

Serologic profiling of D variants in donor routine: unveiling the impact on false-negative results and alloimmunization

Carine P. Arnoni,^{1,*} Tatiane A. Vendrame,¹ Flavia S. Silva,¹ Nayara M. Silva,¹ Afonso Cortez,¹ Flavia Latini,¹ and Lilian Castilho²

¹ Colsan-Associação Beneficente de Coleta de Sangue, São Paulo, SP, Brazil

² Hemocentro-Unicamp, Campinas, SP, Brazil

*Corresponding author: carine.arnoni@colsan.org.br

The high number of D variants can lead to the unnecessary use of Rh immune globulin, overuse of D– RBC units, and anti-D alloimmunization. D variant prevalence varies among ethnic groups, and knowledge of the main variants present in a specific population, their behavior in serologic tests, and their impact on clinical practice is crucial to define the best serologic tests for routine use. The present study aimed to explore the serologic profile of D variants and to determine which variants are most associated with false-negative D typing results and alloimmunization. Donor samples were selected in two study periods. During the first period, D typing was performed on a semi-automated instrument in microplates, and weak D tests were conducted in tube or gel tests. In the second period, D typing was carried out using an automated instrument with microplates, and weak D tests were performed in solid phase. Samples from patients typed as D+ with anti-D were also selected. All samples were characterized by molecular testing. A total of 37 *RHD* variants were identified. Discrepancies and atypical reactivity without anti-D formation were observed in 83.4 percent of the samples, discrepant D typing results between donations were seen in 12.3 percent, and D+ patients with anti-D comprised 4.3 percent. DAR1.2 was the most prevalent variant. Weak D type 38 was responsible for 75 percent of discrepant samples, followed by weak D type 11, predominantly detected by solid phase. Among the D variants related to alloimmunization, DIVa was the most prevalent, which was not recognized by serologic testing; the same was true for DIICc. The results highlight the importance of selecting tests for donor screening capable of detecting weak D types 38 and 11, especially in populations where these variants are more prevalent. In pre-transfusion testing, it is crucial that D typing reagents demonstrate weak reactivity with DAR variants; having a serologic strategy to recognize DIVa and DIICc is also valuable. *Immunohematology* 2024;40:47–53. DOI: 10.2478/immunohematology-2024-007.

Key Words: *RHD* variants, weak D, partial D, D typing discrepancy, anti-D, D variants

The Rh blood group system is the most clinically relevant system after ABO and is also highly complex.¹ Among the 56

antigens carried by the Rh system, D is the most immunogenic, and anti-D causes severe hemolytic transfusion reactions and hemolytic disease of the fetus and newborn.² Therefore, to prevent the formation of anti-D, it is standard practice to avoid transfusing D+ red blood cells (RBCs) to D– patients, especially women of childbearing age. However, the high number of D variants sometimes hinders correct management of these patients, leading to unnecessary use of Rh immune globulin (RhIG), overuse of D– RBC units, and anti-D alloimmunization.³

Since 1946, when the first D variant was described, many D variants have been reported.⁴ The majority of these variants exhibit weak D reactivity in serologic testing ($\leq 2+$ in direct agglutination testing), which may indicate weak D variants, with no risk of forming anti-D, or partial D variants lacking D epitopes, making anti-D alloimmunization possible.³ Serologic tests cannot distinguish between weak and partial D variants. Additionally, there are partial D types that do not show reduced reactivity in D typing or react stronger than 2+ in direct agglutination testing, often recognized only after anti-D formation. On the other hand, some D variants have very low antigen density, such as DELs or certain types of weak D, which are not detected in routine D typing.⁵

The management of these D variants differs between donors and patients. A highly sensitive test is suitable for routine donor testing to cover D variants with low antigen density and avoid alloimmunization of D– recipients. In contrast, it is essential to detect partial D variants in the patient routine. Therefore, a D typing test that intensifies the reaction can hinder their recognition. Additionally, *RHD* genotyping is recommended for samples demonstrating a “serologic weak D phenotype” to determine which patients are at risk of alloimmunization.^{3,6}

D variant prevalence varies among ethnic groups, and the knowledge of the main variants present in a specific population, their behavior in serologic tests, and their impact on clinical practice is crucial for defining the best serologic tests for routine use, aiding molecular investigation, and managing these cases. In the present study, we explored the serologic profile of D variants detected with two methods used in routine donor testing. Additionally, we determined which D variants are most associated with false-negative D testing results and alloimmunization.

