The Conundrum over D

Who needs Rh negative blood and Rh immune globulin?

Harold A. Oberman Memorial Lecture
Current Topics in
Blood Banking 2015
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Dr. Connie M. Westhoff, SBB, PhD
Director,
Immunohematology and Genomics
New York Blood Center and Adjunct Assistant Professor
University of Pennsylvania,
Department of Laboratory Medicine, Division of Transfusion Medicine
Why is typing for RhD sometimes problematic?

- Large number of variables
  - Variation in D strength of antigen expression on some RBCs
  - Variation in test methods
  - Variation in the specificity of antibody clones and reagent formulations
  - Variation in interpretation
Why is typing for RhD sometimes problematic?

- The D antigen is NOT a single epitope on a red cell protein, unlike for example, Jka/b
- D typing detects the presence or absence of a entire red cell protein

Example: Most blood group antigens are single change

\[ \text{Aspartic acid at position 280 = Jk(a+)} \]
\[ \text{Asparagine at position 280 = Jk(b+)} \]
**RH Blood Group Locus – 2 Genes**

Rh “positive”

RHD

5’ \[\text{D antigen}
32-35 amino acid changes\] 3’

RhD

RhCE

C/c and E/e

5’

Rh “negative”

x deletion x

No RhD

RHCE

C/c and E/e

5’

D antigen
32-35 amino acid changes

C or c and E or e antigens

C/c

E/e

RhCE

RhD

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Gene conversion and rearrangement

- hair-pin loop structure
- genetic exchange common in duplicated genes/linked

Donor is not changed
New hybrid alleles and proteins
- part of RhD into RhCE
- part of RhCE into RhD
Partial D examples: \textit{RHD/RHCE} hybrid alleles

<table>
<thead>
<tr>
<th>RHD exons replaced with \textit{RHCE exons}</th>
</tr>
</thead>
<tbody>
<tr>
<td>RHD</td>
</tr>
<tr>
<td>DIIIa</td>
</tr>
<tr>
<td>DVI type 3</td>
</tr>
<tr>
<td>DIIIc</td>
</tr>
<tr>
<td>DIVa</td>
</tr>
<tr>
<td>DIVb</td>
</tr>
<tr>
<td>DIVbIII</td>
</tr>
<tr>
<td>DIVbIV</td>
</tr>
<tr>
<td>DV</td>
</tr>
<tr>
<td>DVI type 1</td>
</tr>
<tr>
<td>DVI type 2</td>
</tr>
<tr>
<td>DFR1</td>
</tr>
<tr>
<td>DFR2</td>
</tr>
<tr>
<td>DBT1</td>
</tr>
<tr>
<td>DBT2</td>
</tr>
<tr>
<td>New antigens</td>
</tr>
<tr>
<td>DAK</td>
</tr>
<tr>
<td>BARC</td>
</tr>
<tr>
<td>Go\textsuperscript{a}</td>
</tr>
<tr>
<td>Evans</td>
</tr>
<tr>
<td>DW</td>
</tr>
<tr>
<td>BARC</td>
</tr>
<tr>
<td>FPTT</td>
</tr>
<tr>
<td>Rh32</td>
</tr>
<tr>
<td>Rh32</td>
</tr>
</tbody>
</table>

Many type as D+ but patients can make anti-D
Partial DVI – associated with majority of cases of fatal HDFN (Caucasians)
Females (under age of 50) should receive Rh- blood; are RhIG candidates
I. Variation in D antigen expressed on RBCs

• More than 200 different RHD alleles in populations
  • single or multiple amino acid changes in RhD
  • could potentially be >200 different antigens or “D subgroups”

• Two Primary Categories
  • **Weak D**
    – changes decrease antigen expression level
    – definition: react weaker than expected (<2+ OR require IAT)
    – **Not at risk for anti-D** (rare exceptions, but no HDFN or HTR)
      • weak D types 1, 2, 3 most common
  • **Partial D**
    – changes alter the epitopes or epitopes are missing
    – **At risk for clinically significant anti-D**

Cannot be distinguished by routine serologic D typing
How many patients have altered RHD gene?

