Partial D & Weak D
Picking Up the Rhesus Pieces

Heart of America Association of Blood Banks
April 24, 2012

Susan T. Johnson, MSTM, MT(ASCP)SBB
Director, Clinical Education
BloodCenter of Wisconsin
Objectives

• List the reasons for RhD typing discrepancies
• Discuss the biochemical and molecular characteristics of RhD & \textit{RHD}
• Understand the differences among partial, weak, D_{el} variants and D epitopes on RhCe protein
• Describe the advantage of a molecular resolution of Rh discrepancies
Rh DESIGNATION

Rh Positive
85%

Rh Negative
15%
RhD Typing Discrepancies

- RhD antigen expression
- \textit{RHD} gene mutations
- Reagent differences
- Method variability
# Variables Impacting Rh Typing

<table>
<thead>
<tr>
<th>CONTRIBUTORS OF VARIABILITY</th>
<th>VARIABLES</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RHD Gene</strong></td>
<td>Weak D</td>
</tr>
<tr>
<td><strong>D epitopes on RhCE Protein</strong></td>
<td>ceCF</td>
</tr>
<tr>
<td><strong>Anti-D Reagents</strong></td>
<td>Polyspecific Slide and Modified Tube Human IgG</td>
</tr>
<tr>
<td><strong>Testing Platform</strong></td>
<td>Test Tubes IS &amp; IAT</td>
</tr>
<tr>
<td><strong>Individual being Rh Typed</strong></td>
<td>Transfusion Recipient</td>
</tr>
</tbody>
</table>

Transfusion Technology Report Vol. #013 Immucor, Inc.
What is D?
Rh DESIGNATION

Rh Positive
85%

Rh Negative
15%
Rh Protein

Multi-pass membrane protein

- Crosses RBC membrane 12 times
- No sugars attached
**RH Genes – Rh Positive**

Chromosome 1

**Locus 1**

- **RHD**

Exons

- **1**  **2**  **3**  **4**  **5**  **6**  **7**  **8**  **9**  **10**

**Locus 1** - presence of **RHD** codes for the presence of D or no D. Differs from *RhCE* by 34 to 37 amino acids (C or c)

**Locus 2**

- **RHCE**

Exons

- **1**  **2**  **3**  **4**  **5**  **6**  **7**  **8**  **9**  **10**

**Locus 2** - presence of **RHCE** codes for Ce, CE, cE, ce.
RH Genes – Rh Positive

Chromosome 1

Locus 1
RHD

Locus 2
RHCE
Rh (D) Negative

• Deletion of \textit{RHD} – in European ancestry

• Inactivating mutations of \textit{RHD}
  • \textit{RHD}_\psi in African Americans

• Hybrid \textit{RHD-CE-D} in African backgrounds
**RH Genes in Rh Negative Caucasians**

Chromosome 1

Locus 1

Locus 2

RHCE

1 2 3 4 5 6 7 8 9 10

Exons

No D antigens  ce antigens

Locus 1 **deletion of RHD** therefore, no D antigen.
Rh (D) Negative – African Background

19%  \( RHD \) deletion
66%  \( RHD_\Psi \)
19%  Hybrid \( RHD-CE-D \)
RH Genes in Rh Negative - African Background

Chromosome 1

Locus 1

\[ \text{RHD}_\psi \]

Locus 2

\[ \text{RHCE} \]

Exons

No D antigen

Exons

C/c and E/e antigens

Locus 1 – 37 bp insertion & several mutations in \( \text{RHD} \) results in no product

66% of AAs have \( \text{RHD}_\psi \)
Rh (D) Negative – African Background

Chromosome 1

Locus 1

Locus 2

RHD

RHCE

1 2 3 4 5 6 7 8 9 10

1 2 3 4 5 6 7 8 9 10

No D antigen

C/c and E/e antigens

Locus 1 – RHCE inserted in RHD results in no D antigen and weak C.

