Our understanding of the Rh blood group system has been greatly advanced since the genes were cloned in the late 1990s. We have witnessed the explosion of information about the Rh blood group antigens with the development of PCR and the rapid elucidation of the genetic basis for the antigens and phenotypes. This new genetic information explains some of the longstanding questions about the Rh system, especially the D antigen, leading to reemergence of discussions about appropriate D testing approaches.

Serologic Foundation of Rh
Recent genetic information has confirmed many of the predictions of the serologists whose primary, and often only, tools were the antibodies made by immunized individuals. Exploiting adsorption and elution approaches, along with selected RBC testing strategies, they uncovered many of the details concerning the specificity and complexity of the Rh blood group system. For example, the prediction that Rh antigens are encoded by two genes, not one or three, was adeptly forecast by Patricia Tippett based solely on serologic observations. Many investigators contributed to knowledge of the variability of the D antigen and variants of the e antigen were elucidated by Issitt and others. That work is the foundation of our understanding today, as the new genetic information builds upon the serologic footing. Serologic reactivity is the basis for blood groups important in blood transfusion practice because serology defines an antigen. That fact will not change as we use different testing methods in the future, including those that are DNA based. The suggestion that agglutination of RBCs is still the mainstay of blood bank technology because the field is reticent to change and stuck in the early 1900s is shortsighted; without a serologic relationship, a variation in a blood group gene at the DNA level is just another single nucleotide polymorphism (SNP) that occurs once in every 100 to 300 bp in the human genome. These polymorphisms are of only academic interest until associated with a phenotype and found to be relevant to transfusion medicine, by stimulation of an antibody, or to RBC function, as when they result in a null phenotype.

Rh Background
Two genes (RHD, RHCE) located on chromosome 1p34–p36 encode the Rh proteins designated RhD and RhCE; one carries the D antigen and the other carries CE antigens in various combinations (cc, Ce, cE, or CE). The genes are 97 percent identical and each has 10 exons, but they encode proteins that differ by 32 to 35 of 416 amino acids (shown as circles on the RhD protein in Figure 1). Both proteins are predicted to cross the membrane 12 times. On the RhCE protein, the E and e antigens differ by one amino acid, Pro226Ala, located on the fourth extracellular loop (Fig. 1). However, the requirement for e antigen expression is more complex than the 226Ala polymorphism and expression can be altered by changes in other regions of the protein. These changes are often encountered in people of African or mixed ancestry and are responsible for weak or altered e expression. C and c antigens differ by four amino acids but only the amino acid change at position Ser103Pro is predicted to be extracellular (Fig. 1). Additionally, three of the polymorphic amino acids in RhC are identical to RhD and explain the expression of G antigen on both proteins.

Why is the Rh System So Complex?
The majority of blood group systems are encoded by single gene loci. In contrast, the Rh system is encoded by two genes that have many identical regions
and the fact that they are in very close proximity on the same chromosome affords the opportunity for numerous exchange events between them. This results in new hybrid proteins that carry portions of RhD and portions of RhCE or vice versa. Most of these exchanges occur by a process called gene conversion. In this process, one member acts as donor template during replication of the other but, unlike in homologous recombination, the donor template remains unaltered. The donated region can span several base pairs, single exons, or even multiple exons. These exchanges between \textit{RHD} and \textit{RHCE} generate new polymorphic proteins and these hybrid proteins are responsible for the myriad of antigens observed in the Rh blood group system (reviewed in Westhoff\textsuperscript{17} and Reid and Lomas-Francis\textsuperscript{18}).

