Serologic Weak D Phenotype to \textit{RHD} Genotyping:

\textit{How will I know?}

Part II

Dr. Connie M. Westhoff, SBB, PhD
Director,
Immunohematology and Genomics
New York Blood Center

Recent Recommendations

- Intra-organizational Task Force
  - September 2013
    - CAP, AABB, ABC, ARC, ACOG, Armed Services
- Response to College of American Pathologists survey* 
  - Lack of standard practice in U.S.
    - for laboratory testing for \textit{RHD}
    - for interpreting the \textit{RHD} blood type when \textit{weak D phenotype} seen


RhD Workgroup Charges

- Develop a recommendation for \textit{RHD} genotyping
  - when a \textit{serological weak D phenotype} is identified
  - OR whenever \textit{D typing} uncertain (discrepancies)
- A recommendation should help
  - clarify clinical issues related to \textit{RhD} typing
    - in pregnant women
    - in transfusion recipients
  - helping to avoid the unnecessary use of \textit{Rh immune globulin}
  - unnecessary transfusion of \textit{Rh-negative RBCs}

Goal: begin to phase-in the use of \textit{RHD} genotyping
Workgroup Did Not Address

- Donor Testing for RhD
  - Well known - current serologic typing fails to detect some donor units with low levels of D antigen
    - less immunogenic and low risk for stimulating anti-D
    - have stimulated anti-D (rare reports)
  - RHD genotyping can detect these donor units
  - Requires high-throughput method to test for presence/absence of RHD gene


Publication

“Draft 50” – April 2014
  - Joint statement for review

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

Transfusion, March 2015:55:680-689

- Goal to BEGIN standardization of practice
  - managing pregnant women
  - transfusion recipients

Objectives from Part I

1. Identify causes for variability in the expression of RhD antigen
2. Describe specificity, sensitivity & intended use of current commercial RhD typing reagents used in test tube and automated methods.
3. List challenges of typing for RhD by serologic methods.
4. Define management & transfusion options for patients with weak or variable RhD typing.
Causes for variability of expression of D antigen?

1.) number of different genetic RHD alleles
2.) number of different epitopes on RhD protein
3.) number of different reagents and methods used
4.) D epitopes can be present on Rhce protein
5.) all of above
6.) none of above. We don’t yet know why.

1. Number of different alleles of RHD gene

• More than 200 different RHD alleles identified to date
  • Encode single or multiple amino acid changes in RHD that can
    1. Cause decrease amount of protein in membrane
       • get weaker than expected reactivity
    2. Can alter protein and abolish or create novel epitopes
       • “conventional” or “normal” or “wild-type” RhD will be foreign
  • Could potentially be >200 different “antigens” or “D subgroups”
  • Prevalence and frequency of specific RHD alleles differs in different populations

2. Large number of different epitopes on RhD protein

• D antigen is NOT a single change on the red cell membrane, unlike for example, Jka and Jkb
  • D typing detects the presence or absence of a entire protein

Most blood group antigens due to single change
2. Large number of different epitopes on RhD protein

Structure of Rh complex in the membrane

12-transmembrane spans
- ~30 epitopes
- If one epitope changed potential to respond to RhD
- Complex antigen


3. Many different methods used

- Manual tube -- with no IAT
- Manual tube -- with IAT for serologic weak D
- Gel card
- Echo/Neo
- PK -- enzyme treated cells for donor testing

What method do you use for typing patients?

1.) Manual tube test, with indirect antiglobulin test (IAT)
2.) Manual tube test, no IAT
3.) Gel Card
4.) Echo/Neo
5.) Combination of methods
Why have many (most) laboratories eliminate IAT for patient testing?

