Introduction

The Rh, or Rhesus, factor was discovered in 1940 by Landsteiner and Wiener, when they observed that an injection of blood from a Rhesus monkey into rabbits caused a reaction in the serum component of the rabbit blood. Later evidence established that the antigen detected by animal anti-Rhesus and human anti-D were not identical, but the Rh blood groups system already had received its name.

Rh Genes and Antigens

- Rh genes are located on the short arm of chromosome 1 (p34.3 to 36.1). Their 3’-ends are oriented to each other and separated by 30,000 base pairs.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>RHD</td>
<td>Encodes the D antigen</td>
</tr>
<tr>
<td></td>
<td>Most Rh-negative phenotypes result from the complete deletion of the RHD gene. Individuals who inherit one or two RHD are considered Rh positive.</td>
</tr>
<tr>
<td>RHCE</td>
<td>Encodes the CE antigens: ce, cE, Ce, or CE</td>
</tr>
</tbody>
</table>

- Inheritance: Rh antigens are inherited as codominant alleles. Rh antigens are present only on red cells, and are not detectable on platelets, lymphocytes, monocytes, neutrophils, or other tissues.
- Rh antigens are fully expressed at birth and can be detected as early as 8 weeks gestation.
- Rh antigens are integral part of the red cell membrane and play a role in maintaining the integrity of the red cell membrane. Red cells that lack Rh antigens have an abnormal shape.
- The term Rh refers not only to the D antigen but also to a complex blood group system composed of over 50 different antigenic specificities.

Terminology

Blood bankers should be able to translate among the different nomenclatures.

<table>
<thead>
<tr>
<th>Terminology</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weiner</td>
<td>One gene at a locus controls the entire Rh system. This theory was incorrect, but for the designation of phenotype, particularly in conversation, a modified version of Wiener’s system is still used.</td>
</tr>
<tr>
<td>Fisher-Race</td>
<td>Three pairs of genes (C/c, E/e, and D) so closely linked that they are passed on genetically as a complex. This theory is also incorrect as there are only two genes, RhD and RhCE. A modified CDE terminology is now commonly used to communicate research and serologic findings. Rh antigens are referred by the letters D, C, c, E, e, and e.</td>
</tr>
<tr>
<td>Rosenfield</td>
<td>Rh antigens are listed in the chronological order of discovery. D=1; C=2; E 3; c=4; e=5. This system is difficult to communicate but is easily computerized.</td>
</tr>
<tr>
<td>ISBT</td>
<td>Alpha-numeric assignment consisting of a six-digit identification number. The first three digits refer to the blood group system and the second three to the antigen itself. For example, the D antigen is 004.001.</td>
</tr>
</tbody>
</table>
Haplotype Frequencies

- Offspring inherit one Rh haplotype from each parent. For example, an individual whose Rh phenotype is D+C+E-c-e+ can have the genotype $R^1R^1$ or $R^1r'$.
- Based on the haplotype frequencies, phenotype frequencies can be calculated.

<table>
<thead>
<tr>
<th>Weiner Haplotypes</th>
<th>Fisher-Race Haplotypes</th>
<th>Whites</th>
<th>Blacks</th>
<th>Asian</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R^1$</td>
<td>DCe</td>
<td>42</td>
<td>17</td>
<td>70</td>
</tr>
<tr>
<td>$R^2$</td>
<td>DcE</td>
<td>14</td>
<td>11</td>
<td>21</td>
</tr>
<tr>
<td>$R^0$</td>
<td>Dce</td>
<td>4</td>
<td>44</td>
<td>3</td>
</tr>
<tr>
<td>$R^Z$</td>
<td>DCE</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>1</td>
</tr>
<tr>
<td>$r'$</td>
<td>Ce</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>$r''$</td>
<td>cE</td>
<td>1</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>$r$</td>
<td>ce</td>
<td>37</td>
<td>26</td>
<td>3</td>
</tr>
<tr>
<td>$r'$</td>
<td>CE</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Antigens Prevalence and D Antigen Sites

Number of D antigens varies, depending on phenotype.
- Common D phenotypes: 10,000 – 33,000
- Exalted D (e.g., D- -) phenotypes: 75,000-200,000

<table>
<thead>
<tr>
<th>Basic Rh antigens Prevalence (%)</th>
<th>Whites</th>
<th>Blacks</th>
<th>Asians &amp; Native Americans</th>
</tr>
</thead>
<tbody>
<tr>
<td>D</td>
<td>85</td>
<td>92</td>
<td>99</td>
</tr>
<tr>
<td>C</td>
<td>68</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>29</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>c</td>
<td>80</td>
<td>96</td>
<td></td>
</tr>
<tr>
<td>e</td>
<td>98</td>
<td>98</td>
<td></td>
</tr>
</tbody>
</table>

Rh Antibodies and Clinical Significance

- D is the most important red cell antigen in transfusion medicine after the A and B antigens. Rh system antigens are highly immunogenic. If a unit of D-positive blood is transfused to a D-negative recipient, the recipient can form anti-D.
- Anti-D is caused by exposure through a transfusion or pregnancy.
- Most Rh antibodies are IgG and react optimally at 37°C or after antiglobulin testing.
- Rh antibodies generally do not bind complement; extravascular hemolysis occurs in transfusion reactions.
- Anti-D can sometimes be detected in the plasma/serum for a very long time after the last known stimulus.