Methods

Sample Selection

Blood samples were selected from the Reference Immunohematology Laboratory at Colsan–Associação Beneficente de Coleta de Sangue. This laboratory receives samples from different regions across São Paulo State, since it supports hospitals and receives donor samples from collection centers.

FIRST PERIOD

From January 2011 to December 2012, D typing in donor routine testing was conducted using a semi-automated instrument (Lyra MP Reader; Bio-Rad, Hercules, CA) with microplates and monoclonal blend reagents (Seraclone Human Monoclonal Blend: BS221, H41 11B7, BS232; Biotest Medical Diagnostics, Dreieich, Germany). D– and weak D samples were confirmed with the same antiserum in tube test, and all samples with ambiguous results were tested with gel card (DiaMed, Lagoa Santa, MG, Brazil). During this period, 367 RBC samples showing atypical D expression, including discrepancies among antisera or reactivity weaker than 2+ in D typing, were selected for molecular investigation. All serology was performed according to the manufacturer's instructions.

SECOND PERIOD

In December 2012, our center implemented the Immucor NEO automated instrument with anti-D clones (IgM RUM-1; Immucor, Dreieich, Germany) and blend D175+D415 (Novacclone, Nova Scotia, Canada). In this system, samples with D– results (score 0–30) or low reactivity in automated microplate direct agglutination testing (score 31–80) were confirmed by solid phase (Capture-R, blend D175+D415). Since then, in non-continuous periods, 237 RBC samples were selected, 80 due to discrepant results from previous donations, and 157 were investigated because they presented atypical reactivity, such as reactivity lower than a score of 90 (4+) in

direct agglutination or discrepant results between both anti-D antisera used in direct agglutination testing.

Samples from patients typed as D+ with anti-D in their plasma have been selected since 2013, totaling 28 samples. Patient samples were tested with gel card DG Gel ABO/Rh 2D: IgM clone P3X61, blend clones: P3x290, P3x35, P3x61, and P3x21223B10 (Grifols, Parets del Vallés, Barcelona, Spain).

In summary, we selected 367 samples showing atypical D expression on a semi-automated instrument using microplates, 237 samples with atypical expression on the Immucor NEO instrument, with 80 involved in discrepant results, and 28 samples from routine patient testing.

Molecular Methods

Molecular investigations were performed during both periods when the samples were selected. Genomic DNA was extracted from the buffy coat of peripheral blood using the DNA blood mini kit (PureLink Genomic DNA Mini Kit; Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. The DNA concentration and purity were determined by the measurement of optical density at 260 and 280 nm using a NanoDrop 2000 Spectrophotometer (Thermal Cycler; Uniscience, São Paulo, Brazil), and DNA samples were stored at –20°C.

D variants were molecularly characterized using multiplex polymerase chain reaction (PCR), PCR–restriction fragment-length polymorphism, and Sanger sequencing, based on an approach that has been previously described,⁷ with some minor adaptations because of the last review of the International Society of Blood Transfusion (ISBT) *RHD* table.⁴ The quantitative multiplex PCR of short fluorescent fragments (QMPSF) protocol was implemented to investigate hybrid alleles.⁸ Briefly, QMPSF consists of two multiplex PCR reactions that amplify *RHD* and *RHCE* exons of different lengths that are fluorescently labeled. By fragment analysis in capillary electrophoresis (3500xL, Applied Biosystems, Waltham, MA), PCR products are separated, and the area of the fluorescent peaks is calculated by computer software (GeneMapper Software Version 4.1; Applied Biosystems). To define the presence of hybrid alleles, a computer spreadsheet (Excel; Microsoft, Redmond, WA) is used to calculate the copy number of *RHD* and *RHCE* exons.

