Policies and Procedures Related to Testing for Weak D Phenotypes and Administration of Rh Immune Globulin

Results and Recommendations Related to Supplemental Questions in the Comprehensive Transfusion Medicine Survey of the College of American Pathologists

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• ***Context.***—**Advances in *RHD* genotyping offer an opportunity to update policies and practices for testing weak D phenotypes and administration of Rh immune globulin to postpartum women.**

Objectives.—**To repeat questions from a 1999 College of American Pathologists proficiency test survey, to evaluate current practices for testing for weak D and administration of Rh immune globulin, and to determine whether there is an opportunity to begin integrating *RHD* genotyping in laboratory practice.**

Design.—**The College of American Pathologists Transfusion Medicine Resource Committee sent questions from the 1999 survey to laboratories that participated in the 2012 proficiency test survey. The results of the 2012 survey were compared with those from 1999. Results from published *RHD* genotyping studies were analyzed to determine if *RHD* genotyping could improve current policies and practices for serological Rh typing.**

Results.—**More than 3100 survey participants responded to the 2012 questions. The most significant finding was a decrease in the number of transfusion services performing a serological weak D test on patients as a strategy to manage those with a weak D as Rh negative (from 58.2% to 19.8%, $P < .001$). Data from *RHD* genotyping studies indicate that approximately 95% of women with a serological weak D could be managed safely and more logically as Rh positive.**

Conclusions.—**Selective integration of *RHD* genotyping policies and practices could improve the accuracy of Rh typing results, reduce unnecessary administration of Rh immune globulin in women with a weak D, and decrease transfusion of Rh-negative red blood cells in most recipients with a serological weak D phenotype.**

(*Arch Pathol Lab Med.* 2014;138:620–625; doi: 10.5858/arpa.2013-0141-CP)

In 1999, the College of American Pathologists (CAP) Transfusion Medicine Resource Committee (TMRC) conducted a survey of policies and procedures for weak D phenotype testing and administration of Rh immune globulin in women with a weak D phenotype.[1] At the time of that survey, there was concern in the transfusion medicine community that laboratory methods for determining Rh (D) types were inadequate because of case reports of persons with a weak D phenotype forming anti-D after exposure to D-positive red blood cells by transfusion or pregnancy.[2–12] In at least 3 cases, a subsequent pregnancy was complicated by fatal Rh hemolytic disease of the fetus or newborn.[13–15] In an effort to decrease this risk of pregnancy, the editors of *Standards for Blood Banks and Transfusion Services* (hereafter *Standards*) of the American Association of Blood Banks (now AABB) advised that a test for weak D was "unnecessary when testing recipient red cells."[16(58)] The intent of this guidance was to encourage transfusion services to use Rh typing methods that would categorize women of childbearing potential (and other patients) who had a weak D phenotype as Rh negative. This practice decreased the potential exposure to D-positive red blood cells by all persons with a serological weak D phenotype and qualified women with a serological weak D phenotype for Rh

Also see p. 585.

Accepted for publication June 13, 2013.

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The authors have no relevant financial interest in the products or companies described in this article.

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immunoprophylaxis. In contrast to this guidance for Rh typing patients, *Standards* required blood donors to be Rh typed by a weak D method if the direct agglutination test with anti-D was negative.[16] This practice for decreasing the risk of persons with a serological weak D phenotype for Rh alloimmunization had been in effect for decades before the 1999 CAP TMRC survey. The results of the 1999 CAP TMRC survey revealed significant variation among transfusion services in their policies and procedures for serological weak D typing and administration of Rh immune globulin for women with a serological weak D phenotype.[1] In the 13 years since that survey was conducted, case reports of Rh-positive persons forming anti-D have continued to accumulate,[17–25] including at least 1 additional case of fatal Rh hemolytic disease of the newborn.[26] Also, there has been considerable progress in elucidating the molecular basis of the weak D phenotype.[27–39] Specific *RHD* genotypes have been identified in persons with a serological weak D phenotype who formed or did not form anti-D after exposure to D-positive red blood cells.[17,27,32,36–39] Investigators have proposed algorithms based on *RHD* genotyping as alternatives to the practice of Rh typing patients and blood donors by various serological methods.[17,32,36,40–42]

