

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



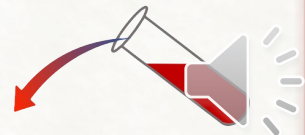
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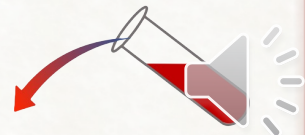
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# Disclosures

➤ None



# Objectives

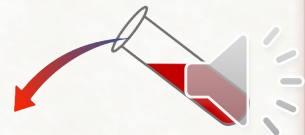
1. Describe different blood groups/ blood group antigens and understand what determines their clinical relevance
2. Understand why pretransfusion testing is performed, the components of pretransfusion testing and the clinical implications of the results

- *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 when patient survived



seems right, as the operation now stands, to confine transfusion to the first class of cases only, namely, those in which there seems to be no hope for the patient, unless blood can be thrown into the veins.

The object of the **Gravitator** is, to give help in this last extremity, by transmitting the blood in a regulated stream from one individual to another, with as little exposure as may be to air, cold, and inanimate surface; ordinary venesection being the only operation performed on the person who emits the blood; and the insertion of a small tube into the vein usually laid open in bleeding, being all the operation which it is necessary to execute on the person who receives it.



# “Rules” for transfusion: Discovery of ABO blood group antigens

## 1901: Landsteiner’s Experiment

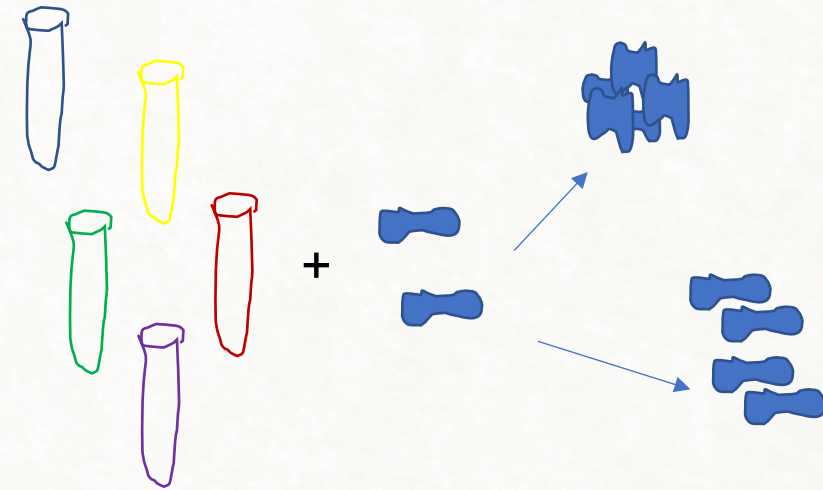
Tabelle III, betreffend das Blut von fünf Puerperae und sechs Placenten (Nabelschnurblut).

| Sera             | + | + | - | - | - | + |
|------------------|---|---|---|---|---|---|
| Lust. . . . .    | + | + | - | - | - | + |
| Tomsch. . . . .  | - | - | + | - | - | - |
| Mittelb. . . . . | - | - | + | - | - | - |
| Seil. . . . .    | - | - | + | - | - | - |
| Linsm. . . . .   | + | + | + | - | - | + |

---

| Blutkörperchen von: | Traum. | Linsm. | Seil. | Freib. | Graupn. | Mittelb. |
|---------------------|--------|--------|-------|--------|---------|----------|
|---------------------|--------|--------|-------|--------|---------|----------|

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



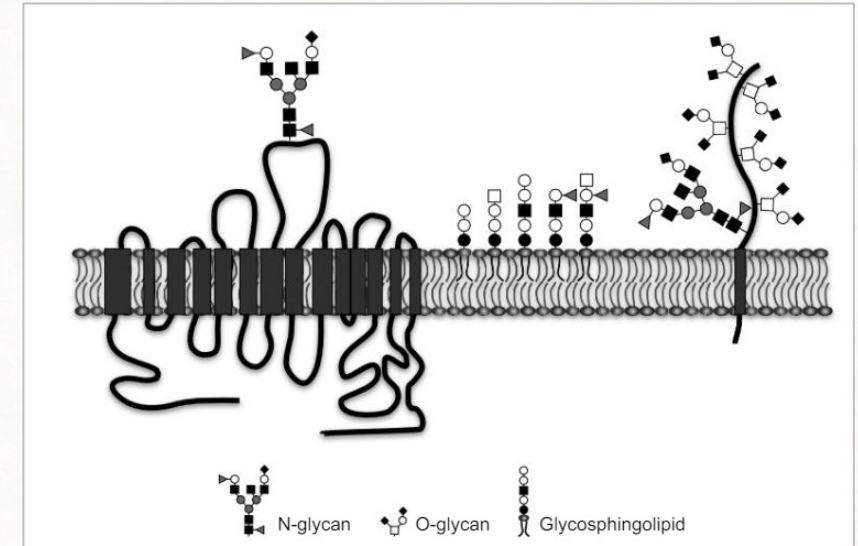
- Recognized a pattern of agglutination
- Blood can be divided into “groups”
- Marked the discovery of the ABO blood group system



# ABO histo-blood group antigens

## ABO antigens

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

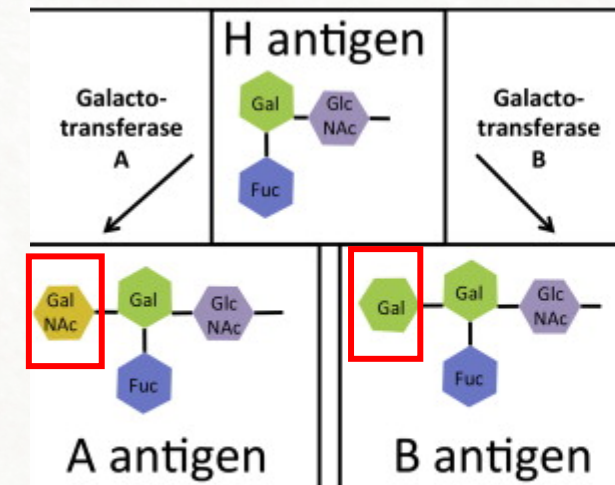


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## The ABO gene locus encodes glycosyltransferases

