# Pre-transfusion testing: Fundamentals of blood typing & cross match


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**Speaker(s) and Co-Authors**

# Disclosures

- None



# Topics To Cover

1. Historical Perspective

2. Erythrocyte Antigens

3. Pretransfusion Testing
   - Routine Serologic Testing
     - Type and Screen
     - Antibody Identification
     - Crossmatch
   - Additional Testing
     - Direct Antiglobulin Test (DAT)
     - Elution
     - Adsorption
     - Phenotyping/Genotyping



# Blood Transfusion: Historical Perspective

1818: First human-to-human transfusion


James Blundell June 13, 1829 *The Lancet*, "Observations on Transfusion of Blood."

# "Rules" for transfusion: Discovery of ABO blood group antigens

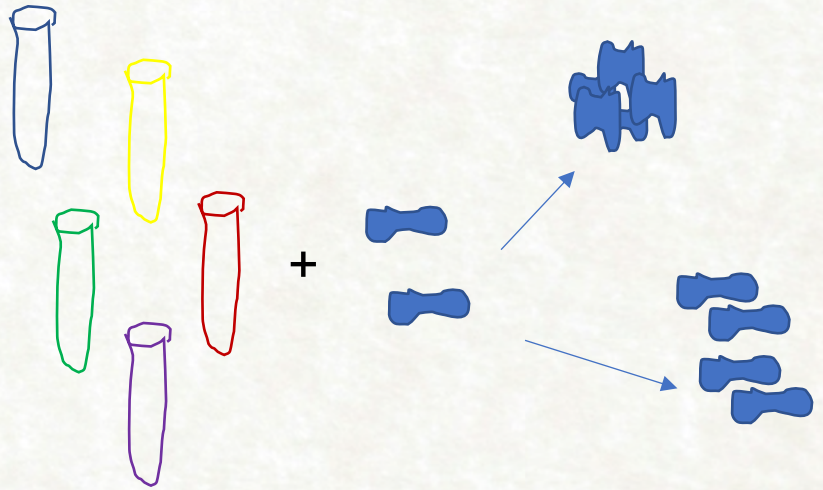
1901: Landsteiner's Experiment

Tabelle III, betreffend das Blut von fünf Puerperae und sechs Placenten (Nabelschnurblut).

| Sera | Trautm. | Linsm. | Seil. | Freib. | Graupn. | Mittelb. |
|---|---|---|---|---|---|---|
| Lust. | + | + | — | — | — | + |
| Tomsch. | — | — | + | — | — | — |
| Mittelb. | — | — | + | — | — | — |
| Seil. | — | — | + | — | — | — |
| Linsm. | + | + | + | — | — | + |

Blutkörperchen von:

Schwarz *et al.* (2003) *British Journal of Haematology*

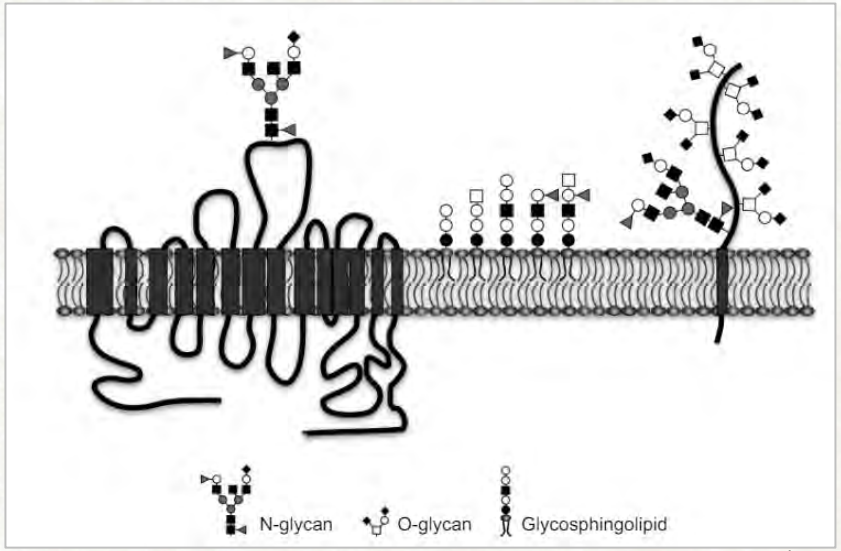


- Recognized a pattern of agglutination
- Blood can be divided into "groups"
- Marked the discovery of the ABO blood group system

# ABO histo-blood group antigens

ABO antigens

- Carbohydrate
- Defined by 3-sugar terminal epitope on glycolipids and glycoproteins
- ~1 million ABO antigens on each human RBC
- Expressed on non-erythroid cells ("histo blood group antigens")
- soluble antigens in saliva/other body fluids (secretors)

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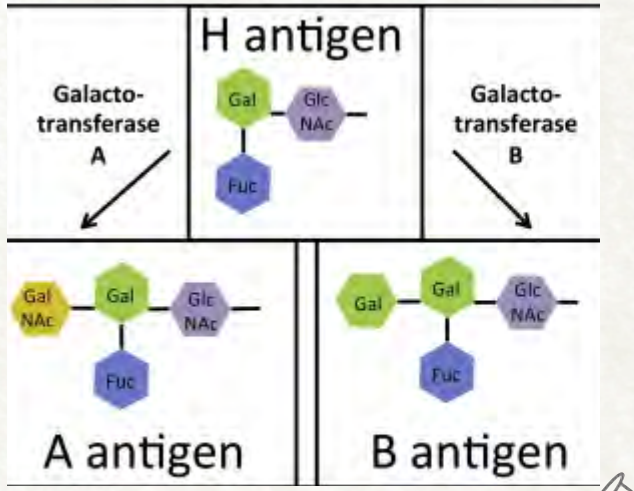
The ABO gene locus encodes glycosyltransferases

- 3 alleles/6 genotypes/4 phenotypes

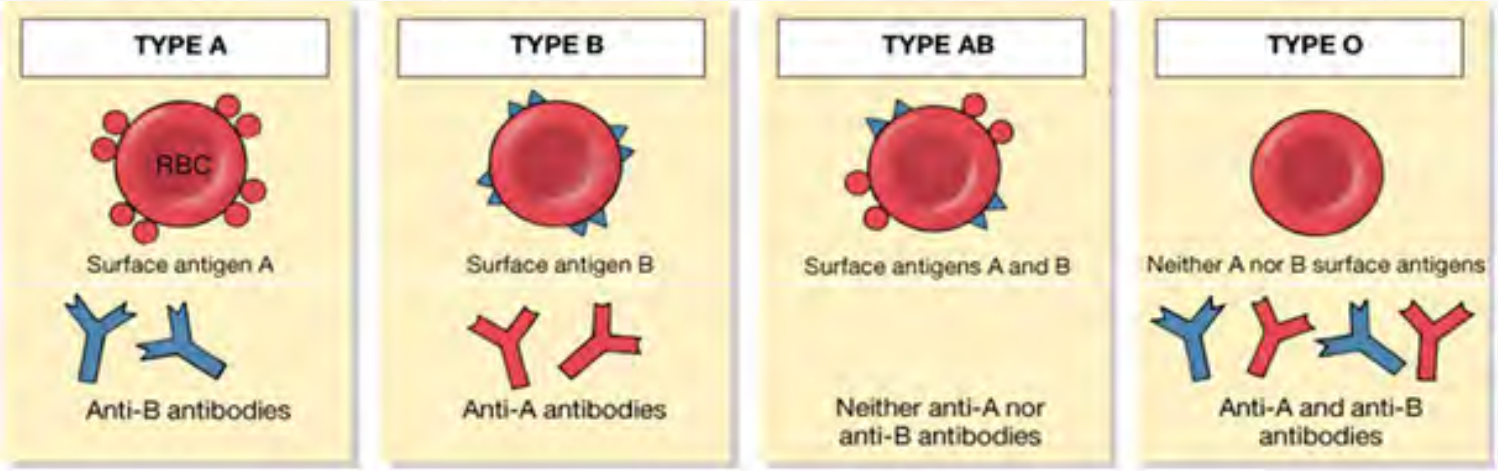



Physiological functions remain unknown

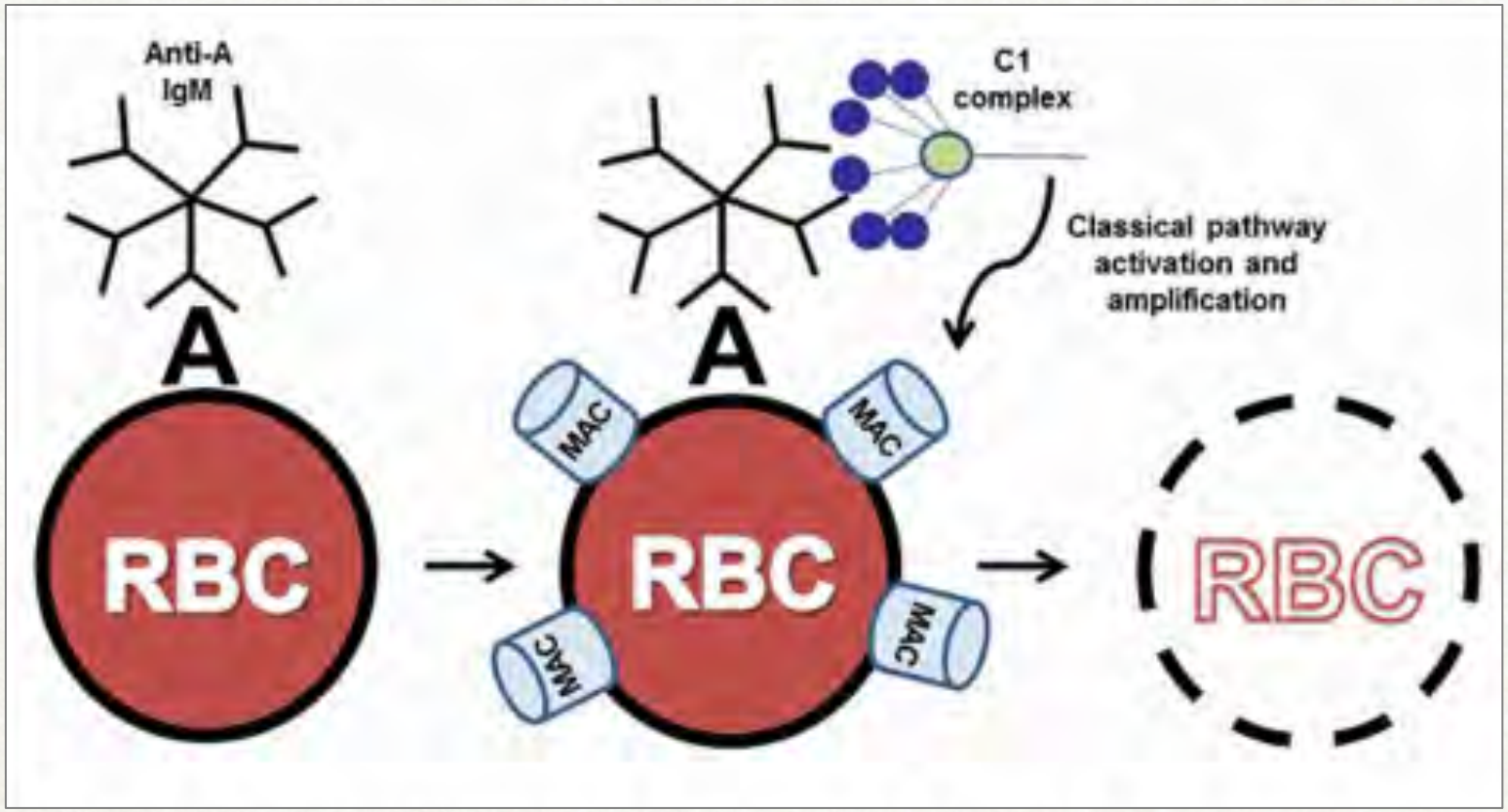
# Antibodies to ABO antigens

Isohemagglutinins (i.e., anti-A, anti-B)

- "Naturally occurring"
- *NOT* in response to foreign RBC exposure (transfusion, pregnancy, etc.)
- exposure response to substances in the environment that resemble non-self RBC antigens
- Formed during the first years of life
- IgM (*except* anti-A,B, IgG); titers vary



