

It's time to phase in *RHD* genotyping for patients with a serologic weak D phenotype

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In 2014, the College of American Pathologists (CAP) Transfusion Medicine Resource Committee (TMRC) reported the results of a survey of more than 3100 laboratories concerning their policies and procedures for testing serologic weak D phenotypes and admin-

istration of Rh immune globulin (RhIG).¹ Among the findings of this survey is the observation that there is a lack of standard practice in the United States for interpreting the RhD type when a serologic weak D phenotype is detected. In some laboratories, an individual with a serologic weak D phenotype, especially if a blood donor, is interpreted to be RhD-positive. In the same or other laboratories, especially if a serologic weak D phenotype is detected in a female of childbearing potential, the individual is likely to be managed as RhD-negative for transfusions and, if pregnant, considered a candidate for RhIG. Also, the performance characteristics of serologic typing methods for RhD vary. For patients, including pregnant women, the majority of laboratories have policies and procedures that do not use the indirect antiglobulin (weak D) test, thereby avoiding detection of a serologic weak D phenotype so that the RhD type will be interpreted to be RhD-negative. Other laboratories typically perform a weak D test for the same category of patients. For blood donors and newborns, it is standard practice for laboratories to have policies and procedures for RhD typing to ensure that serologic weak D phenotypes are detected and interpreted as RhD-positive.¹

ABBREVIATIONS: ACOG = American College of Obstetricians and Gynecologists; CAP = College of American Pathologists; TMRC = Transfusion Medicine Resource Committee.

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The goal of these RhD typing practices is to protect RhD-negative persons from inadvertent alloimmunization to the D antigen by exposure to RhD-positive red blood cells (RBCs), including RBCs expressing a serologic weak D phenotype. Although there has not been a recent prospective study in the United States, it is estimated that current RhD typing practice, together with contemporary obstetric practices for administration of antepartum and postpartum RhIG, is 98.4% to 99% successful in preventing RhD alloimmunization and RhD hemolytic disease of the fetus or newborn.² However, there are unwarranted consequences associated with the practice of not determining the *RHD* genotype of persons with a serologic weak D phenotype, including unnecessary injections of RhIG and transfusion of RhD-negative RBCs—always in short supply—when RhD-positive RBCs could be transfused safely.

CAP's TMRC reviewed the current status of *RHD* genotyping and proposed that selective integration of

RHD genotyping in laboratory practices could improve the accuracy of RhD typing results, reduce unnecessary administration of RhIG in women with a serologic weak D phenotype, and decrease unnecessary transfusion of RhD-negative RBCs to recipients with a serologic weak D phenotype.¹ In response to the findings of the CAP TMRC survey, AABB and CAP convened a Work Group on *RHD* Genotyping and charged it with developing recommendations to clarify clinical issues related to RhD typing in persons with a serologic weak D phenotype. As an initial step for formulating recommendations, the Work Group reviewed the current state of molecular science of *RHD*, including more than 140 publications covering background;¹⁻¹² D variants with anti-D;¹³⁻²⁹ molecular basis of serologic weak D phenotypes;³⁰⁻⁹² reviews, editorials, and commentaries;⁹³⁻¹²⁹ technical resources;¹³⁰⁻¹⁴² and standards and guidelines.¹⁴³⁻¹⁴⁹ This commentary summarizes the proceedings and recommendations of the Work Group.

HISTORY AND ORGANIZATIONAL POLICIES

The first report of a D variant antigen, initially named D^u, was published in 1946.⁴ To ensure that RBCs with a serologic weak D phenotype would not be transfused inadvertently as RhD-negative, the first edition of AABB Standards (1958) required testing for D^u if a donor's blood typed as RhD-negative by direct agglutination using "anti-D typing serum."¹⁴⁴ In contrast, Standards (1958) regarded a direct agglutination method using "anti-D serum" to be "sufficient" for RhD typing for transfusion recipients.¹⁴⁴ This strategy, namely, typing blood donors by a method that interprets a serologic weak D phenotype as RhD-positive, and typing patients by a method that typically interprets a serologic weak D phenotype as RhD-negative, has persisted for more than 50 years. Indeed, the current (29th) edition of Standards (2014) requires a method to detect weak expression of D for blood donor RBCs, but considers a weak D test for transfusion recipients to be "unnecessary," with the exception that testing for weak D is required for RBCs from a fetus or newborn of an RhD-negative mother to determine the mother's candidacy for RhIG.¹⁴⁹ The 10th edition of Standards (1981) addressed recommendations for RhD typing for the administration of RhIG for the first time and recommended that a woman's candidacy for receiving RhIG be determined by the same method as that for RhD typing blood donors.¹⁴⁴ Thus, a woman with a serologic weak D phenotype, that is, a positive weak D test result, was interpreted to be RhD-positive and not a candidate for RhIG. The American College of Obstetricians and Gynecologists (ACOG) addressed the issue of administration of RhIG in women with a serologic weak D phenotype for the first time in a 1981 practice bulletin.¹⁴⁵ ACOG recommended that RhD-negative women "whether Du positive or Du negative" were candidates for RhIG. That

