THE Rh BLOOD GROUP SYSTEM

CONNIE WESTHOFF

OBJECTIVES

After completion of this chapter, the reader will be able to:

1. Describe the Rh system antigens, including:
   a. The alleles inherited at each locus.
   b. The causes of weak D and partial D expression.
   c. Variations in C and e antigen expression.
   d. Compound antigens.
   e. How to determine an individual’s most probable genotype.

2. Discuss causes of the Rhnull phenotype.

3. Describe Rh system antibody reactivity and characteristics.

4. Discuss the administration of Rh immune globulin.

5. Describe the reagents used in Rh typing and the appropriate controls.

6. List causes of discrepancies in Rh typing.

KEY WORDS

- Compound antigen
- Deleted or partially deleted phenotype
- Fisher and Race
- G antigen
- ISBT
- Partial D
- Rh haplotype
- Rh immune globulin
- Rh negative
- Rh positive
- Rhnull
- Weak D
- Wiener terminology

The Rh blood group system is one of the most polymorphic and antigenic blood group systems. It is second only to ABO in importance in blood transfusion and is well known as a primary cause of hemolytic disease of the fetus and newborn (HDFN). The principal antigen is D, and the terms Rh positive and Rh negative refer to the presence or absence of D antigen. Caucasians of European extraction have the highest incidence of the Rh-negative phenotype (15% to 17%), and Rh-negative type is much less common in Africa (5%) and Australia and is considered a rare blood type not routinely tested for in some parts of Asia (<1%). Other common Rh antigens include the antithetical C and c, and E and e antigens. Patients are not routinely typed for these unless they have developed atypical antibodies or are facing long-term transfusion support for diseases such as myelodysplasia or sickle cell disease (SCD). In addition to the five principal antigens (D, C, c, E, and e), more than 50 other Rh system antigens are known. Because they are not often encountered in the routine blood bank, they will not be covered here in detail. References at the end of the chapter provide further information for interested readers.

GENES

Two genes RHD and RHCE encode the Rh proteins: one encodes the D antigen and the other encodes CE antigens in various combinations (Ce, cE, Ce, or CE).
The RhD and RhCE proteins encoded by the two genes differ by 32 to 35 amino acids. This contrasts with most blood group system antigens that are encoded by single genes, with alleles that differ by only one or a few amino acids. Most D-negative (Rh-negative) phenotypes are the result of complete deletion of the RHD gene. The large number of amino acid differences between RhD and RhCE proteins explains why RhD is so antigenic when encountered by the immune system of someone who is Rh negative and has only RhCE.

The RHCE gene encodes C and c antigens, which differ by four amino acids: Cys16Trp (cysteine at residue 16 replaced by tryptophan) encoded by exon 1, and Ile60Leu, Ser68Asn, and Ser103Pro encoded in exon 2. E and e differ by one amino acid, Pro226Ala encoded in exon 5 (Fig. 10-1).

**NOMENCLATURE**

The Rh system was discovered in the 1940s, and several terminologies developed over the years. These reflected differences in thinking regarding the inheritance of the antigens. *Fisher and Race* believed that the Rh system consisted of three closely linked genes or alleles: D at one locus, C or c at the second, and E or e at the third, as reflected in the DCE terminology (Table 10-1). This terminology is used most often in written discussions of the Rh system antigens. The *Wiener terminology* was based on the belief that the Rh antigens were the products of a single gene coding for an “agglutinogen” composed of multiple “blood factors.” The names given to each of the five major Rh antigens were Rh0, rh’, rh”, hr’, and hr”, but the original Wiener terminology is obsolete. A modified...
CHAPTER 10 The Rh Blood Group System

