VII. Rh Blood Group System

A. The Rh system is one of the most complex genetic systems, and certain aspects of its genetics, nomenclature and antigenic interactions are unsettled. The aim of this lecture is to concentrate on commonly encountered observations, problems and solution without exhaustive theoretical considerations.

B. Antigens of the Rh System

1. The descriptive terms D positive and D negative refer only to the presence or absence of the red cell antigen "D". The terms Rh positive and Rh negative are the old terms used. The early name given to the D antigen, "Rho", is less frequently used.

2. Four additional genes are recognized as belonging to the Rh system and they are: C, c, E and e.

3. The major allelic genes are C/c and E/e.

4. Many variations or combinations of the five principle genes and their products antigens) have been recognized.

5. These antigens and their corresponding antibodies characterize the Rh blood group system and account for the majority of Rh antibodies encountered in blood banking.

C. History

1. The first human example of the antibody directed at the D antigen was reported in 1939 by Levine and Stetson, who found it in the serum of a woman whose fetus had fatal hemolytic disease of the newborn.

2. The Rh system was identified by the work of Landsteiner and Wiener who found that human RBCs were agglutinated by an antibody, apparently common to all rhesus monkeys and 85% of humans. This factor was named the Rh factor.

3. Landsteiner and Wiener immunized guinea pigs and rabbits with the RBCs of Rhesus monkeys, the antibody produced by these animals agglutinated 85% of human RBCs.

4. Later the antigens detected by the rhesus antibody and by the human antibody were established as dissimilar, but the system had already been named.

5. This contribution to medical science was the most significant event in blood group systems research since the discovery of the ABO system 40 years earlier.

6. The Rh-hr blood group system is probably the most complex of all erythrocyte blood group systems, with more than 40 different Rh antigens. Only a brief overview of the most important antigens will be presented.

D. Clinical Significance

1. The D antigen is, after A and B, the most important red cell antigen in transfusion practice.
   a. Individuals who lack the D antigen do not have anti-D in their serum.
   b. The antibody is produced through exposure to the D antigen usually as a result of transfusion or pregnancy.
   c. The immunogenicity (ability of antigen to stimulate production of antibody) of D is greater than that of virtually all other red blood cell antigens studied.
2. It has been reported that >80% of D negative individuals who receive a single unit of D positive blood can be expected to develop anti-D.

3. The blood of all potential recipients is routinely tested for D so all D negative recipients can be identified and transfused with D negative blood.

E. Inheritance and Nomenclature

1. Fisher-Race Theory

a. Antigens of the Rh system are determined by three pairs of genes which occupy closely linked loci.

b. According to Fisher-Race, Rh genes are inherited as one gene complex from each parent.

1) Each gene complex carries D or its absence (d), C or c, and E or e.
2) The order of loci on the gene appears to "DCE" but many authors prefer to use the order "CDE" to follow the alphabet.

c. According to the Fisher-Race concept, the gene d appears to be a silent gene or an amorph because there is no demonstrable product of d. The gene d is assumed to be present when D is absent.

(1) Illustration of the Fisher Race theory of three closely linked loci and their possible alleles
(2) Illustration of a DCE/dce individual.

d. The three loci that carry the Rh genes are so closely linked on the chromosome that they never separate but are passed from generation to generation as a unit or gene complex.

e. As illustrated on next page, an offspring of the Dce/dce individual will inherit either DCe or dce from the parent, but not a combination such as dCe. Should such a combination occur it would indicate crossing over. This has never been proven in the Rh system of man.
f. With the exception of the amorph d, each of the allelic genes mentioned so far controls the presence of its respective antigen on the red cell. As seen in the example the gene complex DCe determines the presence of the antigens D, C and e on the red cells.

g. If the same gene complex were on both of the paired chromosomes D, C and e would be the only Rh antigens demonstrable on the cells.

h. If one chromosome carried DCe and the other DcE, the antigens present would be D, C, c, E and e.

j. Each antigen (except d) is recognizable by testing the red cells with a specific antiserum.

