Pretransfusion / Compatibility testing
"Medical Center" instrument for direct transfusion
World War II syringe for direct interhuman blood transfusion
Karl Landsteiner

Did a lot of work in:
- Syphilis
- Haptens
- PCH
- Polio
- ABO blood groups
- Died in 1943 after a heart attack in his lab at Rockefeller Institute

http://nobelprize.org/medicine/laureates/1930/landsteiner-bio.html
Blood Group Antigens

- Inherited, different genes encode for different antigens
- Codominant
- Determine an individual's blood group
- Function of the different antigens unclear
  - Receptor for certain bacteria, viruses and parasites
  - Cytokine receptors
Blood group antigens are either sugars or proteins, and they are attached to various components in the red blood cell membrane.

ABO Blood Group System

Most important, highly immunogenic blood group found on red blood cells and other tissue cells

ABO blood groups

- H gene - H substance
- A gene - A enzyme
- B gene - B enzyme
- O gene - amorphic gene, no enzyme
Formation of the A, B, and H antigen

From: Harmening. Modern Blood Banking and Transfusion Practices
# ABO Genotypes, Phenotypes and Frequencies

<table>
<thead>
<tr>
<th>ABO phenotype</th>
<th>Genotype</th>
<th>Antigen</th>
<th>Frequency %</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>OO</td>
<td>Neither</td>
<td>45</td>
</tr>
<tr>
<td>A</td>
<td>AA or AO</td>
<td>A</td>
<td>41</td>
</tr>
<tr>
<td>B</td>
<td>BB or BO</td>
<td>B</td>
<td>10</td>
</tr>
<tr>
<td>AB</td>
<td>AB</td>
<td>A &amp; B</td>
<td>4</td>
</tr>
<tr>
<td>Blood group</td>
<td>Antigen(s) present on the red blood cells</td>
<td>Antibodies present in the serum</td>
<td>Genotype(s)</td>
</tr>
<tr>
<td>-------------</td>
<td>-----------------------------------------</td>
<td>-------------------------------</td>
<td>-------------</td>
</tr>
<tr>
<td>A</td>
<td>A antigen</td>
<td>Anti-B</td>
<td>AA or AO</td>
</tr>
<tr>
<td>B</td>
<td>B antigen</td>
<td>Anti-A</td>
<td>BB or BO</td>
</tr>
<tr>
<td>AB</td>
<td>A antigen and B antigen</td>
<td>None</td>
<td>AB</td>
</tr>
<tr>
<td>O</td>
<td>None</td>
<td>Anti-A and Anti-B</td>
<td>OO</td>
</tr>
</tbody>
</table>
ABO antibodies

- detectable by age 3 months
- naturally occurring
- IgM antibodies; bind C3 - intravascular hemolysis
  - AB - no antibody
  - A - anti B
  - B - anti A
  - O - anti A, anti B, anti A, B
Antibody response

\- Can occur on exposure to a foreign red cell antigen
  \- Transfusion
  \- Pregnancy

\- Depends on “immunogenenecity” of antigen
  \- ABO and Rh group most immunogenic

\- Naturally occurring antibodies can form
<table>
<thead>
<tr>
<th></th>
<th>IgM</th>
<th>IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naturally occurring</td>
<td>Immune stimulated</td>
<td></td>
</tr>
<tr>
<td>React at “cold” temperatures</td>
<td>React at “warm” temperatures</td>
<td></td>
</tr>
<tr>
<td>No red cell hemolysis</td>
<td>Red cell hemolysis</td>
<td></td>
</tr>
<tr>
<td>Does not cross the placenta</td>
<td>Can cross the placenta</td>
<td></td>
</tr>
</tbody>
</table>

![IgM](http://sprojects.mmi.mcgill.ca)

![IgG](http://sprojects.mmi.mcgill.ca)
Blood Selection

- **donor**
  - identification of donor
  - testing of donor

- **recipient**
  - identification of recipient
  - review of transfusion history
  - compatibility testing
  - selection of appropriate donor units
  - identification of patient before infusion of blood
Goal

- Ensure maximum red cell survival
  - acceptable survival of donor rbcs
  - no destruction of recipient’s rbcs
  - Prevent hemolytic transfusion reactions