recommendation was reversed within a few months to read that "[a] woman who is genetically D^u-positive is Rh-positive and administration of Rh immune globulin is unnecessary."¹³⁰ In 1992, the D^u variant was renamed as weak D.⁷ The ACOG guidance remains unchanged in the most recent ACOG practice bulletin (1999), which recommends that women with a serologic weak D phenotype should be considered RhD-positive and not receive RhIG.¹⁴⁶ The consequence of this guidance is that the RBCs of a pregnant woman who inherited a serologic weak D phenotype may or may not be typed for RhD by a method that detects a serologic weak D phenotype, depending on the laboratory's policy, and she may or may not receive antepartum and postpartum RhIG.

RhD typing practice in the United States changed following the introduction of monoclonal anti-D reagents in the 1980s.¹³¹⁻¹³⁴ Monoclonal antibodies have increased sensitivity and can detect many RBCs with weak D expression as RhD-positive on initial testing. They can also be selected for specificity. Fatal hemolytic disease of the fetus or newborn has been associated with anti-D formed by women with a partial DVI phenotype,^{20,25} which is the basis for the requirement that monoclonal anti-D reagents licensed in the United States not detect RBCs with partial DVI as RhD-positive by initial testing.¹³⁷ The goal of the Food and Drug Administration requirement is to protect females of childbearing potential with a partial DVI phenotype from being typed as RhD-positive. RBCs with a partial DVI phenotype will be typed as RhD-positive as donors when a weak D test is performed.⁸ Hence, the 22nd edition of Standards (2003) recommended that "if the woman's test for D antigen is negative, a test for weak D is not required."¹⁴⁷ That standard persists in the current 29th edition of Standards (2014).¹⁴⁹

SEROLOGIC WEAK D PHENOTYPES AND MOLECULARLY DEFINED WEAK D TYPES

Serologic studies have distinguished three broad categories of D variants, namely, weak D, partial D, and DEL, from wild-type or conventional D. More than 200 *RHD* alleles are categorized by mutations that lead to qualitative and/or quantitative changes in serologic expression of the D antigen (see <http://www.uni-ulm.de/~fwagner/RH/RB2/>; also: <http://www.isbtweb.org/working-parties/red-cell-immunogenetics-and-blood-group-terminology/blood-group-terminology/blood-group-allele-terminology/>).

Serologic weak D phenotypes

A serologic weak D phenotype is defined as reactivity of RBCs with an anti-D reagent giving no or weak ($\leq 2+$) reactivity in initial testing, but agglutinating moderately or strongly with antihuman globulin.^{7,119,136,148} Many

serologic weak D phenotypes are associated with one or more amino acid substitutions in the RhD protein predicted to be within or below the RBC membrane causing decreased antigen expression on the RBC surface.³¹ The prevalence of serologic weak D phenotypes varies by race and ethnicity.¹¹⁹ Serologic weak D phenotypes are the most common D variants detected in Europe and the United States. An estimated 0.2% to 1.0% of Caucasians inherit *RHD* genes that code for serologic weak D phenotypes and the majority are associated with weak D type 1, 2, or 3.^{31,36,102} Reports of the prevalence of serologic weak D phenotypes also vary depending on the method (manual tube vs. automated analyzer), the anti-D reagent (polyspecific serum versus monoclonal blend), and whether potentiating reagents (bromelin) were used.^{103,136}