Results

Table 1 describes all the D variants detected in both study periods. A total of 37 different D variants were identified,

Table 1. Frequency of variant *RHD* alleles segregated by atypical D expression, discrepancies between previous and current donations, and alloimmunized patients.[†]

Variant <i>RHD</i> allele	Atypical D expression	Discrepancies	Patients with anti-D	Total
<i>RHD</i> *DAR1.02	169 (31.2)		4 (14.3)	173 (26.6)
<i>RHD</i> *DAR3.01	104 (19.2)		1 (3.6)	105 (16.2)
<i>RHD</i> *01W.38	37 (6.8)	60 (75.0)		97 (14.9)
<i>RHD</i> *01W.3	89 (16.5)	2 (2.5)		91 (14.0)
<i>RHD</i> *01W.2	48 (8.9)	1 (1.3)		49 (7.6)
<i>RHD</i> *01W.1	27 (5.0)	1 (1.3)		28 (4.3)
<i>RHD</i> *DAR1.01	16 (3.0)			16 (2.5)
<i>RHD</i> *11	1 (0.2)	12 (15.0)		13 (2.0)
<i>RHD</i> *DIVa			9 (32.1)	9 (1.4)
<i>RHD</i> *DAU6	8 (1.5)			8 (1.2)
<i>RHD</i> *DVII	7 (1.3)		1 (3.6)	8 (1.2)
<i>RHD</i> *DAU4	6 (1.1)		1 (3.6)	7 (1.1)
<i>RHD</i> *DAR1.2 (1063A) ⁹	4 (0.7)	2 (2.5)		6 (0.9)
<i>RHD</i> *DMH	5 (0.9)			5 (0.8)
<i>RHD</i> *DAU5	2 (0.4)		3 (10.7)	5 (0.8)
<i>RHD</i> *DIIIc			3 (10.7)	3 (0.5)
<i>RHD</i> *DIIIa	1 (0.2)		2 (7.1)	3 (0.5)
<i>RHD</i> *DAU0	2 (0.4)			2 (0.3)
<i>RHD</i> *DAU3	1 (0.2)		1 (3.6)	2 (0.3)
<i>RHD</i> *DOL2	2 (0.4)			2 (0.3)
<i>RHD</i> *602G,667G,697C,733C,744T,1136T ⁹	1 (0.2)			1 (0.2)
<i>RHD</i> *01W.93	1 (0.2)			1 (0.2)
<i>RHD</i> *841C9	1 (0.2)			1 (0.2)
<i>RHD</i> *DIII.09			1 (3.6)	1 (0.2)
<i>RHD</i> *DAU1	1 (0.2)			1 (0.2)
<i>RHD</i> *DAU2	1 (0.2)			1 (0.2)
<i>RHD</i> *01W.15	1 (0.2)			1 (0.2)
<i>RHD</i> *01W.5	1 (0.2)			1 (0.2)
<i>RHD</i> *DVI.2	1 (0.2)			1 (0.2)
<i>RHD</i> *DNB			1 (3.6)	1 (0.2)
<i>RHD</i> *DOL4			1 (3.6)	1 (0.2)
<i>RHD</i> *01W.18	1 (0.2)			1 (0.2)
<i>RHD</i> *DV.1	1 (0.2)			1 (0.2)
<i>RHD</i> *DV.2	1 (0.2)			1 (0.2)
<i>RHD</i> *DEL1		1 (1.3)		1 (0.2)
<i>RHD</i> *01W.34	1 (0.2)			1 (0.2)
<i>RHD</i> *01(c.801+1G>T)		1 (1.3)		1 (0.2)
TOTAL	541 (83.4)	80 (12.3)	28 (4.3)	649 (100)

[†]Data in parentheses are %.

including variants common in African individuals as well as in European individuals. Analyzing the six most prevalent D variants, which account for 83 percent of the samples, 42.8 percent were typical of African descendants, while 40.8 percent were of European origin.

The samples were categorized into three groups: (1) atypical reactivity: samples with reduced level of reactivity regardless of the initially performed test but were not involved in discrepancies between donations or anti-D formation; (2) discrepancies: samples with a D– result in previous donations and a D+ result in current testing; and (3) RBC samples from patients typed as D+ with anti-D in their plasma. Samples with atypical reactivity constitute 83.4 percent of the studied samples, while samples with discrepancies represent 12.3 percent, and those with anti-D represent only 4.3 percent.

Comparison of First and Second Periods

Comparing the D variants found in the two periods (Fig. 1A), we observed that DAR1.2 and DAR3.1 were most frequently detected in the first period when the screening was performed using a semi-automated instrument. Figure 1B illustrates the difference in reactivity between both periods, with most DAR samples detected with 1+ reactivity in the first period and 3+ reactivity in the second. On the other hand, weak D type 38 was the variant most frequently detected in the second period, representing 32 percent of the samples investigated in this period compared with 6 percent of the samples studied in the first period (Fig. 1A). Despite the low-antigen density of this variant, the NEO instrument was very sensitive in detecting this variant by solid phase. Similarly, weak D type 11 was detected only by solid phase, and no weak D type 11 donor was identified in the first period. Weak D types 1, 2, and 3 are present in both periods, and types 1 and 3 also react strongly when tested on the NEO instrument. The comparison of *RHD* variant frequencies among the different groups studied is illustrated in Figure 2.