1. Introduction of monoclonal antibodies
   - Increased sensitivity detects as D+ some previously IAT+

2. Avoid false positive D typing if patient has +DAT

3. Save costs $$$$

4. To be conservative and manage those with weak D reactivity as Rh negative

5. All of above

2012 CAP survey: 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 (58.2% to 19.8%, P < 0.001).

3. Many different monoclonal antibodies in use

<table>
<thead>
<tr>
<th>Reagent</th>
<th>IgM monoclonal</th>
<th>IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immucor Series 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immucor Series 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ortho BiClone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ortho Gel (DI-MTS)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B2k Anti-B3k</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alba Biocience alpha</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alba Biocience beta</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alba Biocience delta</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alba blend</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- partial DVI - (fatal HDN). RBCs negative at IS with IgM clones, positive at IAT
- strength of reactivity with altered D antigen often depends on reagent
- majority contain different clones
  - can react with different epitopes on RHD
- even the same clone can react differently
- different potentiators and formulations
- reactivity may differ depending on C or E status of the RBCs

4. D epitopes expressed on Rhce protein !!

<table>
<thead>
<tr>
<th>Reagent</th>
<th>IgM monoclonal</th>
<th>IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gammaclone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immucor Series 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immucor Series 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ortho BiClone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ortho Gel (DI-MTS)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B2k Anti-B3k</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alba Biocience alpha</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alba Biocience beta</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alba Biocience delta</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alba blend</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Patients have no RHD gene or RhD protein
  - associated with hemolytic disease and transfusion reactions
  - cCF (Crawford) – Blacks – Ga232Glu, Leu245Val amino acid changes
    - GAMBA1 – strong positive 3+ or greater
    - all others – negative (or very weak positive)
  - cDR (Crawford) – Whites – one RHD exon inserted into the RHCE gene
    - all other – positive 3+
    - Ortho BiClone – negative

2012 CAP survey: 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 (58.2% to 19.8%, P < 0.001).
Summary of Challenges of Serology for D typing

- Variation in strength of D antigen expression on some RBCs
- Variation in test methods
- Variation in the specificity of antibody clones and reagent formulations
- Variation in interpretation

Variation in Interpretation

Manufacturer Instructions and Cautions

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-”

Variation in Interpretation

- How do you report the Rh type when it is weak or variable?
  - RhD positive
  - RhD negative
  - Weak D positive
  - Du positive
  - If female or OB, report as RhD negative
**Variation in Interpretation**

**CAP Survey of ~3,100 laboratories**
- How do you report the patient Rh type when it is weak or variable?
  - RhD positive (47%)
  - RhD negative (11%)
  - Weak D positive (30%)
  - Du positive (terminology discontinued in 1990's)
  - If female or OB, report as RhD negative (some)

**Variation in interpretation = Variation in treatment**

1. Rh positive blood and no RhIg
   - risk for anti-D

2. Rh negative blood and RhIg candidate
   - conservative approach
   - avoids risk for anti-D
   - females- avoids risk for possible HDFN
   - Results in excess use of Rh immune globulin
   - Results in excess use of Rh negative blood

**Objectives – Part II**

1. Discuss the benefits of using a molecular-genetics approach.

2. Describe an approach for phasing in RHD genotyping for transfusion medicine practice.

3. Discuss the recent recommendations of the Inter-organizational Work Group on RHD Genotyping for
   - managing pregnant women
   - transfusion recipients
   - with a serological weak D (or discordant) type.
How many patients have inherited altered RHD?

- 0.5% - 4% of patients have altered RHD gene
  - prevalence depends on ethnic group
  - alleles often differ between ethnic groups

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

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

RhD expression – Two Categories of Altered RhD

- "weak" D antigen (previously D*u)
  - Historical Definition
  - requires the IAT phase of testing for detection
  - Current Definition
  - reacts £ 2+ by tube method
  - or reacts "weaker than expected"
  - Decreased amount of D antigen; do not appear to lack D epitopes
  - Majority NOT AT RISK FOR CLINICALLY SIGNIFICANT ANTI-D