# ABO antibodies necessitate administration of donor RBCs *lacking* the corresponding antigen



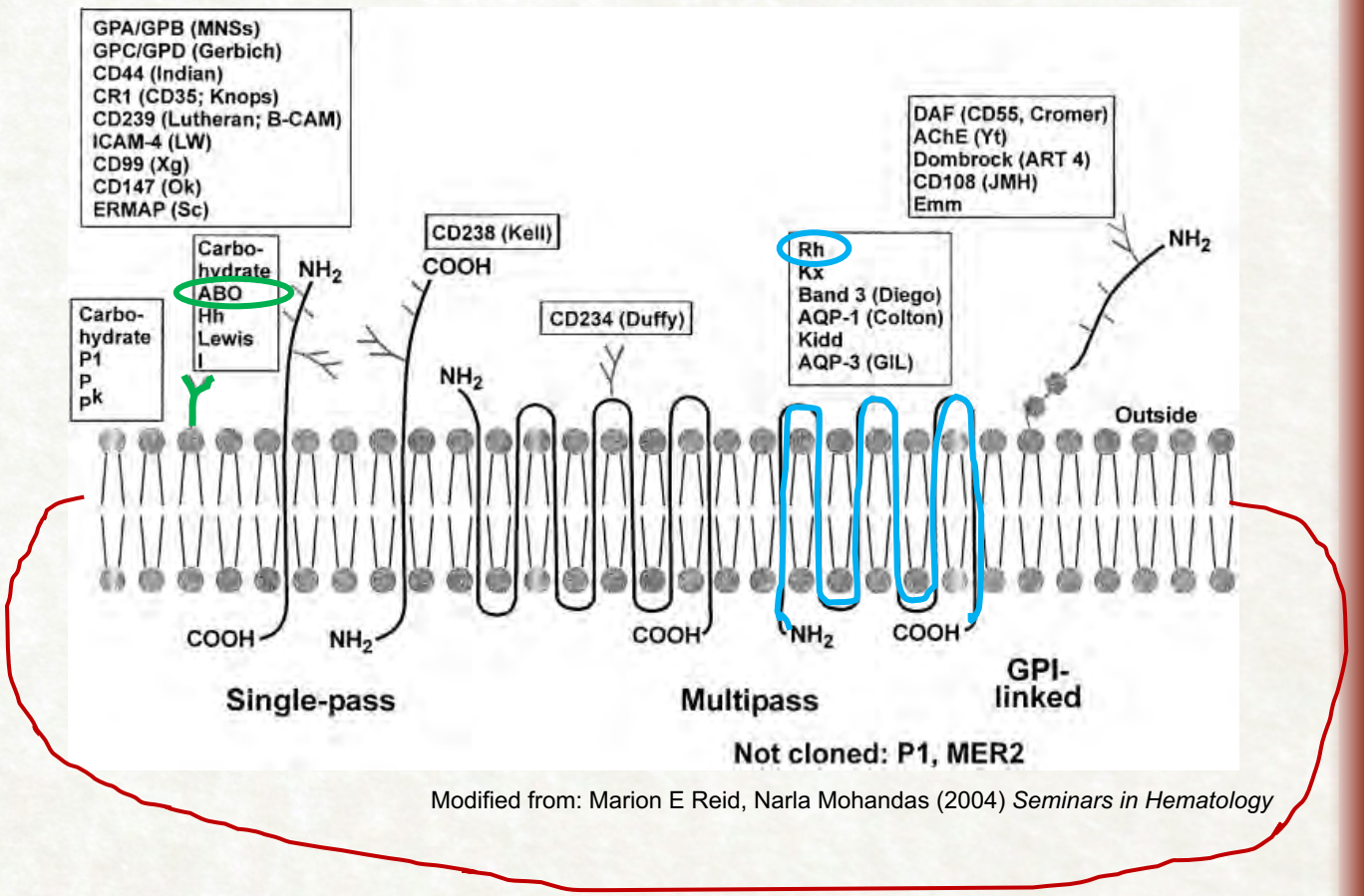

Sharp JA et al (2014) *Frontiers in Immunology*

# Other Blood Group Antigens

**Antigens:**

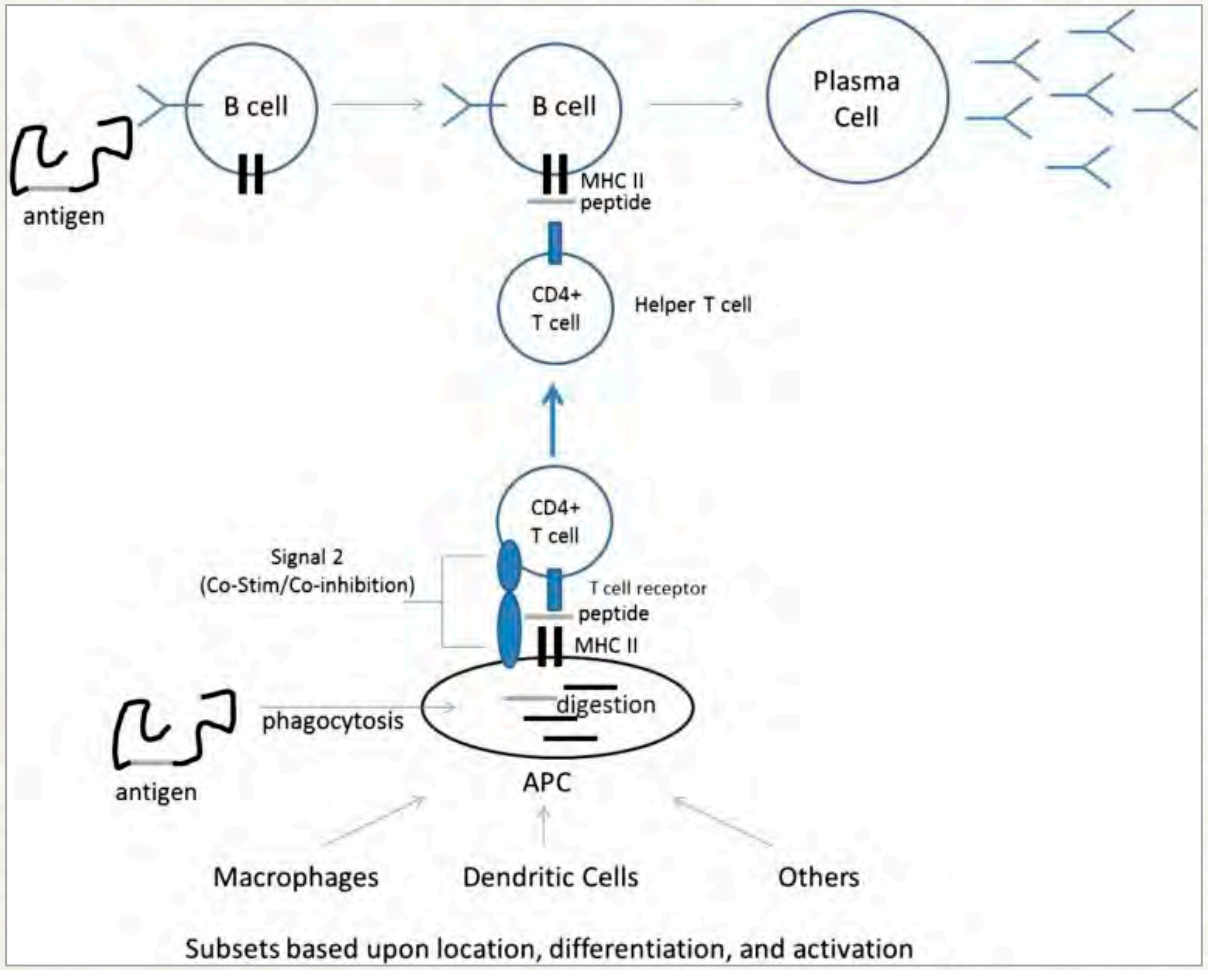
- Polymorphic, inherited, carbohydrate or protein structures located on the extracellular surface of the RBC membrane.

- *>300 antigens* (36 blood group systems) are known

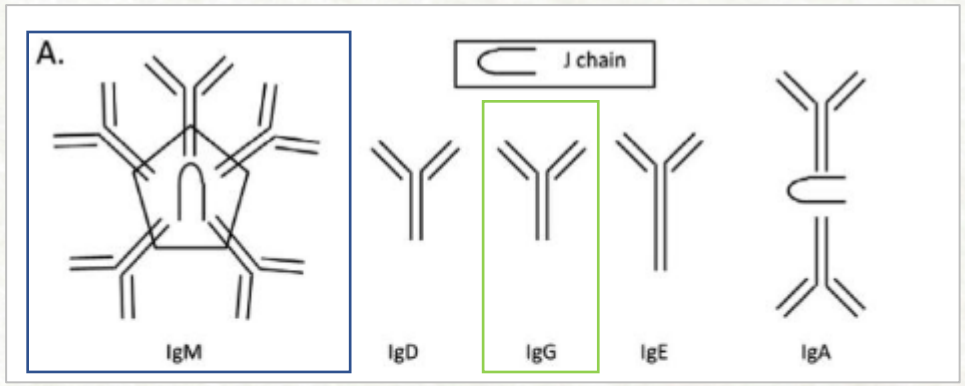



Modified from: Marion E Reid, Narla Mohandas (2004) *Seminars in Hematology*

# Alloantibodies ("unexpected" antibodies)




Zimring JC, Hudson KE (2016) *Hematology Am Soc Hematol Educ Program*.

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- Formed in response to sensitization from a previous exposure event (transfusion or pregnancy)
- Some can develop "naturally" (i.e., Le, P, M, N)
- Clinical significance varies

# Clinical relevance of blood group antigens for transfusion lies in their ability to incite an immune response and the nature of that response

## Immunogenicity of Ag

**Table 3–7 Relative Immunogenicity of Different Blood Group Antigens**

| BLOOD GROUP ANTIGEN | BLOOD GROUP SYSTEM | IMMUNOGENICITY (%)* |
|---|---|---|
| D ($Rh_o$) | Rh | 50 |
| K | Kell | 5 |
| c (hr′) | Rh | 2.05 |
| E (rh″) | Rh | 1.69 |
| k | Kell | 1.50 |
| e (hr″) | Rh | 0.56 |
| $Fy^a$ | Duffy | 0.23 |
| C (rh′) | Rh | 0.11 |
| $Jk^a$ | Kidd | 0.07 |
| S | MNSs | 0.04 |
| $Jk^b$ | Kidd | 0.03 |
| s | MNSs | 0.03 |

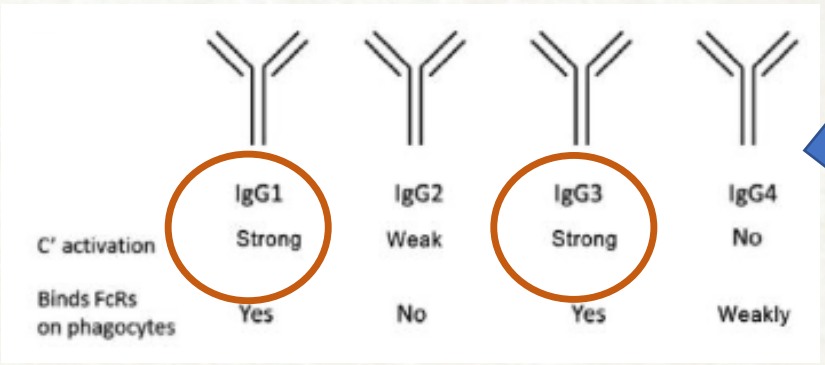
Adapted from Kaushansky, K, et al: Williams Hematology, 8th ed. McGraw-Hill Professional, New York, 2010.
*Percentage of transfusion recipients lacking the blood group antigen (in the first column) who are likely to be sensitized to a single transfusion of red cells containing that antigen.

Harmening, DM. Modern Blood Banking and Transfusion Practices 7th Ed.