- 0.5%- as many as 4% of patients carry RHD genes with mutation(s)
  - incidence depends on ethnic group

- ~25% of sites in CAP survey reported they had seen at least one patient in the past 12 months with a serologic weak D phenotype who made alloanti-D

- Literature: >30 reports of D+ persons, presumed to have partial D, who made anti-D associated with HDFN

II. Variation in Test Methods to type for D

- **Manual tube** – with or without IAT (AHG) for serologic weak D
- **Gel card**
- **Solid phase**
- **PK** – enzyme treated cells for donor testing

### Manual Tube

<table>
<thead>
<tr>
<th>Reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>4+</td>
</tr>
<tr>
<td>3+</td>
</tr>
<tr>
<td>2+</td>
</tr>
<tr>
<td>1+</td>
</tr>
<tr>
<td>0</td>
</tr>
</tbody>
</table>

### Automation – ABO & Rh, antibody screening, identification

- **Gel Card**
- **BioTest TANGO™ Benchtop Blood Bank Analyzer**
- **Immucor Gamma’s Capture solid phase Echo and Neo**

### Solid Phase Capture

**Donor centers**

- PK 7600

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**New York Blood Center**
## II. Variation in Test Methods to type for D

### Donors

- **Goal:** prevent anti-D by detecting any D expression as positive
- **AABB requires** “method designed to detect weak expression of D”
  - U.S. – use of enzyme treated cells and two anti-D (PK instrument)
  - OR use of indirect antiglobulin test (IAT)

### Patients

- **Goal:** to prevent anti-D alloimmunization
- **AABB “testing for weak D expression by IAT not required/optional”**
  - exception: newborns when evaluating D- mothers for RhIG
II. Variation in Test Methods to type for D

• Donors
  – AABB requires “method designed to detect weak expression of D”

• Patients
  – AABB “testing for weak D expression by IAT not required/optional”

Why differ?
  – Patients
    • some partial D are only detected in the IAT
      • females and OB’s “may be better served as Rh negative”
      • Partial DVI only detected by IAT
    • concern for “false positive” (RBCs with +DAT, rouleaux, etc)

CAP survey: majority of hospitals do not do IAT for weak D for patients
History of D typing in U.S.

• Long recognized that donor and patient typing goals may differ
  – Donor: need more sensitive testing to avoid stimulating anti-D
  – Patient: no harm in treating patient as Rh negative

• 1960-70’s Polyclonal anti-D reagents (detect multiple epitopes)
  – Peter Issitt “tradition in blood banking demands that before a donor can be regarded
    as Rh negative he/she be shown not only to lack D antigen, but also C and E”
  – based on fact that weak D antigen expression is often inherited with C+ or E+
  – anti-CDE reagent was in wide use for donor testing

• 1980’s - Monoclonal anti-D reagents
  – Increased sensitivity IgM clones – many RBCs D+ at IAT - now reactive initial spin
  – Could select clones to specific D epitopes
  – Proposed different reagents: one for typing donors; one for patients
    • Too confusing
    • FDA: anti-D reagents for U.S. market MUST be non-reactive with DVI on initial testing (so these patients type as Rh negative)
    • Must react with DVI on IAT (so donors type as Rh positive)
### III. Variation in FDA licensed anti-D reagents

<table>
<thead>
<tr>
<th>Reagent</th>
<th>IgM monoclonal</th>
<th>IgG</th>
</tr>
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<tbody>
<tr>
<td>Gammaclone</td>
<td>GAMA401</td>
<td>F8D8 monoclonal</td>
</tr>
<tr>
<td>Immucor Series 4</td>
<td>MS201</td>
<td>MS26 monoclonal</td>
</tr>
<tr>
<td>Immucor Series 5</td>
<td>Th28</td>
<td>MS26 monoclonal</td>
</tr>
<tr>
<td>Ortho BioClone</td>
<td>MAD2</td>
<td>Polyclonal</td>
</tr>
<tr>
<td>Ortho Gel (ID-MTS)</td>
<td>MS201</td>
<td></td>
</tr>
<tr>
<td>Bio Rad RH1</td>
<td>BS226</td>
<td>BS221, H41 11B7</td>
</tr>
<tr>
<td>Bio Rad RH1 Blend</td>
<td>BS232</td>
<td></td>
</tr>
<tr>
<td>Alba Bioscience alpha</td>
<td>LDM1</td>
<td></td>
</tr>
<tr>
<td>Alba Bioscience beta</td>
<td>LDM3</td>
<td></td>
</tr>
<tr>
<td>Alba Bioscience delta</td>
<td>LDM1/ ESD1M</td>
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</tr>
<tr>
<td>Alba blend</td>
<td>LDM3</td>
<td>ESD1</td>
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- **majority contain different clones**
- often differ in reactivity with RBCs with partial D or weak D
- even the same clone can react differently
  - different potentiators are added
- reactivity with anti-D may differ depending on C or E status of the RBCs
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**Alba delta:**

FDA – this reagent for donor testing only detects partial DVI at initial spin

“**is not recommended for patient testing for transfusion**”
III. Variation in FDA licensed anti-D reagents

Manufacturer instructions & cautions vary

EXAMPLES:
• “Reactions less than 2+ should be evaluated since they may be false positive”