TABLE 10-1 Rh Nomenclature and Incidence of Common Haplotypes

<table>
<thead>
<tr>
<th>Fisher-Race Haplotype</th>
<th>Modified Wiener Haplotype</th>
<th>Incidence (%)</th>
<th>Caucasian</th>
<th>African Black</th>
<th>Asian</th>
</tr>
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<tbody>
<tr>
<td>Rh positive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dce</td>
<td>R₁</td>
<td></td>
<td>42</td>
<td>17</td>
<td>70</td>
</tr>
<tr>
<td>DeE</td>
<td>R₂</td>
<td></td>
<td>14</td>
<td>11</td>
<td>21</td>
</tr>
<tr>
<td>Dce</td>
<td>R₀</td>
<td></td>
<td>4</td>
<td>44</td>
<td>3</td>
</tr>
<tr>
<td>DCE</td>
<td>Rₚ</td>
<td></td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>1</td>
</tr>
<tr>
<td>Rh negative</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ce</td>
<td>r</td>
<td></td>
<td>37</td>
<td>26</td>
<td>3</td>
</tr>
<tr>
<td>Ce</td>
<td>r’</td>
<td></td>
<td>2</td>
<td>2</td>
<td>2</td>
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<tr>
<td>CE</td>
<td>r”</td>
<td></td>
<td>1</td>
<td>&lt;0.01</td>
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<tr>
<td>CE</td>
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<td>&lt;0.01</td>
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</tr>
</tbody>
</table>

The RH genes are designated by capital letters, with or without italics, and include erythroid RHD, RHCE, and RHAG. The alleles are designated by the gene followed by an asterisk (*); for example, alleles of the RHCE gene are designated RHCE*ce, RHCE*Ce, RHCE*cE, and so on, according to which antigens they encode. The proteins are indicated as RhD, RhCE, or according to the specific antigens they carry, Rhc, RhCE, Rhec, or RhCE.

Most Probable Genotype

Typing RBCs for the five major Rh antigens yields the RBC phenotype. The probable genotype coding for the phenotype can be deduced from gene frequency estimates. This is useful when a person is multiply transfused or when typing a father to determine the probability that a fetus may suffer from hemolytic disease when the mother has an Rh antibody. The prevalence of Rh haplotypes by ethnic group is shown in Table 10-1. Knowledge of ethnicity is important when determining the most probable genotype. As an example, if the RBCs phenotype D–C–c–E–e+ and the patient is European American, the most probable genotype would be Dce/ce (R0r); however, if the patient is African American, Dce/Dce (R0R0) would be more likely because the occurrence of the R0 haplotype is higher (44%) than that of the r haplotype (26%) in African Americans. More accurate determination of the RH genotype, specifically RHD zygosity, is of significance when the mother has anti-D. Serologic
testing of the father’s RBCs cannot determine whether they are from a homozygote (D/D) or heterozygote (D/d), as anti-D seldom shows any difference in reactivity between RBCs with a single or double dose of D antigen. RHD zygosity can be determined by DNA molecular testing by assaying for the presence of an RHD gene deletion or an inactive RHD, and testing is available in some specialized blood bank molecular reference laboratories.

**D ANTIGEN**

D antigen is very antigenic and before the routine use of Rh immune globulin, anti-D was the most frequently encountered unexpected and clinically significant antibody seen in pretransfusion testing. As indicated above, it represents the presence (D+) or absence (D−) of an entire RBC protein rather than a single amino acid difference (i.e., K/k, Jka/b, etc.) and is composed of many antigenic epitopes. Approximately 2% of individuals of European extraction, and even more with African ethnicity, have changes in the RHD gene. These encode changes in the protein, which often cause variations in the expression of D antigen and include weak D, D, and partial D phenotypes.