## Type of response

| | IgM | IgG |
|---|---|---|
| Biologic $t_{1/2}$ | 5 d | 21 d |
| Complement Fixation | +++ | + |
| Placental Transfer | No | Yes |
| Reactivity | <22 C** | 37 C |
| Clinically Significant | Usually not** | Usually |

**exception: ABO

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# Alloimmunization

- RBC Allo-Antibodies are found in 0.3-2% of population.
- SCD pts have higher rates (15 - 40%) of alloimmunization compared to other groups receiving multiple transfusion
- Alloimmunization risk is 1-1.6% per RBC unit transfused
- Immunization to RBC antigens may result from:
  - Pregnancy
  - Transfusion
  - Passively acquired – produced in another individual and then transfused to the patient – plasma-containing blood products or derivatives like IVIG

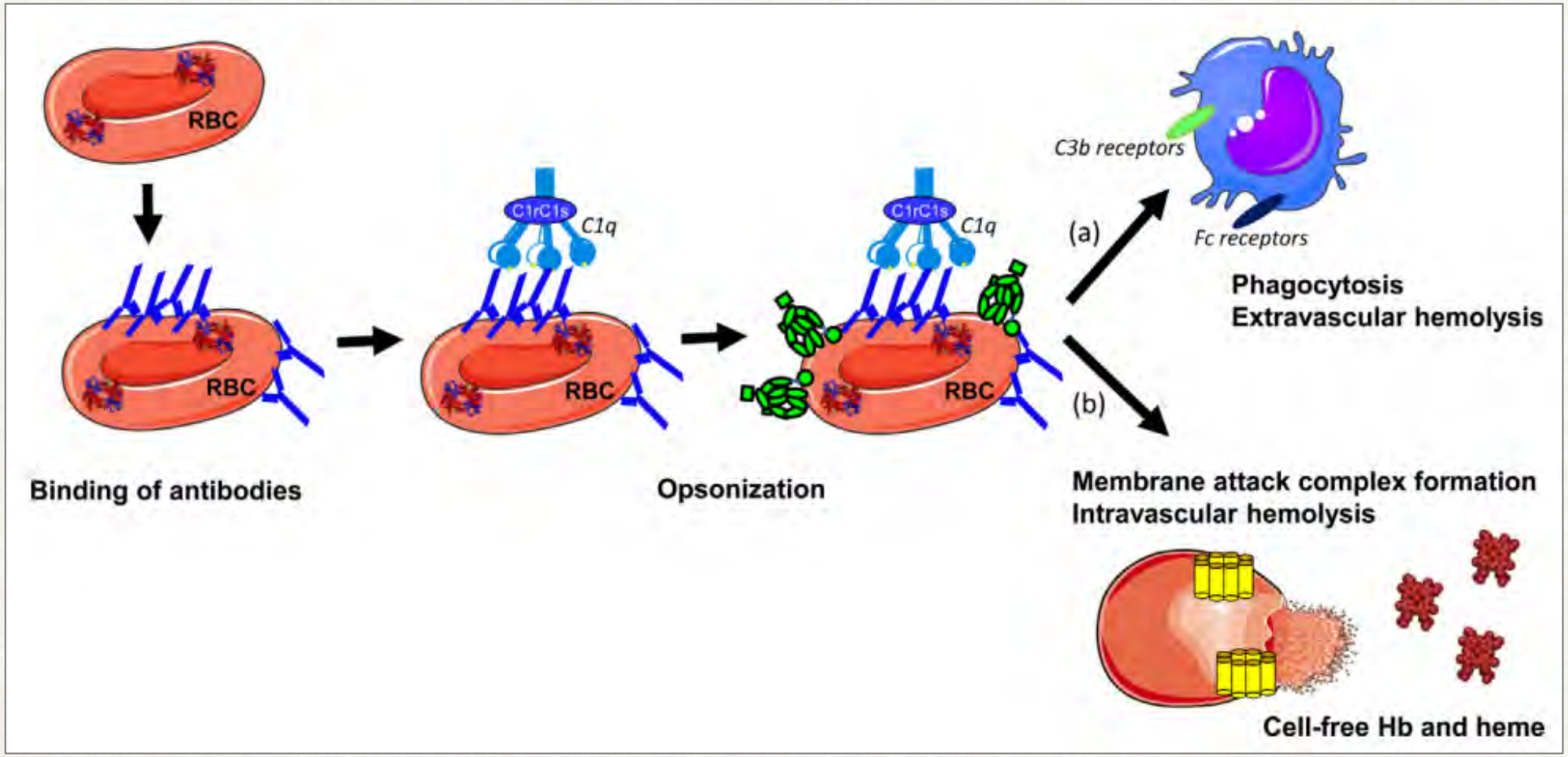
**The importance of obtaining patient history**

-If a patient has no history of Transfusion, Pregnancy or Transplant it is **unlikely** that they have Allo-Antibodies

-If a patient **has ever** had a clinically significant antibody identified then antigen negative blood **must** be provided



# Transfused RBCs do not need to be phenotypically identical to the recipient's RBCs, but they do need to *lack antigens to which the recipient has alloantibodies*




N.S. Merle et al. (2019) *Transfusion Clinique et Biologique*

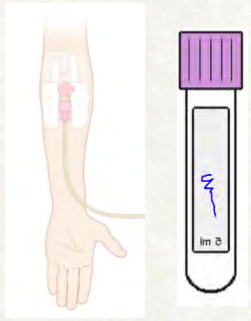
# Why do we need to perform serologic pre-transfusion testing?

*Selection of appropriate blood units for recipient, ensuring RBCs given are compatible with recipient plasma in order to prevent premature destruction of transfused RBCs and prevent harm to patient*



# Serologic Testing Overview

Collect/labeling specimen



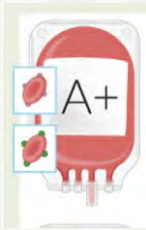
ABO and Rh Type

Antibody Screen

Antibody ID

Select units and crossmatch

Additional Testing
DAT
Elution
Adsorption
Pheno/Genotyping




Release unit

# Routine Serologic Pre-transfusion Testing

## I. Type

What is the ABO and Rh**D** type? (ensure ABO compatibility)

## II. Screen

Are there *unexpected* antibodies in the patient's serum that may react with other antigens on the donor RBC?

## III. Antibody Identification

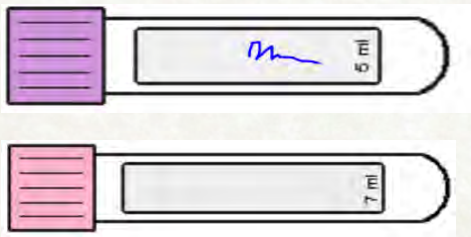
*What* are those unexpected antibodies – Ab ID?
Performed only if screen is positive

## IV. Crossmatch

Ensure all antigen compatibility



# Specimen Requirements



Pink or Purple EDTA tubes (usually 2)

- Signed and Dated
- 2 unique identifiers
- 5-10 mL whole blood aliquot is usually enough for simple antibody identification
- 2 different specimens

**Specimen Expiry**

Outpatient:

- 30 days if NOT Pregnant/transfused within the last 3 months
- 3 days if Pregnant/transfused within the last 3 months

Inpatient:

- 3 days

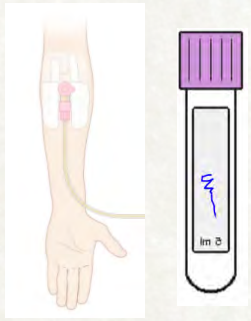
Neonates (<4 mo):

- Valid throughout the same admission
- Maternal sample as an alternative

| Sun | Mon | Tues | Wed |
|---|---|---|---|
| Sample drawn @ 1 pm | Sample used | Sample used | Sample expires @ midnight |
| Day 0 | Day 1 | Day 2 | Day 3 |

# Serologic Testing Overview

Collect/labeling specimen



ABO and Rh Type

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Select units and crossmatch

Additional Testing
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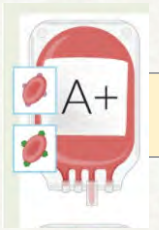
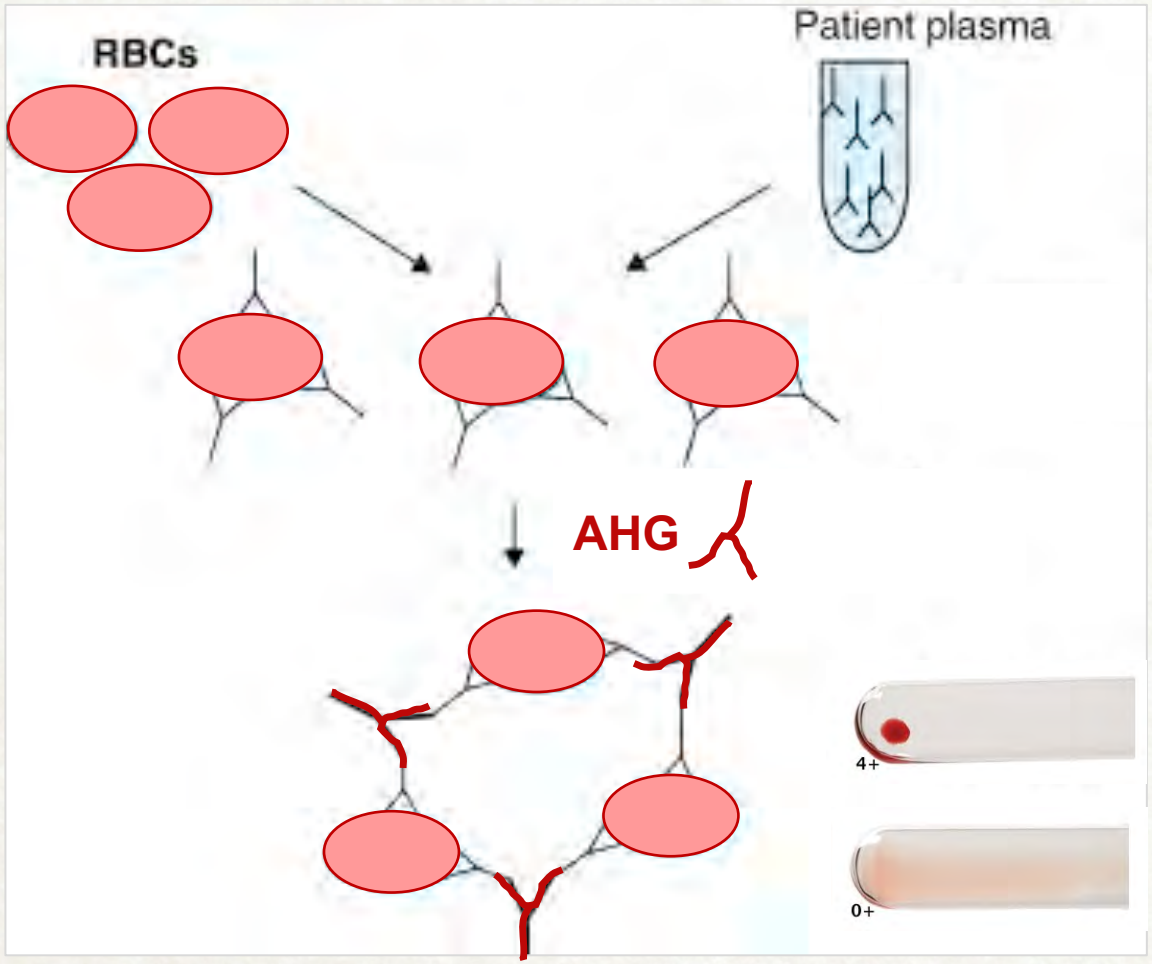



Release unit

# Hemagglutination is the basis for BB testing




- RBCs are bound together by an Ab → visible aggregate ("agglutinates")
- Agglutinates are graded on a scale of 0-4
- Abs vary in ability to agglutinate RBCs (need for AHG reagent)

Indirect Antiglobulin Test (IAT) = Indirect Coombs

- Detects *antibodies in plasma*, unbound to RBCs (*in vitro* antibody reactivity)

# I. Blood Type – ABO

## ABO Grouping

- Antigen typing of patient RBCs (forward type)
- Screening serum for anti-A and Anti-B (reverse type)

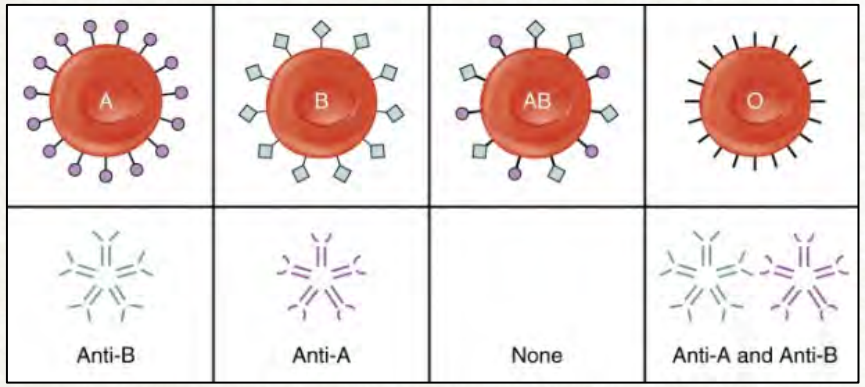
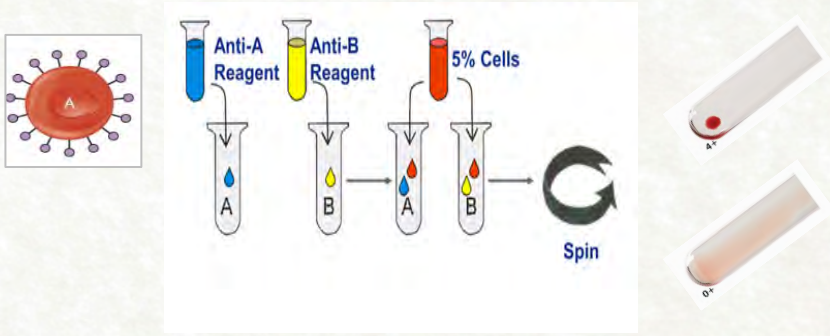



