood group frequencies. The expression of Lutheran antigens is variable, and dosage effects may be seen.

Antibody characteristics

- The antibodies may be stimulated by pregnancy or transfusion.
- Anti-Lu^a is usually an IgM immunoglobulin reacting by saline techniques.
- Reactions with Lutheran antibodies often shows a typical 'mixed field/stringy' form of agglutination when viewed microscopically.
- Anti-Lu^b is usually an IgG immunoglobulin reacting optimally by IAT.
- Anti-Lu^a is seldom seen and rarely causes a problem in the crossmatching laboratory as compatible blood can be easily found.

Clinical significance

- Both anti-Lu^a and anti-Lu^b have been reported to cause mild or delayed transfusion reactions.
- IAT crossmatch compatible blood should be transfused. The provision of Lu(b−) blood may be

Table 22 Lutheran blood group frequencies in percentage

Phenotype	Caucasian	Black
Lu(a+b–)	<1	<1
Lu(a+b+)	7	7
Lu(a–b+)	93	93
Lu(a–b–)	Very rare	Very rare

difficult, but the antibody is seldom seen. In the UK, Lu(a-b-) or Lu(b-) red cell units are readily available from the rare blood stock upon special request.

Lutheran antibodies have not been reported to cause severe HDFN as the antigens are only weakly expressed on cord cells. In addition, even if the maternal antibodies are IgG, which is relatively rare, they are thought to be adsorbed onto fetal Lutheran glycoprotein occurring on the placental tissue.

I Blood group system (ISBT I 027)

Number of antigens: 1(2019)

The I blood group system was promoted to its own system in 2002 after the *I (GCNT2)* gene located on chromosome 6 (6p24.2) was identified as encoding the glycosyltransferase responsible for converting i-active straight oligosaccharide chains to I-active branched chains. Although the I blood group system is not one of the major blood group systems, it is included in this section as it is of practical importance. The I antigen is expressed on all normal adult red cells. The expression of I antigen varies with age and with disease, and the degree of expression varies considerably between individuals.

The I blood group system is associated with "cold-reacting" antibodies that are usually only detected at low temperatures and in some haematological diseases, where the thermal range may broaden.

All cord cells type as I−, because the I antigen is not well developed at birth. Cord cells may therefore be used as a source of I− cells for antibody identification tests. Within 18 months of birth, the I antigen develops and replaces the i antigen, because of the action of a-transferase enzyme using the i antigen as a substrate.

Rare variants of the i antigen exist; for example, the antigen i_1 is found as a rarity in Caucasian individuals, and the antigen i_2 is found as a rarity mostly among Black individuals. Natural antibodies to I are found in the serum/plasma of adults who express high levels of the i antigen; the presence of the i antigen in adults is caused by mutation of the I gene (*GCNT2)*. Initially, no association between the adult i phenotype and disease was detected, but since 1972, it is known that there is a close link between the adult i phenotype and congenital cataracts in the Japanese population.

Antibody characteristics

- Anti-I is usually an IgM immunoglobulin that occurs frequently as a "cold-reacting" autoantibody or a "cold agglutinin". It is seldom seen as an alloantibody.
- However, wide thermal amplitude auto-anti-I can cause cold auto-immune haemolytic anaemia, if the antibody can be detected at 30°C and above and may also be present in mixed type cold and warm reactive auto-immune haemolytic anaemia. Auto-anti-i is occasionally found in patients recovering from diseases such as infectious mononucleosis (glandular fever). Anti-I may be associated with anti-H, forming antibodies with anti-HI specificity.

Clinical significance

- Although most anti-I antibodies are clinically insignificant, some examples of auto-anti-I, have a wide thermal range (see above**)**. Should these patients require blood transfusions, they may be given the "least incompatible" blood, warmed in a validated blood warmer before infusion. The patient's samples may be difficult to type if the patient's cells are auto-agglutinated. The red cells may need to be washed with warm saline before testing.
- Crossmatching must be very carefully performed to ensure that the auto-anti-I is not masking clinically significant antibodies.

I system antibodies have not been implicated in HDFN.