Most data concerning *RHD* alleles and the risk of alloimmunization associated with serologic weak D phenotypes are derived from observational studies in central Europe.^{32,33,55,75} These studies indicate that transfusion recipients with weak D type 1, 2, or 3, in the homozygous or hemizygous state, are not at risk for forming alloanti-D when exposed to conventional RhD-positive RBCs.^{55,75} Approximately 95% of Caucasians in central Europe with a serologic weak D phenotype have weak D type 1, 2, or 3 and can be managed safely as RhD-positive. The absence of alloimmunization in persons with weak D type 1, 2, or 3 is likely due to the fact that the distinct *RHD* allele codes for all epitopes of RhD, although present at lower surface density than on wild-type RBCs.³¹ Alloimmunization has been observed with certain other weak D types, including weak D type 4.2,^{33,55} (DAR),³¹ type 11,^{33,55} type 15,^{33,55} type 21,⁷¹ and type 57.⁶⁰

The Work Group reviewed published and unpublished reports to determine whether weak D types 4.0 and 4.1 should be recommended as weak D types that can be managed safely as RhD-positive. There are no published reports of allo- or autoanti-D in persons with weak D type 4.1 in large European cohorts^{55,75} despite their prevalence^{33,56} and frequent transfusions with RhD-positive RBCs.⁵⁶ The Work Group identified published exceptions for weak D type 4.0, notably, three cases with anti-D in Germany,⁵⁵ nine in France,⁷⁵ one in Tunisia,⁹¹ and three in the United States.⁸⁹ Only one patient in Europe among these 16 case reports has been shown to form an alloanti-D;⁷⁵ the 15 other patients formed autoanti-D. There are additional unpublished observations of alloanti-D in the racially and ethnically diverse patient populations in the United States (C. Westhoff, unpublished data, 2014). The Work Group limited its recommendation to manage serologic weak D phenotypes as RhD-positive to weak D types 1, 2, and 3 until more data are available.

Partial D phenotypes

Partial D phenotypes are associated with amino acid substitutions in the RhD protein on the RBC surface and lack

D epitopes.³¹ Many RBCs expressing a partial D antigen agglutinate by immediate spin using an anti-D reagent and are interpreted to be RhD-positive. Commercially marketed panels of monoclonal reagents are available to differentiate a number of partial D types. The percentage of persons with a partial D phenotype who form anti-D after transfusion with RhD-positive RBCs or delivery of an RhD-positive newborn is not known. However, there are many reports of RhD-positive persons, presumed to have a partial D phenotype, who formed anti-D.¹³⁻²⁹ The presence or absence of anti-D does not distinguish between serologic weak D and partial D phenotypes.¹²⁷ The most common partial D phenotypes in Europe are DNB,⁴⁰ DVI,⁸ and DVII.¹²⁸ In the United States, most persons expressing partial D phenotypes are of African ancestry. The Work Group did not address management of partial D phenotypes, except if the RhD variant is expressed as a serologic weak D phenotype, since the Work Group's charge focused on clinical management of patients with a serologic weak D phenotype.

DEL phenotypes

DEL phenotypes present by conventional blood typing as RhD-negative and are not detected serologically unless adsorption and elution studies are performed.^{41,148} The Asian-type DEL displays the complete repertoire of RhD antigen epitopes.^{38,72,92} DEL phenotypes, which are found more commonly among persons of Asian ancestry, do not present as a serologic weak D phenotype. Therefore, the Work Group did not formulate a recommendation for managing DEL phenotypes, although DEL phenotypes have been implicated as the cause of RhD alloimmunization when RhD-negative/DEL-positive RBCs were unknowingly transfused to RhD-negative recipients.^{48,50,142}

POTENTIAL BENEFITS OF *RHD* GENOTYPING FOR PATIENTS WITH SEROLOGIC WEAK D PHENOTYPES

Pregnant women

The Work Group recommends that *RHD* genotyping be performed when discordant RhD typing results are encountered and/or when a serologic weak D is identified in a female of childbearing potential. The Work Group estimates that if *RHD* genotyping were performed in RhD-negative women with a serologic weak D phenotype in the United States, approximately 13,360 of these women could be managed as RhD-positive, avoiding the injection of approximately 24,700 doses of RhIG annually. To calculate this estimate, the Work Group extrapolated data from studies of the prevalence of serologic weak D phenotypes and the number predicted to genotype as *RHD* weak D type 1, 2, or 3 in RhD-negative individuals, as follows (Fig. 1). There are an estimated 3,812,000 pregnancies annually in the United States, based on the prediction of