- 3 alleles/6 genotypes/4 phenotypes

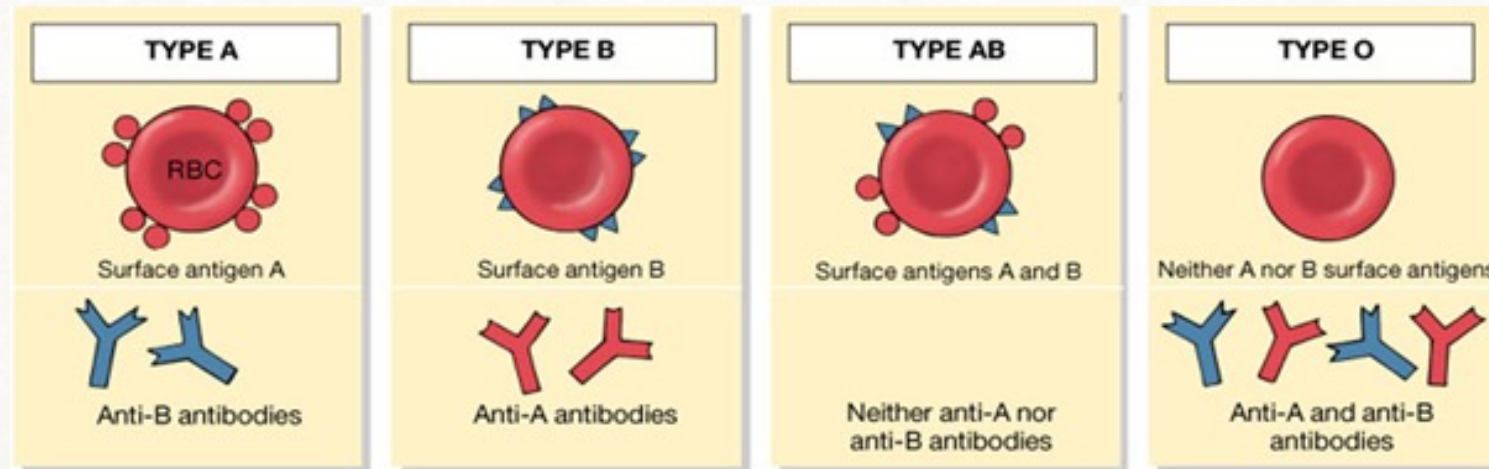
Physiological functions remain unknown



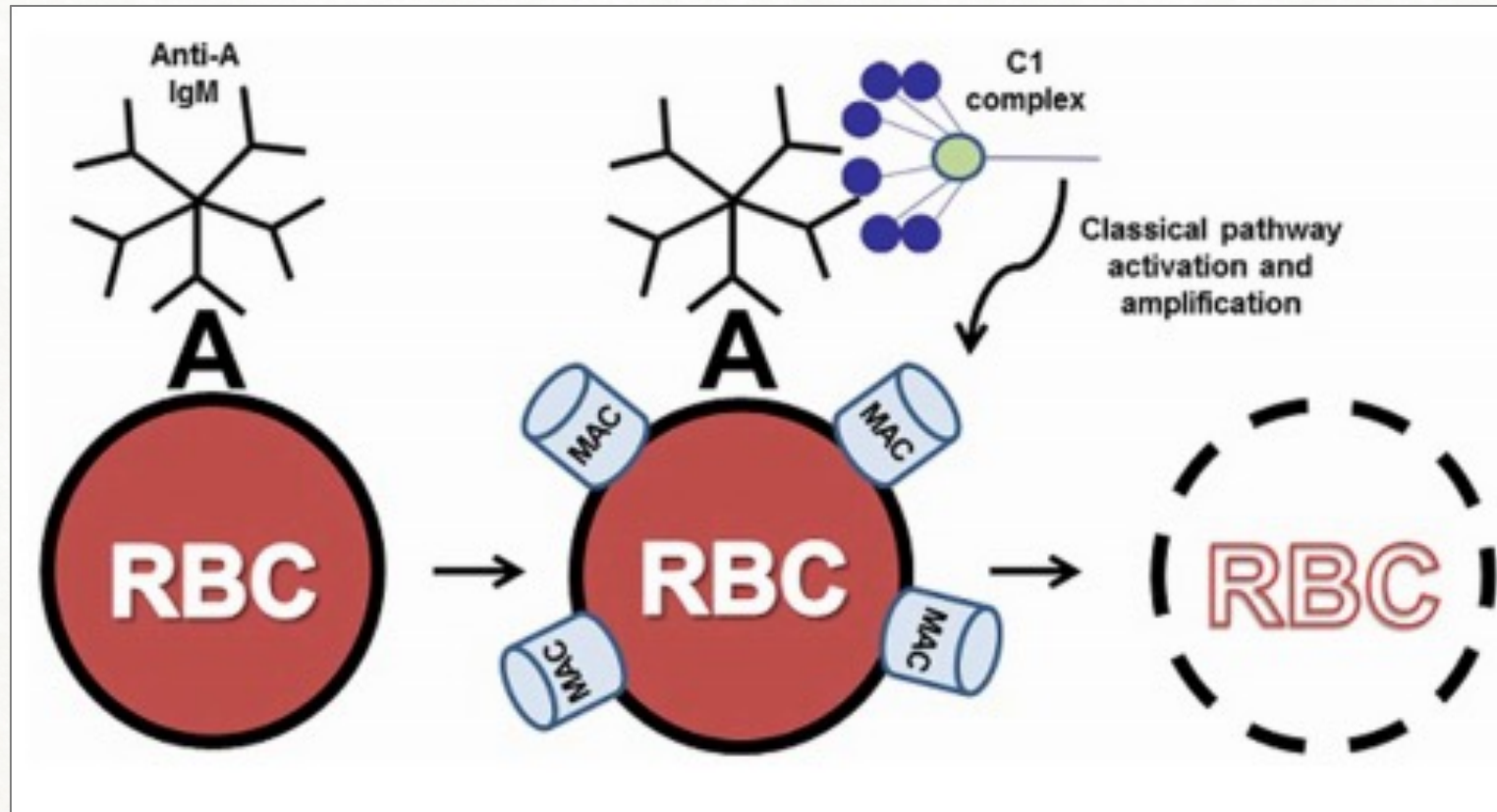
# Antibodies to ABO antigens

## 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*

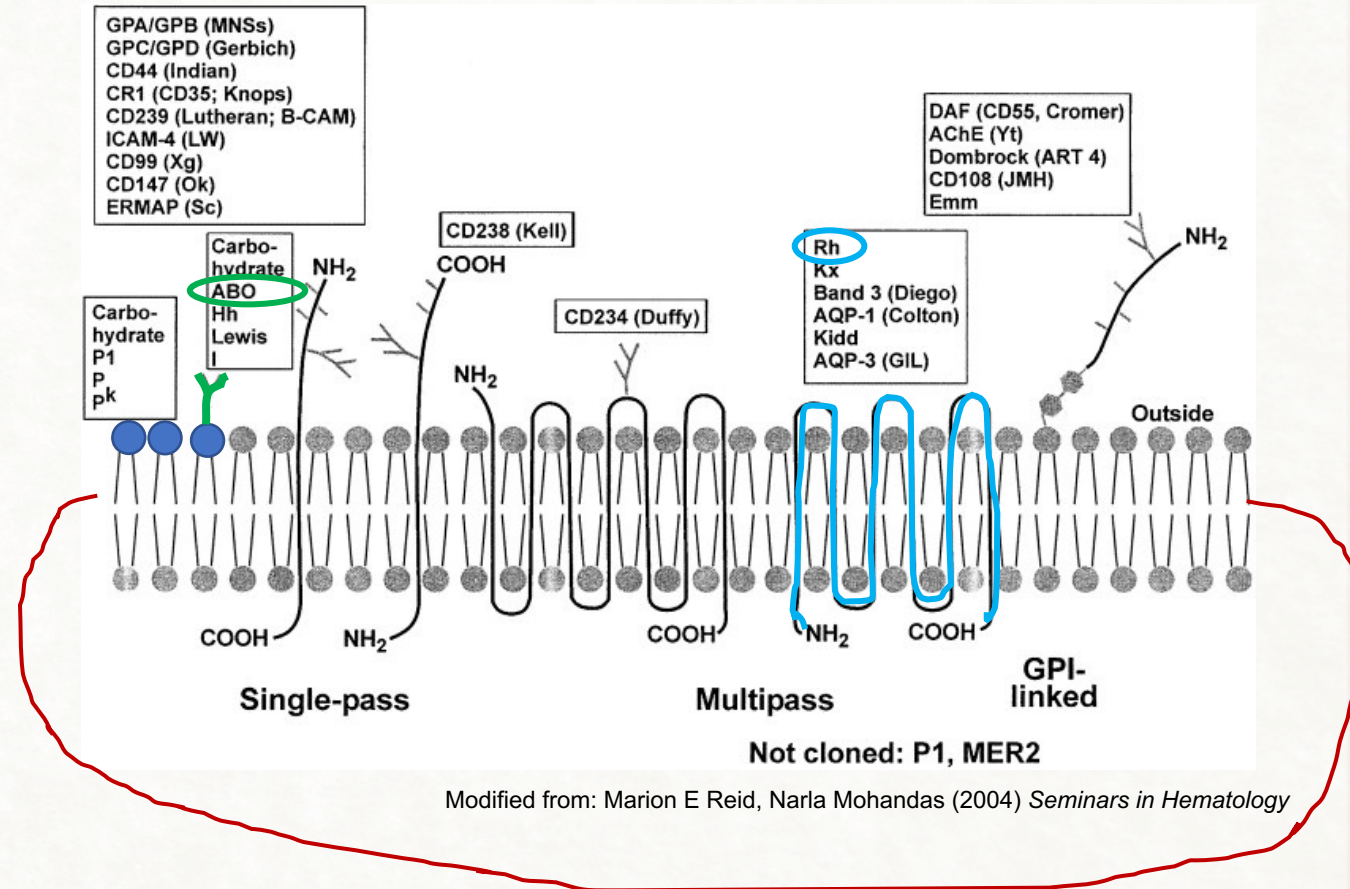