• “Agglutination <1+ at IS should be tested using alternative reagent by IAT prior to final determination”

• “Patients should not be classified as D+ on basis of a weak reaction with a single anti-D”

• “If a clear positive not obtained it is safer to classify the patient as D-”
IV. Variation in interpretation and practice

2014 CAP Survey of ~3,100 laboratories

• Reporting  (1992 D⁺ was renamed weak D and should no longer be used)
  – 47% as D positive        - 30% as “weak D”
  – 11% as D negative        - females or OB’s as D negative

• Treatment
  – D positive
    • Rh positive blood and no RhIG
    • risk for anti-D
  – D negative
    • conservative approach; avoids risk for anti-D
    • females- avoid risk for possible HDFN
    • results in excess use of Rh negative blood
    • results in excess use of Rh immune globulin
RHD genotyping (DNA testing) can distinguish

- **Weak D alleles**
  - **Types 1-76** (76 different point mutations)
  - Weak D type 1, 2, or 3 most common in Caucasian (~95%)
    - ARE NOT AT RISK

- **Partial D alleles**
  - >100 alleles with multiple changes
  - appear to lack epitopes
    - AT RISK

Weak D alleles more common in Caucasians
Partial D alleles more common in African-Americans
2014 Charge to Rh workgroup

Members: CAP, AABB, ACOG, ABC and ARC

- Develop a recommendation for RHD genotyping when a serological weak D phenotype is identified

- Goal: to begin phase-in the use of RHD genotyping

- A recommendation should help
  - clarify clinical issues related to RhD blood typing in pregnant women and transfusion recipients
  - while helping to avoid the unnecessary use of Rh immune globulin and transfusion of Rh-negative Red Blood Cells
Impact on the Blood Supply

O- RBC/WB Distribution

Number of Rh negative units needed to meet demand
Overall blood use declining, Rh negative usage increasing
Brigham and Women’s *RHD* genotyping for OB’s

- To guide RhIG prophylaxis and selection of blood for transfusion
  - OB women with D typing discrepancies
    - positive previously and now negative: or the reverse
    - Rh type from physician office different than hospital
  - D typing weaker than expected

### Table: Brigham and Women’s *RHD* Genotyping for OB’s

<table>
<thead>
<tr>
<th><em>RHD</em></th>
<th>weak D type 1</th>
<th>weak D type 2</th>
<th>weak D type 3</th>
<th>weak D type 4.0</th>
<th>Partial DAR</th>
<th>No RHD RHCE*ceCF</th>
<th>New alleles</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td># OB patients</td>
<td>16</td>
<td>9</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>36</td>
</tr>
<tr>
<td>% of total tested</td>
<td>44%</td>
<td>25%</td>
<td>5.5%</td>
<td>5.5%</td>
<td>11%</td>
<td>2.8%</td>
<td>5.5%</td>
<td>100%</td>
</tr>
<tr>
<td>Risk for anti-D</td>
<td>NO</td>
<td>Majority not at risk</td>
<td>YES</td>
<td>YES</td>
<td>UNKNOWN</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RhIG</td>
<td>Not candidate for RhIG</td>
<td>Candidate for RhIG</td>
<td>Candidate for RhIG</td>
<td>Candidate for RhIG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

75%  
25%

Patients are managed based according to their *RHD* genotype

Algorithm for Resolving Serologic Weak D Test Results by RHD Genotyping for Determining Candidacy for RhIG and Rh Type for Red Cell Transfusions

**Result of RhD typing by Manual Tube or Automated Methods**

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

- **Positive** (and concordant with patient history if available)
  - Not candidate for RhIG

- **Discrepant or Inconclusive or strength of reaction weaker than expected (serologic weak D phenotype)**
  - send for RHD genotyping for weak D type
    - **Weak D type 1, 2, or 3**
      - Not at risk for anti-D
        - Not candidate for RhIG
          - D positive for transfusion
    - **Weak D type 1, 2, or 3 Not detected**
      - May be at risk for anti-D
        - Candidate for RhIG
          - D negative for transfusion

- **D Positive**
  - Not candidate for RhIG
  - D positive for transfusion
Potential Benefits of *RHD* Genotyping Pregnant Women

- 3,953,000 Live births
- 3,812,000 Pregnancies
- 556,500 RhD-negative
- 16,700 Serologic Weak D
- 13,360 weak D types 1, 2 or 3
- 24,700 unnecessary ante- and postpartum RhIG injections

*RHD* Genotyping
Why be concerned about excess usage of RhIG?