As the first step in a comprehensive review and evaluation of current options for updating policies for weak D testing and administration of Rh immune globulin in women with a weak D phenotype, the CAP TMRC conducted a survey in 2012, repeating the questions of the 1999 survey. The present article summarizes the results of the 2012 survey, compares them with those of the 1999 survey, and comments on the opportunities for updating policies and procedures based on changes that have occurred.

MATERIALS AND METHODS

Supplemental questions related to laboratory testing for weak D and administration of Rh immune globulin were included in the CAP 2012 J-B Transfusion Medicine (Comprehensive) survey. These were the same supplemental questions related to testing for weak D phenotypes and administration of Rh immune globulin in women with weak D phenotypes that were sent to survey participants in the 1999 J-A Transfusion Medicine (Comprehensive) and Educational Survey Set. The 2012 survey was sent to more than 3500 participating institutions and hospital transfusion services. The results of the 2012 survey were tabulated by CAP staff. Comparisons of the responses between the 1999 and 2012 surveys and interpretations of the results were performed by us. Pearson χ^2 test was used to test for differences between the 1999 and 2012 survey results. This analysis was performed with SAS version 9.2 (SAS Institute Inc, Cary, North Carolina). $P < .01$ was considered significant.

After reviewing the results of the 2012 survey, the CAP TMRC met and deliberated whether comparable protection against Rh alloimmunization in persons with a weak D phenotype could be achieved by transitioning from current practice to one based on *RHD* genotyping. In this evaluation, the TMRC also considered the possibility of incorporating monoclonal anti-D Rh typing reagents as an alternative or a supplement to current Rh typing procedures. The TMRC reviewed the history of the current practice for Rh typing and evaluated the implications of the trend toward increased implementation of different procedures for Rh typing patients and blood donors. A summary of the information reviewed by the TMRC and the committee's recommendations is given in the Comment section.

RESULTS

More than 3100 participants responded to the 2012 survey, and more than 2900 submitted responses to at least 6 questions. Not all respondents responded to every question. Therefore, the number of responses differs for various questions. A copy of the questions in the 2012 survey and the response rates are included as digital online content (see supplemental digital content at www.archivesofpathology.org in the May 2014 table of contents).

Table 1. Number of Patients or Donors With the Weak D Phenotype and Anti-D Alloantibody Identified by Transfusion Services in the Past 12 Months

No. of Patients or Donors Identified	% of Transfusion Services[a]	
	1999	2012
0	68.1	74.6
1	10.9	10.6
2	6.4	5.9
3	3.7	2.9
4	1.8	0.9
5	1.6	0.7
>5	7.4	4.4

[a] Total of 3255 responses in 1999 and 2826 responses in 2012. The individual comparisons for 1999 versus 2012, as well as the aggregated total, were significantly different ($P < .001$, χ^2 test).

In 1999, a total of 58.2% (2087 of 3588) of participants responded that they routinely performed an antiglobulin test for the weak D phenotype on patients who tested negative by direct agglutination with anti-D reagents. In 2012, only 19.8% (634 of 3198) responded that they would routinely perform an antiglobulin test for weak D on patients who tested negative with anti-D by direct agglutination. There was a corresponding increase in routine testing for institutions that only test pregnant women, women of childbearing age, and other groups (eg, newborns). In 1999, this testing rate was 26.6% (954 of 3588) and increased to 33.7% (1079 of 3198) in 2012. This difference in the practice is statistically significant ($P < .001$).

