Use of RHD Genotyping for Serologic Weak D Phenotypes

Trina Horn, MS, MLT(ASCP)SBB<sup>CM</sup>
Laboratory Manager
National Molecular Laboratory
American Red Cross
Philadelphia, PA
Trina.Horn@redcross.org

The need is constant.
The gratification is instant.
Give blood.™
Objectives

- To review the Joint Statement on *RHD* genotyping in cases of serologic weak D
- To use case examples to illustrate how *RHD* genotyping can be used
- To review *RHD* genotyping results in a cohort of patients tested by the American Red Cross
- To provide a global context to the use of *RHD* genotyping in Transfusion medicine
What's all the fuss?

More Precise Blood Typing Tests Improve Care for Mothers and Babies

Rh immune globulin, or RhIg, has been successful in preventing RhD alloimmunization and hemolytic disease of the fetus or newborn. However, the practice of interpreting serologic weak D phenotypes as Rh-negative for female patients of childbearing years may lead to unnecessary injections of RhIg. An interorganizational work group has now recommended integrating RHD genotyping into laboratory practice to improve the accuracy of RhD typing. The recommendation supports the use of genomic techniques to provide more precise medical care.
What’s all the fuss?

Groups urge phase-in of RHD genotyping

Approximately 0.2–1.0 percent of Caucasians inherit a weak D phenotype, and under conventional laboratory practices of long standing, patients have been Rh-typed using methods that interpret weak D phenotypes as Rh-negative. But, as Anne Eder, MD, PhD, explains, current practice is inconsistent. In most clinical laboratories, pregnant women are not tested for weak D.

Anne Eder, MD, PhD

Read the full story »
Joint Statement on Phasing-In RHD Genotyping for Pregnant Women and Other Females of Childbearing Potential with a Serologic Weak D Phenotype

Background
AABB and the College of American Pathologists sponsored an Interorganizational Work Group on RHD Genotyping that was charged with developing recommendations to clarify clinical issues related to Rh blood typing in persons with a serologic weak D phenotype. Red blood cells that express a weak D phenotype, formerly D[Superscript *], agglutinate weakly or not at all using anti-D typing reagents, but agglutinate moderately or strongly after the addition of an antihuman globulin reagent, i.e., a positive weak D test. An estimated 0.2% - 1.0% of Caucasians inherit a weak D phenotype. In a racially and ethnically diverse urban population in the United States, approximately 80% of persons with a weak D phenotype were identified to have a weak D type 1, 2 or 3 when additional testing included RHD genotyping. Persons with a weak D type 1, 2 or 3 can be managed safely as Rh-positive and such women, if pregnant, do not require Rh immune globulin. For more than 50 years, the recommended practice in the United States has been to Rh type patients using laboratory methods that interpret weak D phenotypes as Rh-negative. The intent of this practice has been to protect Rh-negative persons, particularly Rh-negative women of childbearing potential, from inadvertent exposure and alloimmunization to Rh-positive red blood cells. RHD genotyping methods are now available that can identify those persons with a weak D phenotype who can be managed as Rh-positive (weak D types 1, 2 or 3).
What’s all the fuss?

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

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

S. Gerald Sandler, MD; Susan D. Rosell, MD; Ronald E. Domen, MD; Beth Shaz, MD; Jerome L. Gottschall, MD; for the College of American Pathologists Transfusion Medicine Resource Committee

Survey of >3100 laboratories participating a CAP proficiency test exploring the management of a serological weak D phenotype
AABB BBTS Standards, 29th Ed.

- **AABB Std 5.8.2 - Donors:**
  - If the initial test with anti-D is negative, the blood shall be tested using a method designed to detect weak D.

- **AABB Std 5.14.2 - Recipients:**
  - Rh type shall be determined with anti-D reagent. The test for weak D is unnecessary when testing the patient.