2. **Wiener Theory**

   a. The Wiener theory postulates that two genes, one on each chromosome of the pairs, control the entire expression of the Rh system in one individual.

   b. The two genes at the two loci may be alike (homozygous) or different (heterozygous) from each other.

   c. As shown in the following table, the eight major alleles are called R', R', R', R', r, r', r" and r'.

\begin{itemize}
  \item \textbf{(1)} \textit{Illustration of the Wiener hypothesis of a single gene locus and the possible alleles at the locus}
  \item \textbf{(2)} \textit{Illustration of an Ro/r individual.}
\end{itemize}
d. According to Wiener's hypothesis, each gene produces a structure on the red cell called an agglutinogen (antigen), and each agglutinogen can be identified by its parts or factors that react with specific antibodies (antiserums).

e. As illustrated below, the gene R\(^1\) has been inherited on one chromosome and the gene r at the same locus on the other chromosome.

1) The gene R\(^1\) determines the agglutinogen Rh\(^1\) on the red cell and this agglutinogen is made up of at least three factors: Rho (D), rh'(C) and hr'' (e).

2) The gene r determines the agglutinogen rh on the red cell distinguished by its factors hr' (c) and hr'' (e).

f. These two theories are the basis for the two notations currently in use for the Rh system. The table below compares Fisher-Race and Wiener notations. Immunohematologists use combinations of both systems when recording the most probable genotype. **You must memorize and be able to convert from the Fisher-Race notation to the Wiener Gene complex (or shorthand) notation.**

<table>
<thead>
<tr>
<th>Fisher-Race Notations</th>
<th>Wiener Notations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene Complex</td>
<td>Antigens</td>
</tr>
<tr>
<td>----------------------</td>
<td>----------</td>
</tr>
<tr>
<td>Dce</td>
<td>D, c, e</td>
</tr>
<tr>
<td>Dce</td>
<td>D, C, e</td>
</tr>
<tr>
<td>DcE</td>
<td>D, c, E</td>
</tr>
<tr>
<td>DCE</td>
<td>D, C, E</td>
</tr>
<tr>
<td>dce</td>
<td>c, e</td>
</tr>
<tr>
<td>dCe</td>
<td>C, e</td>
</tr>
<tr>
<td>dcE</td>
<td>c, E</td>
</tr>
<tr>
<td>dCE</td>
<td>C, E</td>
</tr>
</tbody>
</table>
3. **Rosenfield System**

a. Proposed a system of nomenclature based only on serologic (agglutination) reactions. Antigens are numbered in order of their discovery or recognition of the antigen as belonging to the Rh system.

b. The following table is a partial list of the numbers assigned. The phenotype of a given cell is expressed by the base symbol Rh followed by a colon and a list of the numbers of the specific Rh antisera used.

<table>
<thead>
<tr>
<th>Numerical notation as suggested by Rosenfield</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rosenfield</strong></td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>Rh 1</td>
</tr>
<tr>
<td>Rh 2</td>
</tr>
<tr>
<td>Rh 3</td>
</tr>
<tr>
<td>Rh 4</td>
</tr>
<tr>
<td>Rh 5</td>
</tr>
<tr>
<td>Rh 6</td>
</tr>
<tr>
<td>Rh 7</td>
</tr>
<tr>
<td>Rh 8</td>
</tr>
<tr>
<td>Rh 10</td>
</tr>
<tr>
<td>Rh 12</td>
</tr>
<tr>
<td>Rh 19</td>
</tr>
<tr>
<td>Rh 20</td>
</tr>
</tbody>
</table>

c. To write a genotype in this system the presence or absence of the antigen is noted: Rh:1, D antigen is present, Rh:-1, D antigen is absent.

d. This system is very difficult for oral communication, but very precise for written or computer use.

e. Today recently discovered Rh antigens have been given a number only from the Rosenfield system.

4. **Tippett**

a. The most recent genetic model.

b. Proposed that two closely linked structural loci, D and CcEe, are proposed as the basis for Rh antigen production.

1) For the 8 common Rh phenotypes, two alleles are found at the first locus, D and non-D.

2) At the second or CcEe locus, four alleles are needed and include ce, Ce, cE and CE.
### Tippett’s Genetic Model Applied to the Eight Common Rh Gene Complexes

