

## Immunoematology

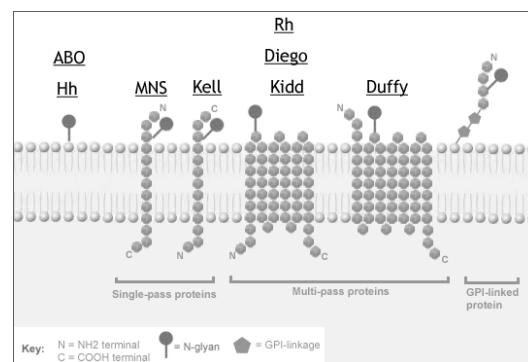
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## Immunoematology

- Demonstration of **red cell antigen-red cell antibody reactions** is the key to immunoematology
- Combination of antibody and antigen can result in observable reactions, most commonly:
  - **Agglutination**
  - **Hemolysis**
  - Precipitation
- **Pretransfusion Testing**
  - ABO/Rh typing
  - other blood group antigen typing
  - detection of red cell alloimmunization (unexpected antibodies)
  - Compatibility testing (crossmatching)
- **Transfusion reaction work up** (Direct Antiglobulin Test, eluate)
- **Immune mediated red cell destruction** (DAT, eluate)

## Blood Group Antigens

- Markers on red cell structures
  - Carbohydrates on proteins or lipids
  - proteins
- Over 250 antigens in 23 Blood Group Systems
  - E.g. ABO, Rh, Kell, Duffy, Kidd, MNSs
- Detected by serologic techniques
  - However, genotypes can be determined by molecular techniques
- Multiple alleles within each system/dominant/codominant
- Red cell phenotypes are highly individualized

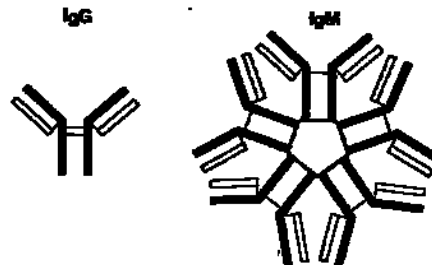


Blood Groups and Red Cell Antigens NCBI Laura Dean, MD

## Characterizing Red Cell Antibodies

- Immunoglobulin Class
  - IgG vs. IgM
- Antigen they are directed against
  - Carbohydrate vs. protein
- Method of stimulation
  - Natural vs. Irregular
- Optimum temperature of reaction
  - Cold vs. Warm
- Optimum Medium (high protein, saline, antiglobulin)
- Complement fixation
- In vitro vs. in vivo effect
  - Intravascular Hemolysis vs. Extravascular Hemolysis vs. Non-hemolytic
  - Agglutinating

## Characterizing Red Cell Antibodies



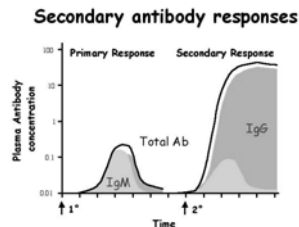
## IgG vs IgM

- | IgG   | IgM  |
|---|--|
| <ul style="list-style-type: none"> <li>• Binds at warm temperature (37°C)</li> <li>• Fc portion carries macrophage receptor</li> <li>• Only 2 Fab sites</li> <li>• High concentration required to activate complement</li> <li>• Extravascular hemolysis</li> </ul> | <ul style="list-style-type: none"> <li>• E.g. anti-A and anti-B</li> <li>• Binds at room or cold temperatures</li> <li>• 10 Fab sites per molecule</li> <li>• Efficient at activating complement</li> <li>• Intravascular hemolysis</li> </ul> |

## Blood Group Antibodies

- Naturally Occurring Antibodies
  - E.g. ABO Blood Group System
  - Combination of A and B antigens make up the ABO Blood Groups (A,B,AB,O)
  - “naturally” occurring antibody will be made against antigens that the individual does not have
  - Usually IgM
- Irregular Antibodies
  - There are many other red cell antigens
  - Exposure by pregnancy, transfusion or transplant can result in an alloantibody if the person does not possess that antigen
  - Usually IgG
  - E.g. anti-D formation in a D negative woman who gives birth to a D-positive infant

## Primary vs. Secondary Response



## What do we do with that tube of blood?

*Do not harm the patient!*  
Draw and label the sample correctly for the right patient.