**TABLE 2. Distribution (%)* of ABO phenotypes by race/ethnicity**

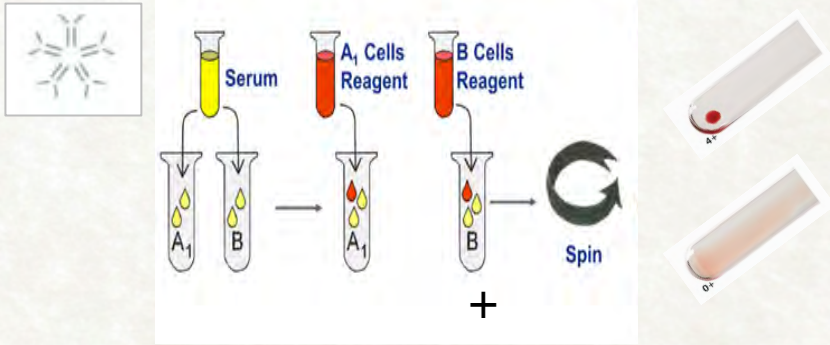
| Race or ethnicity | Number | Phenotype O | A | B | AB |
|---|---|---|---|---|---|
| White non-Hispanic | 2,215,623 | 45.2 | 39.7 | 10.9 | 4.1 |
| Hispanic† | 259,233 | 56.5 | 31.1 | 9.9 | 2.5 |
| Black non-Hispanic | 236,050 | 50.2 | 25.8 | 19.7 | 4.3 |
| Asian‡ | 126,780 | 39.8 | 27.8 | 25.4 | 7.1 |
| North American Indian | 19,664 | 54.6 | 35.0 | 7.9 | 2.5 |
| All donors | 3,086,215 | 46.6 | 37.1 | 12.2 | 4.1 |

Garratty G et al. (2004) *Transfusion*

**1. Is A or B antigen on the surface of RBCs?**




**2. Is Anti-A or Anti-B present in the patient's plasma?**



# RhD Typing

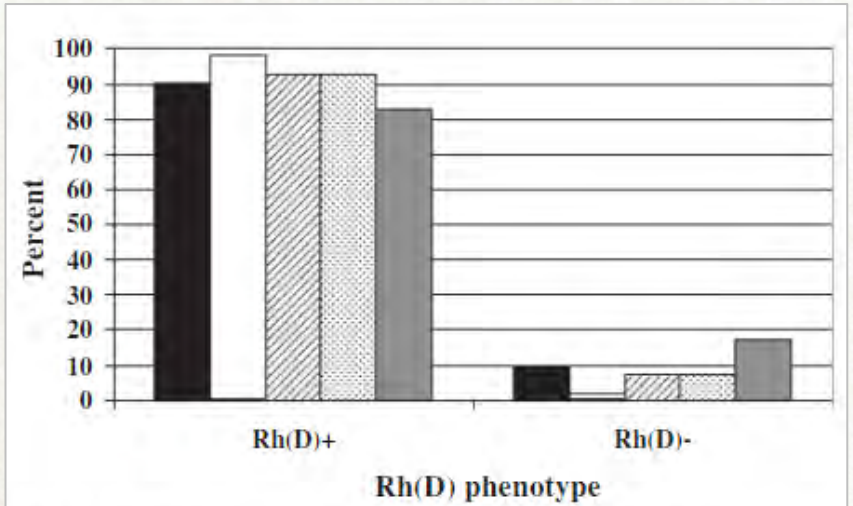
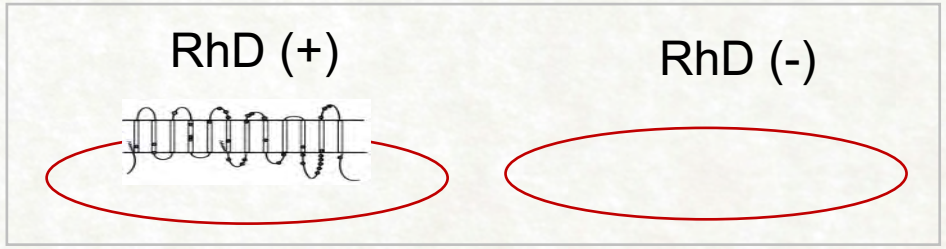
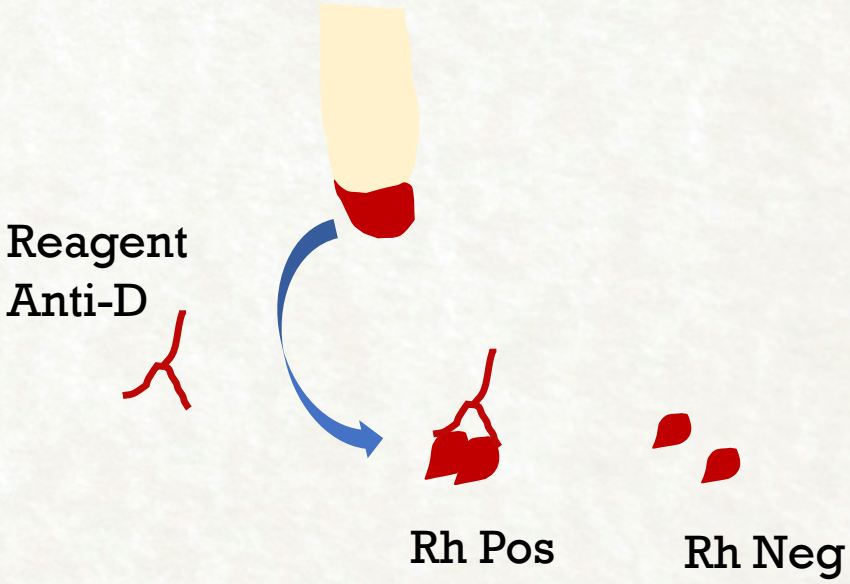



Fig. 2. Rh(D) phenotype by race/ethnicity. (■) North American Indian; (□) Asian; (▨) black non-Hispanic; (▩) Hispanic; (▩) white non-Hispanic.

Garratty G et al. (2004) *Transfusion*




Is D antigen present on patient cells?



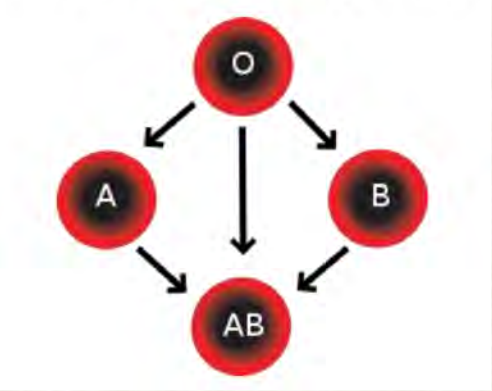

*Why are RBCs routinely typed for D?*

- D antigen is highly immunogenic
- anti-D antibodies can cause significant HDFN
- Rh compatible units should be provided

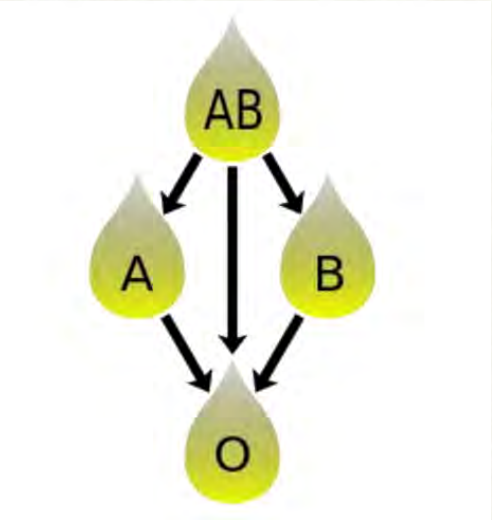
# ABO compatibility rules for blood products

RBCs
- ABO matched/compatible with recipient plasma

Granulocytes
- ABO matched/compatible with recipient plasma




*Per AABB Standards, if **> than 2 mL of RBCs** are present in any product, those RBCs must be **compatible with the recipient's plasma** antibodies.*




**Plasma-Containing blood products:**

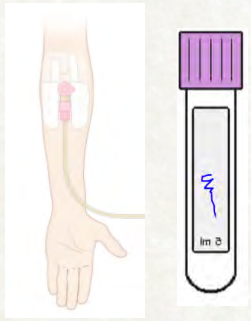
FFP – ABO matched/compatible *with recipient RBCs*

Platelets – Rh matched; typically not ABO matched due to inventory

Cryo – no need to match

# Serologic Testing Overview

Collect/labeling specimen

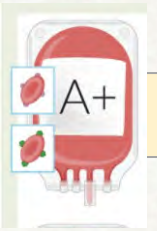


ABO and Rh Type

Antibody Screen

Antibody ID

Select units and crossmatch




Release unit

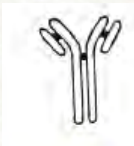
# RBC antibodies

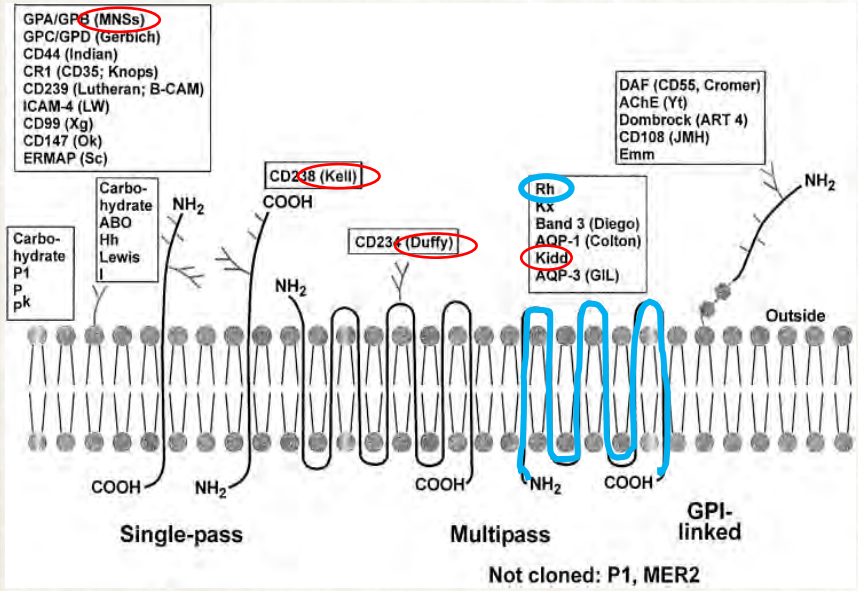
RBC Antibodies

"Expected antibodies"

Isohemagglutinins: Anti-A, Anti-B, Anti-A,B ("naturally occurring")

"unexpected antibodies"






Modified from: Marion E Reid, Narla Mohandas (2004) *Seminars in Hematology*

## *Allo*antibodies

- (react ONLY with reagent cells)

-pregnancy
-transfusion
-transplantation
-exposure

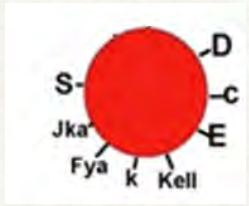
## *Auto*antibodies

(react with reagent and autologous cells)

- 14-50% of chronically transfused patients (Sickle Cell/Thalassemia)
- Rare cases with no known exposure: bacteria, environment, viral antigens that are similar to blood group antigens
- Passively acquired antibodies Abs detected in serologic testing – IVIG, donor plasma, Passenger Lymphocytes in transplanted organs, HPCs

# II. Antibody screen

**Screening Cells:** 2 cells; reagent RBCs




Type O = will not detect any ABO antibodies

Per FDA requirement, collectively express the following 18 antigens:

D, C, E, c,e, M, N, S,s, P1, Lea, Leb, K, k,Fya, Fyb, Jka, Jkb

3 cell panels typically homozygous for D, C, E, c, e, k, M, N, S, s, Fya, Fyb, Jka, Jkb

CAPTURE-R READY-SCREEN (3)

Master List

R407

IMMUCOR, INC. Norcross, GA 30071 USA

US LICENSE NO: 886

LOT NO: R407

EXPIRES: 2014/07/01

| CELL | Donor | Rh - Hr | | | | | | | Kell | | | | | | Duffy | | Kidd | | Lewis | | P | MN | | | | Lulh-eran | | $X_g$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | D | C | c | E | e | V | $C^w$ | K | k | $Kp^a$ | $Kp^b$ | $Js^{a*}$ | $Js^b$ | $Fy^a$ | $Fy^b$ | $Jk^a$ | $Jk^b$ | $Le^a$ | $Le^b$ | $P_1$ | M | N | S | s | $Lu^a$ | $Lu^b$ | $Xg^{a*}$ |
| I | R1R1 B8314 | + | + | 0 | 0 | + | 0 | 0 | + | + | 0 | + | 0 | + | + | + | + | + | + | 0 | 0 | 0 | + | 0 | + | 0 | + | 0 | + | 0 |
| II | R2R2 C4205 | + | 0 | + | + | 0 | 0 | 0 | 0 | + | 0 | + | 0 | + | 0 | + | + | 0 | 0 | + | + | 0 | + | 0 | + | 0 | + | 0 | + | 0 |
| III | rr H555 | 0 | 0 | + | 0 | + | 0 | 0 | 0 | + | 0 | + | 0 | + | + | 0 | 0 | + | 0 | 0 | + | + | 0 | + | 0 | + | 0 | + | + |
| | Positive Control | | | | | | | | | | | | | | | | | | | | | | | | | | | |

* Indicates those antigens whose presence or absence may have been determined using only a single example of a specific antibody.