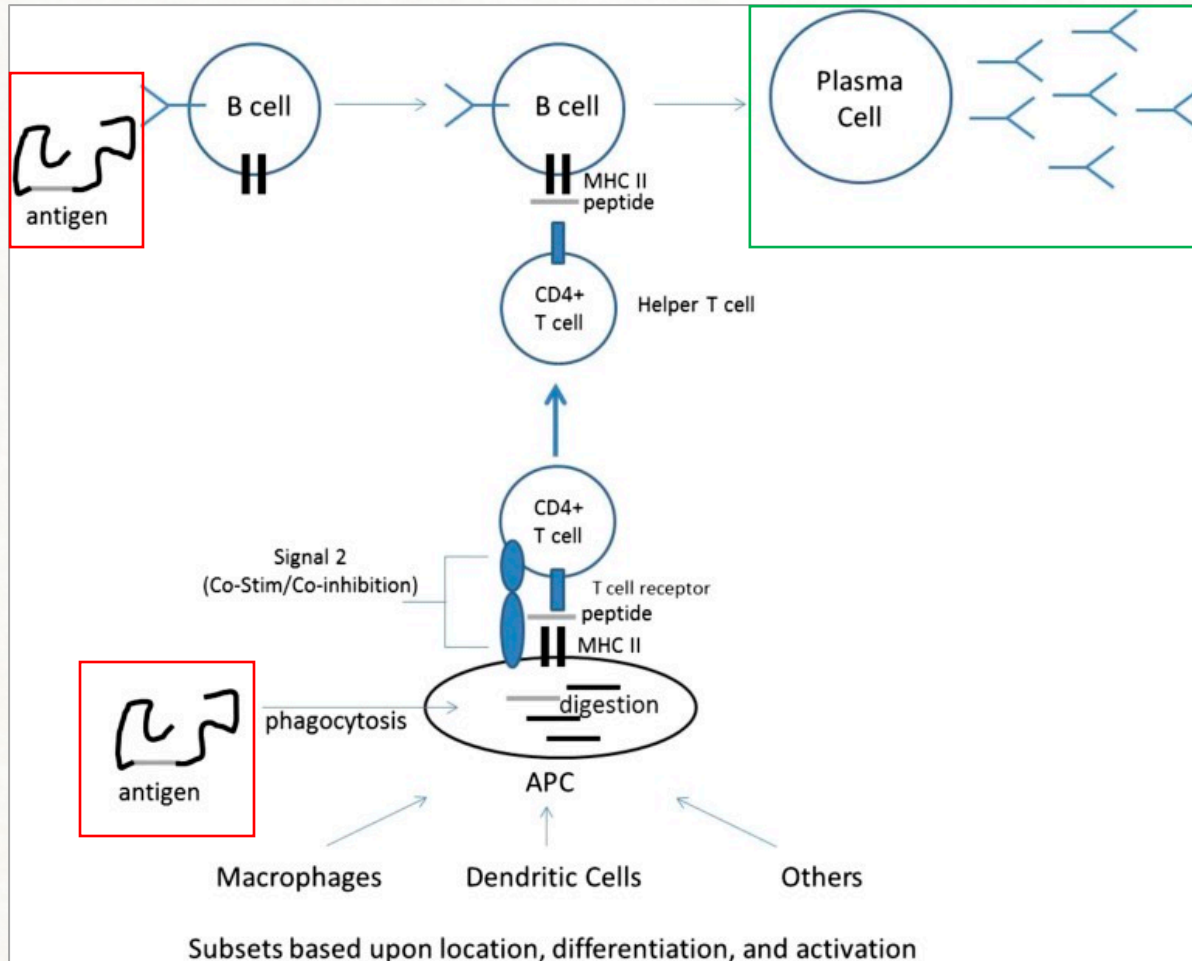
# Other Blood Group Antigens

## Antigens:

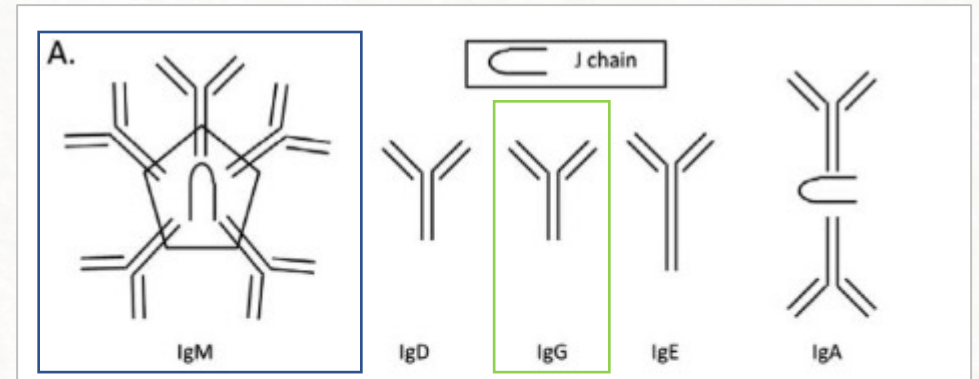
- >300 antigens (36 blood group systems) are known
- Polymorphic, inherited, carbohydrate or protein structures located on the extracellular surface of the RBC membrane.