- **AABB Std 5.30.2 - Newborns of D− moms**
  - Weak D testing is required when the test for D is negative.
Anti-D Reagent Information

With current monoclonal reagents:

- Most D+ RBCs react at Immediate Spin (IS)
- Many partial D RBCs react at IS
  - Partial D status is often not known until anti-D detected
- IgG component is needed in tube test for “weak D” detection
- No single monoclonal anti-D reagent will detect all D+ RBCs
- Polyclonal anti-D by AHG phase detects most partial and weak D, except D_{el}
Challenges of Serologic Typing

- **Serologic methods vary**
  - Tube
  - Gel
  - Solid phase
- **Serologic reagents vary** or are unavailable
  - Monoclonal
  - Polyclonal
  - Blends
  - Patient source
- Simple (eg. $Fy^a/Fy^b$) vs **complex** (eg. RhD) epitopes
- **Antigen variants can be missed** (eg. partial D)
- **Expression level can hamper detection** (eg. weak D)
- **Cross-reactivity** (eg. $RHCE^{*}ceHAR$, $RHCE^{*}ceCF$)
Strengths of Molecular Typing

- RBC genotyping not hampered by
  - Recent transfusion
  - Antibody coated RBCs
  - Expression levels
- Genotyping can determine zygosity (eg, RhD)
- Genotyping can distinguish variants (eg, C, e)
- Genotyping can detect silenced alleles (eg, Fy\textsuperscript{b}/GATA)
- Genotyping can be multiplexed and batched
  - Multiple antigens
  - Multiple individuals

Genetic Variation in *RHD* gene
...more than 100 variants characterized

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The Blood Group Antigen FactsBook 2012
Weak D

- 76 distinct alleles
- Many shown to have low D antigen copy number
- Requires weak D test (indirect antiglobulin test) previously called the D\textsuperscript{u} Test to detect
- Reactivity is varied
  - According to sample (#D sites per RBC)
  - According to method (sensitivity of test)
  - Most detected by Blood Bank Automation which detects \( \geq 1+ \) weak D test (AHG phase in tube)
- 0.2 to 1% of the Caucasian population
Partial D

- 11 alleles with SNPs that alter RhD epitopes
- 45 different hybrid genes
- May not be distinguished from weak D serologically
- Often not recognized until they make anti-D
- D antigen lacks defined epitope(s)
- Serologic Identification
  - Monoclonal anti-D patterns identify the subtypes
**D<sub>el</sub>**

- 10 alleles identified
- Negative in IS and IAT tube tests
- Negative in automated tests
- Very low levels of D antigen detected by adsorption/elution
- Most frequently found in East Asians (~25-30% of D–)
- Few case reports of anti-D formed when blood from D<sub>el</sub> donors transfused to D– recipients
Weak D Types 1, 2 and 3

How I manage donors and patients with a weak D phenotype

Willy A Flegel

Institute for Clinical Transfusion Medicine and Immunogenetics, Ulm, Germany.


DOI: 10.1097/01.moh.0000245694.70135.c3

Source: PubMed

ABSTRACT

Since the adoption of molecular blood-group typing, the considerable heterogeneity of the serologic entities weak D and DEL at the molecular level has come to light. I offer an approach to the management of donors and patients expressing D antigen weakly and carrying any of the various molecular types of weak D and DEL.

More than 60 distinct weak D alleles have been described. An internet-based survey of anti-D immunizations occurring in D-positive transfusion recipients reveals that no allo-anti-D has been observed in patients carrying prevalent weak D types. Anti-immunizations are documented for weak D types 4.2 (also known as DAB1.11 and 15). Anti-D immunizations have been reported in D-negative persons transfused with Patients carrying any of the prevalent weak D types 1 immunization and may safely be transfused with D-positive blood units that are labelled D-negative. The units from our supply of D-negative red blood cell units

Anti-D investigations in individuals expressing weak D Type 1 or weak D Type 2: allo- or autoantibodies?