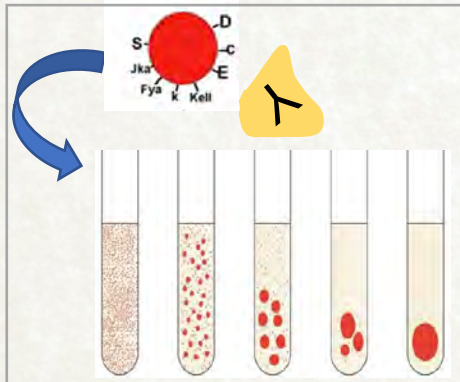
An antigen designated with a 'w' represents a weakened expression of the antigen that may or may not react with all examples of the corresponding antibody.

Patient Plasma

Reactivity

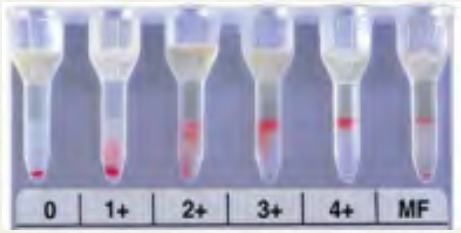
# Antibody detection methodology

## Tube Testing



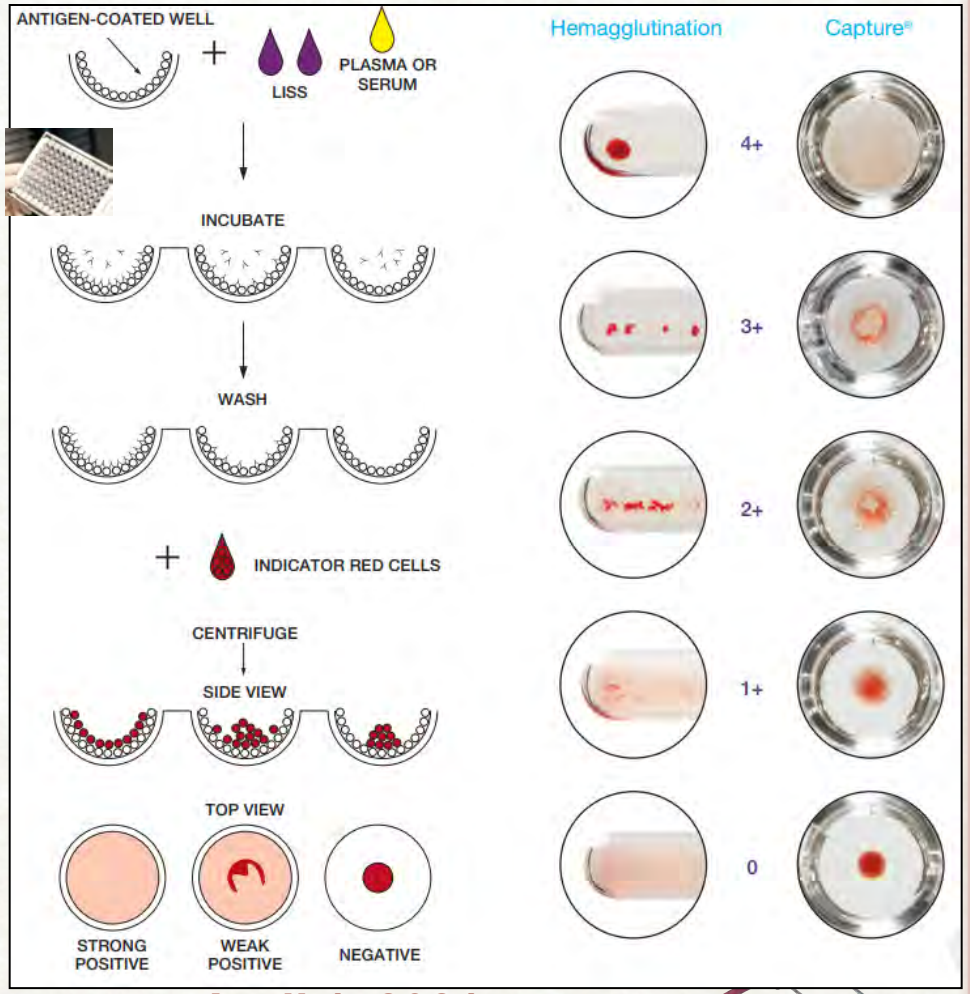

- Plasma and test cell interaction in tube
- Assess for agglutination

## Gel Testing


- Plasma and test cell interaction in chamber
- Centrifugation of RBCs through gel column
- Agglutinates remain on top

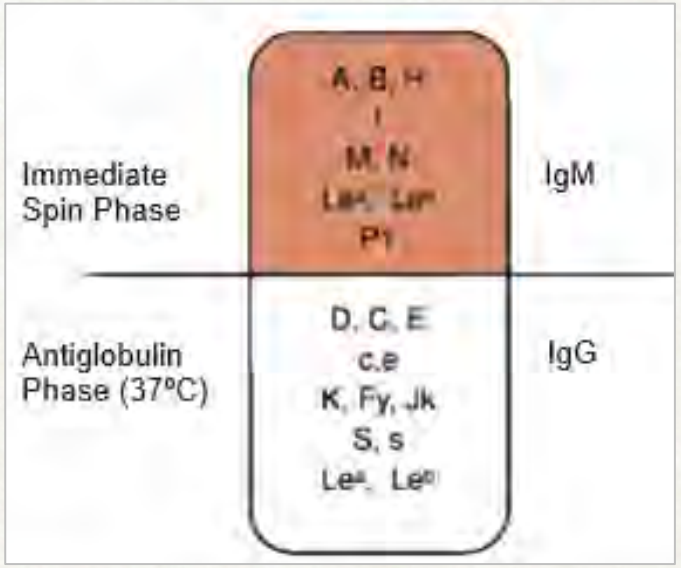
## Solid Phase

# Phases of Reaction

| Donor | Cell number | D | C | c | E | e | $C^w$ | K | k | $Kp^a$ | $Kp^b$ | $Js^a$ | $Js^b$ | $Fy^a$ | $Fy^b$ | $Jk^a$ | $Jk^b$ | $Le^a$ | $Le^b$ | $P_1$ | M | N | S | s | $Lu^a$ | $Lu^b$ | $Xg^a$ | IS | 37 | AHG | CC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $R_1r$ | 1 | + | + | + | 0 | + | 0 | 0 | + | 0 | + | 0 | + | + | + | + | + | 0 | + | + | + | + | + | + | 0 | + | + | 0 | 0 | 2+ | |
| $R_1R_1$ | 2 | + | + | 0 | 0 | + | + | + | + | 0 | + | 0 | + | 0 | + | + | 0 | 0 | 0 | + | + | 0 | + | 0 | 0 | + | + | 0 | 0 | 0 | 3+ |
| $R_2R_2$ | 3 | + | 0 | + | + | 0 | 0 | 0 | + | 0 | + | 0 | + | + | 0 | 0 | + | 0 | + | + | 0 | + | 0 | + | 0 | + | + | 0 | 0 | 3+ | |


| Phase of Rxn | Ab type detected | Mechanism | Types of Abs Detected |
|---|---|---|---|
| IS | IgM | IgM react best at *lower* temp | ABO, cold-reacting Allo/Auto |
| 37 C | IgG | IgG react best at *warm* temp | Warm-reacting "unexpected" Abs |
| AHG/37 C (IAT) | IgG | AHG displays specificity for the Fc portion of the heavy chain of IgG or complement<br>*"bridges" IgG molecules* | Warm-reacting "unexpected" Abs |

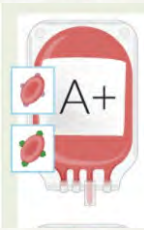


# Serologic Testing Overview

Collect/labeling specimen → ABO and Rh Type → Antibody Screen

| | | Rh | | | | | | | | Kell | | | | | | Duffy | | Kidd | | Lewis | | P | MNS | | | | | Lutheran | | Xg | Results | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | D | C | E | c | e | f | V | C w | K | k | Kp a | Kp b | Js a | Js b | $Fy^a$ | Fy b | Jk a | Jk b | $Le^a$ | Le b | P1 | M | N | S | s | $Lu^a$ | $Lu^b$ | Xg a | IS | IAT |
| I | $R_1R_1$ | + | + | 0 | 0 | + | 0 | 0 | 0 | 0 | + | 0 | + | 0 | + | + | + | 0 | + | 0 | + | 0 | 0 | + | + | 0 | 0 | + | + | 0 | 0 |
| II | $R_2R_2$ | + | 0 | + | + | 0 | 0 | 0 | 0 | 0 | + | 0 | + | 0 | + | + | 0 | + | + | + | 0 | + | + | 0 | + | + | 0 | + | 0 | 0 | 0 |
| III | rr | 0 | 0 | 0 | + | + | + | 0 | 0 | + | + | 0 | + | 0 | + | 0 | + | + | 0 | 0 | + | 0 | + | 0 | 0 | + | 0 | + | + | 0 | 0 |

Select units and crossmatch




Release unit

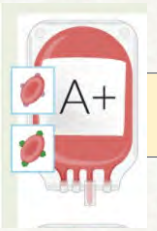
# Serologic Testing Overview

Collect/labeling specimen → ABO and Rh Type → Antibody Screen



Antibody Screen → Antibody ID → Select units and crossmatch

| | | Rh | | | | | | | | Kell | | | | | | Duffy | | Kidd | | Lewis | | P | MNS | | | | | Lutheran | | Xg | Results | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | D | C | E | c | e | f | V | C w | K | k | Kp a | Kp b | Js a | Js b | $Fy^a$ | Fy b | Jk a | Jk b | $Le^a$ | Le b | P1 | M | N | S | s | $Lu^a$ | $Lu^b$ | Xg a | IS | IAT |
| I | $R_1R_1$ | + | + | 0 | 0 | + | 0 | 0 | 0 | 0 | + | 0 | + | 0 | + | + | + | 0 | + | 0 | + | 0 | 0 | + | + | 0 | 0 | + | + | 0 | 0 |
| II | $R_2R_2$ | + | 0 | + | + | 0 | 0 | 0 | 0 | 0 | + | 0 | + | 0 | + | + | 0 | + | + | + | 0 | + | + | 0 | + | + | 0 | + | 0 | 0 | 0 |
| III | rr | 0 | 0 | 0 | + | + | + | 0 | 0 | + | + | 0 | + | 0 | + | 0 | + | + | 0 | 0 | + | 0 | + | 0 | 0 | + | 0 | + | + | 0 | 3+ |




Release unit

# III. Antibody Identification (AbID)

## An antibody is detected "(+) Screen"…now what?

1) Is it an allo or an auto?

2) What is the specificity (AbID)?

3) Is it clinically significant? (i.e., a/w HDFN, hemolytic transfusion reactions, result in notable decrease in transfused RBC survival)

*Caveats:*

- Clinical significance varies even with antibodies of the same specificity
- Some antibodies are known to cause HDFN, while others may result in a (+) DAT in the fetus but no clinical HDFN
- Red Cell Antigen Expression can vary – reaction strength can vary and so reactivity of these antibodies can be less apparent



# Antibody Panel = extended antibody screen to determine AbID

- Test against several reagent RBCs of known phenotype
- Pattern of reactivity aids in identification
- "Rule out" antibodies *not* present in patient's plasma = no reaction
- "Rule in" antibodies present in patient's plasma = reaction

| Donor | Cell number | D | C | c | E | e | C$^{w}$ | K | k | Kp$^{a}$ | Kp$^{b}$ | Js$^{a}$ | Js$^{b}$ | Fy$^{a}$ | Fy$^{b}$ | Jk$^{a}$ | Jk$^{b}$ | Le$^{a}$ | Le$^{b}$ | P$_{1}$ | M | N | S | s | Lu$^{a}$ | Lu$^{b}$ | Xg$^{a}$ | IS | 37 | AHG | CC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| R$_{1}$r | 1 | + | + | + | 0 | + | 0 | 0 | + | 0 | + | 0 | + | + | + | + | + | 0 | + | + | + | + | + | + | 0 | + | + | 0 | 0 | 2+ | |
| R$_{1}$R$_{1}$ | 2 | + | + | 0 | 0 | + | + | + | + | 0 | + | 0 | + | 0 | + | + | 0 | 0 | 0 | + | + | 0 | + | 0 | 0 | + | + | 0 | 0 | 0 | 3+ |
| R$_{2}$R$_{2}$ | 3 | + | 0 | + | + | 0 | 0 | 0 | + | 0 | + | 0 | + | + | 0 | 0 | + | 0 | + | + | 0 | + | 0 | + | 0 | + | + | 0 | 0 | 3+ | |
| R$_{0}$r | 4 | + | 0 | + | 0 | + | 0 | 0 | + | 0 | + | 0 | + | + | 0 | + | + | + | 0 | 0 | + | 0 | + | + | 0 | + | 0 | 0 | 0 | 3+ | |
| r'r | 5 | 0 | + | + | 0 | + | 0 | 0 | + | 0 | + | 0 | + | 0 | 0 | 0 | + | 0 | + | + | + | 0 | + | + | 0 | + | 0 | 0 | 0 | 0 | 3+ |
| r"r | 6 | 0 | 0 | + | + | + | 0 | 0 | + | 0 | + | 0 | + | + | + | 0 | + | 0 | + | + | 0 | + | 0 | + | 0 | + | + | 0 | 0 | 2+ | |
| rr K | 7 | 0 | 0 | + | 0 | + | 0 | + | + | 0 | + | 0 | + | + | + | + | + | 0 | + | 0 | + | + | 0 | + | 0 | + | + | 0 | 0 | 2+ | |
| rr | 8 | 0 | 0 | + | 0 | + | 0 | 0 | + | 0 | + | 0 | + | 0 | + | + | 0 | + | 0 | + | + | + | + | 0 | + | + | 0 | 0 | 0 | 0 | 3+ |
| r'r" | 9 | 0 | + | + | + | + | 0 | 0 | + | 0 | + | 0 | + | 0 | + | + | 0 | 0 | + | + | 0 | + | 0 | + | 0 | + | + | 0 | 0 | 0 | 3+ |
| rr | 10 | 0 | 0 | + | 0 | + | 0 | 0 | + | 0 | + | 0 | + | + | 0 | + | + | 0 | + | + | + | 0 | + | 0 | 0 | + | + | 0 | 0 | 3+ | |
| R$_{1}$r | 11 | + | + | + | 0 | + | 0 | 0 | + | 0 | + | 0 | + | + | + | 0 | + | 0 | + | + | + | + | + | + | 0 | + | + | 0 | 0 | 2+ | |
| | Patient Cells | | | | | | | | | | | | | | | | | | | | | | | | | | | 0 | 0 | 0 | 3+ |

Harmening, DM. Modern Blood Banking and Transfusion Practices 7th Ed.

