Blood Group: Advances in Phenotyping and Genotyping

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Scientific Overview of Blood Group Characterization

- **Antigens**
  - are present on RBCs as glycolipids, proteins, or glycoproteins
  - are inherited characteristics
  - have biological function

- **Antibodies**
  - also known as an immunoglobulin
  - are proteins produced by the immune system following exposure to antigen
  - are usually found in plasma, react with cells carrying foreign antigens.

The secret to complex serology is **in the genes**
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**Antigens**
- Determine A, B, AB or O blood groups
  - A antigen
  - B antigen
  - O is an expression of H (H is precursor to A and B)
- Rh (Rhesus)
  - D positive commonly referred to as Rh Positive
  - D negative commonly referred to as Rh Negative

**Antibodies**
- also known as an immunoglobulin
- are proteins produced by the immune system following exposure to antigen
- are usually found in plasma, react with cells carrying foreign antigens
# Blood Group Serology

<table>
<thead>
<tr>
<th>Red blood cell type</th>
<th>Antibodies in Plasma</th>
<th>Antigens in Red Blood Cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>Anti-B</td>
<td>A antigen</td>
</tr>
<tr>
<td>Group B</td>
<td>Anti-A</td>
<td>B antigen</td>
</tr>
<tr>
<td>Group AB</td>
<td>None</td>
<td>A and B antigens</td>
</tr>
<tr>
<td>Group O</td>
<td>Anti-A and Anti-B</td>
<td>None</td>
</tr>
</tbody>
</table>
ABO and Rh typing is determined by mixing the subject’s blood with specific antibodies; if the antigen for blood group type are present on the patient’s red cells the antisera will agglutinate.
Phenotype and Genotype

- **Phenotype**
  - Greek phainein, ('to show' + typos, 'type') is the visible expression of characteristics or traits
  - Serology it is detected by an agglutination of the antigen on the red cell and the antibody in the test reagent

- **Genotype** is the genetic or gene expression of the characteristic
  - H antigen on Chromosome 19 (precursor to ABO antigen)
  - ABO loci located on Chromosome 9
    - A allele encodes a *glycosyltransferase* that bonds α-N-acetylgalactosamine to D-galactose end of H antigen
    - B allele encodes a *glycosyltransferase* that joins α-D-galactose bonded to D-galactose end of H antigen, creating the B antigen.
Chronology of Immunohematology Discoveries and Important Dates

- **Blood Group Systems Discovery**
  - ABO 1901-1931
  - Rh, Lutheran, Kell, Lewis, Duffy, and Kidd 1939-1951

- **Increase sensitivity in antibody detection**
  - Antiglobulin reagents 1945
  - Enzyme treated RBC 1947
  - LISS 1964
  - Polybrene 1977
  - MoAb production (1975) led to Monocolonal anti-A/B approved in clinical use (1989)

- **Blood banks established in North America (1935)**
- **Blood advances through WW-II**
- **US Public Health Services issues permits to manufacture blood (1946)**
- **AABB established and promotes Standards (1947)**
- **Anti-D immunosuppression 1962 led to RhIG prophylaxis in 1965**
- **Automated and semi-automated instruments (1992)**
  - Solid phase (1984)
  - Column agglutination (1990)
Chronology of Immunohematology Discoveries and Important Dates

- Nucleic acid sequences of blood group genes (1987-1995)
- DNA-based genotyping methods and kits (1993-1999)
- Molecular protocols when RBC phenotyping fails (2000)
- Workshop on Molecular Methods in Immunohematology sponsored by HHS (OASH, FDA, NIH, NHLBI) – Sep 2006
- Transfusion selects to move towards Molecular Genotype – Dec 2010
### Transfusions/1,000 Population Comparison in Selected Countries