<table>
<thead>
<tr>
<th>First Locus</th>
<th>Second Locus</th>
<th>Gene Complex</th>
<th>Shorthand Symbol</th>
</tr>
</thead>
<tbody>
<tr>
<td>D</td>
<td>ce</td>
<td>Dce</td>
<td>( R^e )</td>
</tr>
<tr>
<td>D</td>
<td>Ce</td>
<td>Dce</td>
<td>( R^l )</td>
</tr>
<tr>
<td>D</td>
<td>cE</td>
<td>DcE</td>
<td>( R^l )</td>
</tr>
<tr>
<td>D</td>
<td>CE</td>
<td>DCE</td>
<td>( R^e )</td>
</tr>
<tr>
<td>non-D</td>
<td>ce</td>
<td>dce</td>
<td>( r )</td>
</tr>
<tr>
<td>non-D</td>
<td>Ce</td>
<td>dcE</td>
<td>( r' )</td>
</tr>
<tr>
<td>non-D</td>
<td>cE</td>
<td>dcE</td>
<td>( r' )</td>
</tr>
<tr>
<td>non-D</td>
<td>CE</td>
<td>dCE</td>
<td>( r^y )</td>
</tr>
</tbody>
</table>

#### F. Genotyping

1. Five reagent Rh antisera are available, but routine testing involves only the use of anti-D. The other antisera are used to resolve antibody problems or conduct family studies.

2. The agglutination reactions of an individual's RBCs with specific Rh antisera produce a variety of patterns representing the Rh phenotype.

3. Since there is no "anti-d", genotyping cannot be done by simply testing RBCs for D and antigens. One must take advantage of the statistical probability in the relationship of D with C, c, E and e to determine the most probable genotype. The table below depicts the most probable genotypes of the phenotypes obtained.

### Presumptive genotypes based on reactions with five anti-sera

<table>
<thead>
<tr>
<th>Reactions with Anti-D C E c e</th>
<th>Probable genotype</th>
<th>% Freq</th>
<th>Second most likely genotype</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ + 0 + +</td>
<td>DcE/dce</td>
<td>32.7</td>
<td>DcE/Dce</td>
<td>2.2</td>
</tr>
<tr>
<td>+ + 0 0 +</td>
<td>DcE/DCe</td>
<td>17.7</td>
<td>DcE/dCe</td>
<td>0.8</td>
</tr>
<tr>
<td>+ + + + +</td>
<td>DcE/DeE</td>
<td>12.0</td>
<td>DcE/dcE or DeE/dCe</td>
<td>0.3</td>
</tr>
<tr>
<td>+ 0 + + +</td>
<td>DcE/dce</td>
<td>11.0</td>
<td>DcE/Dce</td>
<td>0.7</td>
</tr>
<tr>
<td>+ 0 + + 0</td>
<td>DcE/DeE</td>
<td>2.0</td>
<td>DcE/dcE</td>
<td>0.3</td>
</tr>
<tr>
<td>+ 0 0 + +</td>
<td>Dce/dce</td>
<td>2.0</td>
<td>Dce/Dce</td>
<td>0.1</td>
</tr>
<tr>
<td>0 0 0 + +</td>
<td>dce/dce</td>
<td>15.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 + 0 + +</td>
<td>dcE/dce</td>
<td>0.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 0 + + +</td>
<td>dcE/dce</td>
<td>0.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4. It is essential that the genotype of a person be listed as "presumptive" or "most probable".
5. Every racial group varies to some extent from those listed here. Even within small countries the frequency of certain antigens will vary slightly from one area to another. Among American Blacks the most common gene complex is Dce. Dce, DcE and dce are more common in Caucasians.

<table>
<thead>
<tr>
<th>Gene Complex</th>
<th>Shorthand</th>
<th>% in Caucasians</th>
<th>% in Blacks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dce</td>
<td>R^o</td>
<td>2</td>
<td>46</td>
</tr>
<tr>
<td>Dce</td>
<td>R^1</td>
<td>40</td>
<td>16</td>
</tr>
<tr>
<td>DcE</td>
<td>R^2</td>
<td>14</td>
<td>9</td>
</tr>
<tr>
<td>dce</td>
<td>r</td>
<td>38</td>
<td>25</td>
</tr>
</tbody>
</table>

6. The racial origin of the person concerned should influence deductions about genotype, because the frequencies of Rh genes differ by race.

G. Weak Expression of D (D^+ is the old term)

1. Not all D positive RBC samples react equally well with every anti-D blood grouping reagent.

2. Red cells that are not immediately agglutinated by anti-D cannot be classified as D negative until additional testing is performed (the Du test).
   a. Incubate cells with anti-D at 37 C.
   b. Wash 3 times and add AHG.
   c. If negative, individual is D negative, if positive patient is weak D recorded as "D+w".