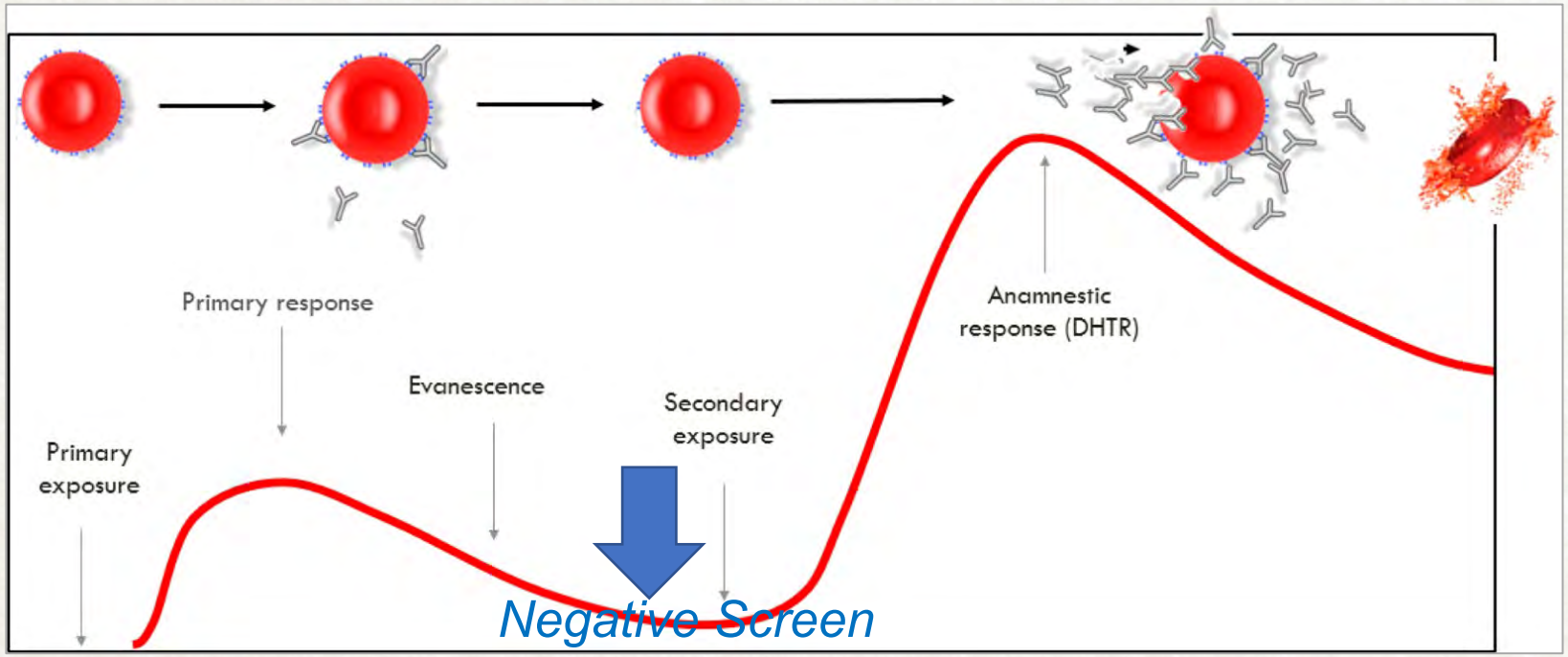
# If an antibody is detected, corresponding antigen negative units must be provided

(+) Ab ID ≠ clinically significant hemolysis

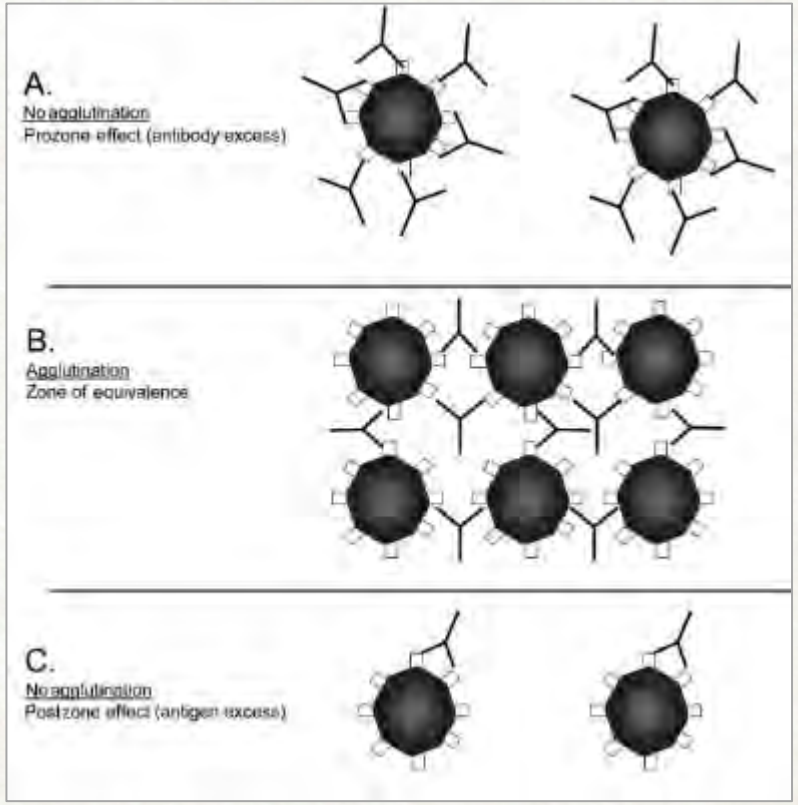
BUT….we treat any potentially clinically significant antibody as if it will induce hemolysis and give *antigen negative units*

# Historical Antibodies

***Historical antibodies are always honored*** independent of whether screen is currently positive or NEGATIVE




Fasano RM *et al* (2019) *Transfusion Clinique et Biologique*




AABB Technical Manual 20th Ed

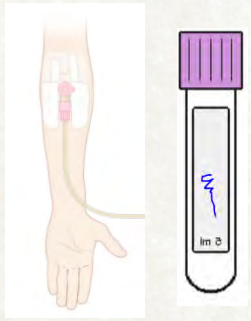
# Timeline for RBC availability

| AVAILABLE TIME | COMPONENT AVAILABLE | RISKS/COMMENTS |
|---|---|---|
| 5 minutes or less | Type O uncrossmatched Rh?????? | 0.2-2% of population has RBC antibodies |
| 45 minutes | Type Specific crossmatched (if antibody screen negative) | Standard Procedure |
| 90 minutes to ??? | Type Specific crossmatched in a patient with a positive screen | If blood is needed before resolution, high risk – EMERGENCY RELEASE. Please clearly communicate urgency w/ BB! |



# Serologic Testing Overview

Collect/labeling specimen

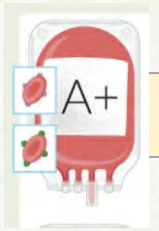


ABO and Rh Type

Antibody Screen

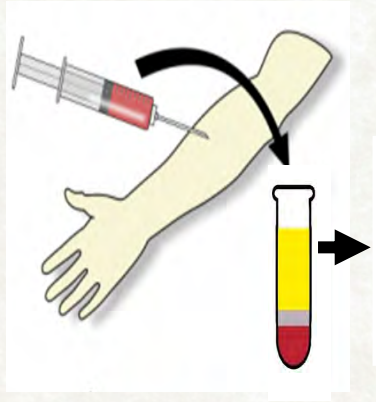
Antibody ID

Select units and crossmatch



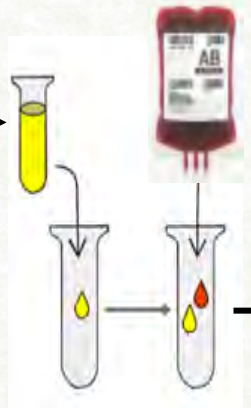

Release unit

# IV. Crossmatch



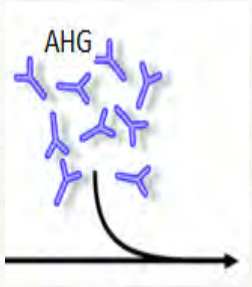
Check patient's transfusion history

Selected RBC unit




IS

IgM




AHG

IgG

Product release

If the current Ab screen is negative and No History of Allo-Abs and tested at **least twice** (with separate samples), can do an **Electronic XM**

If the current Ab screen is positive OR has History of Allo-Abs then **Full XM** must be done



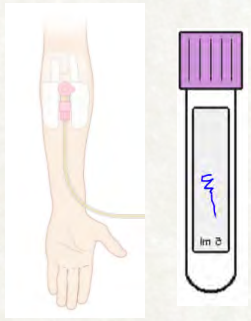
# Types of Crossmatch

| Type of XM | When is it used? | Description |
|---|---|---|
| Immediate Spin (IS) | (-) Ab screen<br>No history of prior (+) screen | Pt plasma + Donor RBC<br>• Agglutination seen at IS<br>• Detects ABO incompatibility |
| IAT ("Full XM" or "Coombs crossmatch") | (+) Ab screen<br>History of (+) screen | Pt plasma + Donor RBC<br>• Incubate at 37 C, wash, AHG used ("bridging")<br>• Detects IgG |
| Electronic XM | (-) Ab screen<br>No history of prior (+) screen<br>2 ABO/Rh types on record<br>Computer software validated | Computer checks ABO compatibility of patient and donor<br>• *No physical testing* |



# Serologic Testing Overview

Collect/labeling specimen

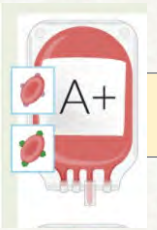


ABO and Rh Type

Antibody Screen

Antibody ID

Select units and crossmatch




Release unit

# Pan-reactive antibody panel

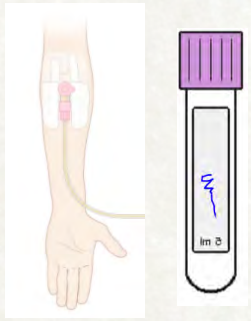
| Donor | Cell number | D | C | c | E | e | C$^{w}$ | K | k | Kp$^{a}$ | Kp$^{b}$ | Js$^{a}$ | Js$^{b}$ | Fy$^{a}$ | Fy$^{b}$ | Jk$^{a}$ | Jk$^{b}$ | Le$^{a}$ | Le$^{b}$ | P$_{1}$ | M | N | S | s | Lu$^{a}$ | Lu$^{b}$ | Xg$^{a}$ | Peg/ IgG |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| R1R1 | 1 | + | + | 0 | 0 | + | 0 | 0 | + | 0 | + | 0 | + | + | 0 | + | + | + | 0 | + | + | + | + | + | 0 | + | + | 2+ |
| R1wR1 | 2 | + | + | 0 | 0 | + | + | + | + | 0 | + | 0 | + | + | + | 0 | + | 0 | + | + | + | 0 | + | + | 0 | + | + | 3+ |
| R2R2 | 3 | + | 0 | + | + | 0 | 0 | 0 | + | 0 | + | 0 | + | 0 | + | 0 | + | 0 | + | + | + | + | + | 0 | 0 | + | + | 2+ |
| R0r | 4 | + | 0 | + | 0 | + | 0 | 0 | + | 0 | + | 0 | + | 0 | 0 | + | + | 0 | + | + | + | 0 | + | 0 | 0 | + | 0 | 2+ |
| r'r | 5 | 0 | + | + | 0 | + | 0 | 0 | + | 0 | + | 0 | + | + | 0 | + | 0 | 0 | 0 | 0 | 0 | + | 0 | + | 0 | + | 0 | 2+ |
| r''r | 6 | 0 | 0 | + | + | + | 0 | + | + | 0 | + | 0 | + | + | + | + | + | 0 | + | + | + | + | + | + | 0 | + | + | 3+ |
| rr | 7 | 0 | 0 | + | 0 | + | 0 | 0 | + | 0 | + | 0 | + | + | + | + | + | + | 0 | 0 | + | + | 0 | + | 0 | + | + | 2+ |
| rr | 8 | 0 | 0 | + | 0 | + | 0 | 0 | + | 0 | + | 0 | + | 0 | + | 0 | + | 0 | + | + | + | 0 | 0 | + | 0 | + | + | 2+ |
| rr | 9 | 0 | 0 | + | 0 | + | 0 | 0 | + | 0 | + | 0 | + | 0 | + | + | 0 | 0 | + | 0 | + | + | + | + | 0 | + | 0 | 2+ |
| rr | 10 | 0 | 0 | + | 0 | + | 0 | + | + | 0 | + | 0 | + | 0 | + | + | + | 0 | + | + | 0 | + | 0 | + | 0 | + | + | 3+ |
| R0r | 1 1 | + | 0 | + | 0 | + | 0 | 0 | + | 0 | + | 0 | + | + | + | 0 | + | 0 | + | + | 0 | + | 0 | + | 0 | + | + | 2+ |
| | Patient Cells | | | | | | | | | | | | | | | | | | | | | | | | | | | 2+ |

- Alloantibody to High Frequency Antigen
- **Autoantibody**
- Multiple Alloantibodies
- Daratumumab (anti-CD38 )

Harmening, DM. Modern Blood Banking and Transfusion Practices 7th Ed.