\textbf{Why is D So Immunogenic?}

Most blood group antigens differ from their antithetical partners by a single amino acid change (e.g., Jk\textsuperscript{+}/Jk\textsuperscript{b}, Fy\textsuperscript{a}/Fy\textsuperscript{b}, E/e, K/k, etc.). An important consideration in the immunogenicity of an antigen is the degree of foreignness to the host. RhD and RhCE differ by 32 to 35 amino acids, which explains why RhD, when seen by the immune system of a D– person, often induces a very robust immune response. Although only nine or ten of the changes are predicted to be extracellular, changes located in the transmembrane and cytoplasmic regions can also affect the topology of the protein in the membrane. Additionally, the large number of amino acid differences explains the numerous epitopes of the D antigen, estimated to range from 9 to more than 30.\textsuperscript{19,20} Exposure to a foreign protein carrying this large number of amino acid changes results in the production of a polyclonal immune response directed at many different parts of the protein.

\textbf{What Causes the Large Number of Variations in Expression of D?}

\textit{D negative}

The RBCs of D– individuals lack RhD protein because of deletion,\textsuperscript{21} or, rarely, a mutation, of the \textit{RHD} gene (Fig. 1). The D– phenotype is much more prevalent in Caucasians of European descent (15%–17%), less likely in individuals with African backgrounds (3%–5%), and rare in Asian populations (< 0.1%).\textsuperscript{6} The D– phenotype has arisen numerous times, as evidenced by the different genetic events responsible for the D– phenotype in different populations. In Caucasians, it is primarily the result of deletion of the entire \textit{RHD} gene.\textsuperscript{21} Some exceptions exist, including \textit{RHD} genes that are not expressed because of a premature stop codon, nucleotide insertions, point mutations, or \textit{RHD/CE} hybrids.\textsuperscript{18,22} Because most of the exceptions arise on DcE (R\textsubscript{1}) or DcE (R\textsubscript{2}) backgrounds and result in the less common Ce (r\textsuperscript{+}) or cE (r\textsuperscript{-}) haplotypes, the presence of these unusual haplotypes is a useful marker for these exceptions.

In contrast to the complete \textit{RHD} deletion found in Caucasians, D– phenotypes in African and Asian persons are often caused by inactive or silent \textit{RHD} genes. Only 3 to 7 percent of South African black persons are D–, of which 66 percent have \textit{RHD} genes that contain a 37-bp internal duplication resulting in a premature stop codon.\textsuperscript{23} The 37-bp insert \textit{RHD} pseudogene is also found in 24 percent of D– African Americans. Additionally, 15 percent of the D–
phenotypes in Africans result from a hybrid RHD-CE-D linked to ce, termed (C) ce, which is characterized by expression of VS, altered C and e (which may appear weakened), normal c, and no D antigen. The weak C phenotype is only observed with polyclonal reagents; monoclonal reagents are strongly reactive with (C) ce RBCs. The prevalence of this haplotype in sickle cell patients can complicate transfusion because this C antigen is altered and these patients may make anti-C when stimulated. In D– African Americans, 54 percent completely lack the RHD gene, while the remaining have either the 37-bp insert RHD pseudogene or the hybrid RHD-CE-D.

Asian D– phenotypes result from mutations in RHD most often associated with Ce, indicating that they originated on a Dce (R1) haplotype. Many Asians whose RBCs type as D– are actually D+.

D+ refers to RBCs with a very low level of D antigen that is detectable only by adsorption and elution, hence the name. Because these RBCs type as D– (including testing by the IAT), they are often only recognized if they stimulate production of anti-D in a D– recipient. This very low level D expression results from several different RHD mutations; currently there are five confirmed DEL alleles. Because these are different mutations, the RBCs can vary in the amount of D antigen expressed and in the effect on D epitope expression. Those resulting from mutations in the intron 3 splice site (designated IVS3+1g>a) have altered D epitopes, as evidenced by epitope mapping and by the production of anti-D after emergency transfusion of D+ units. This mutation appears to cause a partial D phenotype.

Although investigation of the other four D backgrounds did not show epitope alteration, this is not definitive because testing is limited by available reagents and not all D epitopes are known.

D+ RBCs are most often found in Asians, with the most frequent mutation being a 1227G>A change with skipping of exon 9. However, a DEL allele encoding a M295I amino acid change, which has the highest level of D antigen expression, has been found in 1 in 3700 Europeans. The observation that D+ phenotypes may be infrequent, but not rare, along with two recent reports of donor RBCs with a D+ phenotype that stimulated anti-D in D– recipients, have motivated discussions suggesting that these might be an emerging problem.