3. The D antigen may be present in a weak form due to 3 possible mechanisms:
   a. Inheritance of a gene that codes for less D antigen.
   b. Position of the antigens on the chromosomes best know example is "C in trans" position which causes suppression of the D antigen expression.
   c. Absence of a portion or portions of the total material that comprises the D antigen, known as "partial D" (used to be called "D mosaic").

4. Blood donors who are D^+ are considered D positive for transfusion purposes.
   a. It has been shown that D^+ seems to be substantially less immunogenic than normal D.
   b. D^+ blood has caused a severe hemolytic transfusion reaction in a patient with anti-D.

5. The status of the transfusion recipient who is weak D+ is sometimes a topic of debate.
   a. If the weak D+ is due to the partial D phenotype these individuals may make an antibody to the portion of the D antigen they lack.
   b. If the weak D+ is due to suppression or genetic expression then, theoretically, these individuals could receive D pos blood.
   c. It is standard practice in the field to transfuse weak D individuals with D negative blood.

H. Other Rh Antigens

1. Close to 50 Rh antigens have been identified, but most of these are rarely encountered.
2. **Compound antigens** are epitopes that occur owing to the presence of two Rh genes on the same chromosome (*in cis-position*).

   a. The gene products include not only the products of each single gene, but also a combined gene product that is also antigenic.

   b. For example, the r (cde) gene that makes c and e, also makes f, (ce). This only occurs when c and e are in the cis position (on the same chromosome).

   c. The f antigen will not be present if the antigens are in the trans position (opposite chromosomes).

<table>
<thead>
<tr>
<th>Illustration of f antigen on a chromosome</th>
<th>Illustration of c and e present, but no f</th>
</tr>
</thead>
<tbody>
<tr>
<td>c</td>
<td>c</td>
</tr>
<tr>
<td>c</td>
<td>c</td>
</tr>
<tr>
<td>c</td>
<td>c</td>
</tr>
<tr>
<td>c</td>
<td>C</td>
</tr>
<tr>
<td>e</td>
<td>e</td>
</tr>
<tr>
<td>e</td>
<td>e</td>
</tr>
<tr>
<td>e</td>
<td>e</td>
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<tr>
<td>e</td>
<td>e</td>
</tr>
</tbody>
</table>

   d. Antibodies against these compound antigens are not rare but are encountered less frequently than antibodies with single specificities.

   e. The antibody against f (anti-ce), would only react with f positive cells, not cells that were e positive only or c positive only. These are clearly marked on the antigram of screen and panel cells.

3. The **G antigen** cannot be fitted neatly into the concept of three antigenic regions.

   a. It is a gene product of Rh gene complexes that produce C or D.

   b. G is almost invariably present on RBCs possessing C or D, so that antibodies against G appear superficially to be anti-C+D.

   c. The anti-G activity cannot, however, be separated into anti-C and anti-D.

4. **D deletion bloods** are very rare. People may inherit Rh gene complexes lacking alleles at the Ee locus or the Ee and Cc loci, these are called D deletion genes.

   a. These are detected only when the are homozygous for the rare deletion genotype, have two different deletion genotypes (one on each chromosome), or are part of the family study of a person who meets either of the previous two criteria.

   b. D deletion blood is characterized by increases in the number of D antigen sites on the RBCs, resulting in stronger reactions with anti-D antisera than cells having no deletions.
c. The deletion genes that have been described include: cD-, CDw-, -D-, and .D.

d. Homozygous -D- (-D/-D-) individuals may form antibodies to high incidence Rh antigens. This antibody causes agglutination to all RBCs except those of homozygous -D- individuals. These individuals are counseled to donate autologous blood and have it frozen.

5. **Rh null** phenotype fails to react with any or all Rh antisera. The phenotype of these people is ---/---. No C, c D, E or e antigen is detectable on these RBCs.

a. This is caused by genes which are not part of the Rh system and are inherited independently and can interact to **modify** the expression of the Rh structural genes.

b. These modifier (or regulator genes) can totally suppress the structural Rh genes, causing the cells to appear to be devoid of Rh antigens.

c. The Rh genes are normal as shown by the fact that Rh null individuals do transmit functional Rh genes to their offspring.

d. The Rh antigens have been shown to be an integral part of the RBC membrane lipid bilayer. The total absence of Rh system antigens **results in a hemolytic anemia** due to the resulting defect in the RBC membrane. This hemolytic anemia is due to increased destruction of RBCs in the spleen and is usually compensated by increased RBC production in the bone marrow.

