Serologic Weak D Phenotype to *RHD* Genotyping:

*How will I know?*

**Part II**

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*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 RhD
  • for interpreting the RhD blood type when weak D phenotype seen

RhD Workgroup Charges

• Develop a recommendation for $RHD$ genotyping
  – when a serological weak $D$ phenotype is identified
  – OR whenever $D$ typing uncertain (discrepancies)

• A recommendation should help
  – clarify clinical issues related to RhD typing
    • in pregnant women
    • in transfusion recipients
  – helping to avoid the unnecessary use of Rh immune globulin
  – unnecessary transfusion of Rh-negative RBCs

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

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.
<p>| | |</p>
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>1.</td>
<td>number of different genetic RHD alleles</td>
</tr>
<tr>
<td>2.</td>
<td>number of different epitopes on RhD protein</td>
</tr>
<tr>
<td>3.</td>
<td>number of different reagents and methods used</td>
</tr>
<tr>
<td>4.</td>
<td>D epitopes can be present on Rhce protein</td>
</tr>
<tr>
<td>5.</td>
<td>all of above</td>
</tr>
<tr>
<td>6.</td>
<td>none of above. We don’t yet know why.</td>
</tr>
</tbody>
</table>
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, Jk\(^a\) and Jk\(^b\)

- D typing detects the *presence or absence of a entire protein*

\[
\begin{align*}
\text{Jk}^{a/b} &= \text{Asp280Asn} \\
\text{Aspartic acid at position 280} &= \text{Jk}(a+) \\
\text{Asparagine at position 280} &= \text{Jk}(b+)
\end{align*}
\]

Most blood group antigens due to single change

32-35 amino acid changes from Rhce
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

Trimer – 2 RhAG, 1 RhCE or 1 RhD

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

Manual Tube

Solid Phase Capture

Donor centers
PK 7600

Automation – ABO & Rh, antibody screening, identification

Grifols
Gel Card

BioTest TANGO™
Benchtop Blood Bank Analyzer

ImmucorGamma’s
Capture solid phase
Echo and Neo
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 <.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>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>Not recommended for patient testing</td>
</tr>
<tr>
<td>Alba Bioscience beta</td>
<td>LDM3</td>
<td></td>
</tr>
<tr>
<td>Alba Bioscience delta</td>
<td>LDM1/ ESD1M</td>
<td></td>
</tr>
<tr>
<td>Alba blend</td>
<td>LDM3</td>
<td>ESD1</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 potentiatators and formulations
- reactivity may differ depending on C or E status of the RBCs
4. D epitopes expressed on Rhce protein!!

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<td>LDM1/ ESD1M</td>
<td>Not recommended for patients</td>
</tr>
<tr>
<td></td>
<td></td>
<td>detects partial DVI on initial testing</td>
</tr>
<tr>
<td>Alba blend</td>
<td>LDM3</td>
<td>ESD1</td>
</tr>
</tbody>
</table>

- **Patients have no** *RHD* **gene or RhD protein**
  - associated with hemolytic disease and transfusion reactions
- **ceCF (Crawford)** – Blacks – Gln233Glu, Leu245Val amino acid changes
  - GAMA401 – **strong positive 3+ or greater**
  - all others – **negative** (or very weak positive)
- **ceHAR (D^{HAR})** – Whites - one RHD exon inserted into the RHCE gene
  - all other – **positive 3+**
  - **Ortho Bioclone** – negative
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*
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\textsuperscript{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%)

10 years - Observational studies from Central Europe

Weak D type 1, 2, 3 - ARE NOT AT RISK for clinically significant anti-D
**RHD genotyping (DNA testing) can distinguish**

- **Partial D alleles**
  - encode altered proteins or lacking epitopes
  - >100 alleles with multiple changes

- genetic exchange common in duplicated genes that are linked

**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

<table>
<thead>
<tr>
<th></th>
<th>RHD exons replaced with</th>
<th>RHCE exons</th>
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<tr>
<td><strong>RHD</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DIIIa</td>
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<tr>
<td>DVI 3</td>
<td></td>
<td></td>
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<tr>
<td>DIIIc</td>
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<tr>
<td>DIVa</td>
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<tr>
<td>DIVb</td>
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<tr>
<td>DIVbIII</td>
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<td>DIVbIV</td>
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<tr>
<td>DVI 1</td>
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<td>DVI 2</td>
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<td>DBT2</td>
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<table>
<thead>
<tr>
<th></th>
<th>New antigens</th>
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<tbody>
<tr>
<td><strong>DAK</strong></td>
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<tr>
<td>BARC</td>
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<td>Goα</td>
<td></td>
</tr>
<tr>
<td>Evans</td>
<td></td>
</tr>
<tr>
<td><strong>D^W</strong></td>
<td></td>
</tr>
<tr>
<td>BARC</td>
<td></td>
</tr>
<tr>
<td>FPTT</td>
<td></td>
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<tr>
<td>FPTT</td>
<td></td>
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<tr>
<td>Rh32</td>
<td></td>
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<td>Rh32</td>
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</table>

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

**Patients at risk for 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**
1. Women with D typing discrepancies

- Rh positive previously and now Rh negative: or the reverse
- Rh type from physician office different than hospital

2. D typing weaker than expected

<table>
<thead>
<tr>
<th>$RHD^*$</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>
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<td></td>
</tr>
</tbody>
</table>

75 %

25%

Patients are managed according to their $RHD$ genotype

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

**RHD zygosity:** *RHD* hemizygote

**RHD genotype:** *RHD*\textit{weak D type 1} encodes amino acid change Val270Gly.

**D phenotype:** \textbf{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. \textit{(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**
  - Candidate for RhIG
  - D negative for transfusion

- **Discrepant or Inconclusive or strength of reaction weaker than expected**
  - (serologic weak D phenotype)
  - send for RHD genotyping for weak D type

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

- **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

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

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
- 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

• 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

O- RBC/WB Distribution

- Number of Rh negative units needed to meet demand
- Overall blood use declining, Rh negative usage increasing
Potential Benefit of \textit{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

\textit{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*
    - when Rh typing usually done
    - results made part of medical record
  - direct medical costs assessed over 10- and 20-year periods for a simulated population of US women

Financial implications of RHD genotyping of pregnant women with serologic weak D phenotype
### Cost Input Parameters – CMS reimbursement

<table>
<thead>
<tr>
<th>Testing and Product</th>
<th>Cost $</th>
<th>Range</th>
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<tbody>
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<td><strong>Initial Testing</strong></td>
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<tr>
<td>ABO Group</td>
<td>12.12</td>
<td>(9.09-15.15)</td>
</tr>
<tr>
<td>RhD Type</td>
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<td>(9.09-15.15)</td>
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<td><strong>Additional RhD Testing</strong></td>
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<tr>
<td>Rh Immune Globulin (300 μg dose)</td>
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<td>(121.50-202.50)</td>
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<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

Financial implications of RHD genotyping of pregnant women with serologic weak D phenotype
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 - were NOT at risk

3. **Financial implications of RHD genotyping of pregnant women with serologic weak D phenotype**  
   Kacker S, Vassallo R, Keller M, Westhoff CM, Frick K, Sandler S, Tobian A  
   *Transfusion 2015 Early View*
   - 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 DIIIa, 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 $RHD$ genotyping
How will I know……

• ……..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!

New York Blood Center
Immunohematology and Genomics Laboratory