

HOSPITAL/INSTITUTE/CENTER

**Blood Group Antigens
and Antibodies**

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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** **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 $Jk^a Jk^b, Jk^a Jk^a$ or $Jk^a Jk$
 - $Jk^b Jk^b$ or $Jk^b Jk$
 - *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*

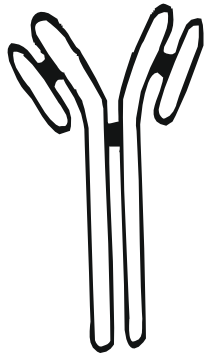
Immunogenicity

- **Chemical composition/complexity**
- **Proteins best, then carbohydrates**
- **Degree of foreignness**
- **Size (>10K daltons better)**
- **Dosage/antigen density**
- **Route of administration (IM/IV)**

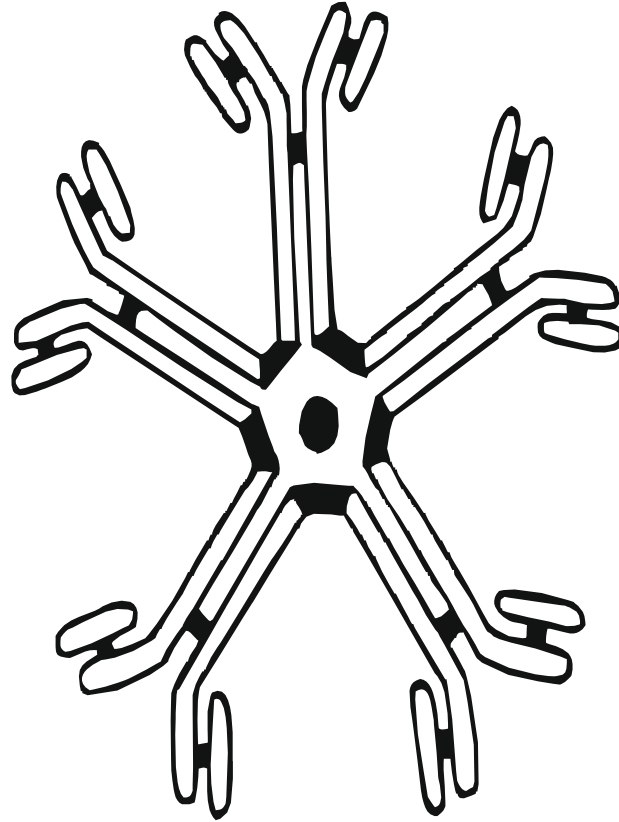
Blood Group Immunization: Most Common Specificities

- Rh
- Kell
- Duffy
- Kidd
- MNSs
- *Antibodies that occur without exposure to*
- *RBC Ag: ABH, Ii, 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