6. The **LW system** was discovered at the same time as the Rh antigen. Landsteiner and Wiener detected the LW antigen on the cells of Rhesus monkeys and on human RBCs in the same proportion as the D antigen.

a. At the time it was thought that the LW and Rho(D) antigens were the same antigen. Later it was discovered that differences existed and that LW and Rh genes segregated independently.

b. The LW system was then renamed in honor of Landsteiner and Wiener, its discoverers.

c. Rare persons exist whose RBCs lack the LW antigen, yet have normal Rh antigens, with or without D. These people can form alloanti-LW and this antibody reacts more strongly with D-positive than with D-negative cells. Keep in mind when a D positive individual appears to have anti-D

7. Other alleles can be inherited at the Cc locus in place of C or c. The antigen C" is an example of a **variant antigen** which may be inherited.

a. This antigen is expressed in approximately 1-2% of some white populations.

b. Anti-C" is infrequently detected in the routine blood bank. In a few cases it has been shown to be the cause of HDN and hemolytic transfusion reactions.

I. **Rh Antibodies**

1. Except for some examples of anti-E and anti-Cw that occur without known stimulus, **most Rh antibodies result from immunization by pregnancy or transfusion and are of the IgG immunoglobulin class**.

2. Although Rh antibodies are associated with both hemolytic transfusion reactions and HDN, **in vitro binding of complement is rare**.
3. Antibody characteristics and frequencies.
   a. IgG antibodies may occur in mixtures with a minor component of IgM. So the Rh system antibodies usually do not agglutinate saline-suspended RBCs unless they have a large amount of IgM.
   b. Rh antibodies can usually be demonstrated when the cells are suspended in a high protein (albumin) media or by the IAT.
   c. **IgG Rh system antibodies react best at 37 C and are enhanced when tested against enzyme treated RBCs.**
   d. **D is the most immunogenic of the common Rh antigens.** Approximately 50% of D negative people who are immunized with as little as 1.0 mL of D positive cells will form anti-D. **This makes the D antigen status of transfusion recipients of secondary importance only to their ABO group.**
   e. In studies of deliberate immunization to induce antibody production the C, c, E, and e antigens were demonstrated to be much less immunogenic than D.
      1) **Anti-E is the most common antibody associated with these four antigens,** followed by anti-c.
      2) Anti-C as a single antibody is rare in both D positive and negative persons.
      3) Anti-e is rarely encountered because only 2% of the population are antigen negative.
   f. Detectable antibody levels usually persists for many years.
   g. Anti-D may react stronger with R^2^R^2^ cells than other genotypes due to the fact that R^2^R^2^ individuals carry more D antigen sites on their cells than other D positive genotypes.

4. **Concomitant antibodies** - It is important to be aware of Rh antibodies which often occur together.
   a. Sera containing anti-D often contains anti-G.
   b. Anti-C is rarely formed as a pure antibody, it may also be found in sera containing anti-D.
   c. Anti-ce (anti-f) is often seen in combination with anti-c.
   d. **The most commonly encountered concomitant antibody pair** is found in R^1^R^1^ individuals who may make anti-E plus -c. If there is detectable anti-E in the serum the individual has most likely been exposed to c as well.
      1) Patients who have detectable anti-E in their serum should be phenotyped for the c antigen also.
      2) Individuals who are R^1^R^1^ with anti-E should be transfused with R^1^R^1^ blood due to the fact the anti-c may be present, but at levels below the sensitivity of the test system.
      3) Sometimes the anti-c will be detected if enzyme treated cells are used.
J. Detection of Rh Antigens

1. Reagents used to detect the D antigen in the slide, tube and microplate tests are available in several types. **It is critical** to read reagent package inserts when using the anti-D typing sera. The instructions will vary depending upon the reagent type used and may or may not require the use of a diluent control.