## Which one is an acceptable specimen?

- Tube labeled with patient name and medical record number, phlebotomist confirms with patient wristband, date on label, labels attached at nursing station
- Tube labeled with the patient name and medical record number, phlebotomist confirms with the medical chart at the foot of the bed, date on label, labels at the bedside
- Tube labeled with the patient name and medical record number, phlebotomist confirms with patient wristband, date on label, signature of phlebotomist on the label, label attached at bedside

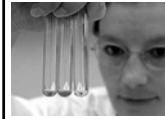
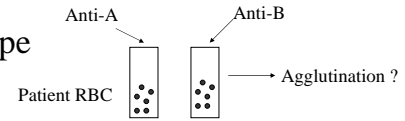
## Why was my sample rejected?

- Need to protect recipient!
- Requisition
- Tube:
  - Full name and MRN
  - Confirm using wristband
  - Signature of phlebotomist
  - Date
  - Labeled at bedside
- Sample good for 3 days if transfused or pregnant
- Good for 30 days if not transfused or pregnant

## Routine Pretransfusion Testing Type and Screen

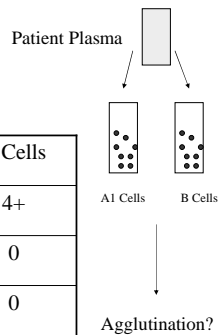
- Type
  - ABO (front and back type)
    - Direct agglutination is seen because anti-A and anti-B are IgM antibodies
  - Rh (D antigen)
- Antibody Screen
  - Screen for irregular antibodies
- Compatibility Testing
  - Crossmatch

## Front Type



	Anti-A	Anti-B
A	4+	0
B	0	4+
AB	4+	4+
O	0	0

## Back Type



	A1 Cells	B Cells
A	0	4+
B	4+	0
AB	0	0
O	4+	4+

- Front and Back Type must match
- Comparison with previous typings must match

## ABO Compatible

- |                           |                   |
|---------------------------|-------------------|
| • <u>Packed Red Cells</u> | • <u>FFP</u>      |
| • A      A,O              | • A      A,AB     |
| • B      B,O              | • B      B,AB     |
| • AB     A,B,AB,O         | • AB     AB       |
| • O      O                | • O      A,B,AB,O |

## ABO Discrepancy Case

- 75 year-old man admitted for colon resection for colon carcinoma

Anti-A	Anti-B	A1 Cells	B Cells
4+	2+	0	4+

### Acquired B phenotype in a A patient

- Front Type: AB
  - Anti-A reacts strongly
  - anti-B reacts weakly with the acquired B
- Back Type: A
  - A type patient make only anti-B regardless of acquired B phenotype
- A antigen is converted to B-like substance by deacetylation of A substance
- Seen in gastrointestinal carcinomas and obstruction
- Resolved by acidifying reaction so that anti-B does not recognize acquired B substance

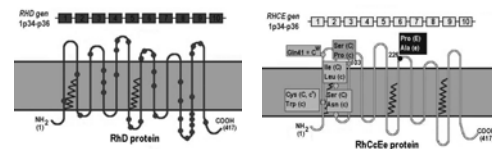
### ABO Discrepancy Case

Anti-A	Anti-B	A1 Cells	B Cells
4+	0	1+	4+

Front Type: A  
Back Type: AB

### Subgroups of A

- Subgroups of A are not uncommon
- Most common subgroup is A2 phenotype
- Lower expression of A substance than A1 phenotype
- Can make an anti-A1 antibody, which is usually clinically insignificant
- Resolve by reacting with A2 cells instead of A1 cells



- The Rh system consists of several antigens (DCCeE....)
- Most commonly known is the D antigen
- Rh typing is performed by adding anti-D reagent to the patient's red cells
- Agglutination: Rh+ (i.e. D+)

### Rh Discrepancy Case

A 24 year-old woman is admitted for elective knee arthroscopy. Routine laboratory tests are ordered. The patient told that her blood type is O negative.