**Weak D**

Some RBCs exhibit a weaker than normal form of the D antigen, giving weaker than expected reactivity with anti-D on initial spin testing or requiring the antiglobulin phase for detection. (Formerly known as Du, that terminology is no longer recognized and should not be used.) Weak D antigen expression is primarily found in persons with a single RHD that has a mutation encoding an amino acid change. Many different RHD changes encode weak D. To date, these are designated types 1 through 57, but the number of different mutations found continues to grow. Importantly, types 1, 2, and 3 are more common and make up 90% of the weak D cases found in Europeans. The number of D antigen sites in RBCs with weak D varies, depending on the severity of the effect of the mutation on the level of protein expression. RBCs with very reduced D antigen numbers, such as weak D type 2, can be missed with some commercial anti-D reagents or with some test methods. This can result in D-typing discrepancies, that is, the patient could be D+ or D− at different institutions or at the same institution after changing reagent manufacturer or typing method. Investigation of D-typing discrepancies is discussed below. Some RBCs with extremely low levels of D antigen are not detected by any routine typing method or reagents. These are revealed only with adsorption–elution studies with anti-D and are designated D₁₁₀₀₂ for elution or by molecular RHD genotyping. D₁₁₀₀₂ are more often found in Asian ethnic groups. Lastly, position effects can also influence D antigen expression. This occurs when a Ce allele is found in trans to RHD and the amount of D antigen in the membrane is reduced. Samples that exhibit this phenomenon have an R₁r (Dce/Ce), R₁r (Dce/Ce), or R₁r (Dce/Ce) haplotype.

**Testing for Weak D**

Testing the RBCs for weak D is not required for patients, unless typing the RBCs of an infant to determine if an Rh-negative mother is a candidate for Rh immune globulin. Weak D testing is also sometimes performed on the Rh-negative mother before the delivery of the infant if the facility uses microscopic reading of the weak D test, or a rosetting test with anti-D, to detect large D+ fetal–maternal bleed after delivery. Testing for weak D in an apparent D-negative patient needing large-volume or long-term transfusion could conserve the use of D− blood supplies.

Testing for weak D is required by the AABB Standards for Blood Banks and Transfusion Services for donor units, and the unit must be labeled “Rh positive” if the test is positive. For AABB-accredited hospitals, the D type of units labeled “Rh negative” must be confirmed from an integrally attached segment before transfusion, but testing for weak D is not required. Discrepancies must be reported to the collecting facility and resolved before issue of blood for transfusion. As mentioned above, serologic methods do not detect some rare RBCs with very low to undetectable levels of D antigen. Blood with very weak D antigen is not as antigenic as blood with normal levels of D antigen, but donor units transfused to a patient who presents with anti-D after receiving Rh-negative blood (anti-G must be ruled out) should be reported to the donor facility so as to investigate the RHD status by adsorption–elution or RH genotyping.

**Partial D**

RBCs with partial D are primarily due to inheritance of hybrid genes in which portions of RHD are replaced by the corresponding portions of RHCE (Fig. 10-1). This results in loss of some D epitopes. The RBCs type as D positive, but individuals make anti-D following transfusion or pregnancy. Note, the new hybrid protein resulting from regions of RhD joined to RhCE can generate antibodies; for example, DVI RBCs carry the Rh antigen called BARC (Rh52).

**Clinical Significance of Weak D and Partial D**

The majority of patients with weak D RBCs are unlikely to make anti-D and can receive D-positive blood. Rare weak D types (11, 15, and 21) have made anti-D, suggesting that they have altered D epitopes.
Patients with partial D RBCs are at risk for production of anti-D, and females should receive D-negative blood and be considered candidates for Rh immune globulin. Unfortunately, serologic reagents cannot distinguish some variant D antigens or differentiate weak D from partial D. RH genotyping is required.

**C AND c ANTIGENS**

C and c are encoded by alleles or alternative forms of the RHCE gene (Fig. 10-1). The C and c antigens are codominant, and if both are present, one on each chromosome, both are expressed on the RBC. The C antigen has an approximate frequency of 68% in the white population, and 80% express the c antigen. The frequency of C antigen is higher in Eastern Asia, but much lower in African Blacks. Both antigens are less immunogenic than D antigen.