2. **High protein antisera** is composed of IgG anti-D potentiated with high protein and other macromolecular compounds to ensure agglutination of D positive cells.
   
a. The high protein media may cause **false positive** reactions when the RBCs tested are coated with immunoglobulin.

b. A diluent control containing all of additives and potentiators in the reagent antiserum but lacking the anti-D component **must** be run simultaneously.

c. The control used **must** be from the same manufacturer as the anti-D serum.

d. The manufacturer's instructions for use must be followed exactly.

e. The Rh control **must exhibit a negative reaction** when tested with the individuals cells, if it is positive the D typing is invalid.

f. Other causes of false positive reactions with this reagent are: strong autoagglutinins, abnormal serum proteins which cause rouleaux formation, antibodies directed against the additive in the reagent or using unwashed RBCs.

g. **Can be used for the weak D (D^w) test.**

3. **IgM anti-D reagents** (low protein)
   
a. Saline-reacting antisera prepared from predominantly IgM antibodies have always been relatively scarce because suitable raw material is difficult to obtain.

b. These reagents are reserved for testing RBC samples that give false positives with high protein antisera.

c. In contrast to the newer saline-reactive antisera, traditional saline tube test reagents require the test to be incubated at 37 C, usually for 15 minutes or longer and are **unsuitable for slide tests.**

d. Antisera of this kind **cannot be used for the Du test.** IgM antibodies generally perform poorly in the IAT.

4. **Chemically Modified IgG Antisera** (low protein)
   
a. The IgG anti-D antibodies in the raw material are converted to direct agglutinins by treating the serum with a sulfhydryl compound that weakens the disulfide bonds at the hinge region of the IgG molecule.

b. Gives the molecule greater flexibility at the hinge regions which allows the antigen combining sites situated at the terminal end of each Fab portion to span a greater distance.
c. Show stronger reactivity than those prepared from IgM antibodies.

d. **Can be used for slide testing.**

e. **Can be used for the weak D (D*) test.**

f. More abundantly available than the IgM kind for routine use.

g. *Negative control not necessary unless patient is AB positive.*

5. **Monoclonal Source Anti-D (low protein)**

a. Prepared from a blend of monoclonal IgM and polyclonal IgG sera.

b. Used routinely in place of high protein or chemically modified sera in rapid tube, slide or microplate tests.

c. The IgM component directly agglutinates D positive RBCs while the IgG component reacts in the antiglobulin phase of the Du test.

d. *Negative control not necessary unless patient is AB positive.*

6. **Control for low protein reagents**

a. Most reagents are in a diluent with a total protein concentration approximating that of human serum.

b. False positive reactions due to spontaneous agglutination of immunoglobulin coated RBCs occur no more frequently with this kind of reagent than with other saline reactive antisera.

c. False positive reactions may still occur. This will become apparent as patient will forward type as AB positive. A saline control must be run (either a manufacturers control or saline suspended RBCs), if it is positive the test is invalid.

d. A negative reaction in the control for anti-D cannot be used as a control for other Rh antigens.

7. **Precautions for Using Rh Typing Reagents**

a. **Whatever antisera are used, the manufacturer's directions must be carefully followed.**

b. The IAT **must not** be used unless the serum is described explicitly by the manufacturer as suitable for this use.

c. Positive and negative controls must be tested in parallel with the test RBCs.

K. **Sources of Error in Rh Antigen Typing**

1. **False positive** reactions can result from the following:

a. Spontaneous agglutination due to heavy coating of patient RBCs with autoantibody, abnormal plasma proteins or rouleaux.

b. Contaminated reagents

c. Use of the wrong antiserum. Antisera for Rh phenotyping are color coded: anti-D is grey, anti-C is pink, anti-c is lavender, anti-E is brown, and anti-e is green.

d. Autoagglutinins (cold agglutinins) or abnormal proteins in the test serum.

e. An unsuspected specificity is present in the reagent or antisera used by test method other than that described by the manufacturer.
2. False negative reactions may result from the following:
   a. Use of the wrong antiserum.
   b. Failure to add antiserum to the test.
   c. Incorrect cell suspension.
   d. Incorrect serum to cell ratio.
   e. Shaking the tube too hard after centrifugation.
   f. Reagent deterioration due to contamination, improper storage or outdating.
   g. Failure of the antiserum to react with a variant antigen.
   h. Antiserum in which the predominant antibody is directed against a compound antigen. This is often a problem with anti-C antiserum.

L. Summary

1. The Rh system is the second most important blood group system when transfusion is considered.

2. Correct interpretation of D typing is essential to a positive outcome of transfusion and is important in protecting infants from HDN due to anti-D.

3. With at least 50 known antigens in the system, it is by far the most polymorphic of the blood group systems routinely studied.

4. Only the five major antigens are tested in most situations, with only D testing required for pretransfusion interpretation unless an unexpected antibody is found in recipient serum.

5. The pitfalls and solutions for Rh typing have been discussed and should provide a starting point for problem solving in most routinely encountered situations.