RBC Samples with Atypical Reactivity

RBC samples with atypical reactivity represent 83.4 percent of the studied samples and are distributed among 32 different D variants. The prevalent D variants detected with reduced expression are from DAR cluster, representing 54 percent of the samples with atypical reactivity. DAU4 showed a significant discrepancy between the antisera used in direct agglutination testing on the NEO instrument, reacting 3+ or 4+ with blend (D175+D415) and 0 or 1+ with IgM antiserum (RUM-1). This information is crucial because these variants

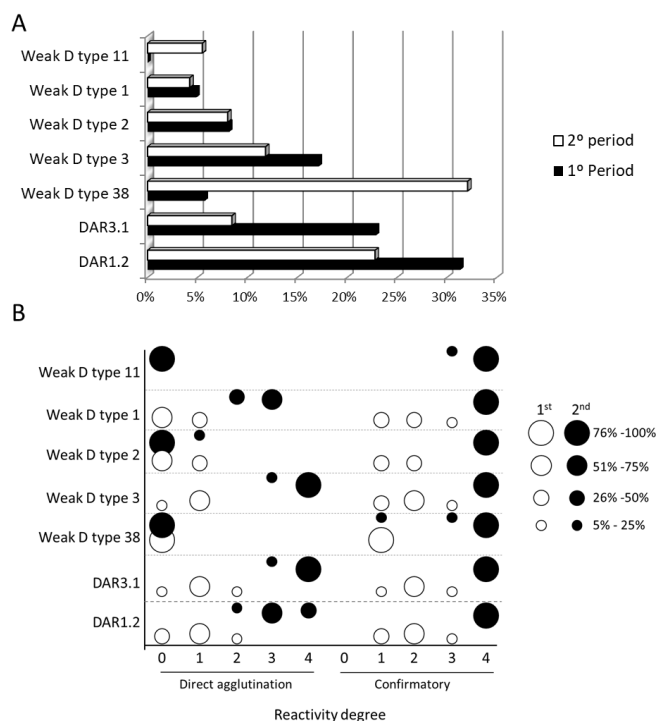


Fig. 1 (A) The percentage of the most prevalent D variants detected in each period. (B) The reactivity degree detected in the direct agglutination test and the weak D confirmatory test during both study periods. During the first period, the direct agglutination test was conducted using a semi-automated instrument with microplates from Bio-Rad (Lyra MP Reader; Bio-Rad, Hercules, CA), and the confirmatory test was performed in the antiglobulin phase by tube testing. In the second period, direct agglutination testing was carried out on the NEO automated instrument (Immucor, Dreieich, Germany), and the confirmatory test was performed using solid-phase testing.

characterize a partial phenotype, including four DAR1.2 samples and one DAU4 sample from alloimmunized patients. Most of the remaining partial D variants, detected due to atypical reactivity, were selected in the first period, when the methodology was less sensitive. Regarding weak D types, in addition to weak D types 1, 2, and 3, which are more common and represent 26 percent of the samples, we found some rare types, such as weak D types 18, 34, and 93.

RBC Samples with Discrepant Results

Among RBC samples with discrepant D typing results between donations, weak D type 38 was the most frequent (75%). Before the implementation of NEO Immucor in routine donor testing, 22 samples were selected due to very weak reactivity in the gel test, ranging from weak (w+) to 1+. After automation of routine donor testing, 60 weak D type 38 samples were investigated molecularly because they presented