Additional blood group systems/collections/ antibodies reacting with high and low frequency antigens

The blood group systems discussed so far in this section are either of clinical or practical importance. However, patients may produce antibodies to many other blood group antigens.

As the frequency of antigens may vary between population groups, it is often important to know the ethnicity of the patient, particularly when trying to find compatible blood for a patient with antibodies directed against an apparent high prevalence antigen or antigens. Such samples are usually referred to a reference laboratory that has rare high prevalence antigen-negative panel cells available.

Antibodies directed against high prevalence antigens are usually detected when the serum/plasma reacts with all samples tested, but the auto-control is negative. This indicates the presence of an alloantibody, not an autoantibody. Further tests are required to identify whether the antibodies are directed against a high prevalence antigen or whether they are a mixture of antibodies of different specificities. On rare occasions, such as anti-At^a in the Augustine blood group system (ISBT AUG 036), the underlying pathology can also give a clue to the probable specificity (in this case, pseudo-gout), as can the sex and

age, in many cases of anti-JMH in the John Milton Hagen blood group system (ISBT JMH 026).

Antibodies to the high prevalence antigen, Kn^a (Knops blood group system, ISBT KN 022) may be referred to as high titre, low avidity (HTLA) antibody. Chido/ Rodgers antibodies directed against the high prevalence antigens Ch and Rg (ISBT CH/RG 017) react with the complement C4 protein, which is adsorbed onto red cells. In the presence of these antibodies, exclusion of other antibodies, that may be clinically significant, is important. Antibodies directed against antigens within the Knops blood group system can be neutralised by CR-1 protein, while anti-Ch/ Rg can be neutralised by ABO compatible pooled plasma. The identification of these antibodies may be time-consuming, and few are clinically significant.

The antibodies may be directed against the high prevalence antigens mentioned previously in the various blood group systems. Many of these antibodies are clinically significant and can cause severe transfusion reactions and HDFN. It may be extremely difficult to find compatible, antigen-negative blood and donations may have to be obtained from a local, national or international rare donor registry/frozen storage facility. For example, in the Ok blood group system (ISBT OK 024), initially a total of eight families with Ok(a-) individuals were identified in Japan. A further two cases, one from an Iranian and another of Hispanic origin, have been identified. Family members who would be suitable donors may also be tested to see if they lack the particular high prevalence antigen, as siblings are more likely to be compatible than donors in the random population.

Such samples are usually referred to a reference laboratory with a supply of rare panel cells of the null phenotypes such as Lan, Vel-, Jk(a-), and U-.

If the patient has an antibody directed against a low prevalence antigen, the provision of compatible blood should not be a problem. Antibodies to low prevalence antigens may be found by chance when crossmatching random units of blood or may be detected when using a panel that includes a panel cell sample positive for a low prevalence antigen.

Table 23 lists some of the rare phenotypes associated with different ethnic groups and the increased likelihood of patients producing antibodies of the corresponding specificity. Note that this should be used as a guideline only as there are always exceptions.

Table 23 Rare phenotypes associated with different ethnic groups and increased likelihood of antibody specificity

	Increased likelihood of rare type		
Ethnic group	**Blood group system**	**Red cell phenotype**	**Antibody specificity**
Black	Rh	hr^S-	Anti-hr^S
	Rh	hr^B-	Anti-hr^B
	MNS	U-	Anti-U
	Kell	Js(a+b−)	Anti-Js^b
Indian	H	O_h	Anti-H, -A, -B, -AB
	Indian	In(a+b−)	Anti-In^b
	Kidd	Jk(a−b−)	Anti-Jk3
Caucasian	Kell	K+k−	Anti-k
	Kell	Kp(a+b−)	Anti-Kp^b
	Vel	Vel-	Anti-Vel
	Yta	Yt(a−b+)	Anti-Yt^a

Polyagglutination

Polyagglutination is the term used to describe red cells that are agglutinated by almost all samples of adult human sera/plasma, but not by autologous serum/plasma or sera/plasma from newborn infants. The polyagglutinable state may be transient or persistent. Transient polyagglutinable results from the exposure of normally crypt antigens by bacterial enzymatic activity (T, Tk, Th) during the course of an infectious process. For example, naturally occurring anti-T is present in the plasma of all individuals. It is formed after the exposure to T-antigens present on many gram-negative bacteria and vaccines. However, in paediatric patients with necrotising enterocolitis (NEC) and atypical Haemolytic Uremic Syndrome (aHUS), neuraminidase removes the sialic acid residues on red cells and exposes T-antigens. Transfusion of blood components with a high level of anti-T may result in severe haemolysis.