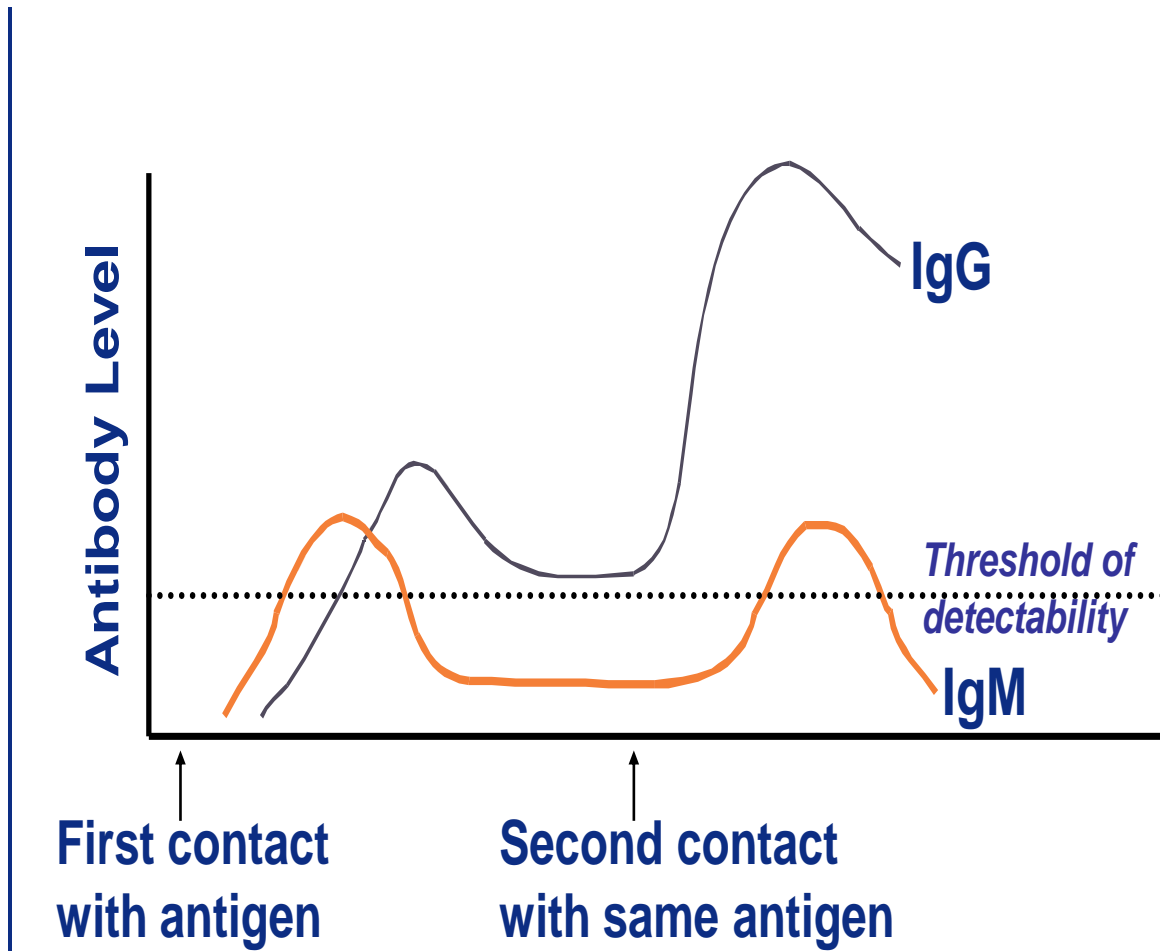
- **Primary differences**

- characteristics of the hinge region
- number of interchain disulphide bonds

- **Ability to activate complement**

- IgG3 ↑ ↑ ↑
- IgG1 ↑

Primary vs. Secondary Antibody Response



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

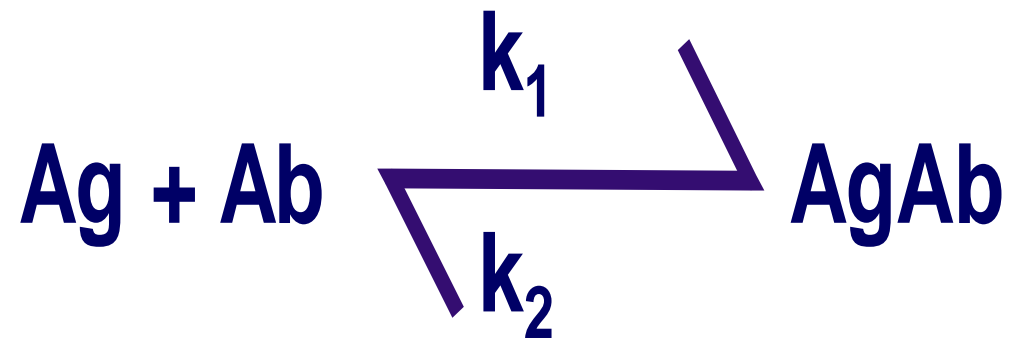
Secondary

- 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



Factors Affecting Agglutination Reactions

- **Sensitization**

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

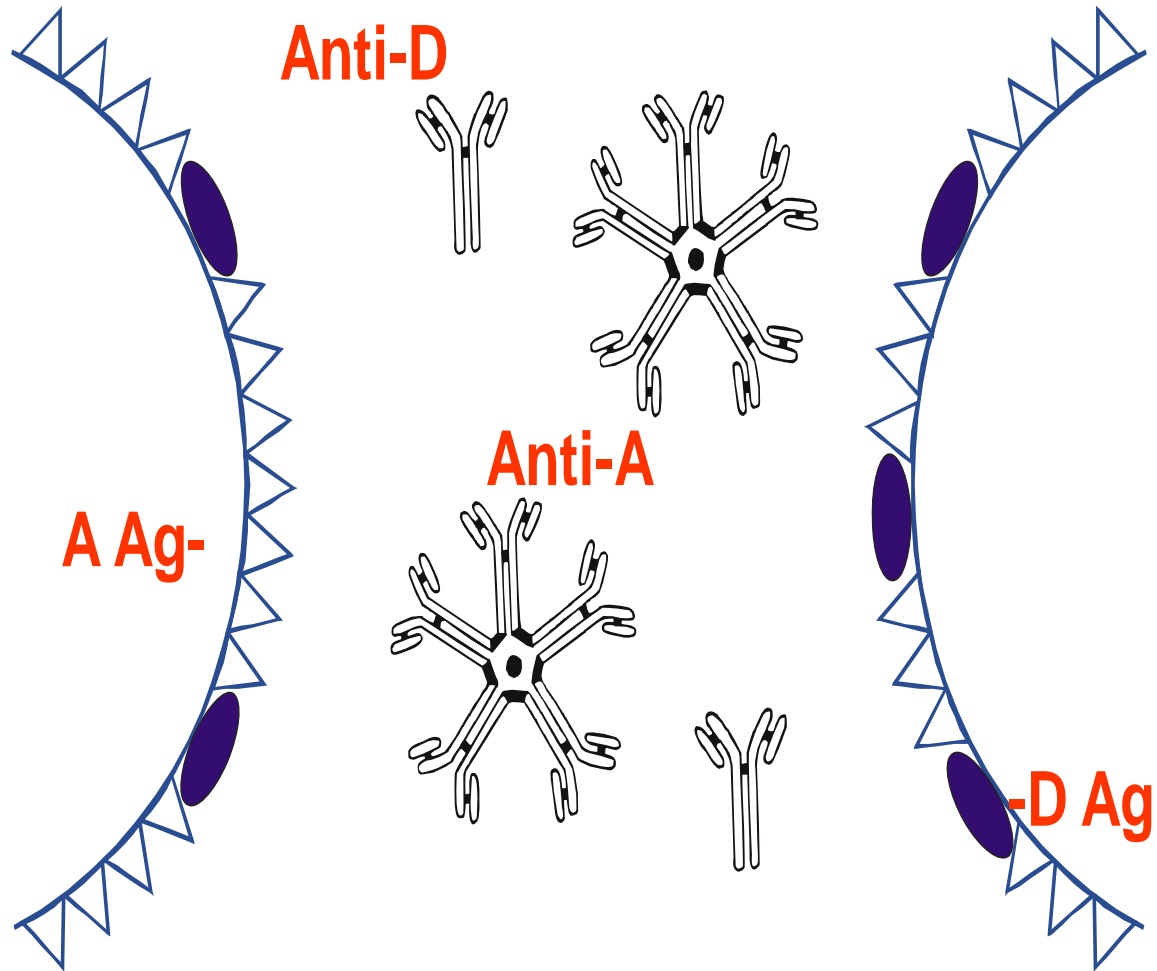
- **Agglutination**

- zeta potential
- antibody class
- antigen density
- antigen/antibody concentration