In 1999, when asked how the patient's Rh type would be reported if found to be positive for weak D, 50.7% (1775 of 3498) indicated Rh positive compared with 46.9% (1348 of 2873) in 2012 ($P = .002$). In 1999, a total of 43.5% (1487 of 3417) of institutions responded that their policy would dictate transfusion with Rh-negative blood components for a person with the weak D phenotype compared with 49.2% (1426 of 2900) in 2012 ($P < .001$).

Responses to the question "In the past 12 months, how many patients or donors with the weak D phenotype and alloanti-D (transfusion- or pregnancy-related) has the transfusion service identified?" are listed in Table 1. The percentages of institutions reporting the detection of at least 1 patient or donor with a weak D phenotype and anti-D alloantibody were 31.9% (1100 of 3450) in 1999 and 25.3% (716 of 2826) in 2012 ($P < .001$).

The percentages of transfusion services that routinely recommended administration of Rh immune globulin to patients with the weak D phenotype who were transfused with various blood components from Rh-positive donors are listed in Table 2. In 1999, a total of 19.9% (237 of 1190) of institutions recommended administration of Rh immune globulin compared with 15.7% (363 of 2305) in 2012 ($P = .002$).

In 1999, a total of 75.4% (2476 of 3282) of transfusion services dispensed Rh immune globulin compared with 16.2% (533 of 3282) by pharmacies. In 2012, a total of 64.4% (1915 of 2972) of transfusion services dispensed Rh immune

Table 2. Percentage of Transfusion Services That Routinely Recommend Administration of Rh Immune Globulin to Women With a Weak D Phenotype

Potential Exposure to RhD-Positive Red Blood Cells	% of Transfusion Services That Recommend Rh Immune Globulin		
	1999[a]	2012[b]	*P* Value
Patients receiving D-positive red blood cells	19.9	15.7	.002
Patients receiving D-positive platelets	12.6	14.1	.21
Patients receiving D-positive plasma components	3.3	1.7	.002
Women of childbearing age who are receiving D-positive blood components	58.2	33.8	<.001
Pregnant women with a possible RhD-positive fetus	71.1	50.4	<.001

[a] Total of 1190 responses.
[b] Total of 2305 responses.

globulin compared with 26.5% (789 of 2972) by pharmacies. These results reflect a significant decrease in the number of transfusion services dispensing Rh immune globulin between the 1999 and 2012 surveys $P < .001$. This question was included in the 1999 survey as a safety issue. Rh immune globulin should be issued for Rh prophylaxis only for Rh-negative women. Most hospitals dispense Rh immune globulin from the blood bank because matching patients' identification and laboratory test results is considered more reliable if performed in 1 versus 2 locations. We interpret the trend for hospitals to centralize dispensing of biologicals and pharmaceuticals in pharmacies to reflect increased reliance on computerized hospital records. Whether in the blood bank or in the pharmacy, matching patients' identification and laboratory test results is performed by accessing computerized medical records and laboratory test results. In one of our hospitals (MedStar Georgetown University Hospital, Washington, DC), the function of dispensing Rh immune globulin was transferred from the blood bank to the pharmacy to centralize purchasing to one distributor of hospital supplies.

Last, the survey asked whether Rh typing is always performed on every pregnant patient at the time of delivery. In 1999, a total of 39.8% (1256 of 3156) of transfusion services answered yes, and 32.3% (1019 of 3156) answered yes but only if no Rh type was already on record. In 2012, a total of 48.0% (1385 of 2886) of transfusion services answered yes, and 21.6% (622 of 2886) answered yes but only if no Rh type was on already on record. This represents a significant change in practice ($P < .001$).

