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Phasing-in *RHD* genotyping

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In this issue, Sandler and colleagues[1] report the results of the College of American Pathologists (CAP) J-B Transfusion Medicine (Comprehensive) and Educational Survey in which more than 3100 institutions describe how they perform Rh typing for blood donors, pregnant women and hospital patients. In accordance with AABB *Standards*,[2] most hospital laboratories reported that they do not routinely perform a serological weak D test on pregnant women or transfusion recipients. This practice results in most pregnant women and hospital patients with a weak D phenotype being categorized and managed as Rh negative (Table 1).[2, 3] In contrast, a weak D test is performed routinely on blood donors whose red blood cells test D-negative by direct agglutination, resulting in most blood donors with a weak D being categorized and managed as Rh positive.[2] This 50-year-old practice appears to be relatively safe[4] and there are only a few published reports of persons with a weak D phenotype forming anti-D.[5–8] However, it confuses patients, blood donors and caregivers, and inappropriately utilizes Rh immune globulin and Rh negative red blood cells for many persons with a weak D who could be safely managed as Rh positive, if their genotypes were known.[3, 9, 10] The CAP Transfusion Medicine Resource Committee (TMRC) reviewed this practice in the context of the current state of science for *RHD* genotyping.[1] The TMRC concluded that selective integration of *RHD* genotyping of weak D phenotypes could improve the accuracy of Rh typing results, thereby reducing unnecessary administration of Rh immune globulin in women with a weak D, and decrease transfusion of Rh negative red blood cells in recipients with a weak D phenotype.[1]

The process of phasing-in *RHD* genotyping in clinical practice has begun in many hospitals, but as the CAP survey indicates, the majority of pregnant women and hospital patients in the United States continue to have their Rh type determined by outdated serological methods.[1]

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None of the serologic anti-D panels or molecular immunohematology tests discussed here have been licensed or approved by the Food and Drug Administration (FDA).

Authorship contributions: SDR recognized the weak D phenotype and followed the patient. WAF and AT evaluated the reference serology and molecular data.

Conflict of interest disclosure: WAF receives royalties and holds intellectual property rights for *RHD* genotyping. The other authors declare no competing interests relevant to this article.

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The first step in phasing-in *RHD* genotyping needs to begin in hospital laboratories. Those laboratories that do not routinely perform weak D tests for patients typing Rh negative by direct agglutination with anti-D should now begin to introduce Rh typing reagents and procedures selected to detect, not to avoid detection of, weak D phenotypes.

We recently encountered a 27-year-old North African woman who was designated as Rh negative for a Caesarean section. Her medical history and laboratory test results are representative of a common subset of patients[11] and illustrate how *RHD* genotyping can improve the management of patients with a weak D phenotype. We have summarized recommended guidance for diagnostic testing and clinical decision making in women with a weak D phenotype after delivery of a D-positive newborn (Table 2).

The woman's routine postpartum blood sample was strongly positive by a rosette fetal bleed screen, suggesting the presence of D+ fetal red blood cells in her circulation (fetomaternal hemorrhage). However, a quantitative acid-elution (Kleihauer-Betke) assay was negative, indicating that the D+ red blood cells in her circulation did not contain a significant amount of hemoglobin F, i.e., the red blood cells were not of fetal origin. A weak D test was positive, confirming the clinical impression that her red blood cells expressed an inherited weak D phenotype. Red blood cells from approximately 0.2% – 1.0% of Caucasians express a weak D phenotype.[12] A weak D phenotype has been reported in 0.1 – 10% of all pregnancies that initially typed as D-negative.[13–15] We estimate that approximately 95% of patients in the United States with a weak D phenotype will have one of the *RHD* genotypes that is prevalent in Caucasians (types 1, 2, 3, or 4.1).[5, 7, 11, 16] Women with one of these prevalent *RHD* genotypes may be managed as Rh positive and do not require Rh immune globulin for prenatal or postpartum Rh immunoprophylaxis.[7, 17] However, that decision can only be made by *RHD* genotyping. Even monoclonal anti-D reagents, which were initially believed to capable of identifying *RHD* genotypes, cannot distinguish among the most prevalent weak D genotypes (Table 3).[6, 18, 19] We performed molecular testing on our patient[20] and established that she had inherited the uncommon weak D type 25,[21] which requires management as Rh negative for purposes of Rh immunoprophylaxis and transfusion of red blood cells.