<table>
<thead>
<tr>
<th></th>
<th>US</th>
<th>England</th>
<th>Australia</th>
<th>Denmark</th>
<th>Sweden</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Units of RBC per 1000 Population</strong></td>
<td>48.7 (2001)</td>
<td>44.9</td>
<td>28</td>
<td>54.08 (2000-2002)</td>
<td>45.3 (1996-20020)</td>
</tr>
<tr>
<td></td>
<td>48.9 (2006)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>48.8 (2008)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Recipient Age</strong></td>
<td>&lt;41 18.8%</td>
<td>&lt;40 14.4%</td>
<td>&lt;40 15.4%</td>
<td>&lt;39 9.4%</td>
<td>&lt;39 9.8%</td>
</tr>
<tr>
<td></td>
<td>41-65 27.8%</td>
<td>40-70 38.4%</td>
<td>40-70 36.7%</td>
<td>40-59 18.2%</td>
<td>40-59 15.1%</td>
</tr>
<tr>
<td></td>
<td>&gt;65 53.3%</td>
<td>&gt;70 47.2%</td>
<td>&gt;70 47.9%</td>
<td>&gt;60 72.4%</td>
<td>&gt;60 75.2%</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td>M 48.5%</td>
<td>M 50.4%</td>
<td>M 52.5%</td>
<td>M 53.2%</td>
<td>M 52.9%</td>
</tr>
<tr>
<td></td>
<td>F 51.5%</td>
<td>F 49.6</td>
<td>F 47.5%</td>
<td>F 46.8%</td>
<td>F 47.1%</td>
</tr>
</tbody>
</table>

2009 National Blood Collection and Utilization Survey

Kamper-Jorgensen Transfusion 2009; 49:888-894

Cobain Transfusion Medicine 2007; 17, 10-15
2009 HHS Blood Collection and Utilization Survey of RBC Use by Hospital Service

- General Surgery: 11%
- Orthopedic Surgery: 6%
- Cardiac Surgery: 7%
- Trauma/ER: 9%
- Hem/Onc: 15%
- Transplant: 1%
- OB/GYN: 2%
- Ped/Neonates: 2%
- Neph/Dialysis: 2%
- ICU: 11%
- General Medicine: 28%
- Other: 6%

Other: 6%
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10| Scientific Affairs BGG | Holmberg | 27 FEB 2012 | Blood Group Genotype and Phenotype | Business Use Only
# 2009 National Blood Collection and Utilization Survey: Cost of Blood

<table>
<thead>
<tr>
<th>Blood Component</th>
<th>Average Cost to Hospitals 2008</th>
<th>Average Cost to Hospitals 2006</th>
<th>HOPPS Reimbursement Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red Blood Cells*</td>
<td>$227.77</td>
<td>$211.50</td>
<td>$185.15 (19%)</td>
</tr>
<tr>
<td>Plasma Frozen Plasma within 24 hours*</td>
<td>$64.87 (w/in 24 hours )</td>
<td>$52.63 (w/in 24 hours )</td>
<td>$77.93 (20%) (between 8-24 hours)</td>
</tr>
<tr>
<td>Fresh Frozen Plasma (8hr)</td>
<td>$63.76</td>
<td>---</td>
<td>$67.03 (5%)</td>
</tr>
<tr>
<td>WB derived Platelets</td>
<td>$81.74</td>
<td>$65.54</td>
<td>$69.50 (-15%)</td>
</tr>
<tr>
<td>Apheresis Platelets*</td>
<td>$549.65</td>
<td>$525.05</td>
<td>$499.53 (-9%)</td>
</tr>
</tbody>
</table>
FDA Fatality Data from 2005 - 2010