COMMENT

The most important finding of the 2012 survey is the decrease in the percentage of transfusion services performing a serological weak D test on patients who test negative by direct agglutination with anti-D reagents (from 58.2% to 19.8%, $P < .001$). The availability of new serological test systems and molecular testing may account for some of the changes observed in the intervening 13 years. However, this change reflects an absolute and significant increase in the number of transfusion services implementing the practice of Rh typing patients by one method and blood donors by another. The immediate intent of this practice is to protect patients, particularly women of childbearing potential, who inherited a serological weak D phenotype from becoming Rh alloimmunized by exposure to D-positive red blood cells. The ultimate goal, reducing the incidence of Rh hemolytic disease of the fetus or newborn complicating pregnancies in women with a weak D phenotype, has been achieved by this practice but not without complications and controversy. The TMRC met and considered whether an equivalent decrease in the risk of Rh alloimmunization in persons with a serological weak D phenotype could be achieved by revising current policies and procedures to increase the role of *RHD* genotyping. The following is a summary of the issues that were discussed.

Historically, the practice of Rh typing transfusion recipients and blood donors by different procedures was introduced after the discovery in 1946 of the first weak D phenotype (D^u),[43] but the occasional practice did not become a formal policy until 1958, when it was adopted in the first edition of the AABB *Standards*.[44] In that initial definition, *Standards* required a weak D test if donors' blood typed as D-negative by direct agglutination using anti-D but regarded a direct agglutination method to be sufficient for Rh typing of transfusion recipients.[44] That standard has remained unchanged for more than 50 years. The current (29th) edition of *Standards*[45] requires a weak D test for blood donors, which protects Rh-negative recipients from exposure by transfusion to the uncommon serological weak D unit, which could cause Rh alloimmunization. The current edition of *Standards* considers a weak D test for transfusion recipients unnecessary, categorizing weak D recipients as Rh negative and protecting them from inadvertent exposure to D-positive red blood cells, which might cause Rh alloimmunization.[45] Regarding the history of administration of Rh immune globulin to pregnant women with a weak D phenotype, the American College of Obstetricians and Gynecologists (ACOG) first addressed the subject in a 1981 practice bulletin.[46] ACOG recommended that "[w]hen an Rho (D) negative (whether D^u positive or D^u negative) woman who is not Rho (D) isoimmunized delivers an Rho (D) or D^u positive infant, she becomes a candidate for Rh immune globulin (RHIG) treatment."[46] The ACOG policy was controversial because most blood bank specialists at the time considered women who were D^u positive to be Rh positive and, therefore, not candidates for Rh immune globulin.[47] According to a contemporary account of the issue, ACOG modified the policy and issued a supplemental statement that "[a] woman who is genetically D^u positive is Rh-positive and administration of Rh immune globulin is unnecessary."[48(66)] The most recent guidance from ACOG is in a 1999 practice bulletin,[48] which recommends that women with a serological weak D should be considered Rh positive and not receive Rh immune globulin. The policy of the AABB for administration of Rh immune globulin to women with a weak D phenotype (then D^u) by determining their Rh phenotype by direct agglutination (only) was introduced in 1981 in the 10th edition of the AABB *Standards*.[49] At that time, a woman's candidacy for Rh immune globulin was determined using the same laboratory

method as that for Rh typing blood donors. Therefore, women with a serological weak D phenotype were categorized as Rh positive and not considered candidates for Rh immune globulin. The current (29th) edition of *Standards* determines a pregnant woman's candidacy for Rh immune globulin using the same Rh typing method as that for a transfusion recipient; that is, "if a woman's test for D antigen is negative. . .a test for weak D is not required."[45]