The patient states that the typing is wrong. The Donor Center where she gives blood has told her that her blood type is O+.

Why is there a discrepancy?

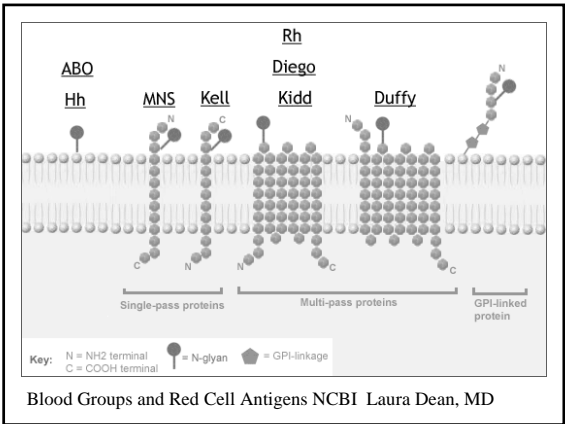
### Weak D phenotype

- Some D+ patient have weak expression of the antigen or only express a portion of the D antigen
- This phenotype may result in a negative test with routine Rh testing with anti-D reagent
- Further testing with anti-D and then antihuman globulin detects the weaker expression

- What does it mean for the donor?
  - Transfusion of blood that has weak expression of D antigen into a D negative recipient → May be immunized
  - Weak D donors are given a D+ typing to protect recipient
  - All D negative donors are tested for weak D
- What does it mean for the recipient?
  - This is a topic of debate
  - Usually, these patients can receive D+ blood without becoming immunized
  - Some can make an anti-D
  - Some institutions will transfuse with D negative for weak D patients or not type for weak D at all
  - Others will transfuse D+ cells into a weak D patient

## Antibody Screen

- There are many blood group antigen systems corresponding to red cell structures
- Exposure to these structures, when not present on the patient’s cells, can result in immunization with the formation of alloantibodies



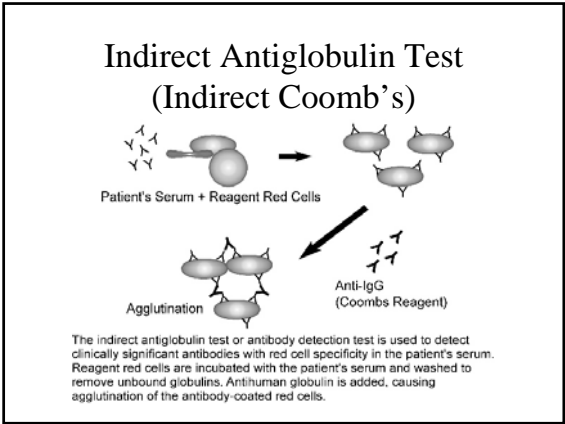
## How are red cell antibodies formed?