Prevent disease transmission
Hemolytic transfusion reactions

Clerical and other human errors are the most common causes of ABO incompatible transfusions

- Preanalytical
- Analytical
- Post analytical
Fatal Adverse Events in the United Kingdom

SHOT: Cumulative Data 1996-2003

- TRALI (7%)
- Infectious (2%)
- Mis-transfusion (66%)
- Post-tx Purpura
- Delayed reaction
- Acute reaction

n = 1451

SHOT Annual Report, April 2004: www.shot.demon.co.uk

Safe transfusion from donor to recipient

Pre-transfusion testing

Compatibility testing
- ABO blood group and D typing.
- Antibody screen and identification.
- Cross-match.
- DAT

Detection of platelet antibodies.

WBC quantitation in leukoreduced products.

Feto-maternal hemorrhage.
AABB requirements

Positive identification of recipient and recipient blood sample

Mismatched Blood Kills Patient at Inova Fairfax
Washington Post (08/29/03) P. B1

At Inova Fairfax Hospital, a patient was given the wrong blood type during surgery after she had switched beds with her roommate to be closer to the window. The blood technician had withdrawn blood from the patient's roommate and failed to verify that the roommate was the correct patient. Technicians are required to check the patient's name, clearly marked on his/her hospital bracelet, or ask the patient to state their name aloud. During the intestinal surgery, the deceased patient was given two pints of the wrong blood, causing her immune system to attack the donated cells--reducing her blood pressure, causing kidney failure, and prompting an acute hemolytic transfusion reaction. Doctors tried desperately to save the patient, but she died shortly after. An internal probe of the incident has prompted the hospital to have two technicians visit patients when blood is withdrawn. However, the family could possibly sue the hospital for malpractice and negligence; the technician has since resigned her post.
AABB requirements contd.

- ABO group and Rh typing of recipient’s blood
- Red cell antibody detection tests for clinically significant antibodies
- Comparison of current findings with records
- Confirmation of ABO group of the red cell components
- Confirmation of the Rh type of the Rh negative units
- Selection of ABO and Rh appropriate components
- Serologic or computer crossmatch
- Labeling products with the recipient’s identifying information
- Dispensing and administering the unit to the patient
Patient id and sample labelling

ID patient in a positive manner
  – State name, birth date or address
  – Wristband
  – Blood bank number
  – Drivers license or other photographic id

Label tube before leaving patient with identifiers, date of collection and id of phlebotomist
Transfusion requests

- Electronic or paper

- Requires
  - Two patient identifiers which should include first and last name, unique id #, DOB
  - Component needed
  - Special requests
  - Other clinical information
  - Name of responsible physician

- All blood banks should have a written policy defining the request acceptance criteria
Blood Orders

- Type
- Type and Screen
- Type and Hold
- Type and Crossmatch
Blood Sample

- Collected in EDTA tube
- Prefer non hemolyzed sample
- Sample should be collected no more than 3 days from intended transfusion
  - To ensure that current sample represents the current immunologic status of the patient
  - Retain for at least 7 days after each transfusion
Serologic Testing

- Tests detecting antigen on recipient cells
  - ABO grouping (most critical test)
  - Rh typing

- Tests detecting antibody in recipient serum
  - Antibody screen
  - Antibody identification
  - Crossmatch

- Tests detecting Antibody on patient red cells

- Other tests
Pretransfusion testing algorithm

Clerical Check

ABO typing

Antibody Screen

Antibody Identification

Immediate spin Crossmatch

AHG crossmatch

Compatible?

Incompatible?

Clerical check, Retype
Repeat antibody screen?
Cold auto?
Crossmatch more units

Issue blood after one more check
ABO Typing

ABO typing is done by testing the patient's red cells with anti-A and anti-B antisera (forward grouping) and testing the patient's serum for the presence of anti-A and anti-B against reagent test A1 and B cells (reverse grouping). This test is done at room temperature and a positive reaction is determined by agglutination of the red cells.