# Serologic Testing Overview

Collect/labeling specimen



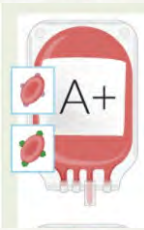
ABO and Rh Type

Antibody Screen

Antibody ID

Select units and crossmatch

Additional Testing:
DAT
Elution
Adsorption
Pheno/Genotyping




Release unit

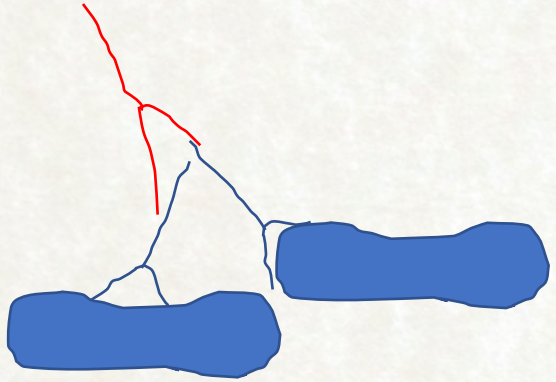
# Additional Blood Bank Tests

- Direct Antiglobulin Test (DAT)
- Elution
- Adsorption
- Phenotyping



# Coombs test: Detecting *in-vivo* antibody coating of RBCs

Anti-human globulin (AHG)



## IN-VIVO ISOSENSITISATION OF RED CELLS IN BABIES WITH HÆMOLYTIC DISEASE

R. R. A. Coombs *
B.Sc. Edin., M.R.C.V.S.
DEPARTMENT OF PATHOLOGY, UNIVERSITY OF CAMBRIDGE
A. E. Mourant
D.Phil., B.M. Oxfd
R. R. Race
M.R.C.S.
MEDICAL RESEARCH COUNCIL, EMERGENCY BLOOD-TRANSFUSION SERVICE

This paper records the results of tests which demonstrate the isosensitisation of the red blood-cells of babies suffering from hæmolytic disease. The sensitisation is shown by the agglutination of these cells by rabbit anti-human-globulin serum. Since the test is rapid, can be performed on a tile, and does not require anti-Rh sera, it promises to be of practical use in the early diagnosis of hæmolytic disease.

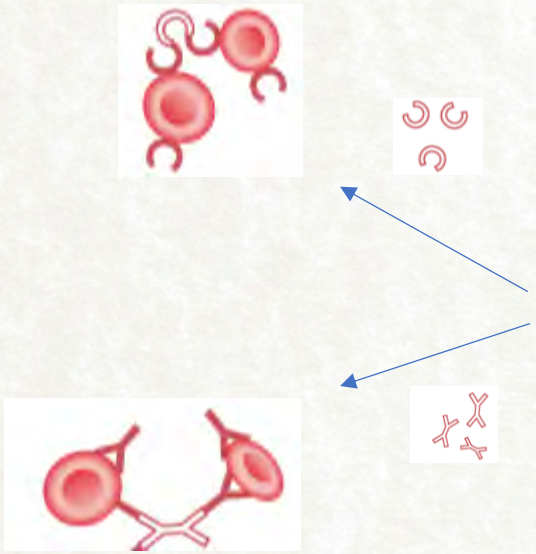
Levine, Katzin, and Burnham (1941) and numerous subsequent workers have shown that hæmolytic disease of the newborn is almost always associated with immunisation of the mother with Rh antigen. In rare cases anti-Rh agglutinin has been demonstrated in the child's serum, and Baar (1945) has shown that "incomplete" Rh antibody is relatively common.

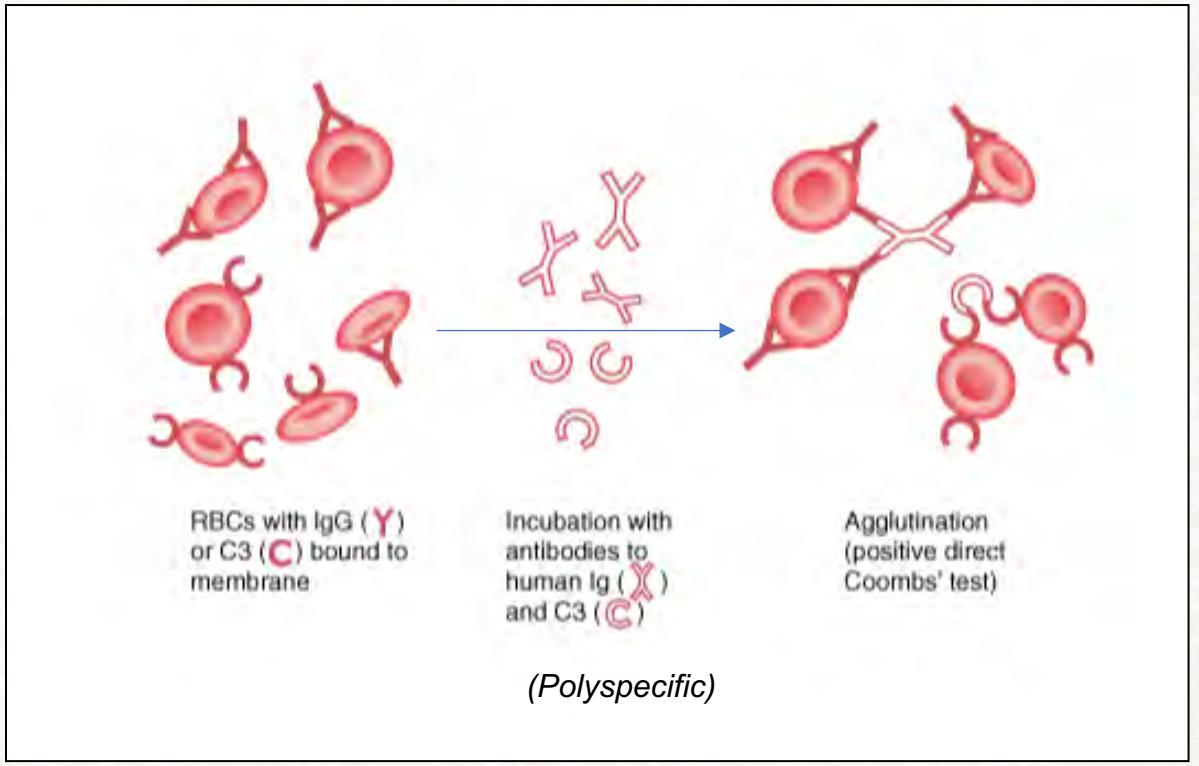
We suggested in our previous papers (Coombs et al. 1945a and b) and have now confirmed that this in-vivo sensitisation can be demonstrated by the use of an anti-human-globulin serum.

COOMBS RR, MOURANT AE, RACE RR. (1946) *Lancet*

# Direct Antiglobulin Test (DAT)

*In-Vivo* antibody coating
Positive DAT = IgG or Complement (C3) is present on the patient's RBCs






May be *suggestive* but not indicative of a hemolytic picture:

IgG reactivity often correlating with a warm-reactive antibody/extravascular hemolysis
C3 reactivity typically correlating with a cold-reactive antibody (IgM)/intravascular hemolysis



Modified from: https://www.merckmanuals.com/professional/hematology-and-oncology/anemias-caused-by-hemolysis/overview-of-hemolytic-anemia#

# (+) DAT

0.1% healthy blood donors, up to 15% hospitalized pts *without* evidence of hemolysis

**(+) DAT ≠ Hemolysis**
**≠ RBC antibodies**
**≠ Immune cause**

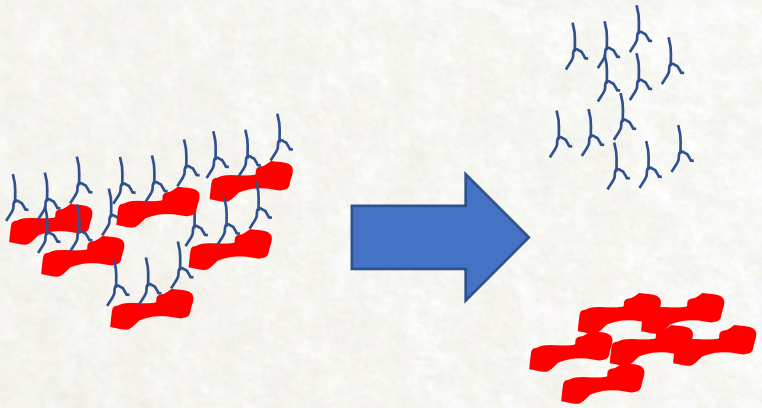
**Other causes of a + DAT**

Drugs – therapeutic monoclonals
Nonspecifically adsorbed proteins – polyclonal hypergammaglobulinemia, IVIG, drugs, rhogam
Severe rouleaux
Drug-induced abs
Complement activation due to infections
Passively transferred Abs – passenger lymphocyte syndrome



# Antibody Elution

*DAT is **positive**, how can we figure out what Ab is coating the RBCs?*



Determine the specificity of the

Run on test

| Donor | Cell number | D | C | c | E | e | $C^w$ | K | k | $Kp^a$ | $Kp^b$ | $Js^a$ | $Js^b$ | $Fy^a$ | $Fy^b$ | $Jk^a$ | $Jk^b$ | $Le^a$ | $Le^b$ | $P_1$ | M | N | S | s | $Lu^a$ | $Lu^b$ | $Xg^a$ | IS | 37 | AHG | CC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $R_1r$ | 1 | + | + | + | 0 | + | 0 | 0 | + | 0 | + | 0 | + | + | + | + | + | 0 | + | + | + | + | + | + | 0 | + | + | | | | |
| $R_1R_1$ | 2 | + | + | 0 | 0 | + | + | + | + | 0 | + | 0 | + | 0 | + | + | 0 | 0 | 0 | + | + | 0 | + | 0 | 0 | + | + | | | | |
| $R_2R_2$ | 3 | + | 0 | + | + | 0 | 0 | 0 | + | 0 | + | 0 | + | + | 0 | 0 | + | 0 | + | + | + | 0 | + | 0 | + | 0 | + | + | | | | |
| $R_0r$ | 4 | + | 0 | + | 0 | + | 0 | 0 | + | 0 | + | 0 | + | + | 0 | + | + | + | 0 | 0 | + | 0 | + | + | 0 | + | 0 | | | | |
| r'r | 5 | 0 | + | + | 0 | + | 0 | 0 | + | 0 | + | 0 | + | 0 | 0 | 0 | + | 0 | + | + | + | 0 | + | + | 0 | + | 0 | | | | |
| r''r | 6 | 0 | 0 | + | + | + | 0 | 0 | + | 0 | + | 0 | + | + | + | 0 | + | 0 | + | + | + | 0 | + | 0 | + | + | + | | | | |
| rr K | 7 | 0 | 0 | + | 0 | + | 0 | + | + | 0 | + | 0 | + | + | + | + | + | 0 | + | 0 | + | + | 0 | + | 0 | + | + | | | | |
| rr | 8 | 0 | 0 | + | 0 | + | 0 | 0 | + | 0 | + | 0 | + | 0 | + | + | 0 | + | 0 | + | + | + | + | 0 | + | + | 0 | | | | |
| r'r'' | 9 | 0 | + | + | + | + | 0 | 0 | + | 0 | + | 0 | + | 0 | + | + | 0 | 0 | + | + | + | 0 | + | 0 | + | 0 | + | + | | | | |
| rr | 10 | 0 | 0 | + | 0 | + | 0 | 0 | + | 0 | + | 0 | + | + | 0 | + | + | 0 | + | + | + | + | 0 | + | 0 | 0 | + | + | | | | |
| $R_1r$ | 11 | + | + | + | 0 | + | 0 | 0 | + | 0 | + | 0 | + | + | + | 0 | + | 0 | + | + | + | + | + | + | 0 | + | + | | | | |
| | Patient Cells | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

| Cause of Positive DAT | Eluate Reactivity |
|---|---|
| Transfusion Reaction (DHTR/DSTR) | Alloantibody pattern (specific) |
| HDFN | |
| Warm Autoantibody | Panagglutanin |
| Drug-induced Antibody | Usually Negative |