# 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



# 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



# 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**

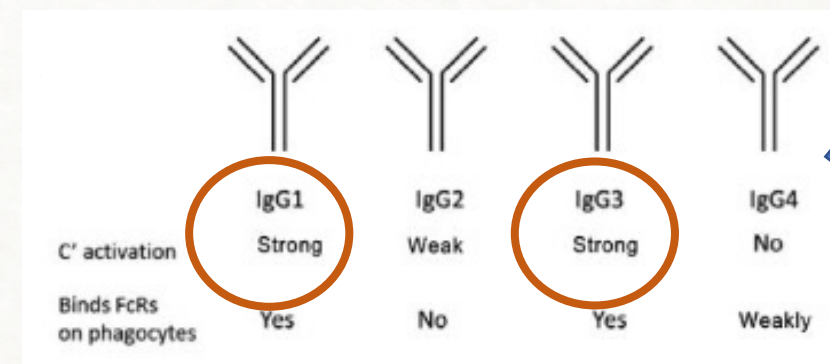
| BLOOD GROUP ANTIGEN  | BLOOD GROUP SYSTEM | IMMUNOGENICITY (%)* |
|----------------------|--------------------|---------------------|
| D (Rh <sub>0</sub> ) | Rh                 | 50                  |
| K                    | Kell               | 5                   |
| c (hr')              | Rh                 | 2.05                |
| E (rh'')             | Rh                 | 1.69                |
| k                    | Kell               | 1.50                |
| e (hr'')             | Rh                 | 0.56                |
| Fy <sup>a</sup>      | Duffy              | 0.23                |
| C (rh')              | Rh                 | 0.11                |
| Jk <sup>a</sup>      | Kidd               | 0.07                |
| S                    | MNSs               | 0.04                |
| Jk <sup>b</sup>      | 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.

## 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   |

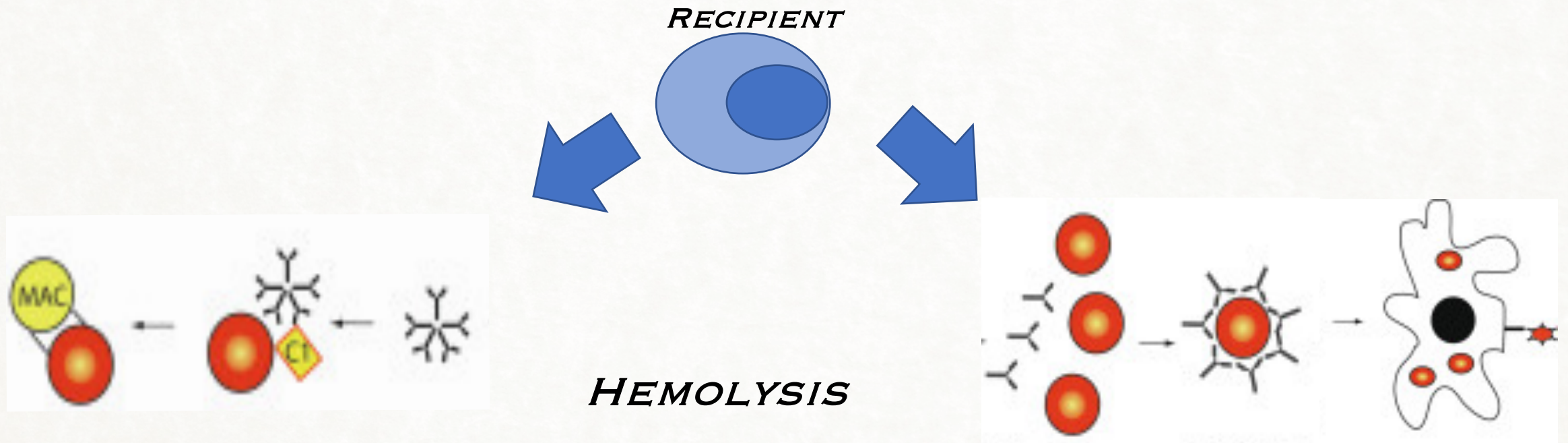
\*\*exception: ABO



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# Intravascular and extravascular hemolysis