Despite the success of decreasing the risk of Rh alloimmunization in persons with a serological weak D phenotype, there are drawbacks to the current practice of Rh typing blood donors by one method and Rh typing transfusion recipients (and pregnant women) by another method. First, as evidenced by the CAP TMRC 2012 survey, 80.2% of transfusion services do not routinely perform an antiglobulin test for weak D on patients who tested negative with anti-D by direct agglutination. As a result, most persons who have inherited a serological weak D phenotype will be typed as Rh positive when presenting as a blood donor, in compliance with *Standards*, but typed as Rh negative when presenting as a patient. Although this policy may be expedient in the short term, it is confusing to patients and caregivers when experienced over a lifetime of blood donation and an occasional pregnancy or hospitalization.[25] Also, approximately 95% of women with a serological weak D are not at risk of forming anti-D,[17,42] but as evidenced by the results of the 2012 CAP TMRC survey, most of them are managed for pregnancies and transfusions as Rh negative. Therefore, most of them will receive a routine, but unnecessary, antenatal injection of Rh immune globulin if pregnant and a second injection of Rh immune globulin postpartum if they deliver an Rh-positive newborn. Last, approximately 95% of potential transfusion recipients with a weak D phenotype do not require Rh-negative red blood cells if they are transfused, but most of them will be transfused with D-negative red blood cells, unnecessarily depleting an already borderline inventory of Rh-negative red blood cells.[17,42] Approximately 15% of persons of white race/ethnicity, 8% of Africans, and 1% of Asians are Rh negative.[50] The number of individuals of white race/ethnicity who have inherited a weak D phenotype is estimated to be 0.2% to 1%.[51] Of these, approximately 95% have an *RHD* allele that has not been associated with forming anti-D.[17,42] The prevalence of weak D phenotypes varies considerably by race, ethnic group, and geography.[52–58] Emigrants (or their descendants) from Africa, Asia, or other distant geographic areas where certain unique *RHD* genotypes occur are increasingly present in blood donor services in the United States. Blood donor services in the United States (and elsewhere) will require policies for detecting and managing *DEL* and other uncommon *RHD* genotypes with a potential for Rh alloimmunization.

The CAP TMRC reviewed the state of science of the molecular basis of weak D phenotypes.[27–42] Genotyping studies of *RHD* have established that the probability a person with a serological weak D phenotype will form anti-D after exposure to D-positive red blood cells is determined by the cellular location of the mutant D antigen.[17,24,27,28,32,42] Most, but not all, persons with a serological weak D phenotype who have formed anti-D have a mutant D antigen located on an exterior (exofacial) loop of the D antigen (ie, on or above the red blood cell membrane). Examples are partial DVI (typing as negative by current licensed reagents) and weak D types 4.2 (*DAR*), 7, 11, 15, and 21. Most people whose mutant D antigens are located on an interior or a transmembrane (intracellular) domain (ie, within or below the red cell membrane) are unlikely to form anti-D. Examples are weak D types 1, 2, 3, and 4.0/4.1. Exceptions to these observations exist, but the principle applies to the majority. There are reports also of anti-D being formed by persons with a Del phenotype, which is not detected by current Rh typing procedures for either patients or blood donors.[20,59,60]

An alternative to the practice of serological Rh typing or *RHD* genotyping includes algorithms using selective monoclonal anti-D reagents that identify D phenotypes (such as DVI) as partial or mosaic D antigens that have been associated with formation of anti-D.[56,61,62] Such algorithms have been implemented in blood centers in Europe and India. In 2005, Denomme and colleagues[34] proposed a 2-monoclonal anti-D reagent algorithm to establish the Rh status of obstetrical patients and potential transfusion recipients that could result in 3% of patients who have a serological weak D phenotype and are currently typed as Rh negative to be safely transfused with Rh-positive red blood cells. Despite these promising reports, clinical experience in transfusion services reveals that currently available monoclonal anti-D reagents have variable reactivity with weak and partial D phenotypes and do not reliably distinguish those D phenotypes that have and have not been associated with formation of anti-D. The gold standard for distinguishing those persons with a serological weak D phenotype who may form anti-D after exposure to D-positive red blood cells from those who will not is *RHD* genotyping.