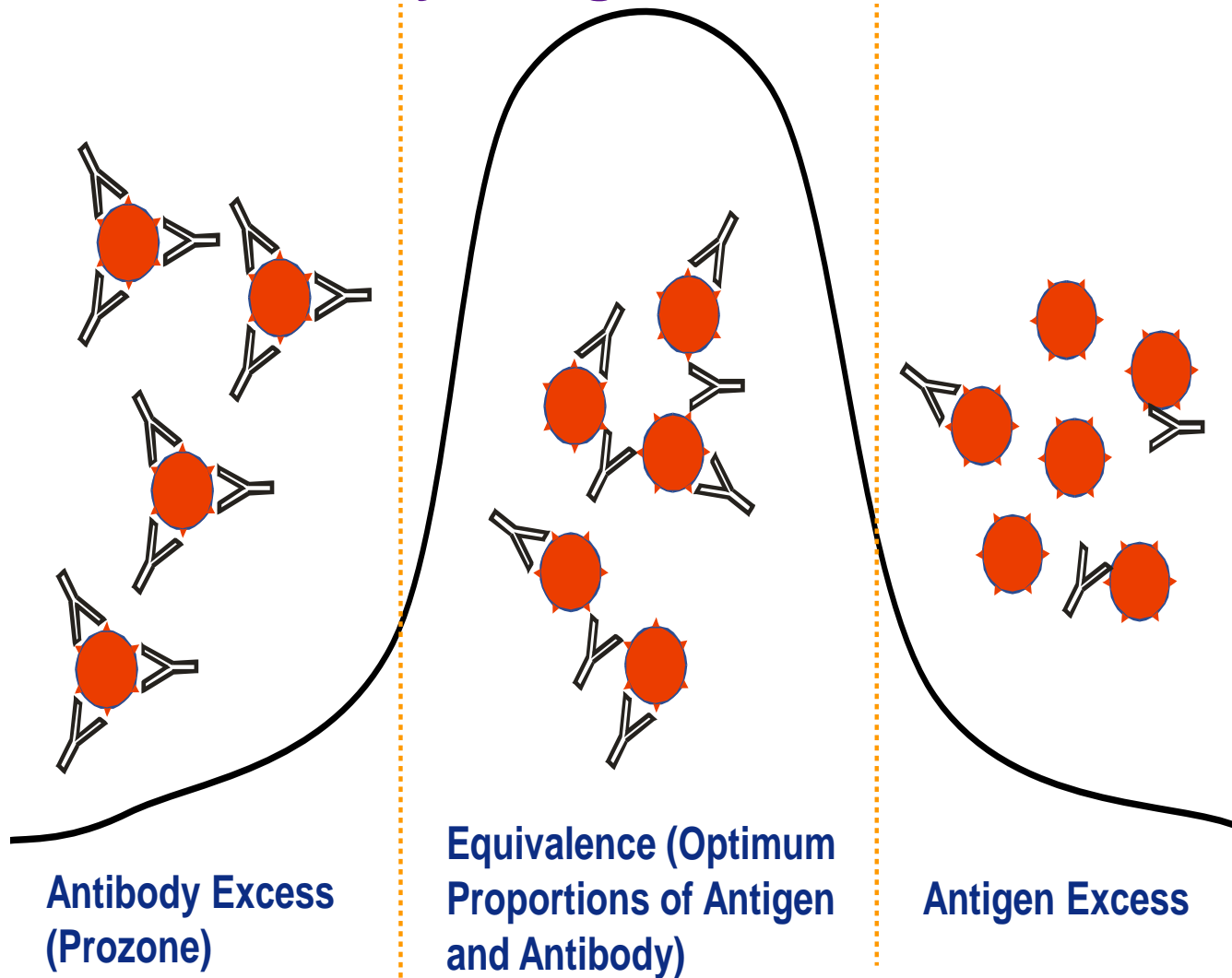
Zeta Potential

- **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



Effects of Antibody-Antigen Ratios



Agglutination Testing

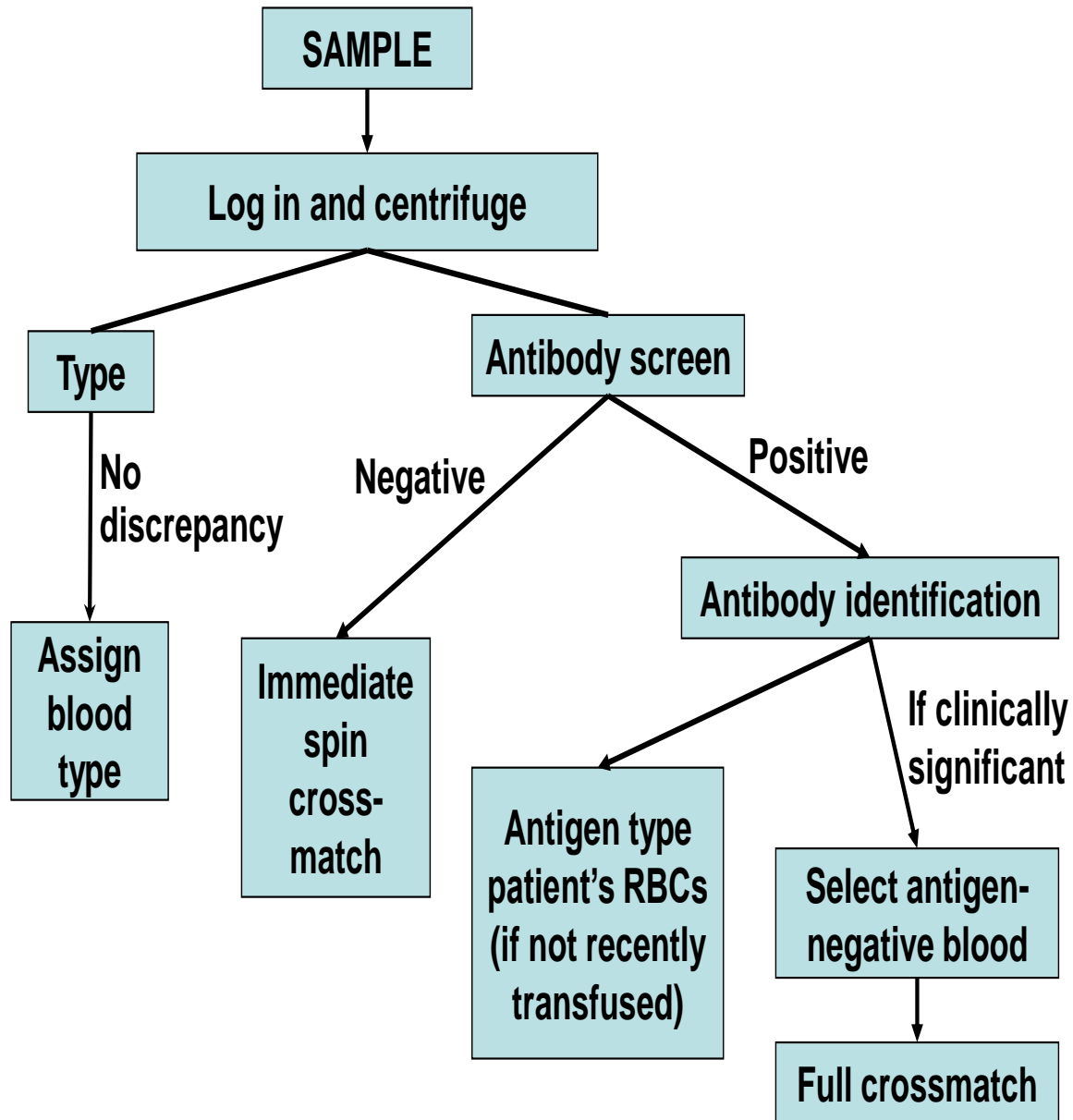


Positive:
Red Cells
Agglutinated



Negative:
Red Cells Not
Agglutinated

Blood Bank Routine Work-Flow



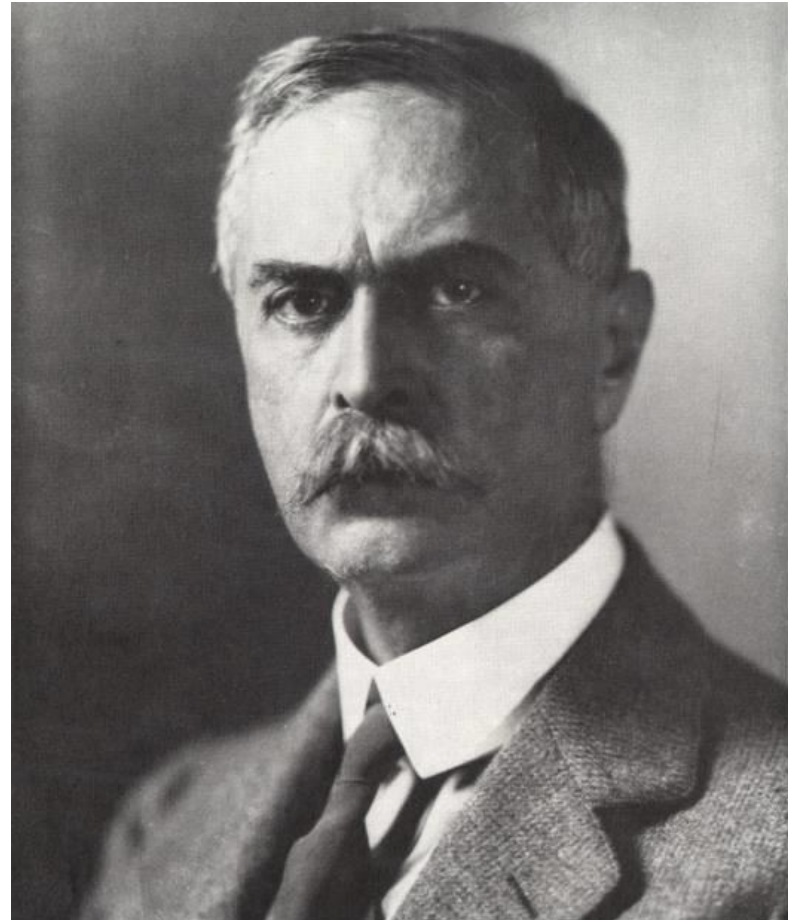
Other Tests Performed

- **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)