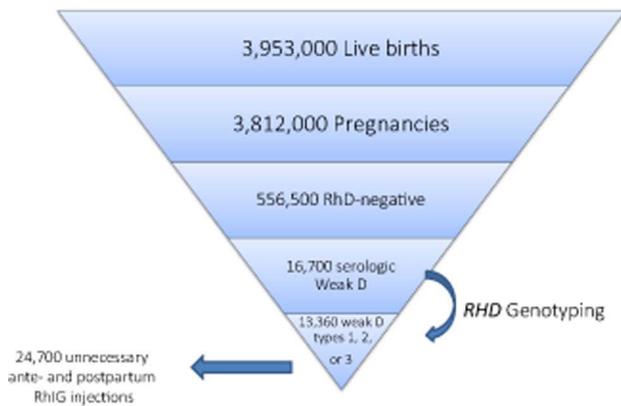


Fig. 1. Unnecessary RhIG injections. Of 3,953,000 live births in the United States each year, an estimated 13,360 mothers have a serologic weak D phenotype, which if confirmed by *RHD* genotyping, could be managed as RhD-positive. Adjusting for antepartum and postpartum dosing, *RHD* genotyping of pregnant women with a serologic weak D phenotype could prevent 24,700 unnecessary injections of RhIG.

3,953,000 births annually and adjusted for multiple births per pregnancy.¹⁴⁰ Of these 3,812,000 pregnancies, approximately 556,500 (14.6% × 3,812,000) are expected to occur in RhD-negative women, as 14.6% represents the overall prevalence of RhD-negative individuals among racially and ethnically mixed populations in the United States.¹³⁵ Extrapolating from data from central Europe,^{8,31,56} the Work Group estimated that of these pregnant women typed as RhD-negative, 16,700 (3.0% × 556,550) will have a serologic weak D phenotype. The 3.0% estimate was calculated using a value of 0.44% for serologic weak D phenotypes among RhD-positive and RhD-negative individuals in central Europe^{8,31,56} and a 14.6% overall prevalence of RhD-negative individuals among racially and ethnically mixed populations in the United States.¹³⁵ Approximately 80% of serologic weak D phenotypes in the ethnically mixed population in the United States are weak D types 1, 2, or 3 (C. Westhoff, unpublished experience, 2014). Therefore, an estimated 13,360 pregnant women, often typed as RhD-negative but having a serologic weak D phenotype (80% × 16,700) are expected to have an *RHD* genotype that could be managed safely as RhD-positive every year in the United States. At present, most pregnant women with a serologic weak D phenotype in the United States are managed as RhD-negative and, therefore, most of these pregnant women will receive at least one unnecessary antepartum injection of RhIG and as many as 25% of these women may receive a second injection because of first-trimester bleeding or an invasive procedure.¹³⁹ An additional 60% (8016) of these women with a serologic weak D phenotype will receive an unnecessary postpartum injection of RhIG because they will deliver a RhD-positive or weak RhD-positive newborn.¹³⁹ Thus, as many

as 24,700 (13,360 + 3340 + 8016) antepartum and postpartum injections of RhIG may be administered annually to 13,360 women with a serologic weak D phenotype who could be managed safely without RhIG as RhD-positive. The number of doses of RhIG saved increases with future pregnancies since, once identified, women with a weak D type 1, 2, or 3 could be managed as RhD-positive without repeat *RHD* genotyping.

While RhIG was one of the greatest medical advances of the 1960s, almost eliminating the mortality and morbidity of Rh hemolytic disease of the fetus or newborn, there are fiscal, safety, and ethical issues when biologic products such as RhIG are administered unnecessarily. Initially, RhIG was manufactured from pooled human plasma from mothers who had stillborns due to RhD alloimmunization. As the need for RhIG rapidly grew, RhD-negative male donors were paid to be injected with RhD-positive RBCs to maintain high anti-D titers as the main source of plasma for RhIG. There are no reports of transmission of hepatitis B virus, hepatitis C virus, or human immunodeficiency virus caused by RhIG manufactured in the United States, but since RhIG is manufactured from pooled human plasma there is a potential risk for transmission of infection. An additional safety issue relates to the lifelong risk, which occurs when an RhD-negative volunteer is intentionally alloimmunized to RhD to manufacture RhIG and subsequently requires a transfusion where RhD-negative RBCs are not readily available. The problem increases with travel to certain regions of Asia and Africa where RhD-negative blood is rare. The ethical issues are not only the unnecessary administration of a blood product, but also the source of human plasma containing high-titer anti-D that is required for manufacturing RhIG. Thus, the paid-donor market for anti-D is supported, in part, by unnecessary injections of RhIG.