**Altered C and c Antigens**

Other alleles inherited at the RHCE locus encode antigens that are of low frequency. C\(\omega\) (Rh8) and C\(\alpha\) (Rh9) antigens are due to single amino acid changes most often encoded by RHCE\(^\omega\)Ce and associated with an R, haplotype. C\(\alpha\) is more often seen than C\(\omega\) and has an incidence of approximately 1% in the American population. The incidence of C\(\omega\) explains why anti-C\(\omega\) is not a rare antibody found in transfused patients in a routine blood bank. C\(\omega\) and C\(\alpha\) are variations of the RhCe protein, and although the RBCs type as C+, some patients make antibodies with anti-C (or −Ce) specificities following transfusion. Another important example of expression of variant C antigen occurs primarily in African Blacks and hence in patients with SCD. This altered C is encoded by a hybrid RHD-CE-D gene and is associated with a haplotype designated (Cce\(^3\) or c\(^{bc}\)). This haplotype encodes an altered C antigen, designated as (C) because the RBCs react more weakly than normal with most polyclonal anti-C (but are strongly reactive with commercial monoclonal anti-C reagents), and also encodes altered e antigen, designated e\(^c\). Hence, the RBCs type as C+ and e+, but patients make anti-C, anti-e, and/or anti-Ce. These multiple and complex Rh antibody specificities are often called anti-hr\(^{8/9}\). The RBCs of patients homozygous for (C)ce\(^3\), or even heterozygous for (C)ce\(^w\) with a second altered Rh haplotype on the other chromosome, type as hr\(^{8/9}\).

**E AND e ANTIGENS**

E and e are encoded by alleles of the RHCE gene (Fig. 10-1). The E and e antigens are codominant, and in all populations e is more frequent than E.

Approximately 30% of the white population express E and 98% have the e antigen. E is a more effective immunogen than e.

**Altered E and e Antigens**

Altered or variant forms of the E antigen, designated E\(i\) through EIV, are rare, and their discussion is beyond the scope of this chapter. Expression of the e antigen is easily altered by other changes in the Rh proteins. For example, as many as 30% of blacks express the Rh antigens V and VS, which result from a Leu245Val amino acid change in the protein that is located close to the e antigen, Ala226. This 245Val change causes a conformation change in the protein and weakens and alters the expression of e antigen on RBCs that are V/VS positive. Although the RBCs type as e+, the patients can make allo anti-e directed to conventional e because their e antigen is altered. Expression of e antigen can be influenced by many other genetic changes in RHCE genes common in individuals of African ancestry. Again, although the RBCs type as e+, patients make alloantibodies with e-like specificities following transfusion. The antibodies, designated anti-hr\(e^{3}\), -hr\(e^{2}\), -RH18, and -RH34, have complex specificities and are difficult to identify serologically. The antibodies can be clinically significant, and rare blood is sometimes needed from the American Rare Donor Program (ARDP). Importantly, because of the multiple and different RH genetic backgrounds, the antibodies produced are not all serologically compatible with donors designated as hr\(e^{3}\) and hr\(e^{2}\) by serologic testing. Molecular genotyping is now being used to find compatible blood for transfusion, and molecular matching is an important tool for transfusion support in these patients.

**Deleted or Partially Deleted Phenotypes**

In rare cases, people may inherit inactivated, or partially inactive, RHCE genes that do not encode E or e and may or may not encode some level of expression of C or c. These are called deleted or partially deleted phenotypes and are found primarily in Caucasians whose parents may be consanguineous or distant cousins. The D antigen is present, usually in increased amounts, and the deletion types that have been described include Dc−, DC\(^{w/\alpha}\), and D−. People homozygous for these haplotypes lack high-prevalence Rh system antigens. When they are exposed to conventional RBCs during transfusion or pregnancy, they form antibodies usually characterized as anti-Rh17 (Hr\(\alpha\)). The presence of these antibodies causes the serum of the individual to agglutinate all RBCs except those of
D— homozygotes or Rhnull, making transfusion very difficult. People who have formed antibodies to conventional RhCE proteins are counseled to donate autologous blood for themselves and to have it frozen to provide for transfusion requirements. These antibodies have been responsible for severe, and sometimes fatal, HDFN.