Allele
H (FUT1)
h

Primary Product
H-specific fucosyltransferase
“silent” allele –

Secondary Product
H antigen
no product

Phenotype
Common
Bombay

Immunodominant
Sugar
L-fucose
Precursor substance

Possible Genotypes
HH, Hh
hh

ABO Blood Group

- *Allele*

- *A*
- *B*
- *O*

Primary Product

A-specific glycosyltransferase
B-specific glycosyltransferase
“silent” allele – no product

Secondary Product

A antigen
B antigen

*Pheno-
Type*

A
B
AB
O

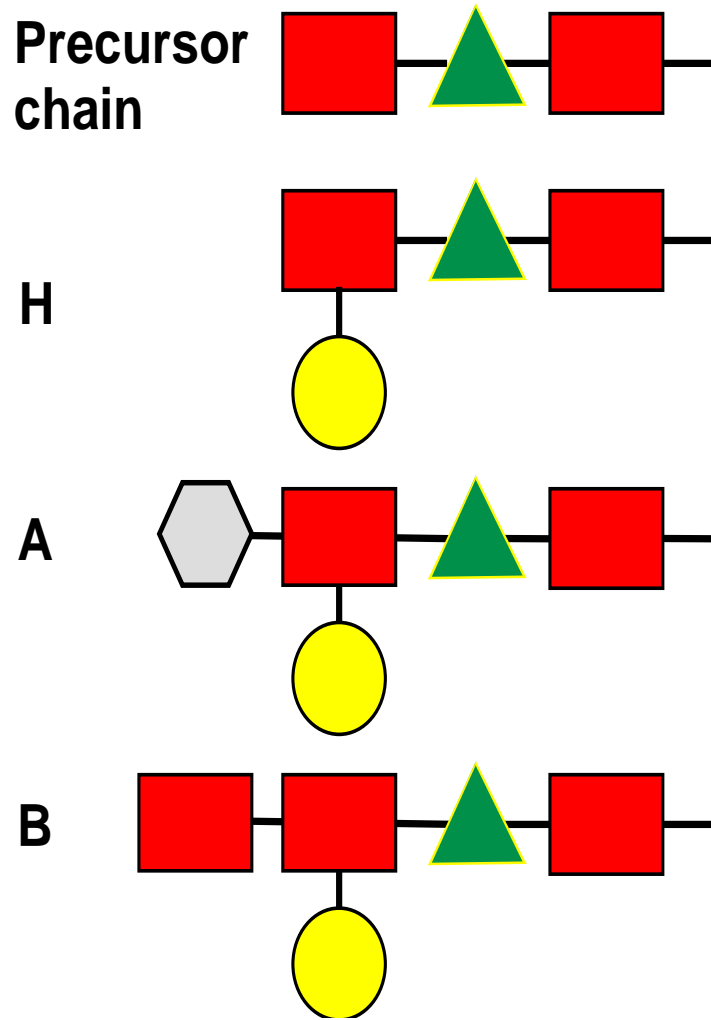
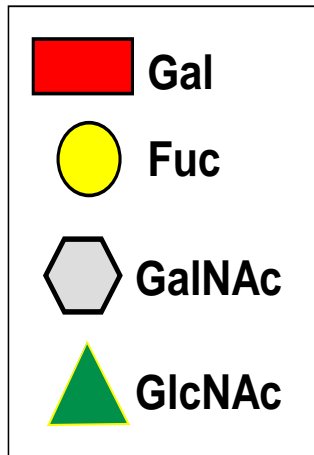
*Immunodominant
Sugar*