Some have suggested consideration of removing D+ RBCs from the D− donor pool by implementation of DNA-based testing methods. Importantly, since all D+ samples reported to date express the C antigen and appear to be r’, the D– C+ phenotype is a useful marker for samples that potentially could be D+.

**Weak D (formerly D+)**

Weak D RBCs are historically defined as having reduced D antigen levels that require the IAT for detection. An estimated 0.2 to 1 percent of Caucasians have RBCs with weak D expression, but the number of samples classified as weak D can depend on the characteristics of the typing reagent. Weak D RBCs were thought to simply have a reduction in the level of D antigen (i.e., a quantitative rather than a qualitative difference), based on the observation that individuals with weak D RBCs generally did not make anti-D when transfused with D+ RBCs. From the work of Wagner et al., it is clear that the majority of weak D phenotypes have at least one amino acid change in RhD.

![Diagram of amino acid changes in RhD proteins](image-url)

**Fig. 2.** Diagram of amino acid changes in RhD proteins. The location is shown as dark circles.

**A. Weak D**

<table>
<thead>
<tr>
<th>Amino Acid Change</th>
<th>Type 1</th>
<th>Type 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMH – L54P</td>
<td>#weakD type 15 – G282D</td>
<td></td>
</tr>
<tr>
<td>DVI – L210P</td>
<td>D+ – T283I</td>
<td></td>
</tr>
<tr>
<td>DFW – H166P</td>
<td>DIM – C283Y</td>
<td></td>
</tr>
<tr>
<td>DHR – R299K</td>
<td>DNU – G353R</td>
<td></td>
</tr>
<tr>
<td>DHO – K235T</td>
<td>D+ – A354R</td>
<td></td>
</tr>
<tr>
<td>DMH – M238T</td>
<td>D+ – M358T</td>
<td></td>
</tr>
<tr>
<td>*DNB – G355S – more common in Europe</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**B. Partial D**

![Diagram of amino acid changes in RhD proteins](image-url)

**Fig. 2.** Diagram of amino acid changes in RhD proteins. The location is shown as dark circles.

**A. Weak D phenotypes**

**B. Partial D phenotypes**

Amino acid changes that cause partial D phenotypes are predicted to be located predominantly in transmembrane and cytoplasmic regions.

**Weak D phenotypes**

Amino acid changes that cause partial D phenotypes are predicted to be located in the extracellular loops.
Because the changes are intracellular or are in the transmembrane regions of RhD and not on the outer surface of the RBC (Fig. 2A), these RBCs do not lack extracellular D epitopes. They affect primarily the efficiency of insertion and, therefore, the quantity of RhD in the membrane. This is reflected in the reduced number of antigen sites on these RBCs and explains why the IAT is required for detection. Weak D expression is caused by a large number of different mutations (classified as Types 1 to 42), with the most common being a Val270Gly substitution designated Type 1. The majority of individuals with weak D phenotypes can safely receive D+ RBCs and will not make anti-D. Nevertheless, because amino acid changes located intracellularly or in the transmembrane regions have the potential to alter surface epitopes, it is still unclear which of the 42 different weak D types may have altered D epitopes. Indeed, individuals with weak D type 4.2 and 15 have been reported to make anti-D. Therefore, they are better classified as partial D.

**Partial D (D categories or D mosaics)**

RBCs with partial D antigens have historically been classified as such because the RBCs type as strongly D+ but the individuals make anti-D when exposed to normal D antigen. It was hypothesized that the RBCs of these individuals lack some part of RhD so that they can produce antibodies to the missing portion. Molecular analysis has shown that this hypothesis was correct and that the altered or missing portions of RHD are actually replaced by corresponding portions of RHCE. Some replacements involve single amino acids but, in contrast to weak D discussed above, these changes are predicted to be located in the extracellular loop regions of RhD (Fig. 2B). Others involve entire exons or large regions of the gene and the novel sequence of amino acids generates new antigens (e.g., D*, BARC, Rh32) (reviewed in Westhoff and Reid and Lomas-Francis). Individuals with partial D antigens can make anti-D and, ideally, should receive D− donor RBCs. In practice, however, most are typed as D+ and are only recognized after the individual makes anti-D.

