Blood Group Antigens and Antibodies

Vivien I Powell, MSc, FIBMS
Operations Manager
Blood Bank
April 18, 2017
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 >30 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 >56, Kell >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  Kidd (JK)
• ISBT  009
  • Antigen  Jk\textsuperscript{a}, Jk\textsuperscript{b}
  • Phenotype  Jk(a+b+), Jk(a+b–), Jk(a–b+)
                Jk(a–b–) null phenotype

• Gene  JK
  • Allele  Jk\textsuperscript{a}, Jk\textsuperscript{b}
            Jk silent allele
  • Genotype  Jk\textsuperscript{a}Jk\textsuperscript{b}, Jk\textsuperscript{a}Jk\textsuperscript{a} or Jk\textsuperscript{a}Jk
               Jk\textsuperscript{b}Jk\textsuperscript{b} or Jk\textsuperscript{b}Jk
               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

IgG

IgM
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

- **IgM**
- **IgG**

First contact with antigen

Second contact with same antigen

Threshold of detectability
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

\[ \text{Ag} + \text{Ab} \xleftrightarrow[\text{k}_2]{\text{k}_1} \text{AgAb} \]
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

**A Ag**

**Anti-D**

**Anti-A**

-D Ag
Effects of Antibody-Antigen Ratios

- Antibody Excess (Prozone)
- Equivalence (Optimum Proportions of Antigen and Antibody)
- Antigen Excess
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

Antigen type patient’s RBCs (if not recently transfused)
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>
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<td>$H$ (FUT1)</td>
<td>H-specific fucosyltransferase</td>
<td>H antigen</td>
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<td>$h$</td>
<td>“silent” allele – no product</td>
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<th>Phenotype</th>
<th>Sugar</th>
<th>Possible Genotypes</th>
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<td>Bombay</td>
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Blood Group Antigens and Antibodies

NYU Langone Medical Center
# ABO Blood Group

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<th>Primary Product</th>
<th>Secondary Product</th>
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<td>A-specific glycosyltransferase</td>
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## Pheno-type

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<th>Phenotype</th>
<th>Sugar</th>
<th>Possible Genotypes</th>
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<tr>
<td>A</td>
<td>N-acetyl-D-galactosamine</td>
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<td>B</td>
<td>D-galactose</td>
<td>BB, BO</td>
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<tr>
<td>O</td>
<td>H substance/Ag</td>
<td>OO</td>
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A, B, and H Antigens

Precursor chain

H

A

B

Gal, Fuc, GalNAc, GlcNAc
ABO Typing: Forward Grouping

- Anti-A Reagent
- Anti-B Reagent
- 5% Cells

Blood Group Antigens and Antibodies
ABO Typing: Reverse Grouping

Serum

A₁ Cells
Reagent

B Cells
Reagent

A₁

B

Spin

Blood Group Antigens and Antibodies
### Routine ABO Typing

Adapted from AABB Technical Manual

<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>
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<td>Anti-A</td>
<td>Anti-B</td>
<td>ABO Group</td>
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<td>+</td>
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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 \textcolor{blue}{blue}\hspace{1cm} anti-B \textcolor{yellow}{yellow}
- IgM Abs allow direct agglutination

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

Reagent QC required \textit{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>
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<th>Anti-D IS</th>
<th>Anti-D IAT</th>
<th>Neg Control</th>
<th>Interpretation</th>
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<td>Rh positive</td>
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<td>+</td>
<td>unresolved</td>
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Blood Group Antigens and Antibodies
5.14.3 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 – 29th Ed
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 \textit{in vivo}
- Diagnostic test
- Performed only when clinical evidence suggests
  - autoimmune hemolytic anemia
  - drug-induced hemolytic anemia
  - hemolytic disease of the fetus/newborn
  - hemolytic transfusion reaction
- Monospecific reagents used to specify immunoglobulin
- One-step test
Direct Antiglobulin Test (DAT)

- 5% Cells
- 3 X Wash
- Anti-globulin reagent
- Spin
Indirect Antiglobulin Test (IAT)

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

Step 1

Serum → 5% Cells → 37°C Incubation

Blood Group Antigens and Antibodies
Indirect Antiglobulin Test (IAT)

Step 2

37°C Incubation → 3 X Wash → Anti-globulin reagent → Spin
Crossmatch Procedure - IS

1. Immediate Spin (IS) Phase

- Patient Serum
- 5% Donor Cells

Spin
2. Antiglobulin Phase

Patient serum + Donor Cells → 37°C Incubation → 3 X Wash → Spin

Anti-globulin reagent
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>
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</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>
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<td>B</td>
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<td>B, AB</td>
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<tr>
<td>AB</td>
<td>AB, A, B, O</td>
<td>AB</td>
</tr>
</tbody>
</table>

Blood Group Antigens and Antibodies
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
Before transfusion at the bedside

• Review of transfusion order and positive identification of the recipient

• Verification of all information matching the blood product with the recipient item by item in the presence of the recipient

• Preferable to use two-person check process
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
## Overview of BB tests

<table>
<thead>
<tr>
<th>Test</th>
<th>Purpose</th>
<th>Known</th>
<th>Unknown</th>
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<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>
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<tr>
<td>Antibody ID</td>
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<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 RBCs</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. Immobilized antigen
2. Add serum
3. Incubate
4. Wash
5. Bound antibody
6. Negative
7. Positive
8. Add anti-IgG-coated indicator cells
9. Spin
Reference

• AABB Technical Manual
• Standards for Blood Banks and Transfusion Services (AABB)
Thank you!
## Antibody Screening

### 3-cell antibody screen

<table>
<thead>
<tr>
<th>cell</th>
<th>Rh-hr</th>
<th>Kell</th>
<th>Kidd</th>
<th>Duffy</th>
<th>Lewis</th>
<th>MNSs</th>
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### Screen for Serum Antibody:

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- **Rh-hr**: Rh, hr
- **Kell**: K, k
- **Kidd**: Jk<sup>a</sup>, Jk<sup>b</sup>
- **Duffy**: Fy<sup>a</sup>, Fy<sup>b</sup>
- **Lewis**: Le<sup>a</sup>, Le<sup>b</sup>
- **MNSs**: M, N, S, s
- **P**: P<sub>1</sub>, 37°C, AHG

**Example - Antibody Screen Result**

Blood Group Antigens and Antibodies
### Ex. Antibody Identification Panel Result

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