Transfusion recipients

The Work Group recommends that *RHD* genotyping be performed when discordant RhD typing results are encountered and/or a serologic weak D is identified in a transfusion recipient. It anticipates that if this practice were implemented in the United States, a potential 17,520 transfusion recipients with a serologic weak D phenotype could be managed as RhD-positive, avoiding the transfusion of as many as 47,700 units of RhD-negative RBCs annually. The potentially decreased requirement for RhD-negative RBCs, if a complete phase-in for *RHD* genotyping could be achieved, was calculated as follows (Fig. 2). Approximately 13,600,000 units of RBCs were transfused to 5,000,000 recipients in the United States in 2011.¹³⁸ Of these 13,600,000 units, a minimum of 1,985,600 (14.6% × 13,600,000) are RhD-negative,¹³⁵ and of the 5,000,000 recipients, approximately 730,000 (14.6% × 5,000,000) are RhD-negative.¹³⁵ Thus, an average of 2.72

units of RhD-negative RBCs are transfused to each RhD-negative patient (1,985,000 ÷ 730,000). The same data for calculating the estimate for pregnant women (above) was used to calculate the 21,900 RhD-negative transfusion recipients who will express a serologic weak D phenotype (730,000 × 3.0%) and the 17,520 who will have a weak D type 1, 2, or 3, which could be managed safely as RhD-positive (730,000 × 3.0% × 80%). Also, those estimates were applied to calculate the 47,700 units of RhD-negative RBCs that would be transfused to these recipients who could be managed as RhD-positive (17,520 × 2.72). The Work Group appreciates that these calculations provide only a good faith estimate of the potential long-term impact of *RHD* genotyping on the requirements for RhIG and for collecting RhD-negative RBCs. The calculations do not account for the different requirements by geography, race, and ethnicity, as well as the many other factors that determine the requirements for RhD-negative RBCs for transfusion. Currently, recipients of emergent transfusion may require RBCs before the results of *RHD* genotyping would be available. However, for patients requiring chronic transfusion, at greatest risk for alloimmunization, the results of a once-in-a-lifetime *RHD* genotyping would ideally be available for all subsequent transfusions. The Work Group anticipates that manufacturers and reference laboratories will recognize the need for a more rapid turnaround of *RHD* genotyping results such that methods with quicker processing will evolve. Intra- and interorganizational integration of electronic medical records will facilitate recognition of persons with a serologic weak D phenotype and allow application of prior *RHD* genotyping results to selecting the most appropriate RhD type for transfusing RBCs.

CONCLUSION AND RECOMMENDATIONS

The Work Group recommends that *RHD* genotyping be performed whenever a discordant RhD typing result and/or a serologic weak D phenotype is detected in patients, including pregnant women, newborns, and potential transfusion recipients. It is anticipated that the immediate benefit will be fewer unnecessary injections of RhIG and increased availability of RhD-negative RBCs for transfusion.

Persons whose *RHD* genotype is weak D type 1, 2, or 3 should be managed as RhD-positive with regard to administration of RhIG and/or selection of blood components for transfusion. To facilitate implementation of this recommendation, the Work Group developed

an algorithm for resolving serologic weak D phenotype test results (Fig. 3). Phasing in *RHD* genotyping in clinical practice will be most effective if manufacturers of *RHD* genotyping assays and systems offer basic cost-effective tests designed to identify the most prevalent and clinically relevant *RHD* genotypes. The Work Group recognizes that most hospitals and health care facilities will not encounter a sufficient number of persons with a serologic weak D phenotype to support in-house *RHD* genotyping. In these situations, samples for *RHD* genotyping should be referred to a reference laboratory to promote standardization of laboratory methods and the economy of