Transfused RBCs should *lack* antigens to which the recipient has antibodies

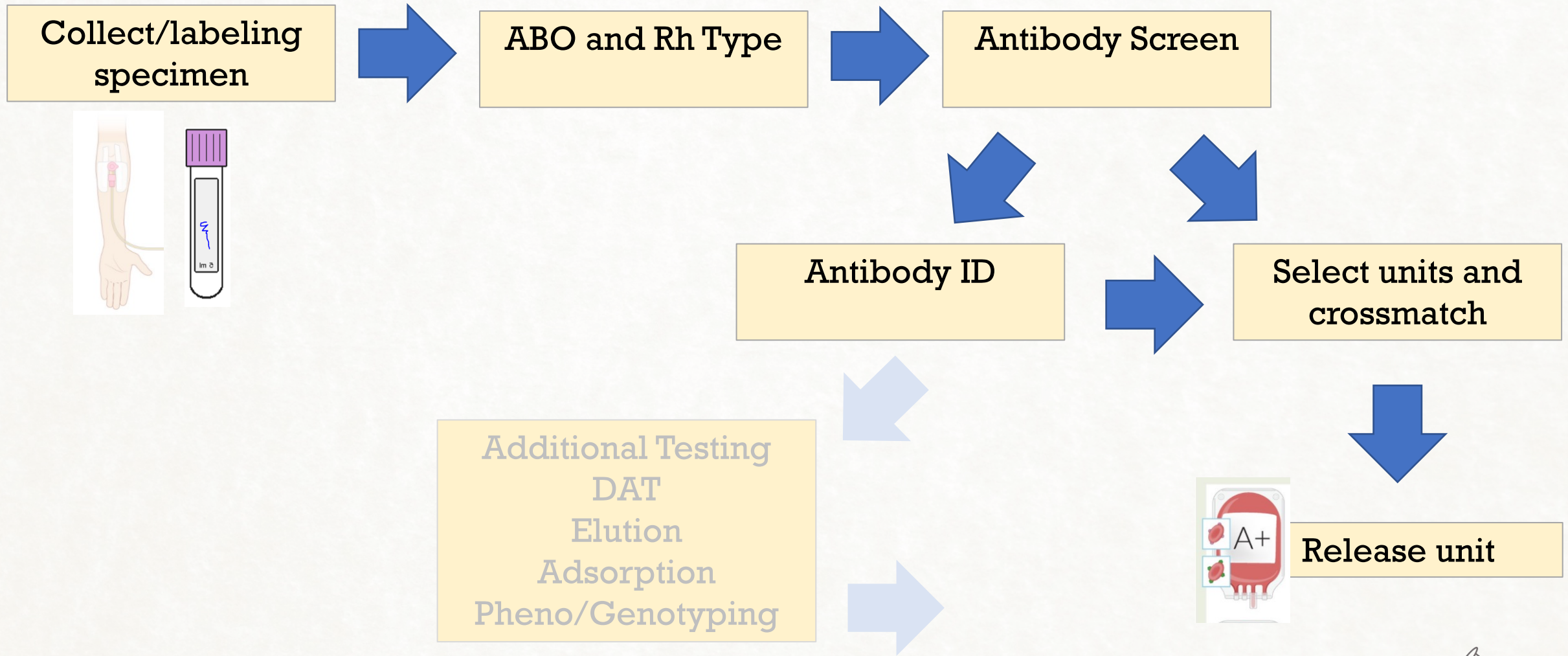


# Why do we need to perform serologic pre-transfusion testing?

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

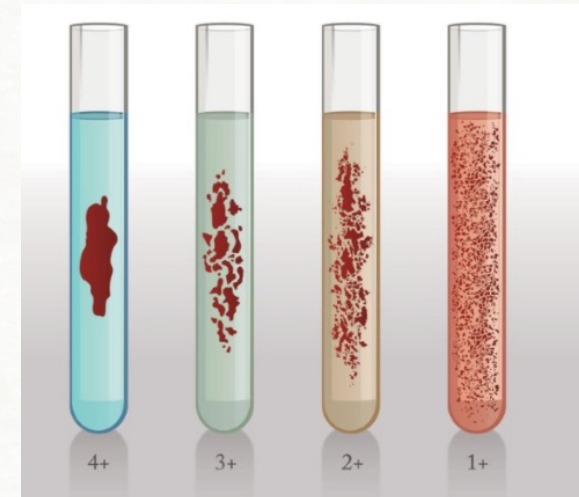
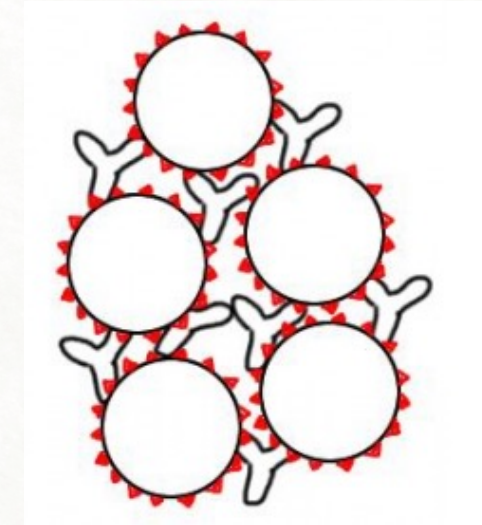


# Serologic Testing Overview



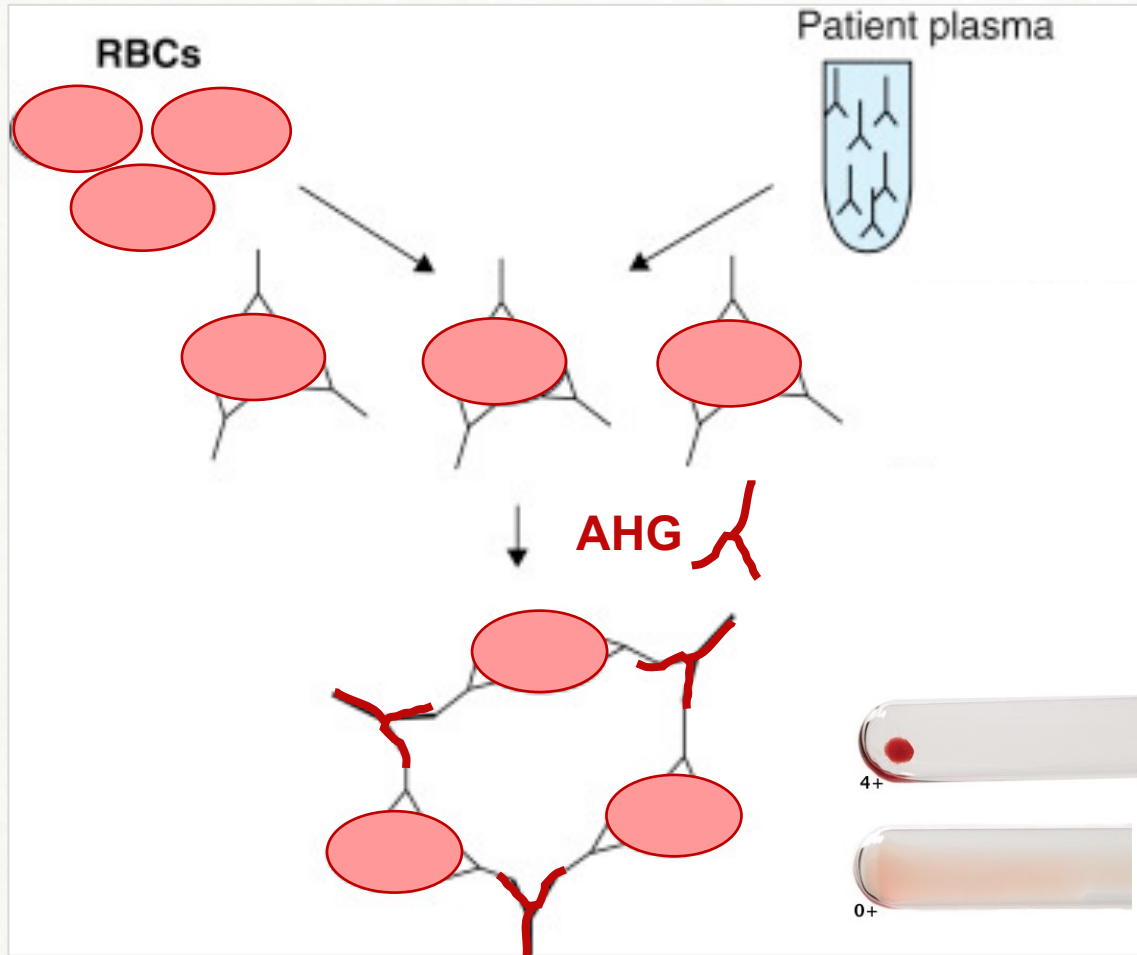
# Hemagglutination is the basis for all BB testing

- Agglutination = clumping
- RBCs are bound together by an Ab → **visible** aggregates (“agglutinates”)
- Agglutinates are graded on a scale of 0-4
- Abs vary in ability to agglutinate RBCs





# Direct vs Indirect Antiglobulin Test



## Direct Antiglobulin Test (DAT) = Coombs

- Detects **antibodies bound to RBCs** (*in vivo* antibody reactivity)

## Indirect Antiglobulin Test (IAT) = Indirect Coombs

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



# Routine Serologic Pre-transfusion Testing

## I. Type

What is the ABO and RhD 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

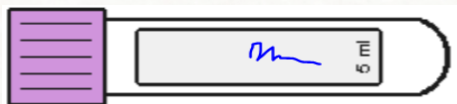
*What* are those unexpected antibodies – Ab ID?  
Performed only if screen is positive

