Blood Group Antigens and Antibodies

• Blood Group Immunology/Pre-transfusion Testing

• ABO & Rh Blood Groups
Blood Group Antigens & Antibodies

• General review of blood group immunology

• Requirements for pre-transfusion testing

• Serologic characteristics of specific antibodies and their clinical significance
Blood Group Antigens and Antibodies

• Blood Group Immunology
  • Immunogenicity
  • Characteristics: IgM and IgG
  • Factors influencing hemagglutination

• Pre-transfusion Testing
  • ABO/Rh and antibody screen
  • Direct and indirect antiglobulin tests
  • Crossmatch
  • Automated testing
What is a blood group?

• “...inherited variations in human red cell membrane proteins, glycoproteins, and glycolipids. These variations are detected by alloantibodies, which occur either ‘naturally’... or as a result of alloimmunization...”

• G. Daniels, Human Blood Groups, 2nd ed.
Blood Group Antigens

- Markers on various red cell structures
- Detected by serologic techniques
  - Discovered when patient serum reacts with donor RBCs
Blood Group Antigens

- Antigens organized into 34 blood group systems that segregate independently
  - >350 known antigens (Ags)
  - Ags within system mark single structure and are part of gene sequence that codes for that structure
  - Genes responsible for systems mapped to locations throughout human genome
Blood Group Antigens

- Multiple alleles within each system
  - Some systems are polymorphic, e.g. Rh has 56, Kell has 34
  - RBCs may express many ags within single system

- Complete red cell phenotypes are highly individualized
ISBT Nomenclature

- ISBT Working Party on Terminology for Red Cell Surface Antigens
- 6 digit unique identifier
- Systems also have an alphabetical symbol
Example of Blood Group Notation

• System
  • ISBT
    • Antigen
    • Phenotype
  • Gene
   • Allele
   • Genotype
  • "Kidd (JK)"
    • ISBT 009
    • Antigen Jk^a, Jk^b
    • Phenotype Jk(a+b+), Jk(a+b–), Jk(a–b+)
    • Jk(a–b–) null phenotype
    • Gene JK
    • Allele Jk^a, Jk^b
    • jk silent allele
    • Genotype Jk^aJk^b, Jk^aJk^a or Jk^aJk
    • Jk^bJk^b or Jk^bJk
    • JkJk null genotype
Blood Group Immunization: Determining Factors

- Immunogenic potential of antigen
  - Rh and Kell most potent
- Dose of antigen
  - amount and frequency of exposure
- Immunocompetence of recipient
  - diagnosis; 20% non-responder rate

- Alloimmunization risk is 1-1.6% per RBC unit transfused
Immunogenicity

• Chemical composition/complexity
• Proteins best, then carbohydrates
• Degree of foreignness
• Size (>10K daltons better)
• Dosage/antigen density
• Route of administration (IM/IV)
Blood Group Immunization: Most Common Specificities

- Rh
- Kell
- Duffy
- Kidd
- MNSs
- Antibodies that occur without exposure to
- RBC Ag: ABH, li, Lewis, P₁, M, N
Blood Group Antibodies
Blood Group Antibodies

IgG
- binds with Ag at 37 °C
- Fc portion carries macrophage receptor
- 2 Fab sites
- monomer requires high concentration to activate complement; only to C3
  - amplifies extravascular hemolysis

IgM
- binds with Ag at ambient temperature or colder
- No macrophage receptor
- 10 Fab sites
- polymer allows complement activation to C9
  - intravascular hemolysis if reactive at 37 °C
IgG Subclasses

- 4 IgG Subclasses
  - IgG1, IgG2, IgG3, IgG4
- Primary differences
  - characteristics of the hinge region
  - number of interchain disulphide bonds
- Ability to activate complement
  - IgG3 ↑ ↑ ↑
  - IgG1 ↑
Primary vs. Secondary Antibody Response

- First contact with antigen
- Second contact with same antigen

Threshold of detectability

IgM
Primary vs. Secondary Antibody Response

Primary
- Occurs over period of weeks
- Requires large antigen dose
- Produces small amount of antibody
- Produces IgM and IgG antibody
- Antibody titer drops shortly after reaching its peak

Secondary
- Occurs over period of days
- Requires small antigen dose
- Produces large amount of antibody
- Produces mostly IgG antibody
- Antibody titer is sustained
Blood Group Antibodies:
Determinants of Hemolytic Potential