15% of AAs have hybrid RHD-CE-D
What About Weak Expression of D?
WEAK EXPRESSION OF RhD HISTORY

- $D^u$
- D mosaics
- Weak D – general term used
- Partial D
- Weak $D$
  - Specific group of RhD variants
- D-elution alleles
WEAK D HISTORY

- Described by Stratton (1946)
- D antigen not detected by all anti-D
- Mistakenly called the $D^u$ antigen
  - $D^u+$ blood to a D- person causes production of anti-$D$ not anti-$D^u$
WEAK D Reactivity with Anti-D

- Agglutinated with some anti-D on direct agglutination (IS)

- Negative on direct agglutination (IS)
  - D antigen detected by IAT only
<table>
<thead>
<tr>
<th>Frequency of Weak Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hopkins</td>
</tr>
<tr>
<td>Garretta</td>
</tr>
<tr>
<td>Beck</td>
</tr>
<tr>
<td>Jenkins</td>
</tr>
<tr>
<td>Flegel</td>
</tr>
</tbody>
</table>
WEAK D
Variation in RhD Expression

• Do not make anti-D

• Able to make anti-D
Weak Expression of D
Do Not Make Anti-D

- C in \textit{trans} with \textit{RHD} (Ceppellini effect)
  - r’ haplotype
- Weak D “Types”: single amino acid changes
- Weak D Type 2
  - Very weak(+) when in \textit{trans} with r’
Ceppelini Effect

\[ D^u \]

\[ DCe/Ce \]
\[ Ce/ce \]
\[ Ce/ce \]
\[ DCe/ce \]

\[ + \]

\[ Ce/ce \]
\[ Ce/ce \]
\[ DCe/Ce \]
\[ Ce/ce \]
\[ DCe/ce \]
Weak D Types

_Do Not Make Anti-D_

- Missense mutations in regions of RHD encoding **transmembrane/cytoplasmic portion of D**
- Less protein inserted into RBC membrane
- Can type as Rh-positive or Rh-negative by direct agglutination with monoclonal (IgM) anti-D reagents

<table>
<thead>
<tr>
<th>IS</th>
<th>D IAT</th>
<th>Ct. IAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-D 3+</td>
<td>0</td>
<td>3</td>
</tr>
</tbody>
</table>

**IS** - Immunosuppression
**Anti-D** - Anti-D Reagents

_BloodCenter of Wisconsin_
Some Weak D Types

- Type 1
- Type 2
- Type 3
- Type 4.0
  - Account for 90% of Weak D;
  - Do not produce Anti-D

- Type 4.2
- Type 5
- Type 11
- Type 15
- Type 19
- Type 20
  - Known to form Anti-D when exposed to D+ RBCs
Molecular Basis of Weak D

Type 1 (V270G)
Type 2 (G385A)
Type 3 (S3C)
Type 4 (T201R; F223V)
Type 5 (A149D)
Type 6 (R10Q)
Type 7 (G339E)
Type 8 (G307R)
Type 9 (A294P)
Type 10 (W393R)
Type 11 (M295I)
Type 12 (G277E)
Type 13 (A276P)
Type 14 (S182T; K198N; T201R)
Type 15 (G282D)

Del (Exon 9 deletion)
Weak D

Plasma membrane

Exterior

Interior

CM Westhoff

<table>
<thead>
<tr>
<th></th>
<th>IS</th>
<th>D IAT</th>
<th>Ct. IAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-D</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>
Weak D Types 1 and 2

- Most common weak D types
- Weak D Type 1
  - $R_1r$ (D+C+E-c+e+)
- Weak D Type 2
  - $R_2r$ (D+C-E+c+e+)
Weak Expression of D Able to Make Anti-D

- Partial Ds: hybrid \textit{RHD} alleles
  - DVI
  - DIIIa
  - DIVa, DIVb, others
- D\textsubscript{el}: detection by adsorption/elution
- D epitopes on \textit{RHCE} gene
RHESUS PIECES
PARTIAL D

- Partial D
  - Lack exofacial epitopes
    - Hybrid proteins
    - Missense mutations affecting exofacial protein
<table>
<thead>
<tr>
<th></th>
<th>IS</th>
<th>D IAT</th>
<th>Ct. IAT</th>
<th></th>
<th>IS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-D</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>Anti-D</td>
<td>3+</td>
</tr>
</tbody>
</table>
One example of Partial DVI gene where 3 exons of RHCE gene are inserted into RHD gene.
Deletion of exon 9 in Asians occurs in 10-30%.

