Reference Lab Case Studies

Virginia Hare, MT(ASCP)SBB
American Red Cross Southern Region
Douglasville, Georgia
Today’s Cases and Presenters

- Rh typing discrepancy
  - by Grace Lee
- An Unusual Phenotype: Getting the type right!
  - by Mellonie Powe
- Anti-D in Rh positive patient with Warm-reactive autoantibody: Is it autoantibody or alloantibody?
  - by Nancy Mullis
Objectives

- Demonstrate the importance of obtaining patient history
- Demonstrate the pros and cons of utilizing special serologic procedures during resolution of serologic investigations.
- Demonstrate methodical process for resolution of patient antibody problems.
- Discuss unusual D phenotypes, how to identify them and how to select blood for transfusion.
Rh Blood Group System

- Number of antigens: 52
- Genes: \textit{RHD} and \textit{RHCE}
  - Many similarities between the two genes
  - Arranged in a hairpin formation; most gene recombination occurs gene conversion
- Nomenclature
  - \textit{RHD*01} for the normal D gene
    - Number changes for genes resulting in partial or weak
  - \textit{RHCE*01} = \textit{RHCE*ce}; \textit{RHCE*02} = \textit{RH*Ce}
  - \textit{RHCE*03} = \textit{RHCE*cE}; \textit{RHCE*04} = \textit{RHCE*CE}
D and anti-D

- After ABO, D is the most important RBC Ag
  - Highly immunogenic
  - Capable of causing transfusion reactions and Hemolytic Disease of Fetus and Newborn (HDFN)
- Usually, easily detected using the high quality, commercially available anti-D reagents
- Anti-D is also readily detectable in most cases
- May present as an autoantibody

*However, sometimes we get test results that are not so easy to interpret and manage.*
Complexities of D (RH1)

- Many epitopes:
  - Usually all of these epitopes are present = Conventional D +
  - Some individuals lack 1 or more epitopes
- ~200 alleles that encode unconventional D protein
  - Result in variation in D antigen expression
    - Some weaker, some stronger than conventional
- Partial vs Weak: Qualitative vs Quantitative
## Partial D vs. Weak D

<table>
<thead>
<tr>
<th>Partial D</th>
<th>Weak D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Qualitative difference</td>
<td>Quantitative difference</td>
</tr>
<tr>
<td>1 or more epitopes missing; external changes</td>
<td>All immunogenic D epitopes-internal changes</td>
</tr>
<tr>
<td>Patient should be considered Rh Negative</td>
<td>Patient should be considered Rh Positive</td>
</tr>
<tr>
<td>Patient can make anti-D</td>
<td>Patient cannot make anti-D</td>
</tr>
</tbody>
</table>
And now let’s hear some interesting cases about D and anti-D
D: Weak, partial, variant???

- D categories: defined by Dr. Tippett;
  - Cat. II-VI were sufficient for decades
- Monoclonal Abs have shown us that many more D epitopes are present on most D + cells
- D terminology can be confusing…. until definitive testing is performed to determine if an individual has a qualitative or quantitative change in the D antigen,
- Is “variant” the best term?
Partial D phenotypes

- Over 90 alleles identified that encode partial D—protein lacks one or more epitopes of D
- How are they identified?
  - When alloanti-D is produced
  - By the reactivity pattern of anti-Ds produced by others with known partial D
  - By their reactivity with well characterized monoclonal anti-Ds (Ex.: Partial D typing kit)
  - Others only by molecular testing.
- Terminology: Names, usually 3 or 4 letters
  - Examples: DBT, DAR, and DHAR
Weak D Phenotypes

- Classification is challenging
- Reagent and method dependent
- Serology cannot define all weak D phenotypes
- DNA analysis is required
- Terminology: Given numbers
  - “Weak D type 1”, type 2, type 3, etc.
  - Up to at least 76; some with subtypes, Ex.- 4.1
What is the right anti-D reagent?

- What is your patient population?
  - Patients vs. donors
  - Prenatal samples
  - Newborns

- What test method(s) will be performed?
  - If tube: I.S. only? Or IAT also?
  - Gel?
  - Solid phase?
  - What are the limitations of the test method?
Get to know your reagent

- Read the manufacturer’s instructions
- What is the reagent capable of detecting?
  - And not detecting?
    - Example: Do you want to detect D Cat. VI phenotypes?
- Is the methodology compatible with your lab?
- Use your resources
  - Literature
  - Manufacturer’s representatives and specialists

Yes, there are lots of considerations…….