• Thermal amplitude
• Ability to activate complement – dependent on titer
• Immunoglobulin class and subclass
• Antibody binding force
• Antigen density
Blood Group Serology

\[ \text{Ag} + \text{Ab} \xrightarrow{k_1} \text{AgAb} \xleftarrow{k_2} \]
Factors Affecting Agglutination Reactions

- **Sensitization**
  - antigen/antibody concentration
  - pH
  - temperature
  - ionic strength

- **Agglutination**
  - zeta potential
  - antibody class
  - antigen density
  - antigen/antibody concentration
Zeta Potential

• Measurement of electrostatic repulsion between red cells
• Directly proportional to distance between red cells
• Must be reduced to support agglutination in some serological tests
  • Albumin and other additives
  • Enzyme treatment of RBCs
ABO and Rh Typing

Anti-A

-D Ag
Effects of Antibody-Antigen Ratios
Agglutination Testing

Positive:
Red Cells Agglutinated

Negative:
Red Cells Not Agglutinated
Blood Bank Routine Work-Flow

SAMPLE

Log in and centrifuge

Type

- No discrepancy
  - Assign blood type

Antibody screen

- Negative
  - Immediate spin cross-match

- Positive
  - Antibody identification
    - If clinically significant
      - Select antigen-negative blood
        - Full crossmatch
  - Antibody identification
    - If clinically significant
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- Positive
  - Antibody identification
    - If clinically significant
      - Select antigen-negative blood
        - Full crossmatch
Other Tests Performed

- Direct Antiglobulin Test (DAT)
- Elution studies
- Auto/allo-adsorption studies – send to Ref. Lab
- Transfusion reaction work-up
- Titers
  - Hemolytic Disease of the Fetus/Newborn
  - Cold agglutinin
  - Anti-A, Anti-B – for kidney transplants
Routine Pre-transfusion Testing

• ABO and Rh typing
• Blood group antibody detection
• Compatibility testing (crossmatch)
• Check previous admission record for typing results and antibody history

• Must be repeated every three days with ongoing transfusions
1900: Landsteiner discovered polymorphisms in human blood (ABO blood groups)
## H Blood Group (Precursor for ABO)

<table>
<thead>
<tr>
<th>Allele</th>
<th>Primary Product</th>
<th>Secondary Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>$H$ (FUT1)</td>
<td>$H$-specific fucosyltransferase</td>
<td>$H$ antigen</td>
</tr>
<tr>
<td>$h$</td>
<td>“silent” allele –</td>
<td>no product</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Immunodominant</th>
<th>Possible Genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Common</td>
<td>Sugar</td>
<td>$HH$, $Hh$</td>
</tr>
<tr>
<td>Bombay</td>
<td>L-fucose</td>
<td>$hh$</td>
</tr>
<tr>
<td></td>
<td>Precursor substance</td>
<td></td>
</tr>
</tbody>
</table>
# ABO Blood Group

<table>
<thead>
<tr>
<th>Allele</th>
<th>Primary Product</th>
<th>Secondary Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>A-specific glycosyltransferase</td>
<td>A antigen</td>
</tr>
<tr>
<td>B</td>
<td>B-specific glycosyltransferase</td>
<td>B antigen</td>
</tr>
<tr>
<td>O</td>
<td>“silent” allele – no product</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Phenotypic Type</th>
<th>Immunodominant Sugar</th>
<th>Possible Genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>N-acetyl-D-galactosamine</td>
<td>AA, AO</td>
</tr>
<tr>
<td>B</td>
<td>D-galactose</td>
<td>BB, BO</td>
</tr>
<tr>
<td>AB</td>
<td>both GalNac &amp; Gal</td>
<td>AB</td>
</tr>
<tr>
<td>O</td>
<td>H substance/Ag</td>
<td>OO</td>
</tr>
</tbody>
</table>
A, B, and H Antigens

GalNAc
GlcNAc
ABO Typing: Forward Grouping

A → spin → B → spin → A → spin → B → spin
ABO Typing: Reverse Grouping

1. Serum
2. A1 Cells Reagent
3. B Cells Reagent

A → B → A → B

Spin
## Routine ABO Typing

<table>
<thead>
<tr>
<th>Reaction of cells tested with</th>
<th>Reaction of serum tested against</th>
<th>Interpretation</th>
<th>Incidence (%) in U.S. population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-A</td>
<td>Anti-B</td>
<td>A&lt;sub&gt;1&lt;/sub&gt; Cells</td>
<td>B Cells</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>+</td>
<td>0</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>0</td>
<td>+</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
ABO Typing: *Background*

- A and B Ag are not restricted to RBCs.
- Not fully developed at birth

- Environmental Ag will provoke anti-A and/or anti-B in individuals who lack the corresponding Ag(s).