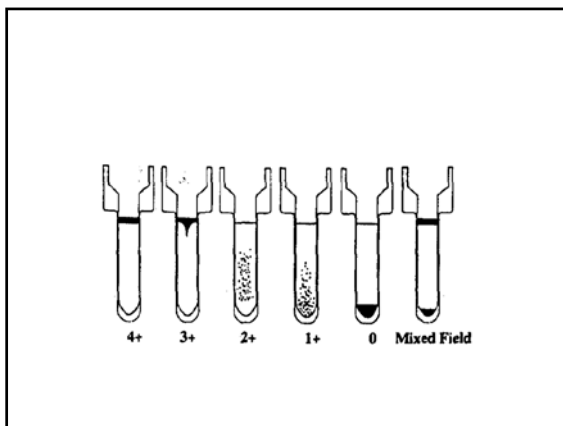
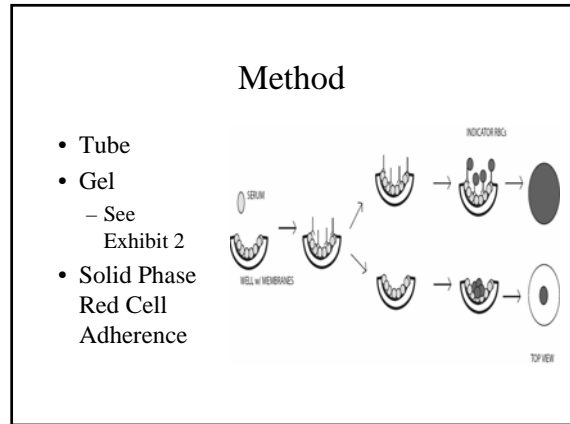
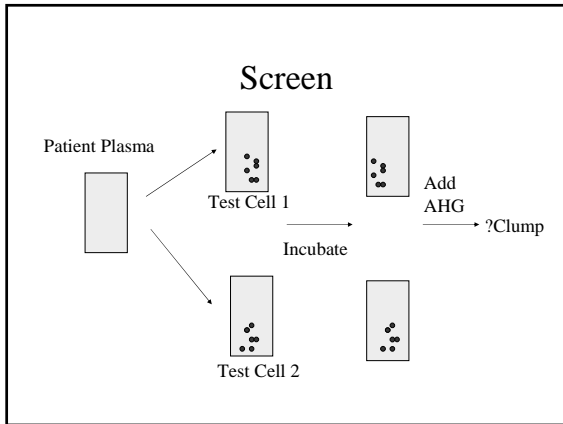
- No expression of the antigen on patient cells
- Exposure to the antigen from:
  - Pregnancy
    - Fetal red cell antigens from a fetomaternal bleed or at delivery
  - Transplant
  - Transfusion

### Anti-human Globulin (Coomb’s Reagent)



- Anti-IgG reagent prepared by immunizing rabbits
- Anti-IgG reagent prepared as a monoclonal antibody
- Anti-IgG will “bridge” IgG attached to red cells





### Antibody Panel

- See Exhibit 1
- An anti-Fy(b) antibody

### Alloantibody Case

- A 24 year old patient with sickle cell disease has a past history of CVA. He is on chronic red cell exchange.
- The laboratory informs you that the antibody screen is positive. You will have to wait several hours for the antibody to be identified and crossmatch units.
- You ask your friendly neighborhood pathologist to explain.

An O negative patient has a positive antibody screen. Antibodies against E,c,K antigens are identified. If the frequency of E antigen is 25%, c antigen 70% and K 10%

How many units will have to be tested to find 2 compatible units?

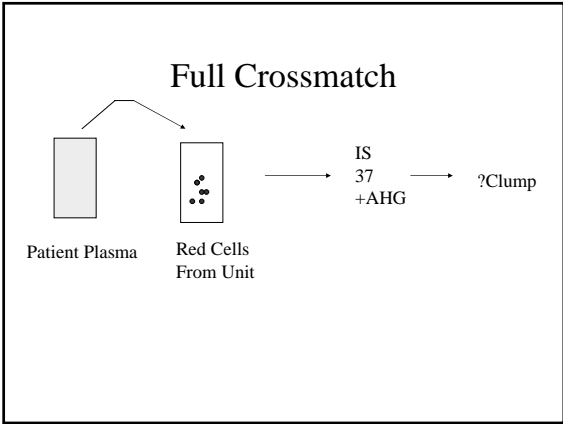
$0.15 \times 0.75 \times 0.3 \times 0.9 = 0.03$  (3 in 100 units)