IgM leads to the agglutination of red blood cells. Source: Med4you

Commercial monoclonal reagents available

Tube, slide, gel, microwell methods available

Tube most commonly used
## Forward and Reverse Grouping

<table>
<thead>
<tr>
<th>Patient cells</th>
<th>Patient Anti A</th>
<th>Anti B</th>
<th>Interpretation:</th>
<th>A1 cells</th>
<th>B cells</th>
<th>Interpretation</th>
<th>Reverse grp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td></td>
<td></td>
<td>Forward grp</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>O</td>
<td>+</td>
<td>+</td>
<td>O</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>-</td>
<td>A</td>
<td>-</td>
<td>+</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>+</td>
<td>B</td>
<td>+</td>
<td>-</td>
<td>B</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>+</td>
<td>AB</td>
<td>-</td>
<td>-</td>
<td>AB</td>
<td></td>
</tr>
</tbody>
</table>
85% of population are positive for the D antigen present on Rh molecule.

D antigen is highly immunogenic.

IgG antibody results following exposure.

IgG antibody is an important cause of hemolytic disease of the newborn.
## Antibodies produced against Rh antigens

<table>
<thead>
<tr>
<th>Antibody type</th>
<th>Mainly IgG, some IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>The majority of Rh antibodies are of the IgG type.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Antibody reactivity</th>
<th>Capable of hemolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rh antibodies rarely activate complement. They bind to RBCs and mark them up for destruction in the spleen (extravascular hemolysis).</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Transfusion reaction</th>
<th>Yes typically delayed hemolytic transfusion reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Anti-D, anti-C, anti-e, and anti-c can cause severe hemolytic transfusion reactions. Hemolysis is typically extravascular</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Hemolytic disease of the newborn</th>
<th>Yes the most common cause of HDN.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>The D antigen accounts for 50% of maternal alloimmunization</td>
</tr>
<tr>
<td></td>
<td>Anti-D and anti-c can cause severe disease.</td>
</tr>
<tr>
<td></td>
<td>Anti-C, anti-E, and anti-e can cause mild to moderate disease.</td>
</tr>
</tbody>
</table>
Rh Testing

- is done using anti D blood grouping serum
- tube or slide method
- Weak D
  - Decreased D antigen
  - Partial expression of D antigen
  - Can confirm by AHG testing
Antibody screen

To detect as many clinically significant red cell antibodies as possible

- antibodies reactive at 37°C
- known to cause transfusion reactions or shortened red cell survival
- incidence 0.78 - 1.64%
Antibody Screen

- is performed using selected group O rbcs that carry most of the common red cell antigens
- Testing on pooled cells not recommended
  - Only reading at AHG phase is required
  - Tube, gel, red cell solid phase
  - Enhancement media may be used
  - Use method that detects most clinically significant and few insignificant antibodies in a timely manner
Indirect antiglobulin test (IAT)

- Detects IgG antibodies in the patient’s serum
- This is the methodology behind the antibody screen or identification
Indirect antiglobulin test

Test RBCs + Patient serum → IgG bound to test RBC + Anti IgG → agglutination
Gel Test

Illustration of a microtube.

ADD REACTANTS
Serum/Plasma and/or Red Cells

Reaction Chamber

Gel and Reagent

The gel test yields reactions that are graded from 0 to 4+. This is similar to the grading used in traditional tube testing.
Ortho gel
# Antibody Screen

<table>
<thead>
<tr>
<th>Cell #</th>
<th>Rh-hr</th>
<th>Donor Number</th>
<th>D</th>
<th>C</th>
<th>E</th>
<th>c</th>
<th>t'</th>
<th>CW</th>
<th>V</th>
<th>K</th>
<th>Kp</th>
<th>Kp</th>
<th>Js</th>
<th>Js</th>
<th>Fy</th>
<th>Fy</th>
<th>Jk</th>
<th>Jk</th>
<th>Xg</th>
<th>Le</th>
<th>Le</th>
<th>S</th>
<th>M</th>
<th>N</th>
<th>P</th>
<th>Lu</th>
<th>Lu</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>R1R1</td>
<td>101692</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>+</td>
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<td>2R2R2</td>
<td>42591</td>
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<td>+</td>
<td>0</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Shaded columns indicate those antigens which are destroyed or depressed by enzyme treatment.