- Type as D-negative (IS & IAT), only adsorb & elute anti-D
- Severely reduced protein
- 2 individuals have made anti-D after receiving D+ blood
D Epitope on RHCE Genes

• Crawford (ceCF) phenotype

• $R_0^{Har}$, also known as $D^{HAR}$
D Epitope on RHce Gene - $D^{CF}$

$D^{CF}$ results from 3 nucleotide changes, $48G>C$, $697C>G$, $733C>G$ in RHce gene.
## Anti-D Reagents: Reactions with Crawford Phenotype RBCs

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Anti-D</th>
<th>RBCs</th>
</tr>
</thead>
<tbody>
<tr>
<td>GammaClone</td>
<td>IgM: GAMA401</td>
<td>IgG: F8D8</td>
</tr>
<tr>
<td>Immucor-4</td>
<td>IgM: MS201</td>
<td>IgG: MS26</td>
</tr>
<tr>
<td>Immucor-5</td>
<td>IgM: TH28</td>
<td>IgG: MS26</td>
</tr>
<tr>
<td>Ortho Bioclon</td>
<td>IgM: MAD2</td>
<td>Human polyclonal</td>
</tr>
<tr>
<td>Ortho (ID-MTS)</td>
<td>IgM: MS201</td>
<td></td>
</tr>
</tbody>
</table>

Reactive clones in some European reagents: RUM-1, D175-2, F5S, H2D5D2F5, MCAD-6
D Epitope on *RHCE* Gene - $D^{HAR}$

$D^{HAR}$ results from one *RHD* exon inserted into the *RHCE* gene.

<table>
<thead>
<tr>
<th>IS</th>
<th>Anti-D</th>
<th>3+</th>
</tr>
</thead>
</table>

No D antigens

cE antigens
**R$_0^{Har}$ Phenotype: Reactivity with Reagent Anti-D**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Anti-D</th>
<th>RBCs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gamma-Clone</td>
<td>IgM: GAMA401, IgG: F8D8</td>
<td>Pos*</td>
</tr>
<tr>
<td>Immucor-4</td>
<td>IgM: MS201, IgG: MS26</td>
<td>Pos*</td>
</tr>
<tr>
<td>Immucor-5</td>
<td>IgM: TH28, IgG: MS26</td>
<td>Pos*</td>
</tr>
<tr>
<td>Ortho Bioclone</td>
<td>IgM: MAD2, IgG: Human polyclonal</td>
<td>Neg</td>
</tr>
<tr>
<td>Ortho (ID-MTS)</td>
<td>IgM: MS201</td>
<td>Pos</td>
</tr>
<tr>
<td>Biotest (Bio-Rad)</td>
<td>IgM: BS232, IgG: BS221 H41 11B7</td>
<td>Pos</td>
</tr>
<tr>
<td>Quotient - Alpha</td>
<td>IgM: LDM1</td>
<td>Pos</td>
</tr>
<tr>
<td>Quotient - Delta</td>
<td>IgM: LDM1, IgG: ESD1M</td>
<td>Pos</td>
</tr>
</tbody>
</table>

*Positive reactions often weaker at IAT*
<table>
<thead>
<tr>
<th>Method</th>
<th>Manufacturer</th>
<th>IgM</th>
<th>IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tube</td>
<td>Ortho</td>
<td>MAD2</td>
<td>Human</td>
</tr>
<tr>
<td>Tube</td>
<td>Gamma</td>
<td>GAMA 401</td>
<td>F8D8</td>
</tr>
<tr>
<td>Tube</td>
<td>Immucor-4</td>
<td>MS201</td>
<td>MS26</td>
</tr>
<tr>
<td>Tube</td>
<td>Immucor-5</td>
<td>Th28</td>
<td>MS26</td>
</tr>
<tr>
<td>Tube</td>
<td>Alba (Quotient BD) alpha</td>
<td>LDM1</td>
<td></td>
</tr>
<tr>
<td>Tube</td>
<td>Alba (Quotient BD) delta</td>
<td>LDM1</td>
<td>ESD1M</td>
</tr>
<tr>
<td>Tube</td>
<td>Biotest (Bio-Rad)</td>
<td>BS232</td>
<td>BS221 H41 11B7</td>
</tr>
<tr>
<td>Gel</td>
<td>ID-MTS</td>
<td>MS201</td>
<td></td>
</tr>
</tbody>
</table>
Human IgG Anti-D
MONOCLONAL IgM/IgG ANTI-D
MONOCLONAL IgM/IgG ANTI-D #1
Direct Agglutination - IS
MONOCLONAL IgM/IgG ANTI-D #1
Weak D Test - IAT
MONOCLONAL IgM/IgG ANTI-D #2
### Confusion Over Weak Expression of D

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Donor</strong></td>
<td><strong>Rh+</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Recipient</strong></td>
<td><strong>Rh-</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Prenatal</strong></td>
<td><strong>RhIG?</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Newborn</strong></td>
<td></td>
<td><strong>Postpartum RhIG?</strong></td>
</tr>
<tr>
<td><strong>Autologous Donor</strong></td>
<td></td>
<td><strong>@#!&amp;*~?</strong></td>
</tr>
</tbody>
</table>
Reasons to Resolve Weak Expression

• Conserve Rh-negative blood for D-negative recipients (high risk of making anti-D).