Bach-Nga Pham, Michèle Roussel, Thierry Peyrard, Marylise Beolet, Véronique Jan-Lasserre, Dominique Gien, Maryline Ripeaux, Sébastien Bourgouin, Sandrine Kappler-Gratas, Philippe Rouger, and Pierre-Yves Le Pennec
**Work Group Recommendation**

*RHD* genotyping should be performed whenever a serological weak D phenotype is detected in a patient, including pregnant women.

Weak D types 1, 2 or 3 should be managed as RhD-positive with regard to administration of RhIG or for transfusion.

For women with a serological weak D phenotype associated with an *RHD* genotype *other* than weak D type 1, 2 or 3, the Work Group recommends conventional prophylaxis with RhIG.

It’s Time to Phase In *RHD* Genotyping

- **Negative**
  - Candidate for RhIG
  - RhD-negative for transfusion

- **Discrepant/inconclusive**
  - Strength of reaction weaker than expected
  - (serologic weak D phenotype)
  - Send for *RHD* genotyping for weak D types

- **Positive**
  - (and concordant with patient history, if available)
  - Not a candidate for RhIG
  - RhD-positive for transfusion

**Weak D type 1, 2, or 3**
- Not detected
  - May be at risk for forming anti-D
  - Candidate for RhIG
  - RhD-negative for transfusion

**Weak D type 1, 2, or 3**
- Detected
  - Not at risk for forming anti-D
  - Not a candidate for RhIG
  - RhD-positive for transfusion
Potential Benefits of *RHD* Genotyping: Pregnant Women

![Diagram](image)

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.

Objectives

- To review the Joint Statement on RHD genotyping in cases of serologic weak D
- **To use case examples to illustrate how RHD genotyping can be used**
- To review RHD genotyping results in a cohort of patients tested by the American Red Cross
- To provide a global context to the use of RHD genotyping in Transfusion medicine
Case 1

- 79 year old Caucasian female with ovarian cancer
- Types RhD- by hospital A
- Types RhD+ by hospital B
- Requested RHD genotyping
## Case 1 – *RHD* Genotyping

<table>
<thead>
<tr>
<th><strong>TESTING PERFORMED</strong></th>
<th><strong>RESULT</strong></th>
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<tbody>
<tr>
<td><strong>RHD Common</strong></td>
<td><strong>Method</strong></td>
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<td>RHD gene</td>
<td>Multiplex PCR</td>
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<td>PCR</td>
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<td><strong>RHD Variants</strong></td>
<td><strong>Method</strong></td>
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<tr>
<td>wRHD BEADCHIP™</td>
<td>RHD Array*</td>
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<td><strong>RHCE Common</strong></td>
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<td>Multiplex PCR</td>
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<tr>
<td><strong>RHCE Exon 5</strong></td>
<td>RFLP</td>
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</table>

*Array includes 35 markers. Only nucleotides that differ from the consensus sequence are listed.*
Case 1 – Results & Interpretation

Predicted Phenotype: Weak D+

Comments: Weak D type 1 is the most common type of weak D. RBCs have reduced D antigen density and may not be detected at immediate spin. Individuals with weak D type 1 are not considered at risk for alloimmunization by RhD.
Case 2

- 28 year old Caucasian female
- Pregnant
- Types RhD+ at immediate spin (w+ to 3+) with 3 of 4 sources of anti-D.
- Typed RhD- at IS and at AHG with the fourth anti-D.
- Requested RHD genotyping
**Case 2 – RHD Genotype and Phenotype**

Predicted Phenotype: D-, C-, E- c+ e+ DHAR+ FPPT+

The patient is not predicted to express RhD. They carry the RHCE*ceHAR allele. Individuals with a RHCE*ceHAR allele express D epitope(s) that type strongly D+ with some monoclonal anti-D reagents.