**THE G ANTIGEN**

The G antigen is a product of exon 2 of RHD or exon 2 of RHCE*Ce, as these are identical and encode the same amino acids (Fig. 10-1). Therefore, RBCs that are either D positive or C positive are also G positive. In antibody identification studies, anti-G appears to be anti-D plus anti-C on routine antibody identification because of the presence of G antigen on both D+ and C+ RBCs. Anti-G versus anti-D+C can be discriminated by adsorption and elution studies, but this is not usually necessary in the pretransfusion setting, as patients with anti-G must receive D+ and C+ blood. However, for obstetric patients, further testing is important because Rh immune globulin prophylaxis is indicated if the mother does not have anti-D.

**COMPOUND ANTIGENS**

Compound antigens in the Rh system are direct to the shared epitopes of C or c and E or e antigens on the same protein. These are ce (f), Ce (rh), cE (Rh27), and CE (Rh22) (see Table 10-2). The f antigen is encoded by the Rhce protein, rhi by RhCe, and so on. Cells of the genotype Ce/DcE would express the compound antigens Ce (rh) and cE (Rh27). These cells would not express f even though they carry the c and e antigens because c and e are not encoded on the same protein. In the routine laboratory, antibodies to compound antigens are encountered less frequently than single-specificity Rh antibodies and may be components of sera with multiple antibody specificities.

<table>
<thead>
<tr>
<th>Compound Antigens</th>
<th>Haplotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ce (rh)</td>
<td>Dce (R_) and Ce (r')</td>
</tr>
<tr>
<td>cE (Rh27)</td>
<td>DcE (R_) and cE (r')</td>
</tr>
<tr>
<td>ce (f)</td>
<td>Dce (R_) and ce (r')</td>
</tr>
<tr>
<td>CE (Rh22)</td>
<td>DCE (Rz) and CE (r')</td>
</tr>
</tbody>
</table>
anti-D following exposure to D+ RBCs and clearly the RBC dose and immune status of the patient are important variables. Nevertheless, it is generally accepted that D+ RBCs should only be given to D− patients in an emergency, when there is a D− blood shortage, or in massive transfusion. Rh-negative girls and women of childbearing age should always receive D− blood and blood products, but if that is not possible, the use of Rh immune globulin to prevent anti-D must be considered.

When Rh system antibodies are encountered in the routine blood bank laboratory, it is important to be aware of the Rh antibodies that often occur together. For example, sera containing anti-D often contain anti-G as well. R1R1 people who make anti-c have probably been exposed to E antigen as well and may also have low-level anti-E.

**Rh IMMUNE GLOBULIN**

The cause of HDFN was discovered when it was realized that the mother was reacting to a paternal antigen inherited on the RBCs of the fetus. These women often had healthy first babies, but subsequent pregnancies often resulted in severe anemia in the fetus or stillbirth and spontaneous abortion. Because fetal RBCs enter the maternal circulation in small amounts during pregnancy and in larger amounts during childbirth, the first pregnancy with an Rh-positive fetus may sensitize the mother to anti-D. Anti-D is predominantly IgG1 and IgG3 and will cross the placenta, resulting in the destruction of the fetal RBCs. The observation that ABO incompatibility between a mother and the fetus had a partial protective effect against production of maternal antibodies led to the development of Rh immune globulin. The protective effect suggested that maternal, naturally occurring anti-A or anti-B bound to the incompatible fetal A-positive or B-positive RBCs, preventing production of anti-D. By the 1960s, a mere 20 years after the discovery of RhD incompatibility, HDFN due to anti-D could be effectively prevented if the mother received an injection of passive antibody. The incidence of HDFN due to anti-D has decreased dramatically in the Western world owing to treatment with Rh immune globulin. Rh immune globulin is a solution containing human IgG anti-D. In the United States, it is administered to Rh-negative women during the 28th week of pregnancy (antenatal dose) and again after the delivery of an Rh-positive infant (postpartum dose) or at the time of an induced or spontaneous abortion. When testing the mother after delivery for the presence of anti-D, the antenatal Rh immune globulin may still be weakly demonstrable when the serum is tested against D-positive RBCs. It is important to recognize this possibility and to not exclude the woman from receiving postpartum Rh immune globulin. Rh immune globulin must be administered in each pregnancy if the fetus is Rh positive to prevent maternal sensitization and formation of anti-D. Unfortunately, many women in poor or developing countries do not have access to Rh immune globulin prevention, and HDFN due to anti-D is still seen in some parts of the world.