- "partial" D antigen
  - Definition
  - have altered or missing D epitopes
    - can require the IAT phase for detection
    - can react "weaker than expected"
    - can react strongly positive - and go undetected
  - AT RISK FOR CLINICALLY SIGNIFICANT ANTI-D

Cannot be distinguished by routine serologic D typing

RHD genotyping (DNA testing) can distinguish

- Weak D alleles
  - majority are single point mutations
  - Types 1 to Type 80 (~80 different point mutations)
  - Type 1, Type 2, and Type 3 - most common in Caucasian (~90-95%)

- Weak D type 1, 2, 3 - ARE NOT AT RISK for clinically significant anti-D

10 years - Observational studies from Central Europe
**Partial D alleles**
- encode altered proteins or lacking epitopes
- >100 alleles with multiple changes

**New hybrid alleles and proteins**
- part of RHD into RHCE
- part of RHCE into RHD

**AT RISK for clinically significant anti-D**

**Partial D examples: RHD/RHCE hybrid alleles**

**RHD genotyping (DNA testing) can distinguish**

- genetic exchange common in duplicated genes that are linked

- RHD exons replaced with RHCE exons

- New antigens

**RBCs type as D+ (some strong; some only IAT reactive; some variable)**

**Patients are managed according to their RHD genotype**

- Patients with D typing discrepancies
  - Rh positive previously and now Rh negative: or the reverse
  - Rh type from physician office different than hospital

- D typing weaker than expected

- Patients are managed according to their RHD genotype

**Beth Israel Deaconess, Boston - RHD genotyping for OB’s**

<table>
<thead>
<tr>
<th>RHD genotype</th>
<th>DIII</th>
<th>DIV</th>
<th>DIII</th>
<th>DIII</th>
<th>DIV</th>
<th>DIV</th>
<th>DIV</th>
<th>DIV</th>
<th>DIV</th>
<th>DIII</th>
<th>DIV</th>
<th>DIII</th>
<th>DIV</th>
<th>DIV</th>
<th>DIV</th>
<th>DIV</th>
<th>DIV</th>
<th>DIII</th>
<th>DIV</th>
<th>DIV</th>
<th>DIV</th>
<th>DIV</th>
<th>DIV</th>
</tr>
</thead>
<tbody>
<tr>
<td>weak D type 1</td>
<td>18</td>
<td>9</td>
<td>2</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>weak D type 2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Partial D</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>No RhD</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>None</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>38</td>
<td>38</td>
<td>38</td>
<td>38</td>
<td>38</td>
<td>38</td>
<td>38</td>
<td>38</td>
<td>38</td>
<td>38</td>
<td>38</td>
<td>38</td>
<td>38</td>
<td>38</td>
<td>38</td>
<td>38</td>
<td>38</td>
<td>38</td>
<td>38</td>
<td>38</td>
<td>38</td>
<td>38</td>
<td>38</td>
</tr>
</tbody>
</table>

- 75% of patients are not candidates for RhD
- 25% of patients are candidates for RhD
**Example: RHD genotyping Report for Weak D**

**RHD zygosity:** RHD hemizygote

**RHD genotype:** RHD*weak D type 1 encodes amino acid change Val270Gly.

**D phenotype:** D+ (weak)

**COMMENTS:**
Weak D type 1 is the most frequent type of weak D. The RBCs have low D antigen density and may require the antiglobulin phase of testing for detection.
Observational studies indicate that individuals with weak D type 1 RBCs are not at risk for clinically significant alloanti-D and can be transfused with Rh positive donor units and females are not candidates for Rh immune globulin. *(Transfusion 2015:55:680-89)*