Persistent polyagglutination may be a consequence of somatic mutation leading to a cellular lineage characterised by an enzyme deficiency that results in exposure of a normally crypt antigen, Tn. Most human sera contain anti-Tn. Tn polyagglutination is regularly accompanied by leucopaenia and thrombocytopenia and has been associated with leukaemia. Other forms of persistent polyagglutination are due to the inheritance of rare blood groups or are associated with a hematologic dyscrasia.

Polyagglutination can be resolved with the help of a lectin panel. These are carbohydrate binding proteins, which are usually obtained from plant seeds or monoclonal antibodies, for example (anti-T).

Table 24 provides a summary of lectins that may be used to identify the specificity of polyagglutinable red cells.

Human Leucocyte Antigen (HLA) System

The human leucocyte antigen (HLA) system is the most polymorphic and complex region of the human genome.

Table 24 Lectin panel for polyagglutinable RBC

	RBC					
Lectin extracts	**Normal**	**T-positive**	**Tk-positive**	**Th-positive**	**Tx-positive**	**Tn-positive**
Arachis hypoea	0	+	+	+	+	0
Salvia sclarea	0	0	0	0	0	+
Salvia horminum	0	0	0	0	0	+
Glycine soja	0	+	0	0	0	+

The HLA system is part of the Major Histocompatibility Complex (MHC), a region of the human genome found on the short arm of chromosome 6 which contains 47 genes and pseudogenes with 13 023 alleles. The MHC genes and molecules are involved in regulating inflammation, the complement cascade, and the adaptive immune response.

The human MHC may be divided into 3 regions, class I, class II and class III. Figure 4 illustrates HLA locus, antigens and proteins products.

MHC class I genes:

Humans have three major MHC class I genes, known simply as HLA-A, HLA-B and HLA-C. These genes code for proteins found on the cell membranes of all nucleated cells and platelets, but not on red cells. On the cell membrane these proteins bind to viral and other fragments that have been transported from within the cell, and they are presented to the cells of the immune system.

MHC class II genes:

Humans have six major MHC class II genes, known as HLA-DPA1, HLA-DPB1, HLA-DOA1, HLA-DOB1, HLA-DRA, and HLA-DRB1. These genes code for proteins that are present on the surface of some of the cells of the immune system such as macrophages and dendritic cells. On the cell membrane these proteins present viral and other fragments to the cells of the immune system, particularly to Helper T-cells.

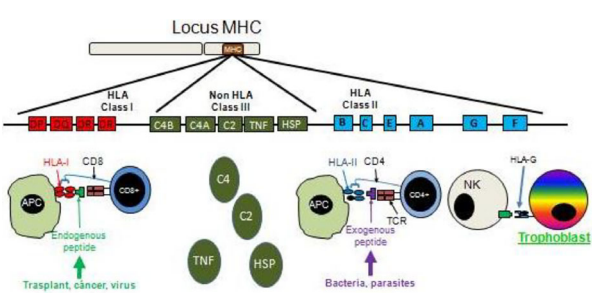

Fig. 4 Human leucocyte antigen (HLA) locus, antigens and proteins products.

MHC class III genes:

This region does not contain any HLA genes, but rather genes that encode different proteins with immune function, such as complement factors (C2, C4, Factor B) and tumour necrosis factor (TNF).