Such individuals should be considered RhD negative.
## Anti-D Cross Reactivity – RHCE*ceHAR

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<th>Reagent</th>
<th>Reactivity at Immediate Spin</th>
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<td>Gamma-clone</td>
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<tr>
<td>Immucor Series 4</td>
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<td>Immucor Series 5</td>
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<td>Ortho BioClone</td>
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<td>Ortho gel (ID-MTS)</td>
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<td>Seraclone IgM</td>
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<td>Seraclone blend</td>
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<tr>
<td>ALBAclone alpha &amp; beta IgM</td>
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<tr>
<td>ALBAclone blend</td>
<td>+</td>
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<tr>
<td>ALBAclone delta IgM</td>
<td>+</td>
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</table>
Anti-D Cross Reactivity – RHCE*ceCF

- Rare allele found in individuals of African descent
- *Does not encode* RhD
- Expresses RH46 (Crawford), a low incidence antigen (~0.1%)
- Reactive with a few anti-D reagents
  - anti-D clone GAMA401
  - Anti-D ALBAclone (?)
- Pregnant patients and transfusion recipients should be considered D negative and receive RhIG and D negative blood
Case 3

- 32 year old Caucasian female, post-partum
- RhD typing discrepancy
  - Patient typed repeatedly RhD negative with negative Ab screen
  - Post-partum, both patient and infant type RhD negative at IS and positive (3+) at AHG.
- Requested *RHD* variant workup
**Case 3 – Targeted Genotyping**

*Array includes 35 markers. Only nucleotides that differ from the consensus sequence are listed.*

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<td>Pst1 site in HRB</td>
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<td><strong>RHD Variants</strong></td>
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<td>w<em>RHD</em></td>
<td><em>RHD array</em></td>
<td>Nucleotide (Amino Acid)</td>
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</table>

**Probable RHD Genotype:** *RHD*01 / *RHD*01N.01

**Predicted RhD phenotype:** D+
Genotyping Methods: Resolution

**Low Resolution**
- Gel-based methods
- SSP-PCR for known SNPs
- PCR-RFLP for known SNPs

**Medium Resolution**
- Arrays such as BeadChip™

**High Resolution**
- DNA sequence analysis
- Exon scanning
- cDNA analysis
- Plasmid cloning and sequence analysis
Ruling out what the array doesn’t…

- cDNA analysis unsuccessful
- Genomic sequence analysis/exon scanning

<table>
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<tr>
<th>RHD Variants</th>
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<td>Exon 10</td>
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Case 3 – Revised Interpretation

Predicted Phenotype: Partial D+

Comments: The patient may be at risk for production of allo-anti-D and is at risk of allo anti-C.
Case 4

- 34 year old African American female with sickle cell disease
- RhD typing discrepancy between two monoclonal IgM-IgG blends
  - Positive with solid phase testing with Immucor’s series 4 anti-D (clones MS201 and MS26)
  - Negative with solid phase testing with Immucor’s series 5 anti-D (clones TH28 and MS26)
- RBCs type C+
- Requested RHD variant workup
Case 4 – RHD Genotype and Phenotype

Predicted Phenotype: Partial D+, Altered C+, E- c+ e+

Comments: The patient may be at risk for production of allo-anti-D and is at risk of allo anti-C.

Characterization of the RHCE genes may be warranted.
Case 4 – RHD Genotype and Phenotype

Predicted Phenotype: Partial D+, Altered C+, E- partial c+ partial e+ V+ VS+ hr^B-

Comments: The patient may be at risk for production of allo-anti-D and is at risk of allo anti-C, -c, -e, -hr^B.
Objectives

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- To provide a global context to the use of \textit{RHD} genotyping in Transfusion medicine
RHD Genotyping Cases: Serologic Weak D or Discrepant Type

Obstetrics (OB) Cases
Or
Women of Child-bearing age (WCBA)

OB or WCBA 67%

Other 33%
**RHD Genotyping Cases:**
Serologic Weak D or Discrepant Type

- **Race NP** 5%
- **non Cauc** 29%
- **Cauc** 32%
- **OB or WCBA**
- **Other** 34%
OB or WCBA by *RHD* Type

- Partial D: 52%
- Weak D type 2: 14%
- Weak D type 1: 15%
- D negative: 1%
- D positive: 10%
- Weak D type 3: 8%
OB or WCBA by RHD Type- Caucasians Only