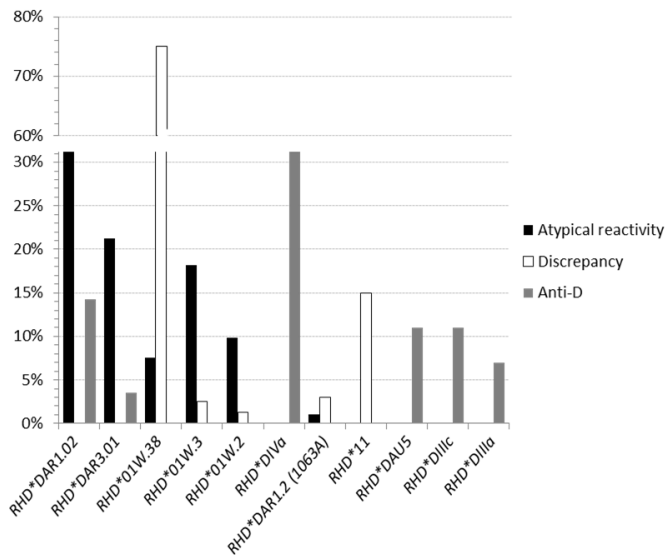


Fig. 2 Comparison of the frequency of the most prevalent *RHD* variants within each study group.

discrepant D typing results with previous donations, and 15 samples were investigated due to weak reactivity. Most weak D type 38 samples (90%) were positive in solid phase with a reactivity of 3+ and 4+ (Fig. 1B). However, six samples showed weaker reactivity (1–2+), including one with a discrepant result on the NEO instrument, detected as D– in the previous donation. Three of those samples with very weak reactivity had all *RHD* exons sequenced, but no additional single nucleotide variation (SNV) to justify the atypical reactivity was found (data not shown). The second most frequent D variant involved in a discrepancy was weak D type 11, totaling 13 samples, which were detected only by solid phase. Despite the low-antigen density, all the samples presented strong reactivity in solid phase (3–4+).

In addition, other interesting D variants were also involved in discrepancies. *RHD*DA1.2(1063A)* presents the polymorphisms of DAR1.2 with the additional c.1063G>A SNV, which alone gives rise to the DNB variant. This variant presents much lower reactivity than DAR1.2 and DNB, being detected only in the confirmatory test in both study periods.

One sample with the DEL phenotype (*RHD*DEL1*) was detected by solid phase; however, it exhibited non-reproducible reactivity. In the second donation, the result was D–, and upon retesting, negative and 1+ reactivities were found. Additionally, a novel allele (*RHD*01[c.801+1G>T]*; GenBank accession number: PP479921) was detected in a sample that tested D– in eight donations before NEO implementation, and a very

weak reaction was observed in solid phase. The serologic result was reproducible on two additional occasions when the donor returned, and an adsorption/elution test was positive. The molecular result showed a change from guanine to thymine at the first base of intron 5. The ISBT table describes a change from guanine to adenine at the same nucleotide, causing a D– phenotype.

RBC Samples of Patients Typed as D+ with Anti-D in Their Plasma

The most frequent D variant involved in anti-D alloimmunization was DIVa, which was found exclusively in the group of patients with anti-D, meaning that such samples are typically typed as D+. Four patients had DAR1.2, which is the most frequent D variant in our population. DAU5, DIIC, and DIIDa were also found in alloimmunized patients, followed by D variants detected in single patients. One patient was detected with DAR3.1 (weak D type 4.0) which does not have sufficient reports to confirm its risk of alloimmunization.⁶ We were not able to perform adsorption studies to accurately classify antibodies as auto- or allo-anti-D.

Discussion

D variants are widely studied, and knowledge of their impact on transfusion and strategies for identification is often a point of discussion. In the present study, we identified common D variants present in the southeastern Brazilian population, explored their serologic profile, and defined those most impactful for transfusion practice. DAR1.2 is the most frequent variant detected by serologic tests in our population, and, although it is a partial D, it was not the most prevalent variant in alloimmunized patients. On the other hand, the DIVa variant was the most prevalent variant in the group of patients with anti-D, but this variant did not present altered expression with the anti-D reagents used for this testing, being exclusively detected only after anti-D formation. Additionally, we detected a significant number of donors with D variants with very low antigen density (weak D types 38 and 11) involved in discrepancies and mistyped blood donors.

In 2013, the Immucor NEO instrument was implemented at our center, marking the first installation of such an instrument in Brazil. Until then, few studies had reported the presence of weak D type 38 in the Brazilian population, and this D type was only documented in donor samples typed as D– that underwent molecular investigation.^{10,11} After the implementation of NEO, particularly in the first year,

many donors previously typed as D– were classified as D+ by confirmatory testing, in which solid-phase methodology in addition to blend D175+D415 antisera showed to be very sensitive. In molecular investigation, donors were predominantly classified as weak D type 38 and, to a lesser extent, as weak D type 11.