## 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

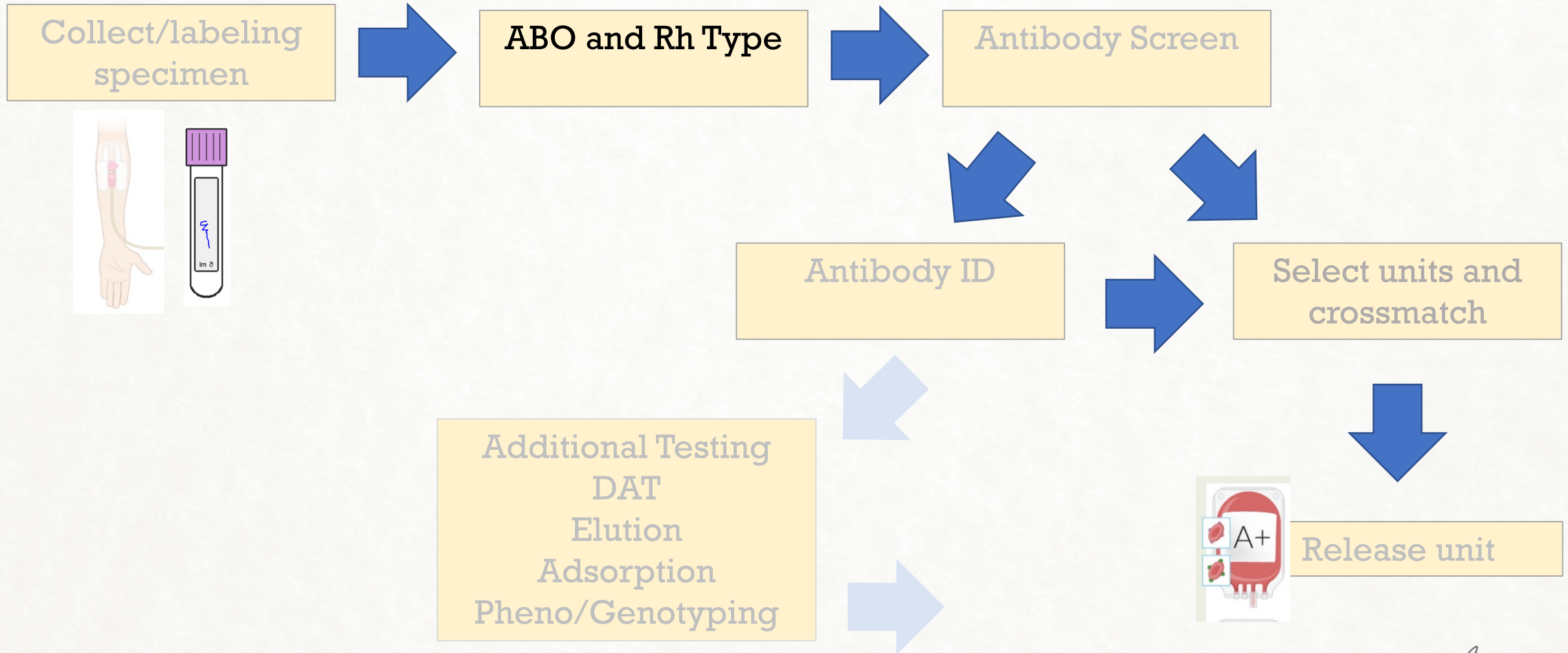
### Neonates (<4 mo):

- 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



# I. Blood Type – ABO

## ABO Grouping

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

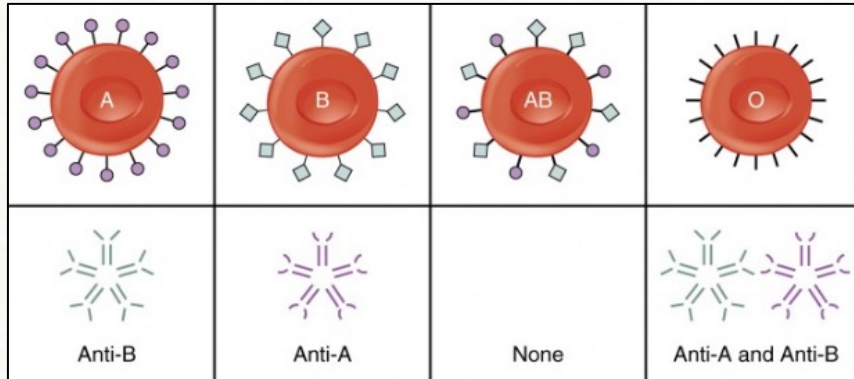
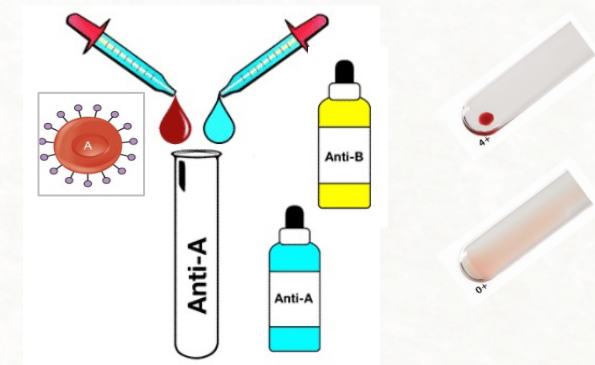


TABLE 2. Distribution (%)\* of ABO phenotypes by race/ethnicity

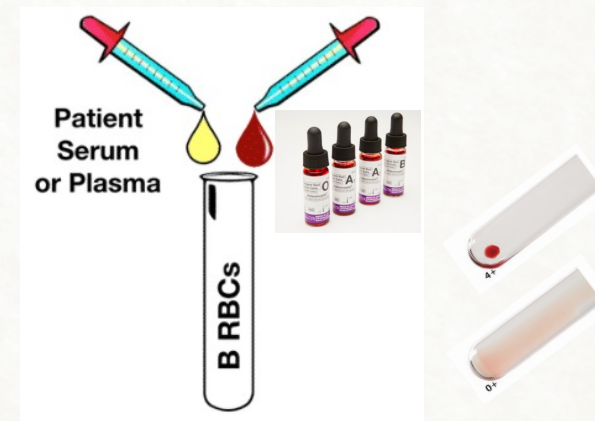
| Race or ethnicity     | Number    | Phenotype |      |      |     |
|-----------------------|-----------|-----------|------|------|-----|
|                       |           | O         | A    | B    | AB  |
| White non-Hispanic    | 2,215,623 | 45.2      | 39.7 | 10.9 | 4.1 |
| Hispanic†             | 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. FORWARD TYPING: Is A or B antigen on the surface of RBCs?



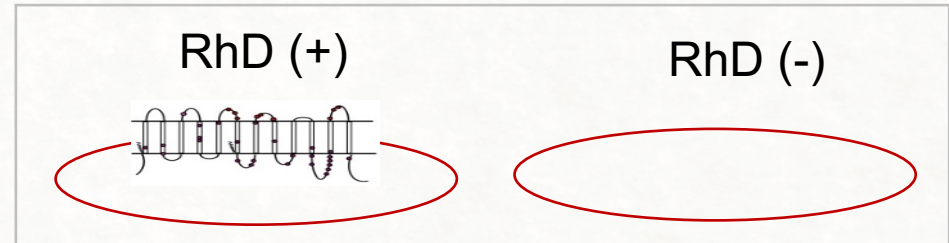
### 2. REVERSE TYPE: Is Anti-A or Anti-B present in the patient's plasma?



# RhD Typing

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



Is D antigen present on patient cells?