N-acetyl-D-galactosamine
D-galactose
both GalNac & Gal
H substance/Ag

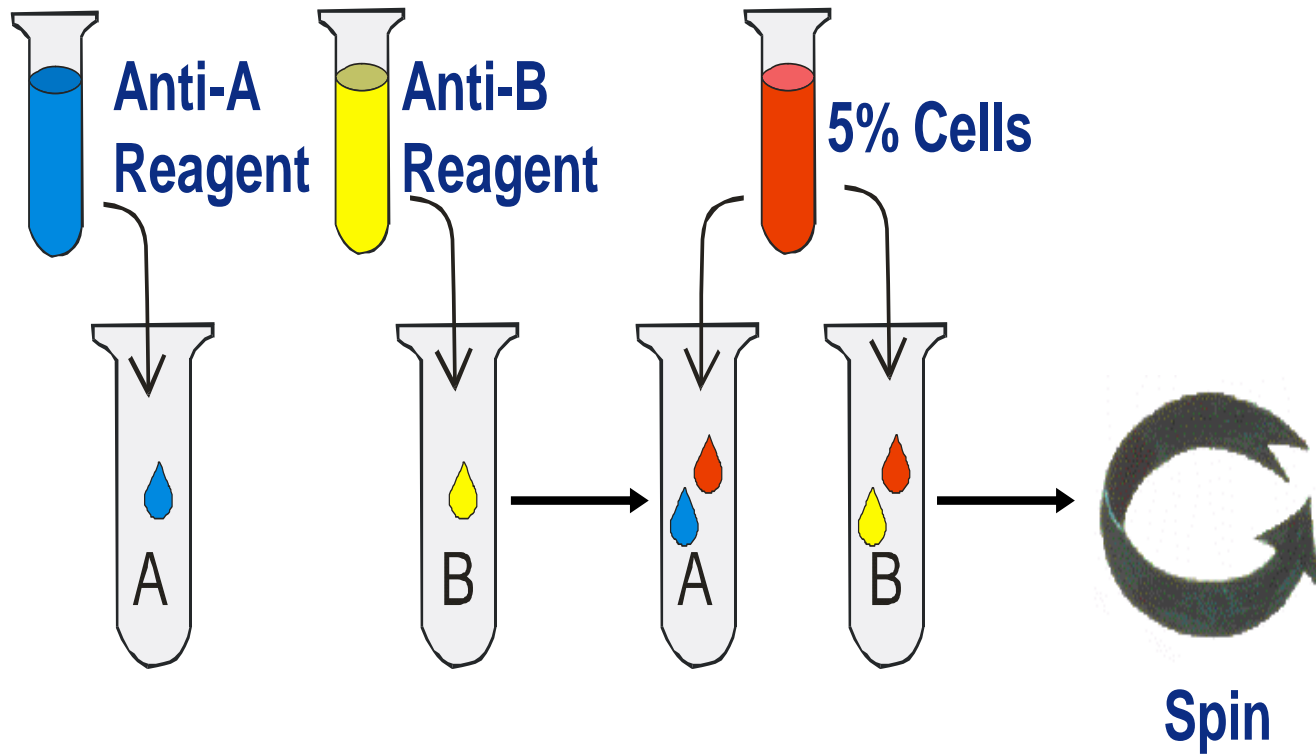
*Possible
Genotypes*

AA, AO
BB, BO
AB
OO

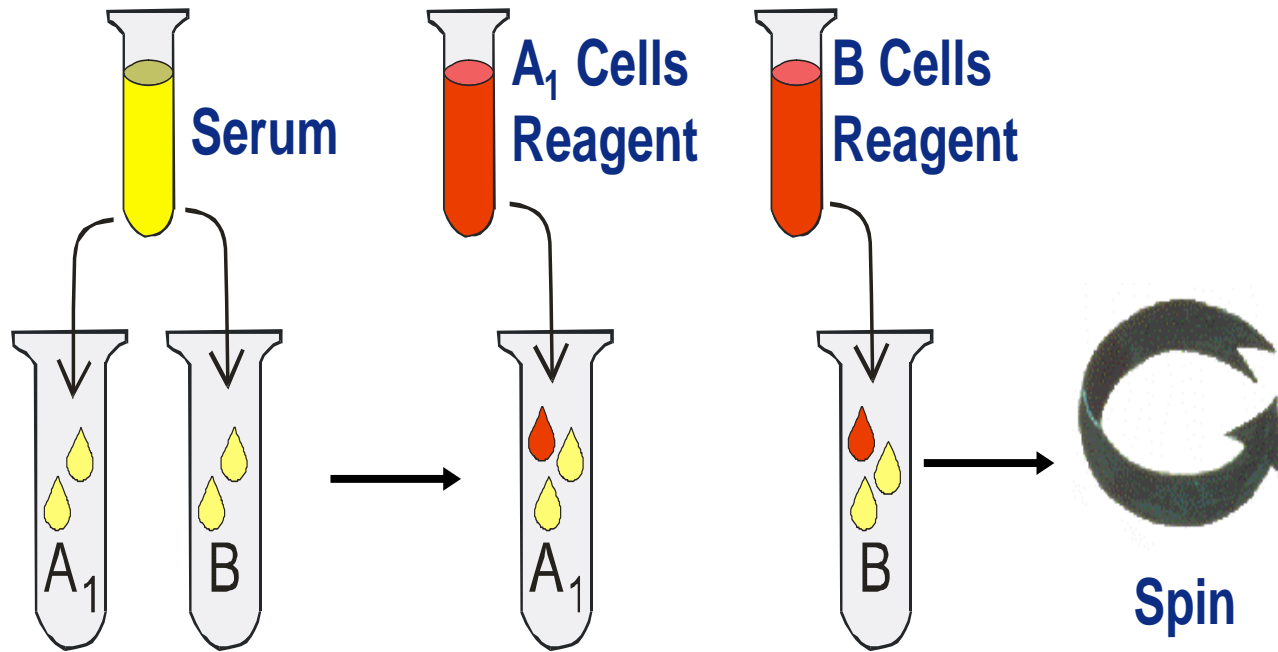
A, B, and H Antigen



ABO Typing: Forward Grouping



ABO Typing: Reverse Grouping



Routine ABO Typing

Reaction of cells tested with		Reaction of serum tested against		Inter-pretation	Incidence (%) in U.S. population	
Anti-A	Anti-B	A ₁ Cells	B Cells		ABO Group	Whites
0	0	+	+	0	45	49
+	0	0	+	A	40	27
0	+	+	0	B	11	20
+	+	0	0	AB	4	4

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

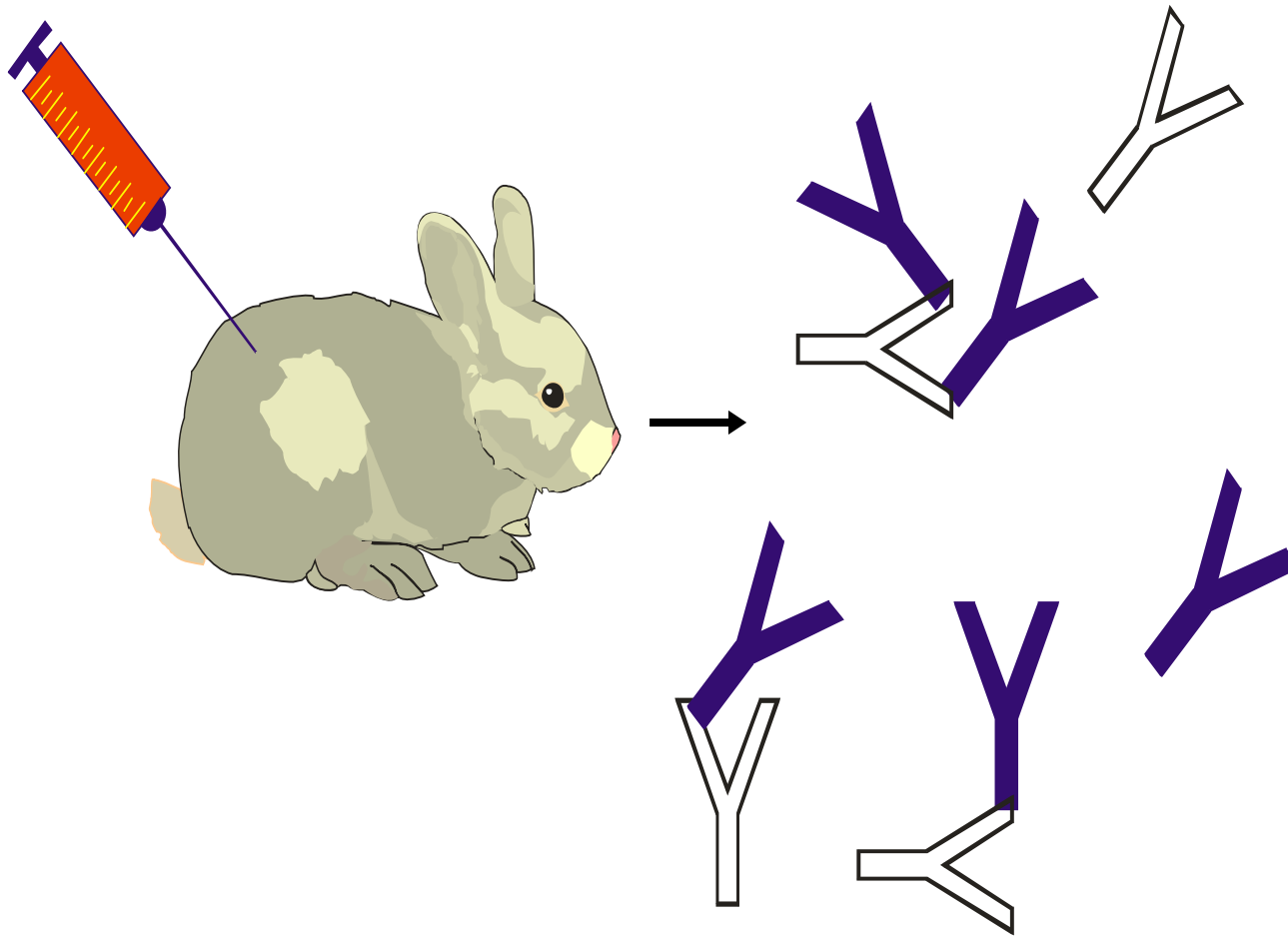
Weak D Typing (donors)

Anti-D IS	Anti-D IAT	Neg Control	Interpretation
+	NA	NA	Rh positive
0	0	NA	Rh negative
0	+	0	Rh positive
0	+	+	unresolved