**D epitopes expressed on Rhce proteins (D∗AR, ceCF, ceRT)**

The discovery that some Rhce proteins carry D-specific amino acids that react with some monoclonal anti-D reagents adds an additional layer of complexity to D typing. Two examples, D∗AR (R^cHar), found in individuals of German ancestry, and Crawford (ceCF), found in individuals of African ancestry, deserve attention because of their strong reactivity (3+–4+) with some FDA-licensed monoclonal reagents and lack of reactivity with others (including the weak D test) (Table 1).

<table>
<thead>
<tr>
<th>Reagent</th>
<th>IgM monoclonal</th>
<th>IgG</th>
<th>DVI IS/AHG</th>
<th>DBT</th>
<th>DHAR (Caucasian)</th>
<th>Crawford (Blacks)</th>
<th>Choice of reagent for</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gamma-clone</td>
<td>GAM401</td>
<td>F8D8 monoclonal</td>
<td>Neg/Pos*</td>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
<td>DONORS</td>
</tr>
<tr>
<td>Immucor Series 4</td>
<td>MS201</td>
<td>MS26 monoclonal</td>
<td>Neg/Pos</td>
<td>Pos</td>
<td>Pos</td>
<td>Neg</td>
<td>-</td>
</tr>
<tr>
<td>Immucor Series 5</td>
<td>Th28</td>
<td>MS26 monoclonal</td>
<td>Neg/Pos</td>
<td>Pos</td>
<td>Vary/Pos</td>
<td>Neg</td>
<td>-</td>
</tr>
<tr>
<td>Ortho BioClone</td>
<td>MAD2</td>
<td>Polyclonal</td>
<td>Neg/Pos</td>
<td>Neg/Pos</td>
<td>Neg/Neg</td>
<td>Neg</td>
<td>RECIPIENTS (No IAT)</td>
</tr>
<tr>
<td>Ortho Gel (ID-MTS)</td>
<td>MS201</td>
<td>-</td>
<td>Neg</td>
<td>Pos</td>
<td>Pos</td>
<td>Neg</td>
<td>-</td>
</tr>
<tr>
<td>Polyclonal</td>
<td>-</td>
<td>-</td>
<td>Neg/Pos</td>
<td>Neg/Pos</td>
<td>Neg/Neg</td>
<td>Neg/Neg</td>
<td>-</td>
</tr>
</tbody>
</table>

*Result following slash denotes anti-D test result by the IAT, as permitted by the manufacturer.

Table 1. Reactivity of FDA-licensed anti-D reagents with some Rh variant RBCs that resulted in D typing discrepancies

Crawford (Rh43) is found in persons of African heritage and was first described in 1980. The antigen was present on the RBCs of 1 in 950 random African Americans in the southeastern part of the United States. Most Crawford+ samples were from persons with an R^cHar haplotype, but exceptions were seen. The Crawford allele, ceCF, encodes the same amino acid changes found in ceS (W16C, L245V) but also carries an additional Q233E change, which is a D-specific residue on the ceS background. The D-specific residue (233E) is responsible for strong direct agglutination (3+) with the GAM401 clone but it does not react with
the anti-D present in Ortho, Immucor, and polyclonal reagents (Table 1). These individuals make anti-D when stimulated (our unpublished observations) and should be treated as D– for transfusion and Rh immune globulin prophylaxis. Although Crawford+ RBCs have not yet been reported to stimulate production of anti-D, most would agree that individuals with this phenotype are better classified as D+ as donors.