---

**Example: RHD genotyping Report for Partial D**

**RHD zygosity:** RHD hemizygote

**RHD genotype:** RHD*DAR encodes amino acid changes 201Arg, 223Val, and 342Thr.

**D phenotype:** D+ (partial)

**COMMENTS:**
The patient has a partial D phenotype associated with risk for anti-D, and the RBCs may have weaker than expected D antigen expression depending on the reagent and method used.
Females of child bearing potential with a partial D phenotype are better served as Rh negative for transfusion and candidates for Rh immune globulin (if they have not produced active anti-D). *(Transfusion 2015:55:680-89)*

---

**Rh Workgroup Recommendations**

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

- **Are not suggesting institutions 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 results to manage clinical decisions**
  - Determine candidates for Rh immune globulin
  - RhD status for blood transfusion
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**
- **Positive** (and concordant with patient history if available)

- **Candidates for RHD**
  - D negative for transfusion
  - D positive for transfusion

- **Weak D type 1, 2, or 3**

- Not detected

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.

**Phasing-in RHD genotyping** will apply modern genomic methods for more precise decision making in obstetrical practice and transfusion medicine.

Potential Benefits of **RHD Genotyping Pregnant Women**

- 3,953,000 Live births
- 3,812,000 Pregnancies
- 556,500 RhD-negative
- 24,700 unnecessary anti- and postpartum RhIG injections
Why be concerned about excess usage of RhIG?

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

BUT
- Is 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 new emerging agents

Impact on the New York Blood Supply
- Number of Rh negative units needed to meet demand
- Overall blood use declining, Rh negative usage increasing

Potential Benefit of RHD Genotyping
- 5,000,000 Individuals Transfused Annually in US
- 730,000 RhD Negative
- 21,900 Serologic Weak D
- 17,520 weak D types
- 47,700 units

Could receive Rh positive RBCs

RHD Genotyping
RHD genotyping

- LDT – Laboratory Developed Test
  - even if using a manufacture kit
- Research Use - RUO testing
- Performed in CLIA regulated laboratory
- AABB accreditation available
- CPT code assigned
  - reimbursement amount requires history of charges

- Cost of testing has not "stabilized"

- Need economy of scale

Financial Implications of RHD genotyping for OB’s

- Cost-Benefit Analysis
  - Goal: evaluate the costs of RHD genotyping for pregnant females with serologic weak D phenotypes
    - using a comparison strategy of managing women as D–
    - RHD genotyping done at first visit/first pregnancy
      - results made part of medical record
    - direct medical costs assessed over 10- and 20-year periods for a simulated population of US women

Cost Input Parameters – CMS reimbursement

<table>
<thead>
<tr>
<th>Testing and Product</th>
<th>Cost $</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Testing</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>Additional RhD Testing</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>Blood Products</td>
<td></td>
<td></td>
</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>

RHD genotyping is cost-savings over treating as Rh negative when genotyping is – $256
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?
     - 25% of women with discrepant or weak D typing - were at risk
     - 75% were weak D type 1, 2, 3 and - were 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 ~$256

**Summary**

- Encounter Rh typing discrepancy or reactivity is weaker than expected
  - Consider referring for RHD genotyping
  - Especially if female of child bearing potential
  - Or patient needing long-term transfusion support
  - Make part of hospital and doctor office medical records
  - Need only be one time testing

**Phasing in RHD genotyping**

- Some women with weak D+ will not be detected (without IAT)
  - Are typed as D negative
  - Get unnecessary RhIg and Rh negative blood
  - Will require testing all Rh negative women by RHD genotyping
- Women with partial D who type strongly D+ (partial DIIa, partial DIVa, etc)
  - Are typed as D positive
  - Do not get 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 \textit{RHD} genotyping

How will I know……..

* \textit{.........when to consider RHD genotyping}?
  
  - When “weaker than expected D typing” seen (serologic weak D phenotype)
  
  - If doing IAT and initial spin is negative and IAT positive (weak D phenotype)
  
  - When variable reactivity with different reagents is seen
  
  - When discrepancy in patient Rh type

When you just don’t know !!!!....what to call the Rh type

Thank You !