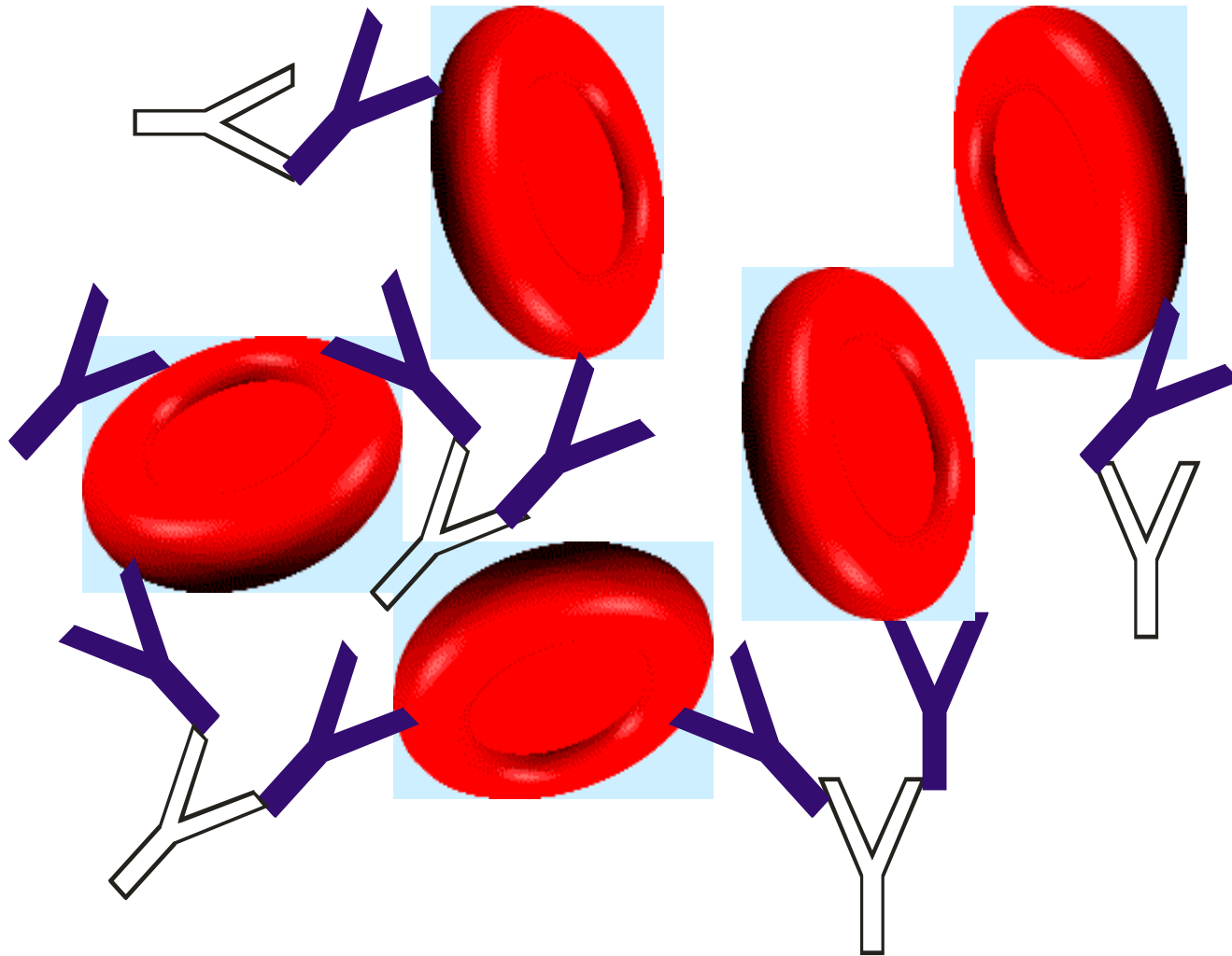
Blood Group Antibody Detection

- **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



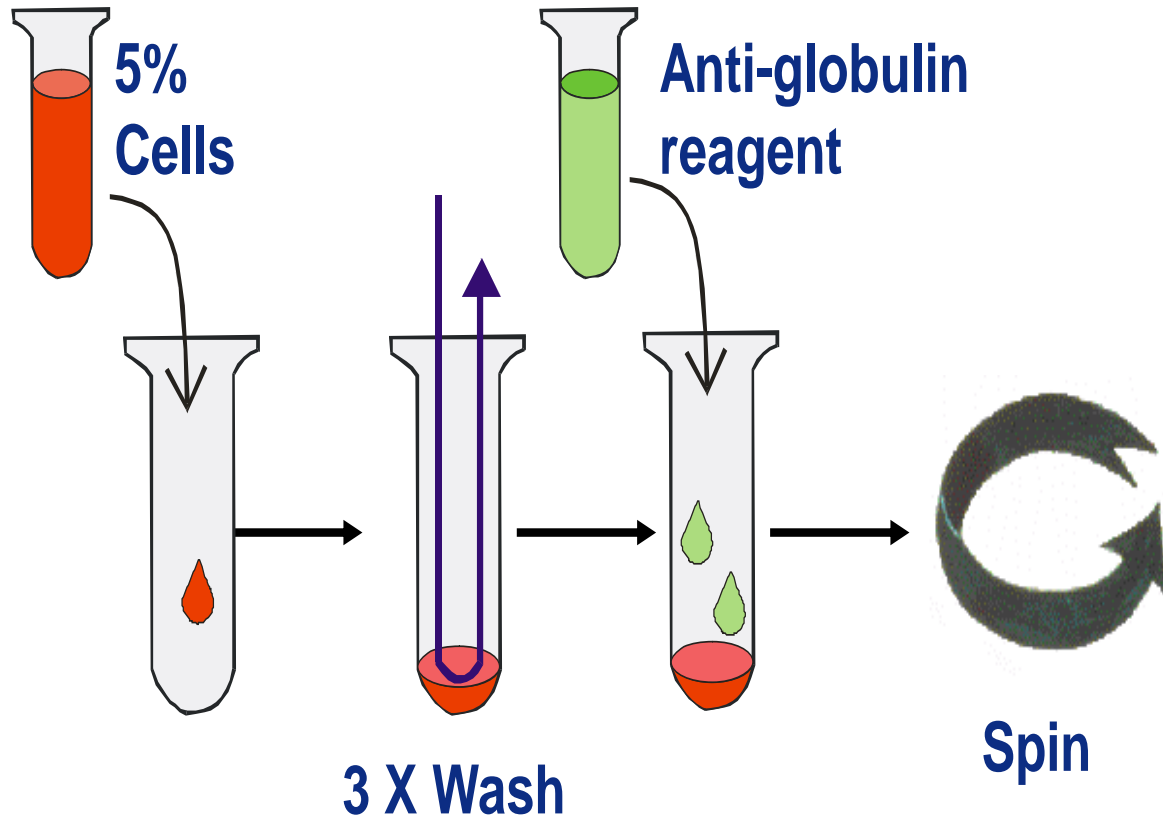
Antihuman Globulin (AHG) Reagents

- **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

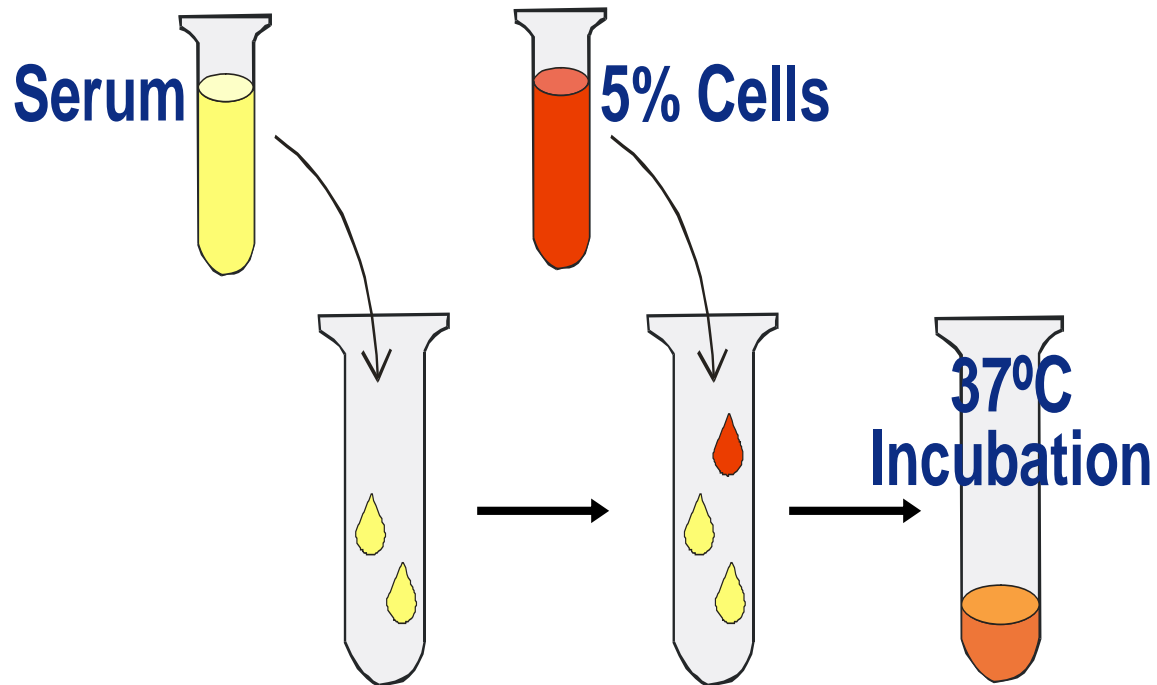
Direct Antiglobulin Test (DAT)



Indirect Antiglobulin Test (IAT)