The HLA system is one of the most polymorphic human systems as it needs to recognise foreign antigens in the form of peptides from a wide variety of different pathogens. The differences between HLA proteins are localised primarily to the extracellular region of these molecules, which bind peptides and interact with T-cell receptors. The high degree of HLA polymorphism is likely the result of positive selection for human survival by enhancing the diversity in the repertoire of HLA-bound peptides. This is reflected in the many different HLA types found throughout the world. Some HLA types are more frequently found in certain populations.

Human leucocyte antigen Alloimmunization

The basis of HLA alloimmunization is the high degree of polymorphism since most individuals are likely to have different HLA molecules on the surface of their cells. Hundreds of HLA alleles have been identified through high-resolution typing, making the chances of two individuals having identical HLA phenotypes extremely low.

Exposure to foreign HLA molecules can occur due to pregnancy, transplantation and transfusion and can result in the production of HLA antibodies and activated lymphocytes in immunologically competent individuals. Female patients that have been primed by pregnancy are more likely to make HLA antibodies in response to transfusion or transplantation if the HLA mismatches are present in the transfusion or allograft.

Human leucocyte antigen alloimmunization is influenced by several patient and blood component factors and can be lessened (but not eliminated) by transfusion of leucocyte-reduced blood components.

Transplantation

The HLA system is one of the main barriers to transplantation between individuals. The immune response produced when a graft containing foreign HLA antigens is transplanted into an unrelated recipient can result in the production of donor-specific HLA antibodies or T-cells by the recipient to reject the graft. Graft survival can be improved when the donor and recipient are HLA matched. For example HLA matched sibling transplants have improved survival when compared to unrelated haemopoietic stem cell donor transplants.

The T-cell activation by the donor's HLA molecules after clinical transplant can be controlled by using appropriate immunosuppressive therapies. However, the major long-term problem is the development of donor-specific antibodies against mismatched HLA antigens. Controlling the antibody responses to mismatched HLA molecules is a challenge and needs continuous monitoring of their development.

Solid Organ Transplantation

Human leucocyte antigen matching has varying degrees of importance in solid organ transplantation depending on the type of organ being transplanted. Three major reasons why organs may be rejected, include:

- Hyperacute rejection where pre-formed antibodies directed against ABO blood group antigens and/or HLA class I antigens on the graft mediate irreversible damage to the transplanted organ within minutes or hours.
- Acute rejection predominately involves a cellular immune response, but donor-specific HLA antibodies can also be involved in binding to HLA molecules on the graft and mediate rejection through complement activation or antibody dependent cell-mediated cytotoxicity, usually weeks or months following transplantation.
- Chronic rejection is a long-term deterioration in graft function due to immune and non-immune causes. The number and severity of acute rejection episodes, infection and drug toxicity are just a few of the factors that can contribute to chronic graft rejection, months to years following transplantation.

Haemopoietic Stem Cell Transplantation

The only potential curative treatment for many patients with bone marrow and some metabolic disorders is haemopoietic stem cell transplantation (HSCT). The stem cells used for transplantation can be obtained from bone marrow, peripheral blood and umbilical cord blood.

The HLA system is the primary immunologic barrier to successful HSCT. Successful transplantation is dependent on optimising the histocompatibility matching between the patient and the donor. This matching requirement is more stringent than for solid organ transplantation. International registries of millions of typed individuals exist to provide a possible matched donor for patients who do not have a matched family donor. Similarly, cord blood banks contain HLA typed cord blood units for use in transplantation of unrelated recipients.

Transfusion

Patients with an intact immune system, who receive multiple transfusions of platelets or leucocyte concentrates, or red cells containing platelets and/or leucocytes, can produce HLA antibodies directed against the mismatched HLA antigens present on leucocytes in the transfused blood product. Anti-HLA antibodies may lead to various types of transfusion reactions, discussed in detail in *Section 14: Risks of transfusion and haemovigilance*. The frequency of alloimmunization is reduced by the use of leucodepleted blood products.

Human Platelet Antigen (HPA) Systems

The molecular basis for the human platelet antigen (HPA) systems has been determined and a total of 39 platelet-specific alloantigens have been defined and their underlying molecular basis has been resolved. The systems are less polymorphic than HLA. Most antigens result from a single nucleotide polymorphism (SNP), which leads to a single amino acid substitution. HPA frequencies vary in different populations. Exposure to foreign platelet antigens following pregnancy, transfusion, or organ transplantation can result in alloimmunization.