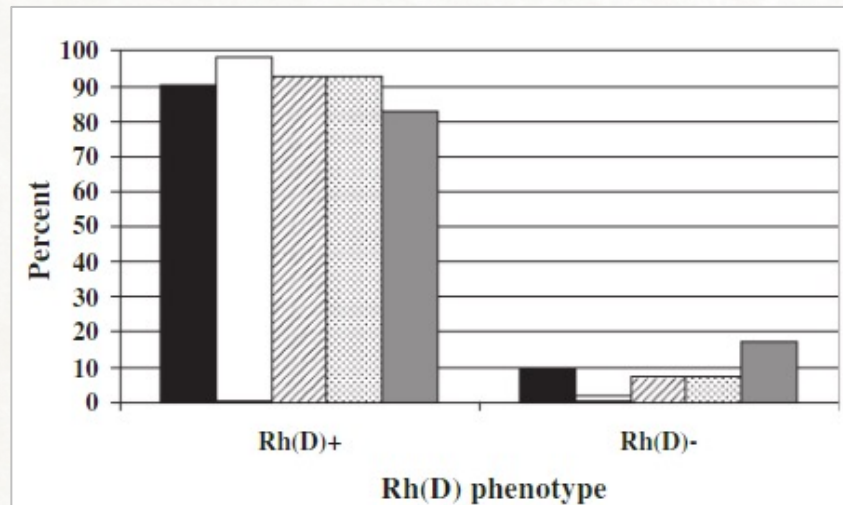
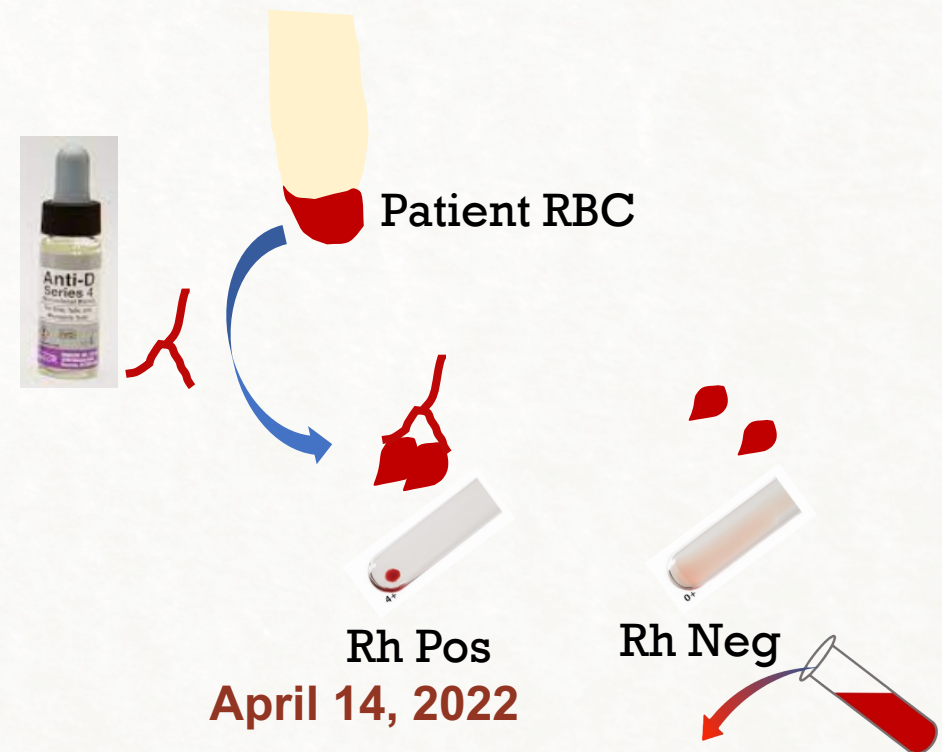


Fig. 2. Rh(D) phenotype by race/ethnicity. (■) North American Indian; (□) Asian; (▨) black non-Hispanic; (▩) Hispanic; (■) white non-Hispanic.

Garratty G et al. (2004) *Transfusion*



# ABO compatibility matching rules for blood products

## RBCs

- ABO matched/compatible with recipient plasma

## Granulocytes

- ABO matched/compatible with recipient plasma

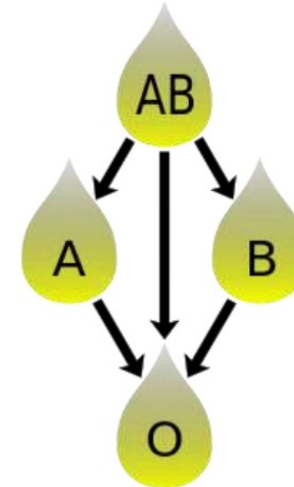
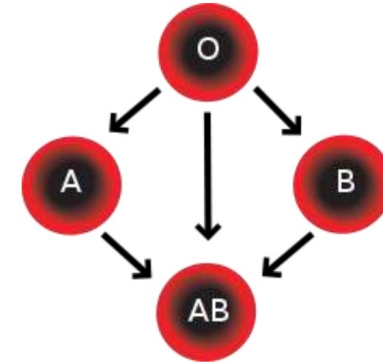
Per AABB Standards, if **> than 2 mL of RBCs** are present in any product, those RBCs must be **compatible with the recipient's plasma** antibodies.

## Plasma-Containing blood products:

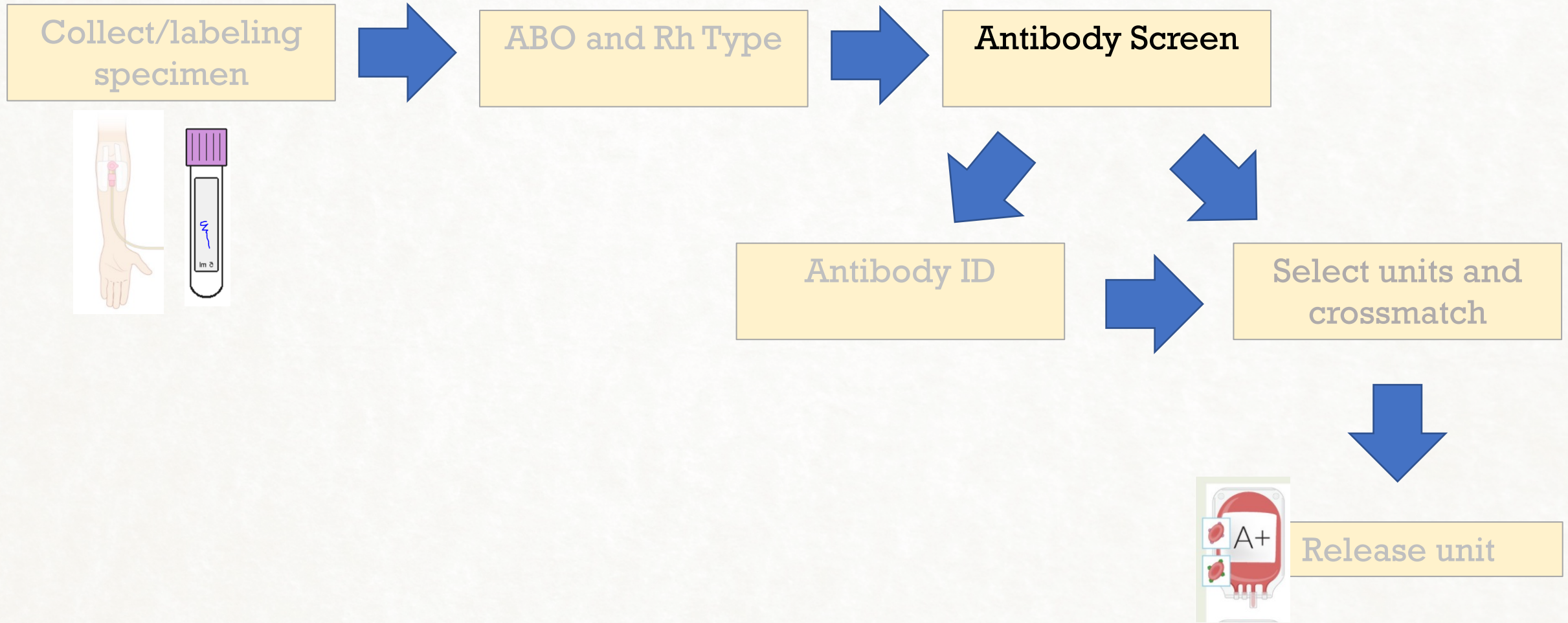
FFP – ABO matched/compatible with recipient RBCs