Lastly, a Rhce protein with a R154T mutation, designated ceR T, demonstrates weak reactivity with some anti-D monoclonal reagents, and the reactivity is enhanced at lower temperatures. Interestingly, this variant does not carry any D-specific amino acid but mimics a D-epitope (epD6) structure.

D Typing Discrepancies

Multiple factors conspire to complicate D typing in the United States. These include the numerous methods used (i.e., slide, tube, solid phase, gel, and automated analyzers using enzyme-treated RBCs) as well as variations in the phases of testing; dissimilar monoclonal antibodies present in manufacturers’ FDA-licensed reagents that can differ in reactivity with variant D antigens; and the large number of different RHD genes present in populations, which affects both the level of expression and, potentially, the structure and epitopes of the D antigen. To date, there are more than 100 different RHD genes known, including 42 that encode different forms of weak D, 40 that result in expression of different partial D antigens, 5 or 6 DEL, and several RHCE genes that encode D epitopes on the Rhce protein. If one takes into account differences in methods, antibody clones, and the variability of D expression, discrepancies are bound to occur and the surprise should probably be that they are not encountered more often.

Methods

Multiple methods for D typing are used in various facilities in the United States. A 2001 to 2004 College of American Pathologists’ (CAP) survey of North American D Testing Practices\(^1\) showed an increase in the use of gel technology (1.1%–7.7%) but multiple methods will continue to be used. Regarding weak D, although testing is the standard practice for donor D determination, there are wide variations in practices in hospital transfusion services. Data from a 1999 CAP survey\(^2\) revealed that weak D testing was performed in 58 percent of responding facilities, although that number has likely dropped since then. A striking feature of the survey was the absence of a standard of practice regarding the D phenotype of units transfused to recipients testing positive for weak D. Forty-four percent indicated that they would give D– RBCs, while 42 percent would give D+ RBCs. About 10 percent would give D– donor RBCs if the woman was of childbearing age. These statistics may reflect uncertainty regarding the significance of weak D and also the conservation and limited availability of D– donor units.

Reagents

Early reagents developed for D antigen testing exploited antibodies produced in D-sensitized women or hyperimmunized volunteers. These polyclonal antibodies were potent and effective because they recognized numerous epitopes of D. Some were IgM antibodies causing direct agglutination but most were IgG. IgG antibodies are unable to cross-link D antigens on adjacent RBCs and cause direct agglutination, probably because of the number of sites and the lack of mobility of the protein. IgG reagents were often subjected to either chemical modification or addition of potentiating agents, with the goal of enhancing cross-linking to produce direct agglutination.

With the advent of monoclonal antibody technology in the 1980s came the promise of freedom from reliance on human source material for anti-D. The fusion of specific antibody-producing B cells with immortalized cell lines allows production of antibodies in cell culture and a potentially inexhaustible source. A large number of IgM, direct-agglutinating, anti-D monoclonals were generated, although it was soon realized that a single monoclonal anti-D specific for a single D epitope did not detect all D+ RBCs. Therefore, D typing reagents in the United States are a blend of monoclonal IgM reactive at room temperature and monoclonal or polyclonal IgG reactive by the IAT for the determination of weak D. The U.S. market offers four different reagents for tube testing and one for gel (Table 1). All but two contain different IgM clones, so
the reactivity of each with variant D antigens may differ. The FDA requires only that manufacturers specify reactivity with category DIV, DV, and DVI RBCs and only limited studies have been carried out with these U.S. reagents to characterize reactivity with other D variants.43 Additional data are needed concerning the reactivity of molecularly characterized variant D antigens with FDA-licensed reagents. The RBCs used for these studies must be well characterized at the molecular level, as the same category D RBCs may have different genetic backgrounds that can impact the data.