*<u>Note:</u> elution studies are most useful for IgG-positive DATs. C3-positive DATs are frequently associated with IgM antibodies (although some IgGs can fix complement); such antibodies are poorly eluted from RBCs and few reagents exist to detect bound IgM*

# Summary of serologic findings in AIHA

**TABLE 17-4.** Typical Serologic Findings in Autoimmune Hemolytic Anemia

| | WAIHA | CAS | Mixed-type AIHA | PCH |
|---|---|---|---|---|
| DAT (routine) | IgG<br>IgG + C3<br>C3 | C3 only | IgG + C3<br>C3 | C3 only |
| Immunoglobulin type | IgG | IgM | IgG, IgM | IgG |
| Eluate | IgG antibody | Nonreactive | IgG antibody | Nonreactive |
| Serum | IAT; 35% agglutinate untreated red cells at 20 C | IgM agglutinating antibody; titer ≥1000 (60%) at 4 C; react at ≥30 C | IgG IAT-reactive antibody plus IgM agglutinating antibody react at ≥30 C | Routine IAT negative; IgG biphasic hemolysin in Donath-Landsteiner test |
| Specificity | Broadly reactive; multiple specificities reported | Usually anti-I | Usually unclear | Anti-P |

AIHA = autoimmune hemolytic anemia; WAIHA = warm AIHA; CAS = cold agglutinin syndrome; PCH = paroxysmal cold hemoglobinuria; DAT = direct antiglobulin test; IAT = indirect antiglobulin test.

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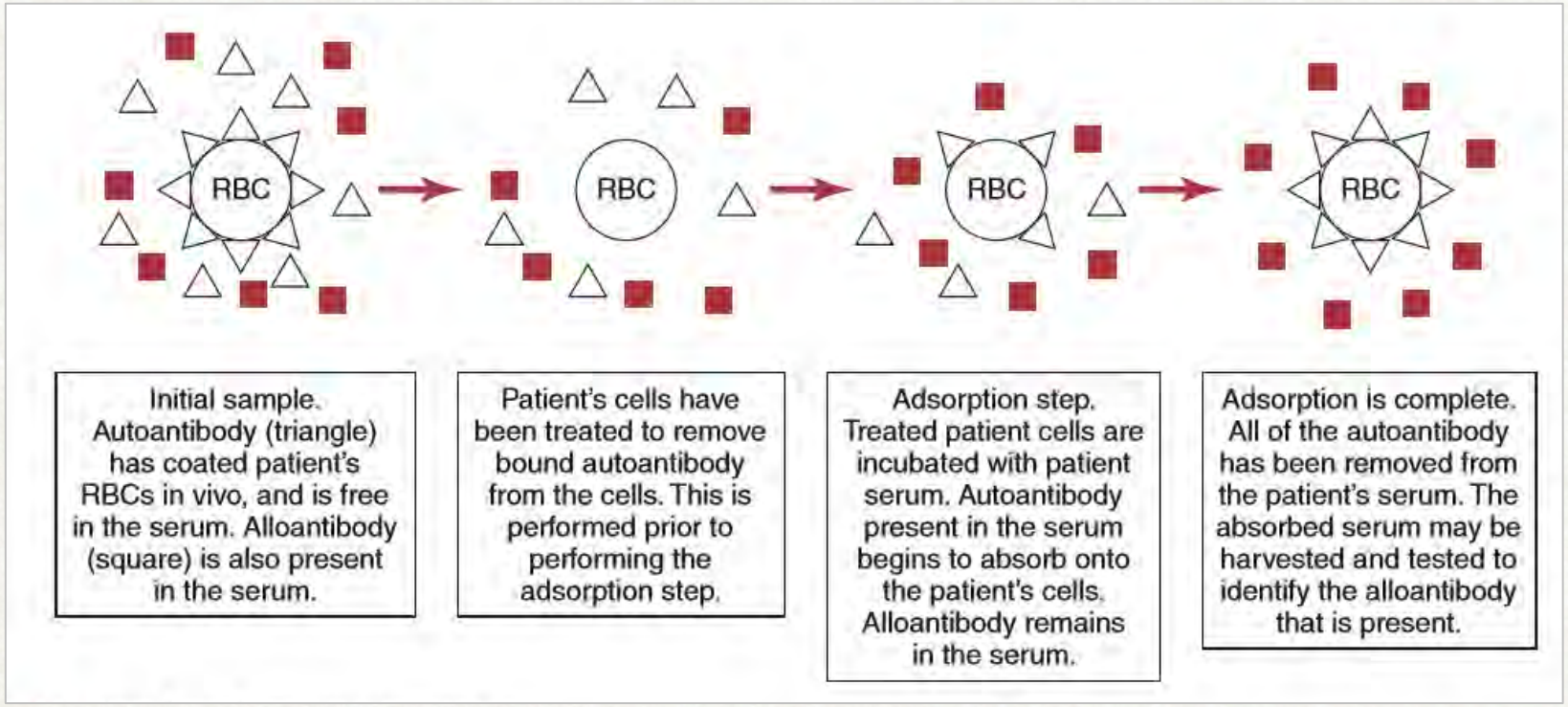
# Removing *auto*-antibodies to detect underlying *allo*-antibodies

## Autoadsorption

- uses patient's own RBCs to adsorb out the autoantibody in the serum → any remaining reactivity?
- In patients not transfused within previous 3 months

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~30% patients with autos will also have allos (*Branch Dr and Petz LD. Transfusion 1999*)

# Alloantibodies may become apparent after autoadsorption

| Donor | Cell number | D | C | c | E | e | $C^w$ | K | k | $Kp^a$ | $Kp^b$ | $Js^a$ | $Js^b$ | $Fy^a$ | $Fy^b$ | $Jk^a$ | $Jk^b$ | $Le^a$ | $Le^b$ | $P_1$ | M | N | S | s | $Lu^a$ | $Lu^b$ | $Xg^a$ | Peg/ IgG | CC | Absorbed serum | CC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| R1R1 | 1 | + | + | 0 | 0 | + | 0 | 0 | + | 0 | + | 0 | + | + | 0 | + | + | + | 0 | + | + | + | + | + | 0 | + | + | 2+ | | 0 | 3+ |
| R1wR1 | 2 | + | + | 0 | 0 | + | + | + | + | 0 | + | 0 | + | + | + | 0 | + | 0 | + | + | + | 0 | + | + | 0 | + | + | 3+ | | 2+ | |
| R2R2 | 3 | + | 0 | + | + | 0 | 0 | 0 | + | 0 | + | 0 | + | 0 | + | 0 | + | 0 | + | + | + | + | + | 0 | 0 | + | + | 2+ | | 0 | 3+ |
| R0r | 4 | + | 0 | + | 0 | + | 0 | 0 | + | 0 | + | 0 | + | 0 | 0 | + | + | 0 | + | + | + | 0 | + | 0 | 0 | + | 0 | 2+ | | 0 | 3+ |
| r'r | 5 | 0 | + | + | 0 | + | 0 | 0 | + | 0 | + | 0 | + | + | 0 | + | 0 | 0 | 0 | 0 | 0 | + | 0 | + | 0 | + | 0 | 2+ | | 0 | 3+ |
| r''r | 6 | 0 | 0 | + | + | + | 0 | + | + | 0 | + | 0 | + | + | + | + | + | 0 | + | + | + | + | + | + | 0 | + | + | 3+ | | 2+ | |
| rr | 7 | 0 | 0 | + | 0 | + | 0 | 0 | + | 0 | + | 0 | + | + | + | + | + | + | 0 | 0 | + | + | 0 | + | 0 | + | + | 2+ | | 0 | 3+ |
| rr | 8 | 0 | 0 | + | 0 | + | 0 | 0 | + | 0 | + | 0 | + | 0 | + | 0 | + | 0 | + | + | + | 0 | 0 | + | 0 | + | + | 2+ | | 0 | 3+ |
| rr | 9 | 0 | 0 | + | 0 | + | 0 | 0 | + | 0 | + | 0 | + | 0 | + | + | 0 | 0 | + | 0 | + | + | + | + | 0 | + | 0 | 2+ | | 0 | 3+ |
| rr | 10 | 0 | 0 | + | 0 | + | 0 | + | + | 0 | + | 0 | + | 0 | + | + | + | 0 | + | + | 0 | + | 0 | + | 0 | + | + | 3+ | | 2+ | |
| R0r | 1 1 | + | 0 | + | 0 | + | 0 | 0 | + | 0 | + | 0 | + | + | + | 0 | + | 0 | + | + | 0 | + | 0 | + | 0 | + | + | 2+ | | 0 | 3+ |
| | Patient Cells | | | | | | | | | | | | | | | | | | | | | | | | | | | 2+ | | | |

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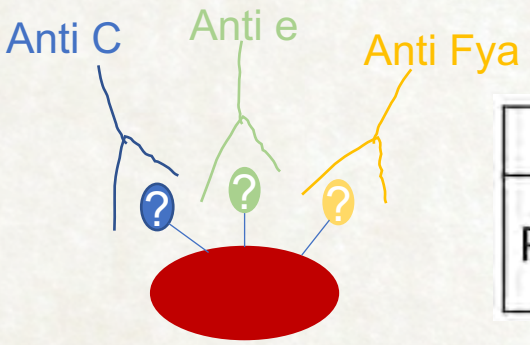
# Phenotyping patient RBCs

Patient is *not* expected to produce an alloantibody to an antigen present on their own RBCs

Determining the RBC antigen expression profile on patient RBCs :

- What antigens are present on patient cells?
- What antigens are missing? → antibodies are they at risk for producing




| | D | C | E | c | e | K | k | $Fy^a$ | $Fy^b$ | $Jk^a$ | $Jk^b$ | M | N | S | s |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Patient RBCs | + | + | 0 | 0 | + | 0 | + | + | 0 | 0 | + | + | + | + | + |

At risk for: Anti-E, anti-c, anti-K, anti-Fyb, anti-Jka

- Phenotypically matched units can be provided in certain clinical situations
- Helpful for serologic workup

# Phenotyping Limitations

Serologic phenotype may be unreliable:

- Recent transfusion
- HSCT
- Positive DAT (antibody must first be removed)

Genotyping:

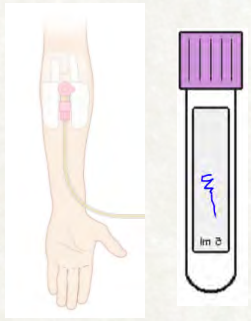
- Used to predict phenotype
- Not available as STAT
- **HEA** (human erythrocyte antigen) **panel**
  Extended typing - 35 antigen profile

| Blood Group | Red Blood Cell Antigens |
|---|---|
| Rh | C (RH2), c (RH4), E (RH3), e (RH5), V (RH10), VS (RH20) |
| Kell | K (KEL1), k (KEL2), Kpa (KEL3), Kpb (KEL4), Jsa (KEL6), Jsb (KEL7) |
| Duffy | Fya (FY1), Fyb (FY2), GATA (FY-2), Fyx (FY2W) |
| Kidd | Jka (JK1), Jkb (JK2) |
| MNS | M (MNS1), N (MNS2), S (MNS3), s (MNS4), Uvar (MNS-3,5W), Uneg (MNS-3,-4,-5) |
| Lutheran | Lua (LU1), Lub (LU2) |
| Dombrock | Doa (DO1), Dob (DO2), Hy (DO4), Joa (DO5) |
| Landsteiner-Wiener | LWa (LW5), LWb (LW7) |
| Diego | Dia (DI1), Dib (DI2) |
| Colton | Coa (CO1),Cob (CO2) |
| Scianna | Sc1(SC1), Sc2 (SC2) |



# Serologic Testing Overview

Collect/labeling specimen



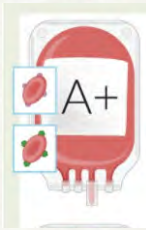
ABO and Rh Type

Antibody Screen

Antibody ID

Select units and crossmatch

Additional Testing
DAT
Elution
Adsorption
Pheno/Genotyping




Release unit