The platelet antigens are located on the platelet membrane. The majority of the HPAs are located on the glycoprotein IIb/IIIa complex (integrin IIIb β_3). The detection of platelet antibodies is complex, and most methods are performed in specialized laboratories. With the development of molecular testing, it is possible to determine the HPA genotype.

In cases where patients develop platelet refractoriness during transfusion therapy, it may be necessary to provide both HLA and HPA matched blood products.

Fetomaternal alloimmune thrombocytopaenia (FMAIT)

- The most common cause of severe thrombocytopaenia in the fetus and newborn is FMAIT.
- Although FMAIT has been regarded as the platelet equivalent of HDFN, it occurs frequently during first pregnancies. In FMAIT, the antigen that stimulates

the maternal antibody response is found on fetal platelets where the fetus has inherited an HPA type from the father, lacking in the mother.

- In Caucasians, the most common antigens to stimulate the production of platelet antibodies are HPA-1a and HPA-5b which are the most immunogenic platelet antigens. FMAIT can also be caused by antibodies to low frequency HPA antigens.
- Paternally inherited platelet antigens, lacking in the mother, may stimulate the production of IgG maternal antibodies as a result of FMH. These IgG antibodies are able to cross the placenta and sensitize fetal platelets, causing thrombocytopenia.
- The diagnosis is usually made after birth when the neonate shows symptoms of thrombocytopenia, and platelet-specific antibodies are detected in the maternal serum/plasma.
- Because of severe thrombocytopaenia, fetal/neonatal intracranial haemorrhage may occur.
- The antenatal administration of intravenous immunoglobulin to the mother may lead to an increase in circulating platelets in the fetus, reducing the likelihood of fetal intracranial haemorrhage. The reason for this is perhaps the modulation of maternal antibody production that may be induced by the administration of immunoglobulin.

Table 25 Some of the main characteristics of blood group systems and the corresponding antibodies

System	Antibody	Predominant type	Clinical significance	Comments
ABO	Anti-A			
	Anti-B	IgM/IgG	HTR and HDFN	Can cause fatal HTR and severe HDFN.
	Anti-A,B			Antibodies may be haemolytic.
	Anti-A_1	Mainly IgM	Rare	
H	Anti-H	Mainly IgM	Low risk	Weakest reactions with group A_1 and B cells.
	Anti-H,-A,-B	IgM/IgG	HTR and HDFN	Bombay O_h type
Rh	Anti-D	Mainly IgG	HTR and HDFN	Main cause of HDFN.
	Anti-C			Often occurs with anti-D.
	Anti-E	IgG/IgM		May be naturally occurring and react with enzymes only.
	Anti-c	IgG		Severe HDFN may be produced with anti-E.
	Anti-e			Not often seen. If individual is a variant e type, antibodies may appear to be anti-e but individual types e+.
MNS	Anti-M	IgM	Rare HTR	Antigen denatured by enzyme treatment. May show dosage.
	Anti-N		Low risk	
	Anti-S	IgG/IgM	HTR and HDFN rare	Antigen denatured by enzyme treatment.
	Anti-s		HTR and HDFN	Rare antibody
	Anti-U	IgG	HTR and HDFN	Antibody to high frequency antigen, compatible blood from rare blood registry.
P_1 antigen	Anti-P_1	IgM	Low risk	Common cold agglutinin produced by P_1- individuals. P_1 substance used in inhabitation tests.
Lutheran	Anti-Lu^a	IgM mainly	Low risk	Low frequency antigen
	Anti-Lu^b	IgG/IgM	HTR and HDFN rare	Antibody reaction and clinical significance variable.
Kell	Anti-K	IgG	HTR and HDFN	Can cause death *in utero*.
	Anti-k			Rare antibody, difficult to find compatible blood.
	Anti-Kp^a			Rare antibody
	Anti-Kp^b			Rare antibody, compatible blood from rare donor registry.
	Anti-Js^a			Very rare antibody
	Anti-Js^b			Antibody to high frequency antigen, compatible blood from rare donor registry.
Lewis	Anti-Le^a	IgM usually	Type IgG: rarely causes HTR	Antigen absorbed from plasma onto red cells.
	Anti-Le^b			Antigen loss in pregnancy.
	Anti- Le^a + Le^b	IgM/IgG		Produced by Le(a-b-) individuals.
Duffy	Anti-Fy^a	IgG	HTR and HDFN	Antigen denatured by enzyme treatment.
	Anti-Fy^b		HTR	Antigen denatured by enzyme treatment, rare antibody.
Kidd	Anti-Jk^a	IgG	HTR, delayed HTR, and HDFN	May require complement for IAT detection.
	Anti-Jk^b			
I	Anti-I	IgM usually	Low risk	Often detected, seldom clinically significant. Soluble I antigen is secreted in human milk.