The second step in phasing-in *RHD* genotyping will be establishing standardized, cost effective *RHD* genotyping protocols for laboratories. Most hospitals will not have a sufficient volume of patients with a weak D phenotype to justify establishing in-hospital *RHD* genotyping services. Hospitals are likely to refer blood samples to regional reference laboratories where high test volumes will support both basic and complex genotyping services. A molecular test in D-negative pregnancies may pay for itself by avoiding the costs associated with often unnecessary multiple administrations of RhIG.[4, 17, 22] Presently, there are no FDA-approved molecular test kits for determining the Rh type, but several unlicensed commercial kits are marketed commercially in the United States. Products utilizing PCR with sequence-specific primers (PCR-SSP) include BAGene Weak D-TYPE and LIFECODES Red Cell EZ Type Weak D (GTI Diagnostics, Waukesha, WI). High throughput methods utilizing multiplex PCR techniques include the BLOODchip v2.0 (Progenika; Balboa, Spain) and the BioArray RHD Beadchip (Immucor; Norcross, GA).

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Any of these test kits can be used for patient care as "tests of high complexity" under the Clinical Laboratory Improvement Act (CLIA).

Based on the results of their 2012 survey and review of the science of *RHD* genotyping, the CAP TMRC has recommended a multi-organizational collaboration among obstetricians, transfusion medicine specialists, serologists, and molecular scientists to update current practice guidelines and establish a nationwide uniform practice.[1] The CAP and AABB have formed a Work Group on Phasing-In *RHD* Genotyping. We believe that the time has come to transition from serological to molecular methods for managing weak D phenotypes. Our case illustrates how easily this transition can be accomplished. We support the CAP TMRC's initiative.

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Table 1

Guidance for weak D typing in the clinical laboratory (standard of care)

Guidance	Document	Text
Requirements	AABB Standards section 5.28.2	Women who are pregnant or who have been pregnant recently shall be considered for Rh Immune Globulin administration when all of the following apply: **1** The woman's test for D antigen is negative. A test for weak D is not required. **2** The woman is not known to be actively immunized to the D antigen. **3** The Rh type of the fetus/infant is unknown or the type of the fetus/infant is positive when tested for D or weak D. Weak D testing is required when the test for D is negative.[2]
	AABB Standards section 5.8.2	Testing of Donor Blood: If the initial test with anti-D is negative, the blood shall be tested using a method designed to detect weak D.[2]
Recommendations	AABB Technical Manual Chapter 22	Women with red cells that are clearly positive on the weak D test should be considered D positive and not receive RhIG, although rarely a positive weak D test can be caused by a partial D antigen.[3]

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Table 1

Diagnostic tests performed for clinical decision making in the mother after delivery of a D positive neonate

Test	Required per	Purpose	Result	Conclusion	Next step
D typing *	AABB Standards	Determine D positive/D negative status of patient	Negative	Patient is D negative	Check for fetal blood in mother's circulation
Rosette fetal bleed screen †	AABB Technical Manual	Detection of D positive RBCs in a D negative mother after delivery	Positive	D positive RBCs are present in mother's circulation	**1** Determine if D positive RBCs are fetal in origin **2** Quantify D positive RBCs to appropriately dose RhIG
Kleihauer-Betke test ‡	AABB Technical Manual	Quantification of fetal RBCs	Negative	**1** No fetal RBCs are present **2** Positive rosette test is caused by mother's RBCs	Use a more sensitive test for D typing
Weak D test §	At discretion of Medical Director	Determine if mother carries a weak D phenotype	Positive	**1** Mother carries a weak D phenotype **2** Serologic weak D test is not conclusive to determine anti-D immunization potential	Use a conclusive test to determine if RhIG is needed
RHD genotyping ‖	At discretion of Medical Director	Test for the prevalent weak D types 1, 2, 3 and 4.1	Negative	**1** Mother carries none of the prevalent weak D types (which are not prone to anti-D alloimmunization) **2** Immunization by the D positive fetus may occur	Administer RhIG