For 1 unit:  $1/0.03 = 33$  units

For 2 units = 67 units

## Electronic Crossmatch

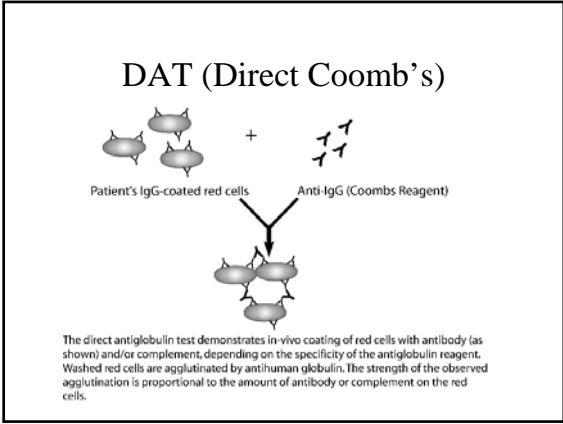
- Validated Computer System
- If ABO typing confirmed and no irregular antibodies



## Emergency Transfusion

- There may not be time for full pretransfusion workups
  - In acute blood loss, the need is more urgent for volume than for oxygen carrying capacity; fluids are probably more critical in early stages.
- Rule #1: Don't harm anyone!
  - High stress !!!

<10 min	Uncrossmatched; O+ male and older female; O neg younger female
10-30 min	ABO/Rh uncrossmatched
>30 min	ABO/Rh XM



## Major Causes of Positive DAT

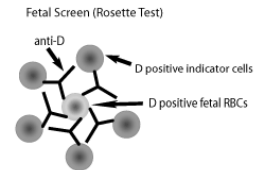
- Alloantibodies
  - E.g. delayed hemolytic transfusion reaction
- Autoimmune Antibodies
- Hemolytic Disease of the Newborn
  - Anti-D
  - Anti-A or anti-B

- A 26 year-old healthy woman gives birth to a 7 lb male infant. At birth, the infant appears jaundiced with elevated bilirubin and a mildly low Hct . The mother is type O neg and the antibody screen is negative. The infant's blood type is A negative.
- What test would confirm the diagnosis of hemolytic disease of the newborn due to ABO antibodies?

## ABO HDN

- Isn't anti-A IgM and doesn't cross the placenta?
  - Mother makes anti-A,B IgG
  - Crosses the placenta and binds to fetal red cells causing hyperbilirubinemia
  - DAT+
    - Antibodies removed from red cells and they react with A cells (fetal cells are coated with anti-A reactive ab)

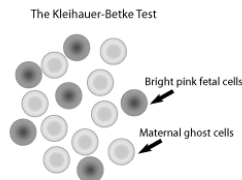
## Fetal Screen



- 36 year old woman with two prior pregnancies presents for her first prenatal visit at 28 weeks during her third pregnancy. The first two pregnancies and deliveries were uneventful. Her blood type is O negative. The father is O positive. The first child is O negative and the second child is O positive.
- Her antibody screen is positive and antibodies against the D antigen are identified. It reacts 4+ and is titrated out to a titer of 1:1024.
- How could this have been prevented?

- Rh HDN
  - Mother does not have D antigen
  - Father passes on D antigen to fetus
  - Delivery or fetomaternal bleed leads to immunization and anti-D is formed
  - Antibodies can cross placenta and hemolyze D positive fetal cells during another pregnancy
- RhoGAM prophylaxis
- At birth, screen for bleed
  - Fetal Screen
- Quantify with Kleihauer-Bethke
- Dose

## Kleihauer-Bethke



## Dose the RhoGAM

- Fetal Screen+
- Kleihauer-Betke 1%
- Mother blood volume = 5000cc
- $5000\text{cc} \times 0.01 = 50\text{cc}$
- $300\text{mcg}/30\text{cc} \rightarrow$  two 300mcg vials + 1 for good measure
- Give 3 doses