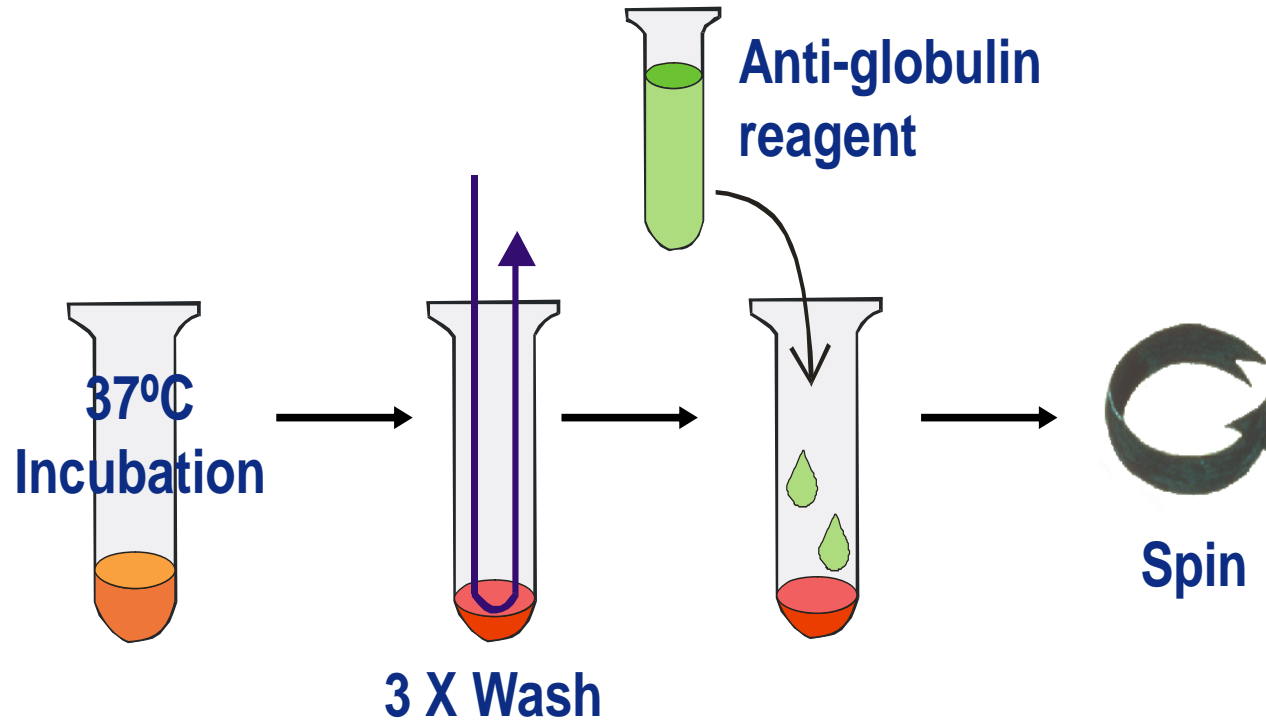
- Detects free antibody in serum
- ***Method for pretransfusion antibody detection***
- AHG reagent must contain anti-IgG
- Two-step test - AgAb binding occurs *in vitro*
- Other applications: antibody identification, crossmatch, extended antigen typing, weak D test

Indirect Antiglobulin Test (IAT)



Step 1

Indirect Antiglobulin Test (IAT)