- Ab appears shortly after birth, peaks in titer at 5-10 yrs, gradually declines over time.

- Anti-A/B in cord blood is maternal IgG.

- Expected Ab may be missing in infants, elderly, or immunocompromised patients.
ABO Typing - Reagents

• Standardized reagent color
  • anti-A blue  anti-B yellow

• IgM Abs allow direct agglutination

• Interpretation
  • forward and reverse group must confirm
  • must match historical record

• Reagent QC required daily
  • test for specificity
  • document vendor, lot no., outdate, test results
  • note appearance
ABO Typing: Clinical Importance

• ABO incompatible transfusions cause more serious clinical consequences than any other blood group.
• Every recipient (except type AB) is at potential risk for ABO incompatibility.

• Note: Most errors are clerical, not technical.
Rh Typing

• Anti-D reagent + 5% RBCs
  • Spin and read
• Manufacturer must adjust reagent to allow direct agglutination:
  • Rh antigen is less accessible and has fewer sites than A/B
  • Rh antibody is IgG
Rh Typing Reagents

• “Modified tube / slide test”
  • Contain additives to reduce zeta potential
  • May cause false positives; test must include Rh control
• Monoclonal blend
  • Contains both IgM and IgG components
## Weak D Typing (donors)

<table>
<thead>
<tr>
<th>Anti-D IS</th>
<th>Anti-D IAT</th>
<th>Neg Control</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>NA</td>
<td>NA</td>
<td>Rh positive</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>NA</td>
<td>Rh negative</td>
</tr>
<tr>
<td>0</td>
<td>+</td>
<td>0</td>
<td>Rh positive</td>
</tr>
<tr>
<td>0</td>
<td>+</td>
<td>+</td>
<td>unresolved</td>
</tr>
</tbody>
</table>
Blood Group Antibody Detection

5.13.3 Unexpected Antibodies to Red Cell Antigens

“Methods of testing shall be those that demonstrate clinically significant antibodies. They shall include incubation at 37°C preceding an antiglobulin test using reagent red cells that are not pooled.”

Standards for Blood Banks and Transfusion Services
Immunization of Rabbits
Anti-human globulin
Antihuman Globulin (AHG) Reagents

- **Polyclonal**
  - multiple cell lines with different specificities

- **Monoclonal**
  - single antibody specificity

- **Polyspecific**
  - contains both anti-IgG and anti-complement

- **Monospecific**
  - contains either anti-IgG or anti-complement
Direct Antiglobulin Test (DAT)

- Detects antibody bound to RBCs *in vivo*
- Diagnostic test
- Performed only when clinical evidence suggests
  - autoimmune hemolytic anemia
  - drug-induced hemolytic anemia
  - hemolytic disease of the newborn
  - hemolytic transfusion reaction
- Monospecific reagents used to specify immunoglobulin
- One-step test
Direct Antiglobulin Test (DAT)
**Indirect Antiglobulin Test (IAT)**

- Detects free antibody in serum
- *Method for pretransfusion antibody detection*
- AHG reagent must contain anti-IgG
- Two-step test - AgAb binding occurs in vitro
- Other applications: antibody identification, crossmatch, extended antigen typing, weak D test
Indirect Antiglobulin Test (IAT)

Step 1

Serum

37ºC Incubation
Indirect Antiglobulin Test (IAT)

Step 2

37°C incubation → Spin
Testing Additives

• Albumin - detects Rh antibodies
  • Binds to phospholipid layer, disrupts repulsion between cells

• Enzymes - differentiates specificity

• Low ionic strength solution (LISS)
  • Rate of Ab uptake increased
  • Reduced incubation
Testing Additives

• Polyethylene glycol (PEG)
  • Concentrates Ab by displacing diluents from cell surface
  • Also increases rate of Ab uptake when combined with LISS
AHG Testing: Sources of Error 1

• False negative results may be due to:
  • inadequate washing
  • failure to add AHG reagent
  • inactive AHG reagent

• Coombs Control Cells ("Check Cells") must be added to all negative tests to ensure presence of active AHG reagent
Crossmatch Procedure - IS