Complication

<table>
<thead>
<tr>
<th>Complication</th>
<th>FY05</th>
<th>FY06</th>
<th>FY07</th>
<th>FY08</th>
<th>FY09</th>
<th>FY10</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRALI</td>
<td>29</td>
<td>35</td>
<td>34</td>
<td>16</td>
<td>13</td>
<td>18</td>
</tr>
<tr>
<td>HTR (non-ABO)</td>
<td>16</td>
<td>9</td>
<td>2</td>
<td>7</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>HTR (ABO)</td>
<td>6</td>
<td>3</td>
<td>3</td>
<td>10</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Microbial Infection</td>
<td>8</td>
<td>7</td>
<td>6</td>
<td>7</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>TACO</td>
<td>1</td>
<td>8</td>
<td>5</td>
<td>3</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>Anaphylaxis</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Other</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>
# Red Cell Serology Typing and Potential Genotyping Application

All Blood Group Genotyping tests commercially available in the U.S. and Canada are for Research Use Only. Not for use in diagnostic procedures

<table>
<thead>
<tr>
<th>Topic</th>
<th>Phenotyping</th>
<th>Potential Genotyping</th>
</tr>
</thead>
<tbody>
<tr>
<td>Techniques</td>
<td><strong>Serology</strong></td>
<td><strong>RUO Molecular</strong></td>
</tr>
<tr>
<td></td>
<td>• Solid phase</td>
<td>• Sequencing</td>
</tr>
<tr>
<td></td>
<td>• Gel cards</td>
<td>• Flow cytometry</td>
</tr>
<tr>
<td></td>
<td>• Manual antisera</td>
<td></td>
</tr>
<tr>
<td>Blood Systems</td>
<td>• ABO and Rh</td>
<td>• Over 30 blood systems</td>
</tr>
<tr>
<td>Patient Populations</td>
<td>• All transfusion and</td>
<td>• Sickle cell patients</td>
</tr>
<tr>
<td></td>
<td>transplantation patients</td>
<td>• Patients with warm antibodies</td>
</tr>
<tr>
<td></td>
<td>• All blood/tissue/organ</td>
<td>• Patients that have formed</td>
</tr>
<tr>
<td></td>
<td>donors</td>
<td>alloantibodies</td>
</tr>
<tr>
<td></td>
<td>• Pregnant women</td>
<td>• Multiply transfused patients</td>
</tr>
<tr>
<td>Objective</td>
<td>• Eliminate ABO-related</td>
<td>• Scientific knowledge to eliminate</td>
</tr>
<tr>
<td></td>
<td>hemolytic transfusion</td>
<td>non-ABO hemolytic transfusion</td>
</tr>
<tr>
<td></td>
<td>reactions (HTRs)</td>
<td>reactions (HTRs)</td>
</tr>
<tr>
<td>Prevalence</td>
<td>• 95% of units go to one-time</td>
<td>• 5% units go to multiply transfused</td>
</tr>
<tr>
<td></td>
<td>transfusions</td>
<td>patients</td>
</tr>
</tbody>
</table>

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In 2000 a German consensus statement was developed on the use of blood group genotyping and its potential application in clinical situations (Legler et al. Infusioinsther Transfusionmed 2000; 27: 215-16)

- In fetus from amniotic fluid or trophoblastic cells (chorionic villi)
- In multiply transfused patients, if standard serology fails
- In case of auto and allo-immunohemolytic anemia, if standard serology fails
- For weak D types and other variant RH alleles, if serology is inconclusive
Blood Group Genotyping – Potential Research Applications

Research Studies to Identify:

- Frequency of rare donors in geographic locations
- Frequency of multiple blood group systems in selected donor populations
- Process improvement for advancing immunohematology
- Research to develop fundamental scientific knowledge related to human disease conditions

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Intended Use

**Red Cell ABO/Rh Typing**

To improve transfusion safety by reliably and accurately identify red blood cell antigens and plasma antibodies in blood samples.

**Blood Group Genotyping**

To genotype antigens for the main allelic variants of red blood cells via selective DNA amplification and solid-phase hybridization.

FOR IN VITRO DIAGNOSTICS

FOR RESEARCH USE ONLY - NOT FOR USE IN DIAGNOSTIC PROCEDURE

Intended Use will be finalized upon regulatory submission
Thank you

The secret to complex serology is in the genes