Ortho-Clinical Diagnostics, Inc.
a Johnson & Johnson company

Reagent Red Blood Cells
0.8% Surgiscreen®

LOT NO.
8SS314

EXP. DATE
2005-02-08

Antigam® Antigen Profile

Memorial Hermann
201 E Loop South
HOUSTON, TEXAS

* 1 antigen status may have been determined presumptively based on Rh-hr phenotype
Screening Panel

Possible Interpretation

- single alloantibody
- two antibodies, antigen present in cell II only
- probable IgG antibody

<table>
<thead>
<tr>
<th>Cell</th>
<th>AHG</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC I</td>
<td>neg</td>
</tr>
<tr>
<td>SC II</td>
<td>2+</td>
</tr>
<tr>
<td>Auto</td>
<td>neg</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>cell</th>
<th>AHG</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC I</td>
<td>3+</td>
</tr>
<tr>
<td>SC II</td>
<td>1+</td>
</tr>
<tr>
<td>Auto</td>
<td>neg</td>
</tr>
</tbody>
</table>

- multiple antibodies
- single antibody
- probable IgG
To determine specificity of antibody
- Tube, Gel, SPECA, methodologies
- Can use enhancement media like LISS, albumin, PEG, AHG, enzymes
- Honor previous antibody even if undetectable
  - 30-35% antibodies become undetectable in 1 year, and 50% are undetectable in 10 or more years
- Autocontrol or DAT not required
Antibody identification panel

Patient’s serum is tested against a panel of blood group O rbc's
Factors affecting agglutination (stage one)
- Chemical bonding
- Equilibrium constant of antibody
- Temperature
- pH
- Incubation time
- Ionic strength
- Antigen-antibody proportion

Factors affecting agglutination (stage two)
- Size and physical properties of antibody
- Concentration of antigen sites
- Distance between cells
  - Zeta potential
  - Water of hydration
  - Van der Waals forces
## Enhancement media

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Action</th>
<th>Antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>22% albumin</td>
<td>Adjusts zeta potential</td>
<td>IgG, may enhance cold abs</td>
</tr>
<tr>
<td>LISS</td>
<td>Increases rate of ab binding to RBC antigens</td>
<td>IgG, may enhance cold abs</td>
</tr>
<tr>
<td>Polybrene</td>
<td>Neutralization of charge</td>
<td>IgG, may be less sensitive for Kell</td>
</tr>
<tr>
<td>enzymes</td>
<td>Enhances some RBC antigens, depresses others</td>
<td>Enhances Rh, Kidd, P₁, I, Lewis</td>
</tr>
<tr>
<td></td>
<td>Lower zeta potential</td>
<td>Destroys Duffy, MNS</td>
</tr>
<tr>
<td></td>
<td>Interfacial tension</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spicule formation</td>
<td></td>
</tr>
<tr>
<td>Polyethylene Glycol</td>
<td>Removes water, promotes ab</td>
<td>IgG, ↓IgM reactivity, false positives, enhances warm auto</td>
</tr>
<tr>
<td></td>
<td>uptake and reaction strength</td>
<td></td>
</tr>
<tr>
<td>AHG</td>
<td>Crosslinks sensitized cells → visible agglutination</td>
<td>Polyspecific or monospecific IgG</td>
</tr>
</tbody>
</table>
Crossmatch

- Final check of ABO compatibility
- May detect antibody not found on screening
Crossmatch

- **SeroLogic**
  - Immediate spin only if antibody screen is negative (abbreviated crossmatch)
  - Antiglobulin if antibody screen is positive

- **Electronic or Computer crossmatch**
  - if antibody screen is negative
## Compatible RBC units

<table>
<thead>
<tr>
<th>DONOR</th>
<th>O</th>
<th>A</th>
<th>B</th>
<th>AB</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>B</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>A</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>O</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>

### Table

- **Compatible RBC units** for various donor and recipient blood groups are indicated by a check mark (✓).
Frequency of + crossmatch and - screen

- **Mintz study**
  - 0.2% patients had positive crossmatches after a negative antibody screen

- **Heddle study**
  - 0.3% patients had incompatible transfusions, no adverse outcome

- **Havemann study**
  - Calculated incidence 0.008%
Causes of Incompatible crossmatches