Interestingly, weak D type 11 was initially associated with a ce haplotype.¹² However, subsequent reports indicated its association with a Ce haplotype, presenting an antigen density borderline between weak D and DEL, likely due to the suppressive effect of C in *cis*.^{13,14} All weak D type 11 samples reported here were C+, and, although a haplotype study was not performed, it is likely that this variant is associated with the Ce haplotype and exhibits reduced expression of D. Similar to weak D type 38, they strongly reacted in the solid-phase test.

Analyzing the reactivities obtained in the discrepant samples, the direct agglutination test was unable to detect them, yielding D– results. However, when tested by solid phase, most weak D type 38 and 11 samples showed 4+ reactivity, demonstrating the high sensitivity of the confirmatory test. In agreement, a sample with the DEL phenotype was detected by solid phase, as well as a sample with a new genotype *RHD*01(c.801+1G>T)*, which was not detected in the gel test and which exhibited weak reactivity in solid phase.

Complementary to these results, in a previous study, we investigated almost 2000 samples typed as D– on the NEO. No sample with an *RHD* allele encoding a weak D phenotype was found when tested by molecular methods.¹⁵ These data indicate that the NEO instrument performs well in routine donor testing. In retrospect, a total of 203 RBC units from donors with weak phenotypes were mistyped as D–. Unfortunately, we were not able to investigate recipients to evaluate the risk of alloimmunization.

Regarding anti-D alloimmunization, despite the fact that DAR1.2 is the most prevalent D variant in our population and is known to be involved in alloimmunization,¹⁶ only 2.3 percent of DAR1.2 samples presented with anti-D. The low rate of alloimmunization may be due to altered expression of this variant in pre-transfusion testing, leading to the selection of D– RBC units for transfusion. Therefore, in contrast to routine donor testing, it is important to choose reagents and methods that do not exacerbate anti-D reactivity in the routine patient testing. On the other hand, DIVa is the main D variant involved in alloimmunization, and it was exclusively detected due to anti-D formation. Similarly, DIIc is exclusively detected in alloimmunized patients. Both D variants have high-antigen

density and are described as partial D detected as D+ mainly in individuals of European descent.¹⁷ In the present study, we found samples with DIVa and DIIc variants at a frequency of 1.4 and 0.5 percent, respectively. However, we do not know the real frequency since they remain undetected until alloimmunization.

As in some European countries, in Brazil, D typing is carried out with two monoclonal D antibodies, one of which does not recognize DVI, to protect individuals with DVI from immunization. von Zabern et al.¹⁷ proposed an interesting strategy that could be applied in the Brazilian population, especially in women of childbearing age and children. It consists of a set of two monoclonal D antibodies, one unable to recognize DVI and another unable to recognize other clinically important D variants, such as DIVa, which lacks the epitopes epD 1, 2, 3, 4, and 9.

On the other hand, 26 percent of donors' samples present weak D types 1, 2, and 3. Extrapolating this finding to patient routine testing suggests that these weak D types could be safely managed as D+, potentially avoiding the unnecessary use of D– RBC units and RhIG prophylaxis.³ Genotyping is recommended when an inconsistency in D typing results and/or a serologic weak D phenotype is identified. However, the cost and time involved in this screening need to be taken into account. Given that most patients with D variants in our population should be managed as D–, meaning that their management will not be altered by genotyping, specific criteria for molecular testing should be considered.

Although serologic tests are unable to distinguish between types of D variants, selecting samples for genotyping based on serologic reactivity and associated haplotypes can restrict the use of genotyping and provide a more precise means of identifying D variants that may be considered D+. Here, we present a strategy to select candidate samples for genotyping, with a focus on limiting molecular testing to identify weak D types 1, 2, or 3. For this strategy, genotyping would be performed only on samples positive for C or E showing 1–2+ reactivity with anti-D in the antiglobulin phase using tube testing.

When applying this strategy to the samples presented in this study, only 5 of 173 would present other weak D types (weak D types 5, 15, 18, 34, and 93). Therefore, by using a simple molecular investigation focused on weak D types 1, 2, and 3, we could identify the patients that could be managed as D+, and all the remaining samples should be managed as D–.