Other Variations in Expression of D

- The D antigen consists of more than 30 different epitopes. Most D-positive cell phenotypes have a conventional RhD protein. Most individuals are either Rh positive or Rh negative.
- However, there is a huge number of different RHD alleles that encode proteins with amino acid changes that give rise to numerous variations in the expression of D. These alleles can cause variations in the expression of D, resulting in red cells with altered D expression.
- Reagent anti-D may not detect all epitopes, and different typing results could be obtained because of reagent variation.
- Four basic categories include weak D, partial D, Dae and nonfunctional RHD. Serologic testing cannot differentiate weak D from partial D. Molecular testing is required.
Weak D
- Weak D phenotypes are defined as having a reduced amount of the D antigen, and are detected by IAT. The patient could type as D+ or D-, depending on the facility and reagent/method used.
- Weak D is caused by amino acid changes that impair the insertion of the protein in the red cell membrane. Many types of weak D have been categorized with Types 1, 2, and 3 accounting for most of the weak D types of individuals of European ethnicity.
- Another cause of weak D is when Ce is trans to RhD (example: R0r'). Blood recipients with C in trans to RH and those with the genetic weak D have the complete D antigen and do not produce alloanti-D, Rh-positive blood may be used for transfusion.

Partial D
- Partial D, historically called category D or D mosaic, is an altered form of D in which red cells typed D positive, but individuals make anti-D when exposed to conventional D antigens.
- Partial D are primarily due to inheritance of hybrid genes, resulting in the replacement of portions of RHD by RHCE, and loss of some D epitopes.
- One example is DVI red cells, which carry the BARC antigen.

\( \text{Del} \)
- \( \text{Del} \) is caused by different RHD mutations. These red cells express extremely few D antigens.
- \( \text{Del} \) is very rare but is found in 10% to 30% of apparent D-negative Asians.
- \( \text{Del} \) red cells will phenotype as D- but can adsorb and elute anti-D.

Nonfunctional RHD
- These are RHD genes that cannot produce a full-length polypeptide.

Rh Testing for Blood Donors vs. Patients

Typing blood donors for D
- For blood donors, use a testing method that is designed to detect weak expression of D.
- Donor units are labeled as Rh positive if the D typing is positive in any phase of testing.

Typing patients for D
- Weak D testing (using IAT) is not required for patients.

Typing obstetrical patients for D
- Weak D testing is not required.
- For patients with partial D, it may be more prudent to interpret them as Rh-negative on direct agglutination testing. DVI is the most common partial D in individuals of European ethnicity. FDA-licensed anti-D reagents are IgM monoclonal selected to be reactive with DIV and DV red cells, but nonreactive with DVI red cells in direct testing. Therefore, women with DVI red cells would type Rh-negative. This provides a safety margin for these women to receive RhIG prophylaxis or to receive Rh-negative RBCs for transfusion.
- At delivery, when the mother is Rh-negative and the newborn is typed Rh-negative, the weak-D status of the mother must be determined to avoid problems in testing for excessive fetal-maternal hemorrhage.
- Weak D testing is required on newborn to access the need for RhIG prophylaxis for the mother.

Obsolete Terms
- Variant refers to an antigen form that reacts differently from the common or normal phenotype.
- \( \text{D}^v \) is a quantitative D variant. This terminology is no longer used.
- D Mosaic lacks components of, altered or a new form of the D antigen. Current terminology more appropriately describes these red cells as partial D.
Molecular Testing
Advances in molecular testing have helped elucidate the complexities of the Rh system, and serve as an adjunct in hemagglutination testing to provide safe patient transfusion management. Advantages of molecular methods include:

- Resolve discrepant D typing due to weak D, partial D, or D- and would help ensure that donor units labeled as Rh negative lacked expression of D antigen.
- Identify blood recipients (including pregnant females) with a serologic weak expression of D who can be safely transfused with D+ red cells.
- Prevent unnecessary RhIG for pregnant women with weak D type 1, 2, 3, and 4.
- Preserve Rh-negative blood for true Rh-negative patients.
- Determine RHD zyosity and facilitate noninvasive fetal typing.
- Provide Rh antigen-matching for sickle cell disease patients.

Transfusion Policy for Patients with weak D expression

- Because blood recipients with C in trans to RHD and those with the genetic weak D clearly have the complete D antigen and do not produce alloanti-D, Rh-positive blood may be transfused.
- Some facilities believe that the number of individuals homozygous for the partial D is so small, the risk of sensitizing a partial D individual so low, and the supply of Rh-negative blood so precious than an intended blood recipient who types weak D, be it truly a weak D or partial D, that an intended blood recipient should be given Rh-positive blood. Since regulatory agencies do not require routine testing for weak D in blood recipients, these patients will receive Rh-positive blood unless anti-D is present in the serum.
- Some facilities are moving into using molecular genotyping to define the types of weakened RhD antigen expression.

Rh Testing Discrepancies

- The large number of RHD alleles resulting in variant D phenotypes can complicates the assignment of the D type.
- Results of serological testing are further complicated due to the variation of reagent anti-D (not all anti-D detect the same partial or weak expression of D antigens) and testing methodologies (e.g., tube, gel, solid phase, and automation).
- For these reasons, interpretation of D antigen typing may be different between laboratories or from the historical records.

Policies Vary

It is up to the facility to establish policies on the interpretation of Rh testing results. Some considerations include the patient population, risk of immunization to D, and the limited supply of D-negative blood supply. For Rh discrepancies, approaches include:

1. Test a new sample to rule out errors in the sample.
2. Test using different anti-D reagents and different methodologies. Follow manufacturer’s directions because D typing reagents have different characteristics.
3. Perform molecular testing.
4. In general, an individual with partial D should be considered D+ as a blood donor and D- as a transfusion recipient.

Scenario
- Donor centers test for weak D. A donor who is classified as Rh positive may be classified as Rh negative as a patient.
- This information should be communicated to the patient, healthcare staff and

Example:
Patient serological testing <2+
- Genotyping performed: RHD gene present. The patient would likely qualify as D positive. Give Rh-positive RBCs.
- Genotyping not available: consider the patient as D negative. Give Rh-negative RBCs.