Table 1 compares the reactivity of different manufacturers’ reagents with some of the more frequently encountered RhD and RhCE variants that cause D typing discrepancies referred to our laboratory for investigation by molecular techniques. This serologic information, along with knowledge of the ethnic background of the donor or patient, can be very helpful in resolving the discrepancy. Additionally, although this comparison only includes four different variant D, it suggests that the Gamma reagent used in the donor setting would successfully exclude DVI, DBT, D\textsuperscript{18ar}, and Crawford phenotype RBCs from the D– donor pool by typing them as D+. This comparison also suggests that the Ortho tube reagent best serves these individuals in the hospital or prenatal setting by classifying the RBCs as D– for transfusion and Rh immune globulin purposes. (Note the importance of eliminating the antihuman globulin [AHG] testing phase on recipients to achieve the desired interpretation for DVI and DBT.)

The suggestion that there should be different anti-D reagents used for donor D determination and for typing patients is not new. This concept was avidly debated in the United States in the early days of implementation of monoclonal antibody reagents.44 Discussions primarily focused on category VI RBCs, the most common D variant in Caucasians. Because DVI RBCs can stimulate production of anti-D in a D– individual, the reagent for typing donors should be reactive with these RBCs. However, since these individuals often make anti-D when exposed to conventional D through transfusion or pregnancy, the reagent for use in typing patients’ samples would be selected to not react with category VI RBCs. At that time, however, it was felt that this was not practical and that being called D+ as a donor and D– as a patient was problematic.44 Blends were judged to be the best answer; this is reflected in the current reagents available in the United States. In all of them, the IgM anti-D component does not react with DVI RBCs but the IgG component reacts with these RBCs in the AHG phase of testing (Table 1). This has prompted the movement away from weak D testing in the hospital and prenatal setting to better serve individuals with DVI RBCs by classifying them as D–. This movement was slow to develop, however, and many hospital transfusion service laboratories were still performing the weak D test in the 1999 CAP survey,42 a decade after implementation of monoclonal reagents.

### Reporting Variable Reactivity With Anti-D Typing Reagents

How does the laboratory report the D type when testing results differ between various manufacturers’ reagents or between transfusion service and donor center D typing? Key to assigning D antigen status should be whether the patient is a blood donor or a transfusion recipient. DNA-based testing is very useful to confirm the molecular basis underlying D typing discrepancies, but is not always necessary if a thorough serologic workup is performed. Most agree that serologically or molecularly confirmed D variants should be considered D+ as blood donors but D– as recipients. Historically it has caused consternation within the profession to label an individual as D+ in one situation and D– in another. Laboratories fear appearing indecisive and do not want to confuse the patient or donor, the physician, or the nursing staff. However, as we move toward an age of well-informed medical care consumers, with the promise of designer health care algorithms and treatments based on genetic polymorphisms, any RHD polymorphism that results in altered D antigen expression is relevant and should be part of the medical record. To begin to move in this direction, one colleague has modified the acceptable Rh typing interpretation fields in the hospital computer system to include POS, NEG, and DEP. DEP is translated to “Negative*” in this hospital setting, with the following explanatory remark.

“The Rh type is dependent on reagents used, tests performed, and/or technical performance. Patient may have been previously reported as Rb Positive or Rb Negative. For Transfusion Service testing, the patient will be treated as Rb Negative, a candidate for Rh Immune Globulin, and will receive Rb Negative blood. As a Blood Donor, patient will be treated as Rb Positive.”* (B. Sipherd, personal communication, 2005)

### European D Testing

In Europe, weak D testing is not performed; two different IgM monoclonal anti-D reagents are used for
initial D typing. For testing recipients, at least one must not react with category DVI RBCs, so that these individuals are properly classified as D– for transfusion and Rh immune globulin prophylaxis. Many more monoclonal anti-D reagents are available in Europe and most do not use the same clones that are licensed in the United States. The wider availability of monoclonals and the fact that a weak D test is not performed are important considerations when reviewing data from recent European publications detailing the frequency of D+ donors not detected by routine testing. One cannot directly extrapolate to the incidence in the United States when the anti-D clones and methods differ.

D Typing Concerns

Issues of concern for the determination of the D status of donors differ from those for transfusion recipients. In donors, the issue is one of detection of any and all D antigen expression. In recipients, it is one of detection of D antigens with altered D epitopes.