• Avoid giving RhIG to women who do not need it (Rh status is confirmed for historical discrepancies)

• Resolve early in pregnancy to eliminate false-positive rosette tests.
Rh Discrepancies - MSH, Toronto

Discrepancy between two anti-D direct tests

- 33,864 RhD phenotypings performed over an 18 month interval
- 55 of 5672 potential Rh-negative patients were tube test positive for one anti-D (0.98%)
  - 54 were tube test negative using one FDA-approved reagent but positive (2+ or less) using another government approved antisera
Summary of the Toronto Study

20 functional *RHD* alleles detected; 1 wildtype (HDN)

- **34 Weak D Types (PCR-RFLP):**
  - 16 weak D Type 1
  - 8 weak D Type 2
  - 1 weak D Type 3
  - 6 weak D Type 4
  - 1 weak D Type 5
  - 2 weak D Type 42

- **7 DAR (exon mapping plus sequencing)**

- **6 D\(^{Va}\) or D\(^{Va}\)-like alleles:**
  - 3 D\(^{Va}\)(Kou.)
  - 1 D\(^{Va}\)HK(E233K)
  - 1 D\(^{Va}\)-like
  - 1 DTO (Novel)

- **DFR, DAU-4, DAU-5 (Novel), DAU-6 (Novel)**

- **DAR/DAU-2, DAU-0/Cde\(^s\) (compound heterozygotes)**

- **1 not identified (possible D\(\text{IIIa, DVa, DAR, DOL}\)**

**57% were Weak D types 1, 2, 3 and 4**
**Impact if deemed Rh-negative**

**Inappropriate use of blood products**

<table>
<thead>
<tr>
<th>RHD Allele</th>
<th>OB</th>
<th>TR</th>
<th>NB</th>
<th>Impact</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weak D Types 1-4</td>
<td>12</td>
<td>8</td>
<td>5</td>
<td>12 OB patients received Rhig 4 transfusion recipients received 12 Rh-neg RBCs</td>
</tr>
<tr>
<td>Weak D Type 42</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>OB patient received Rhig Transfusion recipient received 11 Rh-neg RBCs</td>
</tr>
<tr>
<td>DAR</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>3 OB patients received Rhig Potential transfusion recipient was not transfused.</td>
</tr>
<tr>
<td>DVa and DVa-like</td>
<td>1</td>
<td>1</td>
<td>5</td>
<td>1 OB patient an delivered an Rh-neg infant Potential transfusion recipient not transfused</td>
</tr>
<tr>
<td>DAU, DFR, DTO</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>2 OB patient delivered an Rh+ infant Neither potential transfusion recipient transfused</td>
</tr>
<tr>
<td><strong>Total:</strong></td>
<td></td>
<td></td>
<td></td>
<td>21 RhIG 23 Rh-negative RBCs</td>
</tr>
<tr>
<td><strong>DAU, DFR, DTO</strong></td>
<td></td>
<td></td>
<td></td>
<td>7 Rhig 0 Rh-negative RBCs</td>
</tr>
</tbody>
</table>
# Summary of Alberta Study