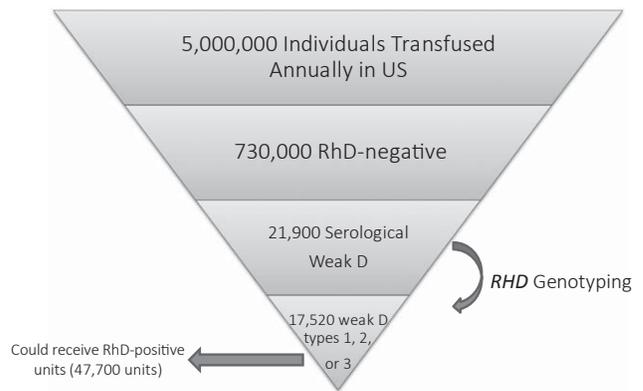


Fig. 2. Unnecessary transfusion of RhD-negative RBC units. Of 5,000,000 individuals transfused annually in the United States, an estimated 17,520 with a serologic weak D phenotype will, in the absence of indirect antiglobulin testing, be typed as RhD-negative. If their *RHD* genotype were determined, they could be managed safely as RhD-positive. Since the average number of RBC units is 2.7 per recipient, *RHD* genotyping could make 47,700 units of RhD-negative RBCs available to patients who require them.

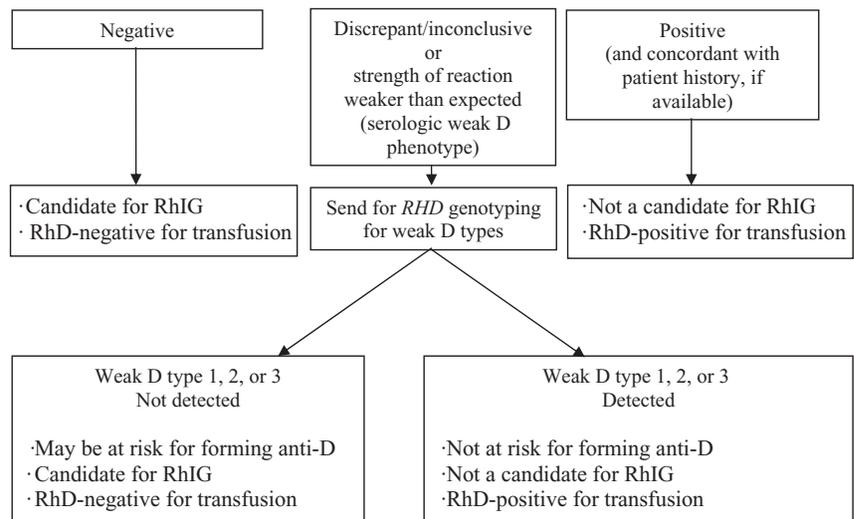


Fig. 3. Algorithm for resolving serologic weak D phenotype test results by *RHD* genotyping to determine candidacy for RhIG and RhD type for transfusions.

large-scale testing. Reference laboratories performing RBC genotyping services should offer tiered services, beginning with affordable first-tier testing, so that the most prevalent and clinically relevant *RHD* genotypes can be detected.

For women with a serologic weak D phenotype associated with *RHD* genotypes other than weak D type 1, 2, or 3, the Work Group recommends that these women receive conventional prophylaxis with RhIG, including postpartum RhIG if the newborn is RhD-positive or has a serologic weak D phenotype. Clinicians and investigators are encouraged to publish alloimmunization outcomes of pregnancies and transfusions of individuals with *RHD* genotypes for which the risk of RhD alloimmunization is unknown.

Finally, members of the Work Group emphasize the concept of a “phase-in” for implementing of these recommendations. We believe that it is time for *RHD* genotyping all patients whose routine RBC typing has resulted in detection of a serologic weak D phenotype. While recognizing the lack of comprehensive cost-benefit analyses, the Work Group concludes that it is time to begin to phase in selective *RHD* genotyping. It is the intent of this commentary to increase awareness of the available molecular science and promise of *RHD* genotyping and, thereby, to shorten the time for achieving the full benefits of a more comprehensive implementation of *RHD* genotyping. Phasing in *RHD* genotyping will apply modern genomic methods for more precise decision making in obstetric practice and transfusion medicine. It will foster the application of genomic science to promote more personalized and accurate medical care.¹²⁹

CONFLICT OF INTEREST

WAF receives royalties for *RHD* genotyping. GAD is named as an inventor of granted RBC genotyping European Patents Serial No. 2298784 and 2298785, currently owned by Canadian Blood Services. MAK receives financial support from Life Technologies for genotyping software development consulting, has received research support from BioArray, and has been on Immucor’s speakers bureau. RV receives research support from Fenwal. SGS, CMW, MD, STJ, LK, JTQ, and CDS have disclosed no conflicts of interest.

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