Step 2

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

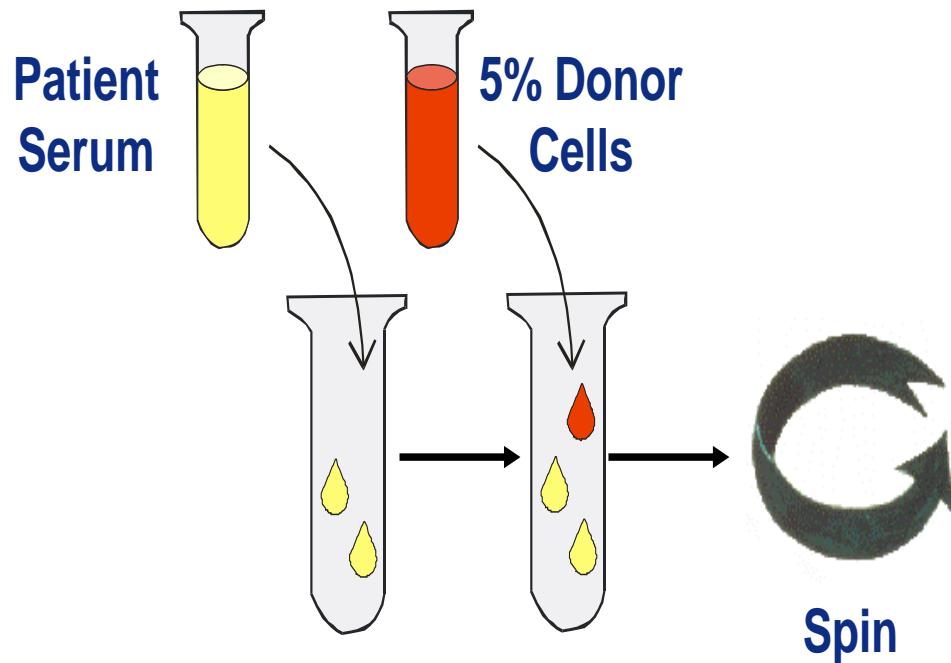
Testing Additives

- **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**

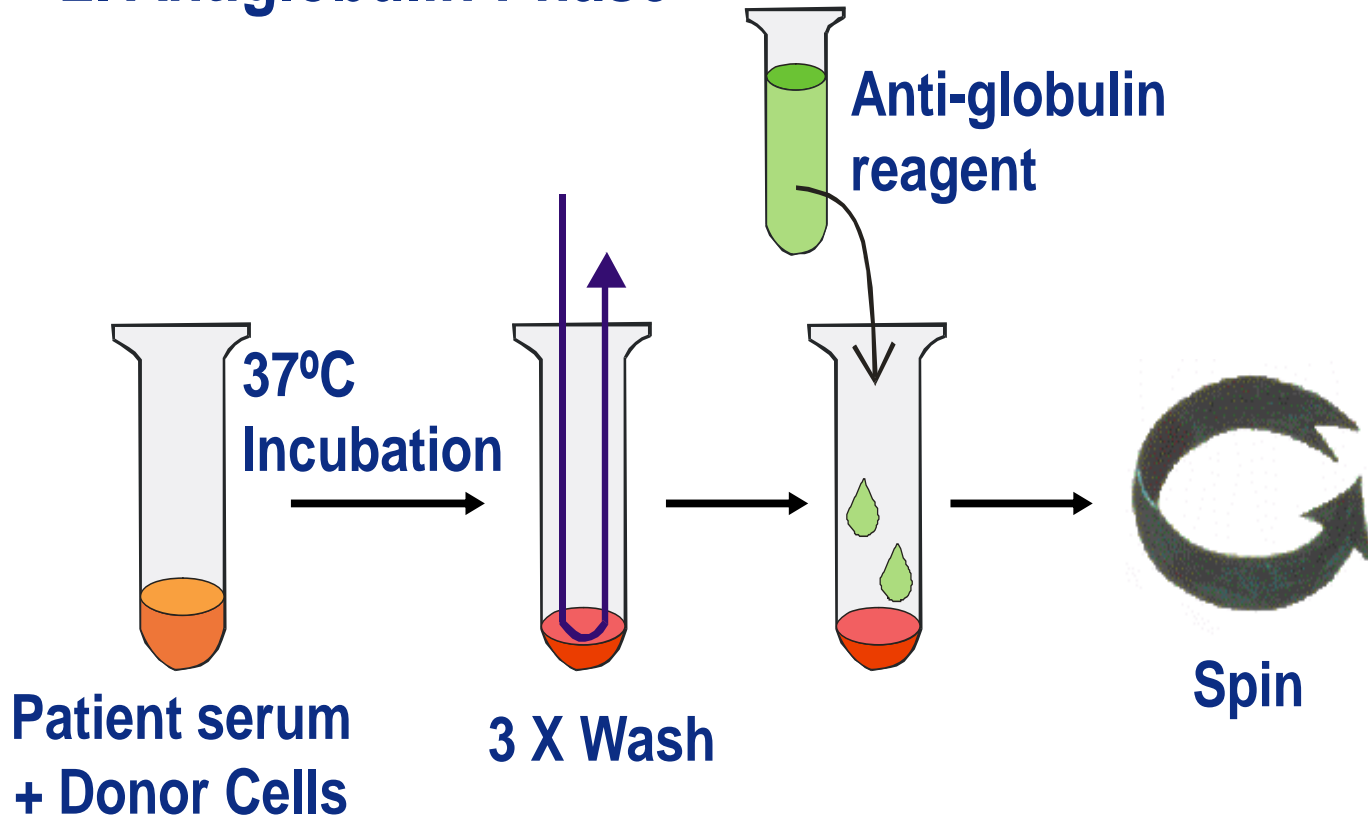
Crossmatch Procedure - IS



1. Immediate Spin (IS) Phase

Crossmatch Procedure - IAT

2. Antiglobulin Phase



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

Patient's ABO Type	Donor RBC Type	Donor Plasma Type
O	O	O, A, B, AB
A	A, O	A, AB
B	B, O	B, AB
AB	AB, A, B, O	AB

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

Test	Purpose	Known	Unknown
ABO/Rh	Test for antigens on RBCs	Commercial antisera (A,B,D)	RBCs
DAT	Test for IgG/C3 on RBCs	Commercial AHG antisera	RBCs
Antibody screen/ Antibody ID	Detect/identify alloantibodies	Commercial reagent RBCs	plasma
Antigen typing	Test for antigens on RBCs	Commercial antisera (anti-K, anti-Jk ^a)	RBCs
Crossmatch	Test for compatibility of donor RBC	Test results on patient and donor	Patient plasma and donor RBCs

Automated/Semi-automated Methods

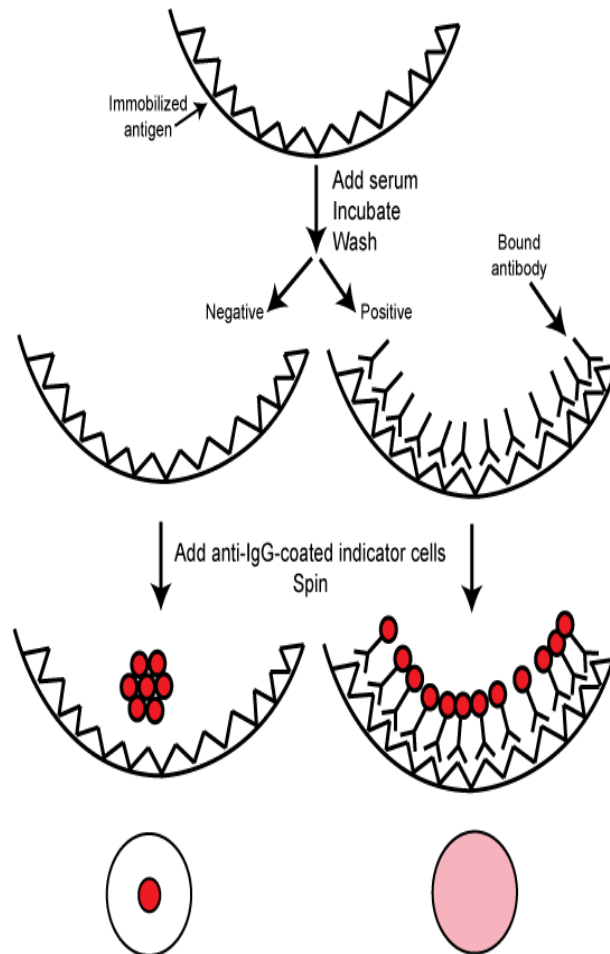
Alternatives to Tube Testing

- 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



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)**