- Although the introduction of antenatal screening has been considered, there is generally a lack of optimal antenatal treatment. The safety of fetal blood sampling and platelet transfusion is also a concern. Most laboratories are unable to predict the severity of FMAIT based on antibody characteristics.
- As in the case of HDFN, premature induction of labour may also be recommended. It appears that caesarean section may be preferred to natural birth, in order to avoid cranial trauma at the time of delivery.

The practical problem is that most cases are diagnosed postnatally. Time is important as the condition should be treated promptly, preferably with HPA compatible platelets although random donor platelets, or (rarely) washed and irradiated maternal platelets, may be used. Most transfusion services do not have HPA matched platelets available as this would require the use of a panel of HPA typed donors donating regularly to provide an available unit in stock or available on call.

Summary of section: Blood group systems

Table 25 summarizes the characteristics of the blood group systems and their corresponding antibodies. Haemolytic transfusion reaction has been abbreviated as HTR.

Key Points

- The major histocompatibility complex (MHC) contains the genes of the highly polymorphic human leucocyte antigen (HLA) system and is located on the short arm of chromosome 6.
- The MHC also contains the genes for some cytokines and various complement components.
- The HLA gene encodes two types of molecule, HLA class I (HLA-A, B, C) and HLA class II (HLA-DR, DQ, DP).
- HLA class I molecules are found on nearly all nucleated cells and both HLA class I and II molecules are found on cells of the immune system which is a major immunological barrier to successful transplantation.
- The role of HLA is to present peptides to the immune system. These peptides may be derived from pathogens, altered self-cells (virus-infected, tumoral) or foreign tissue.
- The HLA system needs to be very polymorphic to be able to recognise the many different types of pathogens that humans may encounter throughout the world. This is why HLA matching between unrelated individuals is difficult.
- Exposure to foreign, mismatched HLA can occur as a result of pregnancy, transfusion or organ transplantation and can lead to the production of antibodies or the development of immune cells that are directed against the foreign HLA.
- The introduction of universal leucodepletion for blood and blood products has reduced the incidence of alloimmunization to HLA in some patient groups.
- The human platelet antigen (HPA) systems are less polymorphic and the frequency of the different genes varies in different populations.
- Exposure to foreign HPA following pregnancy, transfusion, or organ transplantation can result in alloimmunization.
- The HPA-1a and HPA-5b are considered to be the most immunogenic platelet antigens in populations of Caucasian origin and most cases of FMAIT are the result of anti-HPA-1a.
- In cases where patients develop platelet refractoriness during transfusion therapy it may be necessary to provide both HLA and HPA matched blood products.
- The most common cause of severe isolated thrombocytopenia in the fetus and newborn is FMAIT. Although it has been regarded as the platelet equivalent of HDFN, FMAIT occurs frequently during first pregnancies.
- The diagnosis is usually made after birth when the neonate shows symptoms of thrombocytopenia and platelet-specific antibodies are detected in the maternal serum/plasma.
- The practical problem is that most cases of FMAIT are diagnosed postnatally. Time is important as the condition should be treated promptly with HPA compatible platelets. Most transfusion services do not have HPA matched platelets available.