Other Rh antibodies may also cause severe HDFN. Rh immune globulin prevents only anti-D, not the formation of other Rh antibodies such as anti-C, anti-c, anti-E, and anti-e. Women should be screened early in each pregnancy for Rh antibodies as well as other IgG blood group antibodies like anti-Kell, which may cause HDFN.

**Rh MOLECULAR TESTING**

Serologic reagents detect the principal antigens, D, C, e, E, and e, but there are many other Rh antigens, including altered or variant antigens, which are not uncommon in African Black and Hispanic groups. No commercial serologic reagents are available to detect these. DNA-based genotyping has the potential to modernize selection of compatible blood for patients with complex Rh antibodies.

**Rh SEROLOGIC REAGENTS**

Reagents used to detect the D antigen in the slide, tube, microplate, automated, and gel tests often have different formulations and performance characteristics. Each manufacturer’s anti-D reagents may contain different antibody clones, potentiators, additives, or diluents, and reagents that contain the same antibody clone can also vary, for example, in antibody dilution or the preservative present. Hence, instructions for testing may differ and must be consulted and carefully followed for accurate testing.

High-protein polyclonal reagents prepared from pools of human sera and containing high concentrations of protein (20% to 24%) were originally used for Rh typing. These reagents are potent and reliable, but were associated with false-positive reactions when the test RBCs are coated with immunoglobulin. A control consisting of the diluent used by the
manufacturer to prepare the reagent must be tested in parallel for valid results.

Many polyclonal anti-D have been replaced with monoclonal reagents, and in the United States, most Food and Drug Administration (FDA)-licensed anti-D contain a mixture of monoclonal IgM and monoclonal or polyclonal IgG. The IgM anti-D component causes direct agglutination of positive RBCs at immediate spin, and the IgG anti-D is reactive in the antiglobulin phase of testing, making the reagent suitable for weak D testing. Spontaneous agglutination causing a false-positive result is much less frequent than seen with high-protein reagents, but controls performed as described by the reagent manufacturer are still required. Proper controls for false positives are usually a negative test result, performed concurrently, for example, the absence of agglutination of the RBCs with anti-A or -B. If a separate control test must be done (AB sample), 6% albumin is an appropriate control.

Chemically modified IgG antisera have been treated with a sulfhydryl compound that weakens the disulfide bonds at the hinge region of the IgG molecule. This allows greater distance between the two antigen-binding sites in the hypervariable regions of the antigen-binding (Fab) portions of the molecule, converting the IgG anti-D to a direct agglutinin.

Reagents are also commercially available for determining the presence of C, c, E, and e antigens on RBCs. Most of these are monoclonal blends intended for direct testing only and are not appropriate for anti-human globulin (AHG) phase of testing. Follow manufacturer’s instructions. Appropriate negative controls for false-positive typing, as discussed above for anti-D, also apply.

Slide Testing
A glass slide containing a drop of 40% to 50% serum or plasma suspension of RBCs and a drop of anti-D is mixed and placed on a heated Rh view box that is tilted continuously for 2 minutes to observe for agglutination. To achieve rapid warming of the materials on the slide to 37°C, the Rh viewing box is kept at a temperature between 40°C and 50°C. Slide testing is rarely performed owing to the imprecise nature of the test method and risk of biohazard exposure.