Another limitation of current monoclonal antibodies is that, like conventional polyclonal anti-D reagents, they do not identify Del phenotypes by direct agglutination. Del, formerly D_{el}, is the weakest of the D antigens and is routinely detected only by adsorption and elution of anti-D. Typically, Del red blood cells express 200 or fewer copies of the D antigen per red blood cell.[63] The Del phenotype has been detected in approximately 30% of East Asians (Hong Kong, Taiwan, and mainland China).[64] In the current era of frequent migrations, the Del phenotype is of global interest because of an increasing number of reports that it has caused Rh alloimmunization when Del red blood cells were transfused as Rh negative or by pregnancy.[65–68]

Despite the advances in monoclonal antibody technology and *RHD* genotyping, there are no updated organizational guidelines to advise practitioners whether or not to administer Rh immune globulin to pregnant women with a weak D phenotype. Several investigators have proposed using *RHD* genotypes to identify the few persons with a serological weak D phenotype who are at risk of forming anti-D if exposed to D-positive red blood cells. Wagner and colleagues[17] proposed an Rh typing and transfusion strategy based on D antigen density that would interpret approximately 95% of all persons of white race/ethnicity with a weak D phenotype as Rh positive. Legler and colleagues[32] proposed an *RHD* sequencing method for selective application when serological reactions are inconclusive. Flegel[37] proposed managing weak D phenotypes based on the observation that most weak D phenotypes are associated with specific *CDE* haplotypes and supplementing sensitive gel serological testing with monoclonal reagents and selective *RHD* genotyping. In 2011, Flegel[42] reported that more than 10 years after the molecular description of weak D types 1, 2, 3, and 4.0/4.1, which represent more than 95% of weak D phenotypes in European and white populations,

there have been no reported cases of alloimmunization and formation of alloanti-D. Brajovich and colleagues[41] performed *RHD* genotyping on serological weak D samples from 18 379 patients in Argentina and concluded that transfusing weak D types 1, 2, and 3 with D-positive red blood cells could safely save more than 5% of D-negative units of red blood cells.

The CAP TMRC concluded that the results of the 2012 CAP survey indicate a trend among participating institutions that does not incorporate the potential advantages of current evidence-based science of *RHD* genotyping. A scan of the RhesusBase database (www.uni-ulm.de/~fwagner/RH/RB2/ [accessed April 29, 2013]) revealed a listing of more than 200 *RHD* alleles sorted by mutation, including 90 weak D types, 28 D categories, and 30 *DEL* alleles. Organizations interested in establishing policy and clinicians seeking to utilize the benefits of scientific investigation have a challenge to keep pace with the evolving molecular science. The findings of the CAP 2012 survey present an opportunity to update guidelines for Rh typing and qualifications for administration of Rh immune globulin to women with a serological weak D phenotype according to specific *RHD* genotypes. Although the current state of molecular science does not identify all *RHD* genotypes capable of forming anti-D, it does identify approximately 95% who are not at risk of forming anti-D and, therefore, can be safely categorized as Rh positive. It is conceivable that the cost savings from decreased administration of Rh immune globulin to women with a serological weak D who do not require Rh immunoprophylaxis will offset the cost of their once-in-a-lifetime molecular testing.[69] A policy to phase in *RHD* genotyping for potential transfusion recipients with a serological weak D phenotype, estimated to be 0.2% to 1.0% in the United States, could potentially decrease the need for tens of thousands of units of Rh-negative red blood cells annually in the United States (12 million U/year times 15% Rh negative times 1% serological weak D phenotypes times 95% of *RHD* genotypes that do not require Rh negative times 1–2 U transfused per patient). The CAP TMRC believes that the practice for Rh typing blood donors by one method and patients by another method is overdue for a policy review. Because no up-to-date organizational guidelines are in effect, an opportunity exists for a multiorganizational collaboration among obstetricians, transfusion medicine specialists, serologists, and molecular scientists and, thereby, establish a nationwide uniform practice.

We thank the participants in the College of American Pathologists survey who responded to the supplemental questions; Rhona J. Souers, MS, who performed the statistical analyses; and Willy A. Flegel, MD, who reviewed the sections on molecular science.

Supplemental digital content is available for this article at www.archivesofpathology.org.

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