• one of the greatest medical advances of the 1960’s
• Very safe product

BUT

• a human blood product
• manufactured from pooled plasma from paid donors
• must be actively immunized
• ethical issues when biologic products are administered unnecessarily
• are no reports of transmission of hepatitis B virus, hepatitis C virus, or HIV caused by RhIG manufactured in the United States
• always potential for emerging agents
Potential Benefit of RHD Genotyping Transfusion Recipients

5,000,000 Individuals Transfused Annually in US

730,000 RhD Negative

21,900 Serologic Weak D

17,520 weak D types 1, 2 or 3

Could receive RhD positive RBCs 47,700 units

RHD Genotyping
Rh Workgroup Recommendations

- **Definition of weak D serologic result**
  - weaker than expected reactivity (<2+)
  - depends on method, reagent, and local population being tested
  - institution should have policy

- Are not indicating institutions must change methods of typing or do an IAT on all female patients

- **Use RHD genotyping to resolve**
  - D typing discrepancies
  - weaker than expected reactivity

- **Use RHD genotyping to manage clinical decisions**
  - Determine candidates for Rh immune globulin
  - RhD status for blood transfusion
Rh Workgroup Recommendations

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 at this time.

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.

Clinicians and investigators are encouraged to publish outcomes of pregnancies and transfusions of individuals with *RHD* genotypes for which the risk of RhD alloimmunization is unknown.

**Phasing-in *RHD* genotyping** will apply modern genomic methods for more precise decision making in obstetrical practice and transfusion medicine.
Financial Implications of *RHD* genotyping for OB’s

**Cost-Benefit Analysis**
- RHD genotyping is an LDT – Laboratory Developed Test
- Research Use - RUO testing (CPT code)
- Performed in CLIA regulated laboratory
- Cost of testing has not “stabilized”

**Goal:** evaluate the costs of *RHD* genotyping for pregnant females with serologic weak D phenotypes
- using a comparison strategy of managing as D–
- *RHD* genotyping done at first visit/first pregnancy when Rh typing done and results made part of medical record
- direct medical costs assessed over 10- and 20-year periods for a simulated population of US women
- one-way and probabilistic sensitivity analyses used to assess the robustness of conclusions
## Cost Input Parameters – CMS reimbursement

<table>
<thead>
<tr>
<th>Testing and Product</th>
<th>Cost ($)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Initial Testing</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ABO Group</td>
<td>12.12</td>
<td>(9.09-15.15)</td>
</tr>
<tr>
<td>RhD Type</td>
<td>12.12</td>
<td>(9.09-15.15)</td>
</tr>
<tr>
<td><strong>Additional RhD Testing</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RHD Genotyping Assay</td>
<td>250</td>
<td>(100-500)</td>
</tr>
<tr>
<td>Cord Blood RhD Typing</td>
<td>30.33</td>
<td>(22.75-37.91)</td>
</tr>
<tr>
<td><strong>Blood Products</strong></td>
<td></td>
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</tr>
<tr>
<td>Rh Immune Globulin (300 μg dose)</td>
<td>162</td>
<td>(121.50-202.50)</td>
</tr>
<tr>
<td>Rh Immune Globulin Administration</td>
<td>9.60</td>
<td>(7.20-12.00)</td>
</tr>
</tbody>
</table>

Cost-savings over treating as Rh negative when **RHD genotyping** is ~ $256

**Financial implications of RHD genotyping of pregnant women with serologic weak D phenotype**

Limitations

• Did not address *detection* of partial D phenotypes
  – workgroup focus on clinical management of patients with a serologic weak D phenotype

  – some women with weak D+ will not be detected by method used
    • are typed as D negative
    • get unnecessary RhIG

  **Will require testing all Rh negative women by RHD genotyping**

  – women with partial D who type strongly D+ (partial DIIIa, DIVa)
    • are typed as D positive
    • do not get the needed RhIG
      – no cases associated with fatal HDFN in literature
      – but results in costly monitoring of an “at risk pregnancy”

  **Will require testing all Rh positive women by RHD genotyping**
Future for all pregnant women

Rh status will be determined by RHD genotyping
Summary Recent Publications in *Transfusion*

1. It’s time to phase in RHD genotyping for patients with a serologic weak D phenotype  
   - Commentary from RhD workgroup (ABC, AABB, CAP, ARC, ACOG)  
   - Goal to BEGIN standardization of practice

2. How do I manage Rh typing in obstetric patients?  
   Haspel R, Westhoff CM  
   *Transfusion 2015 55:470-74*  
   - 25% of women with discrepant or weak D typing were at risk  
   - 75% were weak D type 1, 2, or 3 and NOT at risk

3. Financial implications of RHD genotyping of pregnant women with serologic weak D phenotype  
   - Rather than managing as D-  
   - Cost-savings when cost of RHD genotyping is below $256
Thank You!

New York Blood Center
Immunohematology and Genomics Laboratory