Donors

Ideally, tests to determine a donor’s D status would detect all RBCs with any amount of D antigen or D epitope expression as D+. Unfortunately, some RBCs with the weak D phenotype may be “missed” serologically, and there are no serologic reagents to detect D_{el} RBCs. It had been suggested that the number of antigen sites, termed the Rhesus index, might be a way to distinguish which RBCs would be immunogenic when transfused to D– recipients, with a possible cutoff of 300 to 400 antigen sites required.45 Antigen dose is one consideration that determines immunogenicity, but the large number of RBCs associated with a unit of blood will compensate for a low number of antigen sites. RBCs with less than approximately 30 sites stimulate anti-D.27,28 Molecular screening for the presence of the RHD gene would detect these and testing could be done as pools,51 lending support for future implementation of DNA testing methods in donor centers. However, the absolute association of the C phenotype with the DEL alleles described to date, as well as the strong association of either C or E phenotypes with weak D types that might not be detected serologically, suggest that elimination of apparent r’ (D– C+) or r” (D– E+) donors from the D– donor pool would be an effective approach for those concerned about screening out donor units capable of immunizing D– recipients.

Alternately, molecular testing could target specifically this group of D– donors to test for the presence of the RHD gene. Indeed, molecular screening for the presence of RHD in D– donors who phenotype as D– and C+ or E+ has been implemented in some Central European blood centers.86

Patients

Ideally, tests to determine a patient’s D status would distinguish those with RBCs that lack, or have altered, D epitopes (and are at risk of immunization to conventional D) from those that carry mutations that simply reduce expression levels of D (and do not confer such risk). Unfortunately, standard serologic anti-D reagents cannot discriminate partial D RBCs. Nor can they identify weak D RBCs that, similar to Type 4.2 and Type 15,34 have altered D epitopes manifest as production of anti-D when transfused with conventional D+ RBCs. On a positive note, our long history of transfusing patients who have weak D RBCs with conventional D+ donor blood strongly suggests that weak D Types 1, 2, and 3, which comprise at least 90 percent of weak D individuals, do not make anti-D, as others have emphasized.40 It is also important to put the “D dilemma” into perspective. We routinely accept that 10 percent of recipients are potentially exposed to c and K antigens. Severe anemia and hemolytic disease of the fetus and newborn have been reported due to maternal anti-c or -K stimulated by transfusion. Most believe that routine matching for c and K is not justified even in females of childbearing age. Perhaps it might be appropriate to revisit this policy; however, the question is one of clinical allocation of resources. Importantly, is D different, in that we will not tolerate any anti-D immunizations?

Will DNA-Based Testing Be the Answer for D Typing?

DNA-based testing strategies can, by sampling multiple regions of the RH genes, determine a specific weak D type or partial D category or the presence of D_{el}. Currently, this can be cumbersome, often requiring complete gene sequencing. The development of automated, high-throughput platforms that sample many regions of both RHD and RHCE, along with detailed algorithms for accurate interpretation, are needed. The significance of many of the other weak D types (Types 4–42) has not been determined because serologic evidence, in the form of immunization and production of antibodies in recipients, is not available.
Because direct experimentation and deliberate immunization cannot be used to answer the question of which RBCs lack, or have altered, D and which mutations simply reduce expression levels of D, it is important to investigate D+ patients who make anti-D. Although anti-D immunization is probably still infrequent or rare, as pointed out previously, the observation that 32 percent of participants in the 1999 CAP survey had observed at least one case per year of a weak D individual who had anti-D and 21 percent had encountered two or more cases might suggest otherwise. To gather the appropriate information relevant to the test methods and anti-D reagents used in the United States, it will be important for laboratories to investigate the following:

1. D– patients who receive D– products but produce anti-D.
2. Products labeled D– that may have stimulated anti-D.
3. D+ patients who make anti-D.

This should ideally include serologic workups combined with molecular DNA testing. It is the powerful combination of serology with genetics that will define our future as we move into the postgenomic era of DNA-based testing methods.

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