- incorrect ABO grouping
- alloantibody in patient serum
- autoantibodies
- positive DAT on donor cells
- abnormalities in patient serum
  - imbalance of albumin/ gamma globulin ratio
  - plasma expanders
  - Antibody to additives
- Contaminants in test system
Direct Antiglobulin testing

tests for sensitization of red cells by antibody or complement

Autoimmune hemolytic anemia
Hemolytic disease of the newborn
Hemolytic transfusion reactions
Drug induced
idiopathic
Direct Antiglobulin test

Patient red blood cells + Anti-IgG (AHG or Coombs serum) → agglutination
Turn around time

‘But I sent in the sample 10 minutes ago!’
Turn - Around time

- RBC
  - emergency - immediately
  - emergency incomplete - 15mins
  - emergency complete - 60 mins
  - elective complete - 2 - 4 hours

- FFP, cryoprecipitate – 30- 60mins
- platelets - 30mins (for pooled)
Emergency Transfusion

Especially seen in trauma and surgical cases

May need to transfuse blood before testing is completed
Emergency Transfusion

- Physician will need to sign an emergency release form, waiving compatibility testing

- The blood bank will immediately issue
  - Uncrossmatched O negative RBCs
    - We need a patient sample ASAP
  - OR
  - Uncrossmatched group specific RBCs
    - If you can wait 5-10 minutes
    - If patient's type is already known
Other Serologic Techniques

- Enzyme treatment
- Elution
- Adsorption
- Neutralization
- Chloroquine diphosphate
- Quantification of antibody
Plasma Products

- Test for ABO group
- no crossmatch required
- plasma or plasma product should be compatible with recipient ABO blood group.
## Compatible Plasma

<table>
<thead>
<tr>
<th>DONOR</th>
<th>AB</th>
<th>O</th>
<th>A</th>
<th>B</th>
<th>AB</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB</td>
<td>✔</td>
<td></td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>B</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O</td>
<td>✔</td>
<td>✔</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Compatibility testing for infants (less than 4 months old)

- ABO, Rh testing

- Alloimmunization to RBC antigens is rare

- Antibody screen
  - using maternal serum or plasma
  - using infants serum or plasma
  - infants eluate
Compatibility testing for infants (less than 4 months old)

Repeat compatibility testing may be omitted during one hospitalization as long as several criteria are met:

- Initial screen is negative
- Transfused cells are group O or ABO identical
- Rh negative or Rh identical
Crossmatch/ Transfusion ratio

- Ratio of crossmatched units to actually transfused units for a patient

- C:T >2.) indicates excessive crossmatch requests

- Ordering guidelines available for different surgical procedures
Other Terminology

- **Maximum surgical blood order schedules (MSBOS)**
  - Use data to determine if a T/S order or a certain number of units is required for different elective surgical procedures

- **Standard blood order system (SBO)** – modification of MSBOS in some institutions
Problems you will encounter

- Multiple antibodies
- Antibodies to high frequency antigens
- Antibodies to low frequency antigens
- Cold or warm autos
- Antibodies to reagents
- Incompatible crossmatch
- Emergency release
- Switch Rh type

Dr Smart, Path resident
How do I determine how many units to screen when a clinically significant antibody is detected?

- **Need to know incidence of antigens**

Example: Pt has anti K and needs 4 units RBC

- K - incidence is 90% = 0.90
- 4 units K- blood needed = 4.4 units
- 0.90
Future of Compatibility testing

- RBC substitutes
- Biochemical modification of all non O blood groups
- Automation
  - Solid phase
  - Galvanic testing
  - Gel testing
- Dipstick tests
- Dry plate testing
Hemoglobin polymerization

Tetramers → Polymerization → Purification → Polymers

Red Blood Cell

http://www.northfieldlabs.com/polyheme

Polyheme

Patient sample
Biochemical modification of all non O blood groups
Automation
Pathogen inactivation

http://www.interceptbloodsystem.com
Future contd

Genomic microarray Technology and Proteomic analysis

Blood molecular genotyping
"Personally, I wouldn't have signed it."

http://www.jwolfe.clara.net
Quiz

How did protein S come about its name?

CLUE: a city

Protein S is named S for Seattle, because the first patient described with protein S deficiency was in this city.