Tube Testing
Tube testing is performed in either 10 × 75-mm or 12 × 75-mm test tubes. The washed RBCs to be tested are suspended in saline at approximately 2% to 5% cell suspension. One drop of the cell suspension is added to the test tube containing one drop of antiserum and centrifuged. The mixture is resuspended and observed for agglutination. Tube testing is relatively easy to read and, for some reagents, can be continued to the indirect antiglobulin phase of testing, depending on the specific manufacturers’ instructions.

Automated and Microplate Testing
Automated testing (e.g., Olympus PK) or microplate testing has been adopted by larger blood centers for donor typing. The antiserum used must state that it is formulated for automation or microplate Rh testing and may require that the blood be collected in a specific anticoagulant. Always consult and follow manufacturers’ instructions.

Gel Testing
Rh typing may also be done using a gel technique. The antiserum is distributed throughout the gel particles. Antigen-positive RBCs react with the antisera, and the agglutinins are trapped and cannot pass through the gel when centrifuged. This method has the advantage that the gel card can be saved for later review of the results.

Technical Considerations for Rh Typing

False-positive Results
False-positive results can result from the following:

- Cold autoagglutinins. Washing the cells, sometimes multiple times, with warm saline and retyping should correct the problem.
- Warm autoagglutinins. Washing the cells multiple times and retesting or using IgM reagents may be required. Treatment of the cells to remove IgG can also be done.
- Positive direct antiglobulin test (DAT). Indirect antiglobulin testing for weak D will not be valid on RBCs coated with IgG with a positive DAT. Treatment of the cells to remove IgG can also be done.
- Using a posttransfusion sample, including D typing in patients who are D− and have received large amounts of D+ blood during massive or emergency transfusion. Always obtain an accurate transfusion history for valid typing.
- Use of the wrong antiserum. Careful reading of labels on each reagent vial is required. Antiserum labels must not be obscured, and those with unreadable labels should be discarded.
**False-negative Reactions**

False-negative reactions may result from the following:

- Failure to add antiserum. Adding the antiserum before the addition of the RBCs aids the worker in seeing whether reagent is present.
- Blocking of antigen sites. In severe HDFN, the RBCs may be so heavily coated with antibody that no antigen sites remain for antisera to bind. The correct Rh type may be obtained if the cells are first heat eluted to remove some of the blocking antibody.
- Incorrect cell suspension. Too heavy or too light a cell suspension can cause false-negative results. If the button after centrifugation is larger or smaller than usual, the test should be repeated.
- Incorrect antiserum-to-cell ratio. Deviation from the manufacturer’s instructions can result in using inappropriate amounts of particular antisera. Two drops are often not better than one, and the manufacturer’s instructions must always be followed.
- Overly vigorous resuspension after centrifugation. Shaking too hard results in dispersal of weak agglutination. Careful resuspension is essential.
- Failure of the antiserum to react with a weak or variant antigen. A specific antibody may not react with weak forms of an antigen or with variant antigens. Using an antiserum from another manufacturer or a known donor or patient antibody may aid investigation. Absorption and elution of the antibody may also be necessary to demonstrate the presence of the antigen.
- Reagent deterioration. Prolonged or incorrect storage of reagents may result in the destruction of the antibody in the reagent. Antiserum should not be used after the expiration date.

**Rh TYPING DISCREPANCIES**

A patient or donor RBC sample that was previously found to be positive but is now determined to be negative, or vice versa, should always be investigated to rule out identification, clerical, or recording errors. A new sample should be obtained and tested. If the discrepancy is between current testing and historical records, it may be due to the method used, phase of testing (direct or IAT), type of reagent (polyclonal vs. monoclonal), or manufacturer. Different reagents often contain different antibody clones that may show variable reactions with RBCs with weak or partial Rh antigens. Knowledge of the ethnicity of the donor or patient can be helpful when investigating a typing discrepancy because some partial and weak phenotypes are more common in a specific ethnic group. Typing with several reagents from different manufacturers may be helpful.