In conclusion, based on our data, we recommend serologic tests for routine donor screening capable of detecting weak D

types 38 and 11, especially in populations where these variants are more prevalent. In pre-transfusion testing, it is crucial that D typing demonstrates weak reactivity in DAR variants, and a serologic strategy to recognize DiVa is also valuable. Additionally, we propose a simple strategy to identify weak D types 1, 2, and 3 to improve the management of patients with D variants.

References

1. Flegel WA. Molecular genetics of *RH* and its clinical application. *Transfus Clin Biol* 2006;13:4–12.
2. Avent ND, Reid ME. The Rh blood group system: a review. *Blood* 2000;95:375–87.
3. Sandler SG, Flegel WA, Westhoff CM, et al. It's time to phase in *RHD* genotyping for patients with a serologic weak D phenotype. College of American Pathologists Transfusion Medicine Resource Committee Work Group. *Transfusion* 2015;55:680–9.
4. International Society of Blood Transfusion. Red cell immunogenetics and blood group terminology. 2023. Available from <http://www.isbtweb.org/working-parties/red-cell-immunogenetics-and-blood-group-terminology/>.
5. Stegmann TC, Veldhuisen B, Bijman R, et al. Frequency and characterization of known and novel *RHD* variant alleles in 37,782 Dutch D-negative pregnant women. *Br J Haematol* 2016;173:469–79.
6. Flegel WA, Denomme GA, Queenan JT, et al. It's time to phase out “serologic weak D phenotype” and resolve D types with *RHD* genotyping including weak D type 4. *Transfusion* 2020;60:855–9.
7. Arnoni CP, Latini FR, Muniz JG, et al. How do we identify *RHD* variants using a practical molecular approach? *Transfusion* 2014;54:962–9.
8. Fichou Y, Le Marechal C, Bryckaert L, et al. A convenient qualitative and quantitative method to investigate *RHD-RHCE* hybrid genes. *Transfusion* 2013;53:2974–82.
9. Arnoni CP, Muniz JG, de Paula Vendrame TA, et al. Identification of four novel *RHD* alleles with altered expression of D in Brazilians. *Transfusion* 2016;56: 1475–6.
10. Costa S, Martin F, Chiba A, Langhi D Jr, Chiatton C, Bordin J. *RHD* alleles and D antigen density among serologically D– C+ Brazilian blood donors. *Transfus Med* 2014;24:60–1.
11. Mota M, Dezan M, Valgueiro M, Sakashita A, Kutner J, Castilho L. *RHD* allelic identification among D– Brazilian blood donors as a routine test using pools of DNA. *J Clin Lab Anal* 2012;26:104–8.
12. Wagner FF, Gassner C, Muller TH, Schonitzer D, Schunter F, Flegel WA. Molecular basis of weak D phenotypes. *Blood* 1999;93:385–93.
13. Wagner FF, Frohmajer A, Flegel WA. *RHD* positive haplotypes in D negative Europeans. *BMC Genet* 2001;2:10.
14. Wagner FF, Flegel WA. The rhesus site. *Transfus Med Hemother* 2014;41:357–63. <https://www.rhesusbase.info/>.
15. Vendrame TAP, Arnoni CP, Muniz JG, et al. Characterization of *RHD* alleles present in serologically *RHD*-negative donors determined by a sensitive microplate technique. *Vox Sang* 2019;8:869–75.
16. Hemker MB, Ligthart PC, Berger L, van Rhenen DJ, van der Schoot CE, Wijk PA. *DAR*, a new RhD variant involving exons 4, 5, and 7, often in linkage with *ceAR*, a new Rhce variant frequently found in African blacks. *Blood* 1999;94:4337–42.
17. von Zabern I, Wagner FF, Moulds JM, Moulds JJ, Flegel WA. D category IV: a group of clinically relevant and phylogenetically diverse partial D. *Transfusion* 2013;53:2960–73.

Free Classified Ads and Announcements

Immunohematology will publish classified ads and announcements (SBB schools, meetings, symposia, etc.) **without charge**.

E-mail information to immuno@redcross.org.

Notice to Readers

All articles published, including communications and book reviews, reflect the opinions of the authors and do not necessarily reflect the official policy of the American Red Cross.

Attention:

State Blood Bank Meeting Organizers

If you are planning a state meeting and would like copies of *Immunohematology* for distribution, please send a request, 4 months in advance, to immuno@redcross.org.