1. Immediate Spin (IS) Phase

- 5% Donor Cells
- Spin
Crossmatch Procedure - IAT

2. Antiglobulin Phase

Spin
Compatibility Testing

• Immediate spin mandatory
  • to detect ABO incompatibility

• IAT required if unexpected antibody detected in current or any previous sample
  • to detect Ag positive donor

• Electronic crossmatch
  • FDA approved information system, validated to detect ABO mismatch
  • two ABO typing tests of donor and recipient
### Selection of Compatible Donor Blood

<table>
<thead>
<tr>
<th>Patient's ABO Type</th>
<th>Donor RBC Type</th>
<th>Donor Plasma Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>O</td>
<td>O, A, B, AB</td>
</tr>
<tr>
<td>A</td>
<td>A, O</td>
<td>A, AB</td>
</tr>
<tr>
<td>B</td>
<td>B, O</td>
<td>B, AB</td>
</tr>
<tr>
<td>AB</td>
<td>AB, A, B, O</td>
<td>AB</td>
</tr>
</tbody>
</table>
Donor Confirmation Testing for RBCs

• Rh positive units: ABO only

• Rh negative units: ABO and Rh
Pretransfusion Record Requirements

• Transfusion order must include at least patient’s full name and unique numeric identifier

• Patient’s wristband must match information on transfusion order

• Patient sample label must be legible and include:
  • First and last name
  • Unique numeric identifier
  • Date
  • Initials of phlebotomist

• Sample must be labeled at the bedside!
Pretransfusion Record Requirements

• Donor unit designated for transfusion

• Label or tie tag must include:
  • Recipient’s first and last name
  • Recipient’s unique numeric identifier
  • Donor unit number
  • Interpretation of compatibility test
Pretransfusion Record Requirements

• **Release of donor unit for transfusion**
• **Visual inspection of donor unit for container integrity and normal appearance**

• **Release records must include:**
  • Recipient’s name, numeric identifier, ABO and Rh type
  • Donor unit number, ABO and Rh type
  • Interpretation of compatibility test
  • Date and time of issue
  • Names of persons issuing and accepting unit
Pretransfusion Record Requirements

- Emergency issue before completion of compatibility testing
- Physician signed release indicating urgent transfusion need
- Select Group O donor unit
  - may be ABO compatible if current sample typed
  - Rh neg? only young female patients?
- Note - release without compatibility testing on donor unit label
Special Transfusion Circumstances

- Emergency issue
- Massive transfusion
- Neonates
# Overview of BB Tests

<table>
<thead>
<tr>
<th>Test</th>
<th>Purpose</th>
<th>Known</th>
<th>Unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABO/Rh</td>
<td>Test for antigens on RBCs</td>
<td>Commercial antisera (A,B,D)</td>
<td>RBCs</td>
</tr>
<tr>
<td>DAT</td>
<td>Test for IgG/C3 on RBCs</td>
<td>Commercial AHG antisera</td>
<td>RBCs</td>
</tr>
<tr>
<td>Antibody screen/</td>
<td>Detect/identify alloantibodies</td>
<td>Commercial reagent RBCs</td>
<td>plasma</td>
</tr>
<tr>
<td>Antibody ID</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antigen typing</td>
<td>Test for antigens on RBCs</td>
<td>Commercial antisera (anti-K, anti-Jk(a))</td>
<td>RBCs</td>
</tr>
<tr>
<td>Crossmatch</td>
<td>Test for compatibility of donor RBC</td>
<td>Test results on patient and donor</td>
<td>Patient plasma and donor RBCs</td>
</tr>
</tbody>
</table>
Automated/Semi-automated Methods

Alternatives to Tube Testing

• MTS gel cards: acrylamide gel particles in microtubules

• Solid phase: immobilized antigens on microplate wells
Ortho ID-MTS Gel Method
Solid Phase Red Blood Cell Adherence

1. Immobilize antigen
2. Add serum
   - Incubate
   - Wash
3. Bound antibody
   - Negative
   - Positive
4. Add anti-IgG-coated indicator cells
   - Spin
5. Result: Positive adherence (red cells)
Automated Testing

- Gel card (Ortho Clinical Diagnostics)
  - ProVue

- Microplates (Immucor)
  - Galileo, Echo
  - Galileo-Neo

- Microplates (Bio-Rad)
  - TANGO
Reference

• AABB Technical Manual

• Standards for Blood Banks and Transfusion Services (AABB)