- D negative: 2%
- D positive: 12%
- Partial D: 12%
- Weak D type 1: 30%
- Weak D type 2: 30%
- Weak D type 3: 14%

- RHD weak partial D type 4.0
- RHD*DAR
- RHD*DOL
- RHD*DFR
- RHD*DAU5
- RHD*DVI type 2
- RHD*DCS1
OB or WCBA by *RHD* Type
Non-Caucasians Only

- **D positive**: 5%
  - **D negative**: 2%
  - **weak D type**: 1%
  - **weak D type**: 3%

- **D positive**: 5%
  - **RHD weak partial D type 4.0**: 1%
  - **RHD*DAR**: 2%
  - **RHD*DIIla-CE(4-7)-D**: 2%
  - **RHD*DAU5**: 3%
  - **RHD*DAU2**: 2%
  - **RHD*DVI type 3**: 3%
  - **RHD*DCS1**: 4%
  - **RHD*DIVa (novel)**: 5%
  - **RHD*DAU4**: 6%
  - **RHD*DAU3**: 7%
  - **RHD*DAU0**: 8%
  - **RHD*DOL**: 9%

- **Partial D**: 89%
Objectives

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- \textbf{To provide a global context to the use of} \textit{RHD} genotyping in \textbf{Transfusion medicine}
  - In blood donors
  - In pregnancy
Brief Report

Frequency of DEL phenotype in RhD negative donor population

Sazia Samdani, Giacomo L. De Matteis

RESEARCH ARTICLE

Molecular basis of DEL phenotype in the Chinese population

Juan Gu, Xue-Dong Wang, Chao-Peng Shao, Jun Wang, An-Yuan Sun, Li-Hua Huang, and Zhao-Lin Pan

Six years’ experience performing RHD genotyping to confirm D– red blood cell units in Germany for preventing anti-D immunizations

Willy A. Flegel, Inge von Zabern, and Franz F. Wagner
NIPD using circulating cell free fetal (ccff) DNA in maternal plasma
- Netherlands
- Denmark
- Iceland
- Sweden
- UK

Fetal Rhesus D typing in Rhesus D negative mothers
NIPD has been used in the UK for the past decade for RhD- women who have been sensitised and have a history of haemolytic disease of the newborn (HDN) or have elevated levels of anti-D antibodies in pregnancy.

When the mother has a RhD- blood type, NIPD can be used to detect whether the fetus has inherited the \textit{RHD} gene from the father. If the woman is found to have a \textit{RHD}- fetus, there is no risk of HDN and they can have standard antenatal care. Women carrying an \textit{RHD}+ baby need to be closely monitored throughout pregnancy to determine optimum management, such as intrauterine transfusions or early delivery if necessary.

Research has shown that fetal \textit{RHD} typing in RhD- mothers is highly accurate from around 11 weeks’ gestation. In the future it may be possible to test all RhD- mothers in early pregnancy to determine the fetal \textit{RHD} type. This will allow the administration of anti-D prophylaxis to be targeted to only those women who are carrying a \textit{RHD}+ fetus.

http://www.rapid.nhs.uk/guides-to-nipd-nipt/introduction/
Conclusions

- The RhD antigen is genetically and structurally complex. RhD variants are often classified as weak, partial or \(D_{e1}\).
- Serologic testing differs in hospitals vs donor centers. Such testing can yield discordant results depending on the methods and reagents. \(RHD\) genotyping can be used to more accurately predict RhD expression.
- The newly released Joint Statement advises that when a patient is identified as weak D by serologic methods, \(RHD\) genotyping can be used to assess alloimmunization risk and appropriateness of RhIG.
- \(RHD\) genotyping can be used to identify D+ individuals who express partial D and are at risk of making allo anti-D. These individuals may also be at risk of making other Rh antibodies.
- The approach of integrating \(RHD\) genotyping into clinical care differs across the globe and as does the frequency and types of \(RHD\) variants.
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