**Analysis ‘07 – ’08 = 88,972**

<table>
<thead>
<tr>
<th>DNA Typing Results</th>
<th># of Patients</th>
<th>Rh Status Assigned</th>
<th>RHIG Recommended</th>
<th>% of DNA Results Received</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weak D Type 1</td>
<td>60</td>
<td>Pos</td>
<td>No</td>
<td>29.0</td>
</tr>
<tr>
<td>Weak D Type 2</td>
<td>19</td>
<td>Pos</td>
<td>No</td>
<td>9.2 64%</td>
</tr>
<tr>
<td>Weak D Type 3</td>
<td>38</td>
<td>Pos</td>
<td>No</td>
<td>18.4</td>
</tr>
<tr>
<td>Weak D Type 4</td>
<td>15</td>
<td>Pos</td>
<td>No</td>
<td>7.2</td>
</tr>
<tr>
<td>DAR</td>
<td>2</td>
<td>Neg</td>
<td>Yes</td>
<td>1.0</td>
</tr>
<tr>
<td>Partial DVI Type I</td>
<td>3</td>
<td>Neg</td>
<td>Yes</td>
<td>1.3</td>
</tr>
<tr>
<td>Partial DVI Type II</td>
<td>1</td>
<td>Neg</td>
<td>Yes</td>
<td>0.5 36%</td>
</tr>
<tr>
<td>DVI Type II</td>
<td>2</td>
<td>Neg</td>
<td>Yes</td>
<td>1.0</td>
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<tr>
<td>DVa partial</td>
<td>1</td>
<td>Neg</td>
<td>Yes</td>
<td>0.5</td>
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<tr>
<td>Partial DVA-like</td>
<td>1</td>
<td>Neg</td>
<td>Yes</td>
<td>0.5</td>
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<tr>
<td>Unclassified</td>
<td>65</td>
<td>Neg</td>
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<td>31.4</td>
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<tr>
<td>Pending</td>
<td>2</td>
<td>TBD</td>
<td>TBD</td>
<td></td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>209</strong></td>
<td></td>
<td></td>
<td><strong>(0.23% of total)</strong></td>
</tr>
</tbody>
</table>
## Monoclonal Anti-D Panel

### Table: Expected patterns of reactivity of different forms of partial D with the different monoclonal anti-D antibodies

<table>
<thead>
<tr>
<th>Anti-D cell line</th>
<th>Weak D type 1&amp;2</th>
<th>DII &amp; DNU</th>
<th>DIII</th>
<th>DIV</th>
<th>DVI</th>
<th>DCC</th>
<th>DVI</th>
<th>DVII</th>
<th>DOL</th>
<th>DFR</th>
<th>DMH</th>
<th>DAR</th>
<th>DAR-E</th>
<th>DHK &amp; DAU-4</th>
<th>DBT</th>
<th>Ro</th>
<th>Pos Cont</th>
<th>Neg Cont</th>
<th>Pt</th>
</tr>
</thead>
<tbody>
<tr>
<td>LHM76/58</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+/0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>(+)/0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>LHM76/59</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>+</td>
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<td>+</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>LHM174/102</td>
<td>(+)/0</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>+</td>
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</tr>
<tr>
<td>LHM50/28</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>0</td>
<td>0</td>
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</tr>
<tr>
<td>LHM169/81</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>+</td>
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<td>0</td>
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</tr>
<tr>
<td>ESD1</td>
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<td>+</td>
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<td>+</td>
<td>+</td>
<td>+</td>
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<td>LHM57/17</td>
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</tbody>
</table>

### Interpretation: DVI
Rh Discrepancy Algorithm

- **Anti-D (method 1)**
  - >2+ agglutination score:
    - Yes: **Rh Positive**
    - No: **Inconclusive**
    - **Matches historical?**
      - Yes: **Report Rh(+)**
      - No: **Tube Test - 'key' anti-Ds**
      - **Both >2+ score**
      - **Grading strength difference of 2 or more between anti-Ds**
- **Anti-D (std method)**
  - Negative:
    - Yes: **Rh Negative**
    - **Matches historical?**
      - Yes: **Report Rh(-)**
      - No: **At least one <2+ score**
      - **Report Rh Indeterminate**
      - Genotyping
# Bagene Weak D Worksheet

## Worksheet und Auswertetabelle / Worksheet and Evaluation diagram

<table>
<thead>
<tr>
<th>Reaktions-Nr. / Reaction No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR-Produkt (Größe in bp) / PCR product (size in bp)</td>
<td>150</td>
<td>126</td>
<td>165</td>
<td>101</td>
<td>130</td>
<td>83</td>
<td>112</td>
<td>198</td>
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<tr>
<td>weak D Allele / weak D alleles</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td>weak D type 1</td>
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<td>+</td>
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<tr>
<td>RHD(M295I) (haplotype CD_{e})</td>
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<td>-</td>
<td>-</td>
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<td>198</td>
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<td>weak D type 15</td>
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<td>weak D type 4, 2, 17</td>
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<td>-</td>
<td>+</td>
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<td>Weak D type 11 / RHD(M295I), 17</td>
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<td>83</td>
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<td>198</td>
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### Genotyp / Genotype