* immediate spin (not incubated and without antiglobulin): using 2 different monoclonal anti-D reagents (clones MS201/IgM and MS26/IgG, Series 4; and clones MS201/IgM and TH28/IgG, Series 5; Immucor, Norcross, GA)

† Fetal Bleed Screening Test (Immucor). This kit was phased out in 2013 and replaced by a method with a shorter incubation time.

‡ Fetal Cell Stain Kit (Simmler, High Ridge, MO)

§ incubated and with antiglobulin: using monoclonal anti-D (clones GAMA 401/ IgM and F8D8/IgG, Gamma-clone; Immucor) and anti-human globulin (anti-IgG or anti-IgG, -C3d; polyspecific; Immucor)

‖ BAGene DNA-SSP WEAK D-Type Kit (BAG Health Care, Lich, Germany)

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Table 3

Serologic reactivity with 21 monoclonal anti-D reagents

Monoclonal Anti-D					Controls				
No.	Clone	Isotype	Epitope*	Patient CcDee †	Weak D type 1 CcDee	Weak D type 2 ccDEe	Weak D type 3 CcDee	Partial DVII CcDee	Normal D CcDee
Panel 1‡									
A	LHM76/58	$IgG_{1\lambda}$	ND	++++	++++	+++	++++	++++	++++
B	LHM76/59	IgG_{1}	ND	++++	++++	+++	++++	++++	++++
C	LHM174/102	$IgG_{3\kappa}$	1.2	+ (w)	+++	++	+++	++++	++++
D	LHM50/2B	$IgG_{1\lambda}$	6.3	++++	++++	++++	++++	++++	++++
E	LHM169/81	$IgG_{3\kappa}$	1.1	++++	++++	++++	++++	++++	++++
F	ESD1	$IgG_{1\kappa}$	ND	++++	++++	++++	++++	++++	++++
G	LHM76/55	$IgG_{1\kappa}$	3.1	++++	++++	+++	++++	++++	++++
H	LHM77/64	$IgG_{1\kappa}$	9.1	++++	++++	+++	++++	++++	++++
I	LHM70/45	$IgG_{1\lambda}$	1.2	0	+++	+	+++	++++	++++
J	LHM59/19	$IgG_{3\kappa}$	8.1	++++	+++	+++	++++	0	++++
K	LHM169/80	$IgG3_{\lambda}$	6.3	+++	++++	+++	++++	++++	++++
L	LHM57/17	$IgG_{1\lambda}$	6.3	+ (w)	+	++	++	++++	+++
Panel 2¶									
1	HM10	IgM	6.6	0	++++	0	++	++++	++++
2	HM16	IgG	6.4	++++	++++	+++	++++	++++	++++
3	P3x61	IgM	6.1	0	++++	++	++++	++++	++++
4	P3x35	IgG	5.4	0	++++	+++	++++	++++	++++
5	P3x21211F1	IgM	8.2	0	+	0	0	0	++++
6	P3x21223B10	IgM	9.1	0	++	0	++	++	+++
7	P3x241	IgG	5.4	++++	++++	++++	++++	++++	++++
8	P3x249	IgG	2.1	++++	++++	++++	++++	++++	++++
9	P3x290	IgG	3.1	++++	++++	++++	++++	++++	++++

*Epitope patterns (epD) as described previously[18]

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† GenBank accession number JX495049

‡ Advanced partial RhD typing kit (Alba Bioscience, Edinburgh, UK)

¶ D-Screen (Diagast, Loos, France)