Reagents licensed by the FDA for D typing have been selected to be nonreactive with partial DVI RBCs in the direct test. This is because girls and women of childbearing age with DVI RBCs are at risk for production of anti-D associated with fatal HDFN and are better treated as D negative for transfusion and as candidates for Rh immune globulin. DVI RBCs will test positive for weak D, which is required for donor testing. Therefore, patients with partial DVI are considered D negative for transfusion but appropriately classified as D-positive donors.

**SUMMARY**

The Rh system is one of the most important systems in transfusion medicine. It is second only to ABO in transfusion importance. Antibodies or combinations of antibodies often result in individuals who are exposed to antigens that they do not possess. Advances in testing have allowed us to learn much about the complexities of the system, and our ability to test and understand variants has increased. Although once a major cause of HDFN, anti-D does not pose as high a risk today, thanks to the use of Rh immune globulin.
Review Questions

1. The term “Rh positive” refers to the presence of which of the following:
   a. D
   b. E
   c. C
   d. none of the above

2. The use of R to denote the presence of “D” in Rh nomenclature is an example of which terminology?
   a. Fisher–Race
   b. Wiener
   c. ISBT
   d. none of the above

3. True or false? Weak D can result from few D-antigen sites on the red cell or from the presence of Ce allele in trans position to D.

4. True or false? For AABB-accredited hospitals, the D type of units labeled Rh negative must be confirmed from an integrally attached segment before transfusion, but testing for weak D is not required.

5. True or false? The E and e alleles are codominant.

6. Anti-G will agglutinate which of the following:
   a. D-positive red cells
   b. C-positive red cells
   c. E-positive red cells
   d. both a and b
   e. both a and c

7. Which of the following is true regarding Rhnull individuals?
   a. their cells lack Rh29
   b. their cells lack DCE but have c and e
   c. they can form a weak but harmless antibody
   d. all of the above

8. True or false? The Rh antibodies do not bind complement except in rare instances.

9. Which of the following is the most antigenic?
   a. D
   b. C
   c. c
   d. E
   e. e

10. HDFN has been dramatically reduced by giving Rh-negative women doses of __________.

   Please indicate if the following can result in a false positive (P), a false negative (N), or both depending upon the circumstances (B)

11. Contaminated reagent
12. Polyagglutinable red cells
13. Blocking of antigen sites
14. Use of wrong antiserum
15. Reagent deterioration

ADDITIONAL READINGS


PROCEDURAL APPENDIX

RAPID TUBE TYPING FOR D ANTIGEN

This procedure is intended to be a general guide for testing. Follow instructions of the manufacturer.

1. Label two 10 × 75-mm or 12 × 75-mm test tubes. Label one tube “D” and the other tube “cont.”
2. Wash the RBCs to be tested in normal saline.
3. Prepare a 2% to 5% suspension of the washed cells in fresh normal saline.
4. Add one drop of anti-D to the tube labeled “D.”
5. Add one drop of 6% albumin to the tube labeled “cont.”
6. Centrifuge for 30 seconds at 1,000 g (3,200 rpm in a serofuge or immunofuge).
7. Gently resuspend the cell button, watching for agglutination as resuspension occurs. Be sure to fully resuspend the entire cell button before deciding that no agglutination has taken place. The Rh control tube must show no agglutination. If there is agglutination in the Rh control tube, the test is invalid and another antiserum must be used.
8. Record the agglutination reaction as 4+, 3+, 2+, 1+, w+, or negative.

WEAK D TESTING

1. If the reaction is less than 2+, incubate the test and the control at 37°C for 15 to 60 minutes.
2. After incubation, wash the cells in each tube with normal saline.
3. Fill the tube with saline, mixing well. Be sure to completely resuspend the cell button in the saline.
4. Centrifuge for 1 minute at 1,000 g.
5. Decant the saline completely.
6. Repeat steps 3 through 5 for a total of three to four washes.
7. Add two drops of antiglobulin serum, and mix well.
8. Centrifuge for 15 seconds at 1,000 g.
9. Resuspend gently and observe for agglutination. The control tube must show no agglutination to validate the test.
10. Record the results.