<table>
<thead>
<tr>
<th>Genotyp / Genotype</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
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</tbody>
</table>
Investigation strategy for RhD typing discrepancies using a combination of PCR-SSP and serological techniques

Lay See Er, MSTM, (ASCP)SBB

• http://www.aabb.org/development/awardsscholarships/scholarships/Pages/pastwinners.aspx
Bagene Weak D Kit Results

Lane 2: DNA ladder
Start reading from lane 3
Lane 1, 11, 12: buffer load (no bands)
Bagene Weak D Kit Results

Lane 2: DNA ladder
Start reading from lane 3
Lane 1, 11, 12: buffer load (no bands)
Summary

- 3-5% RhIG doses go to women with Weak D Types
  - How often do you need to switch Rh status?
    - Molecular test is a permanent solution
    - Weak D Types 1 – 4 are Rh+ as a recipient and donor
  - Informed consent for administration of RhIG?
    - Avoid a blood product where it is not needed!
    - RhIG shortage, new infectious disease
Summary, cont...

- Resolution $\leq$ Molecular Test $< \text{RhIG}$$
  - Rh allele pop’n frequencies
  - # of pregnancies
Guideline for Interpreting Discordant Rh Typing Results

Rh typing results are evaluated at immediate spin (direct agglutination) and Rh typing is repeated with identical results

<table>
<thead>
<tr>
<th>If individual types...</th>
<th>And individual is a...</th>
<th>And...</th>
<th>Then, consider molecular typing...</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rh-negative</td>
<td>Transfusion recipient</td>
<td>Donor record is Rh-positive</td>
<td>Interpret Rh-negative</td>
</tr>
<tr>
<td>Rh-negative</td>
<td>Obstetrical patient</td>
<td>Donor record is Rh-positive</td>
<td>Interpret Rh-neg or Rh-pos?</td>
</tr>
<tr>
<td>Rh-negative</td>
<td>Post delivery</td>
<td>Donor record is Rh-positive</td>
<td>Perform anti-D IAT*</td>
</tr>
<tr>
<td>Rh-negative</td>
<td>Transfusion recipient</td>
<td>Facility history is Rh-positive</td>
<td>Interpret Rh-negative</td>
</tr>
<tr>
<td>Rh-negative</td>
<td>Obstetrical patient</td>
<td>Facility history is Rh-positive</td>
<td>Interpret Rh-neg or Rh-pos?</td>
</tr>
<tr>
<td>Rh-negative</td>
<td>Post delivery</td>
<td>Facility history is Rh-positive</td>
<td>Perform anti-D IAT*</td>
</tr>
</tbody>
</table>

Modified from Transfusion Technology Report Vol. #013 Immucor, Inc.
# Guideline for Interpreting Discordant Rh Typing Results

Rh typing results are evaluated at immediate spin (direct agglutination) and Rh typing is repeated with identical results.

<table>
<thead>
<tr>
<th>If individual types...</th>
<th>And individual is a...</th>
<th>And...</th>
<th>Then, consider molecular typing...</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rh-positive</td>
<td>Transfusion recipient</td>
<td>Rh Negative at another facility</td>
<td>Type with different anti-D reagent</td>
</tr>
<tr>
<td>Rh-positive</td>
<td>Obstetrical patient</td>
<td>Rh Negative at another facility</td>
<td>Type with different anti-D reagent</td>
</tr>
<tr>
<td>Rh-positive</td>
<td>Post delivery</td>
<td>Rh Negative at another facility (regardless of history)</td>
<td>Type with different anti-D reagent</td>
</tr>
</tbody>
</table>

Modified from Transfusion Technology Report Vol. #013 Immucor, Inc.
Conclusions

• Rh discrepancies are better resolved using a molecular approach.
  • MoAb approach is erroneous for some partial Ds
  • MoAb approach does not positively identify Weak D Types 1 and 2 and does not address Weak D Types 3, and Weak D Type 4 versus DAR.

• Laboratories who change methodologies or drop the IAT as a routine test on all patients have the appropriate support to resolve historical discrepancies through molecular testing.
Objectives

• List the reasons for RhD typing discrepancies
• Discuss the biochemical and molecular characteristics of RhD
• Understand the differences among partial, weak, and Del variants
• Outline the advantage of a molecular resolution of Rh discrepancies
References

- Flegel WA. Molecular genetics and clinical applications for RH. Transfusion and Apheresis Science 2011;44:81-91. 2.
Thank You

sue.johnson@bcw.edu