Platelets – Rh matched; typically, not ABO matched due to inventory, although preferably ABO-compatible

Cryo – not matched



# Serologic Testing Overview



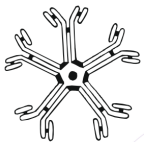


# RBC antibodies

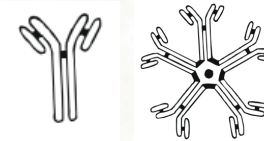
## RBC Antibodies

### “Expected antibodies”

Isohemagglutinins: Anti-A, Anti-B, Anti-A,B (“naturally occurring”)



### “unexpected antibodies”



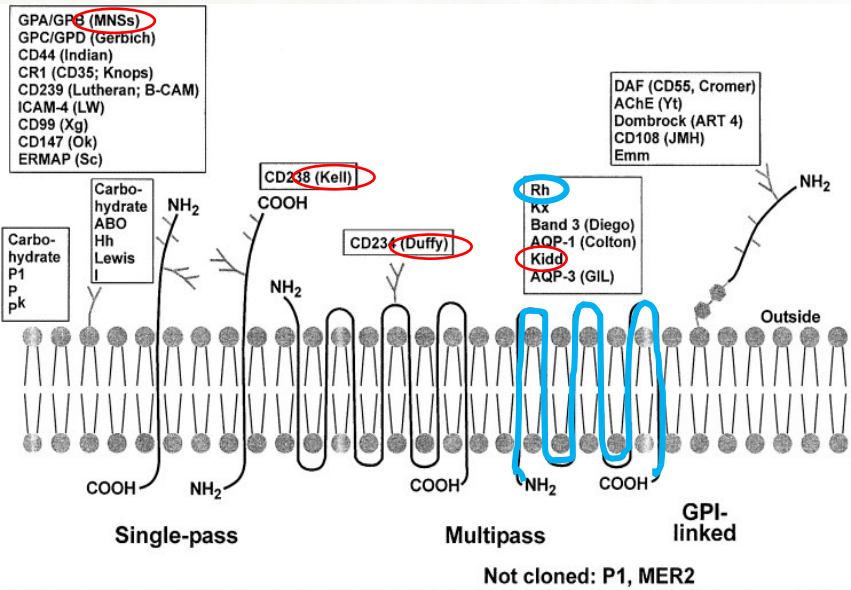
### Alloantibodies

- (react ONLY with reagent cells)
- pregnancy
- transfusion
- transplantation
- exposure

### Autoantibodies

(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



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



# II. Antibody screen

## Screening Cells:

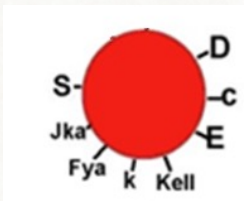
2 cells; reagent RBCs

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



06/06/2014 4173

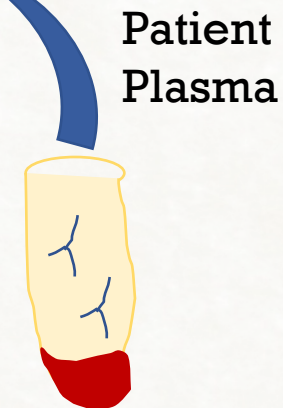
**CAPTURE-R READY-SCREEN (3)**  
Master List

Case Study  
Medical Center Echo

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              |                 |                 | Lutheran        | Xg              | Reactivity |                |   |   |   |   |                 |                 |                 |  |
|------|-------------------------|---------|---|---|---|---|------|----------------|---|---|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|------------|----------------|---|---|---|---|-----------------|-----------------|-----------------|--|
|      |                         | D       | C | c | E | e | V    | C <sup>a</sup> | K | k | Kp <sup>a</sup> | Kp <sup>b</sup> | Js <sup>a</sup> | Js <sup>b</sup> | Fy <sup>a</sup> | Fy <sup>b</sup> | Jk <sup>a</sup> | Jk <sup>b</sup> | Le <sup>a</sup> | Le <sup>b</sup> |            | P <sub>1</sub> | M | N | S | s | Lu <sup>a</sup> | Lu <sup>b</sup> | Xg <sup>a</sup> |  |
| 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               | +               | 0               |  |
| III  | rr H555                 | 0       | 0 | + | 0 | + | 0    | 0              | 0 | + | 0               | +               | 0               | +               | 0               | 0               | 0               | 0               | +               | +               | 0          | +              | 0 | + | 0 | 0 | +               | +               |                 |  |
|      | <b>Positive Control</b> |         |   |   |   |   |      |                |   |   |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |            |                |   |   |   |   |                 |                 |                 |  |

\* 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.

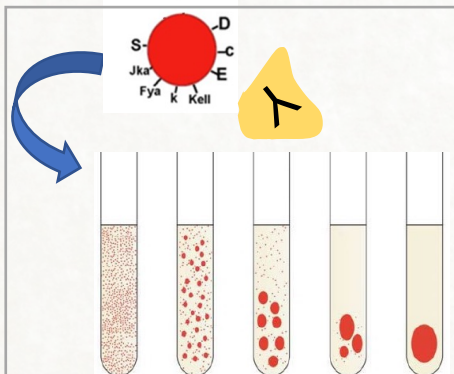


Reactivity



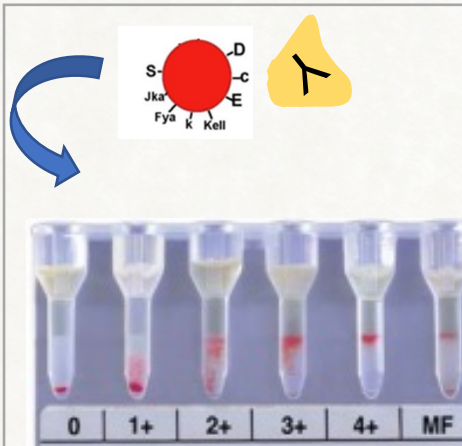
# Antibody detection methodology

## Tube Testing



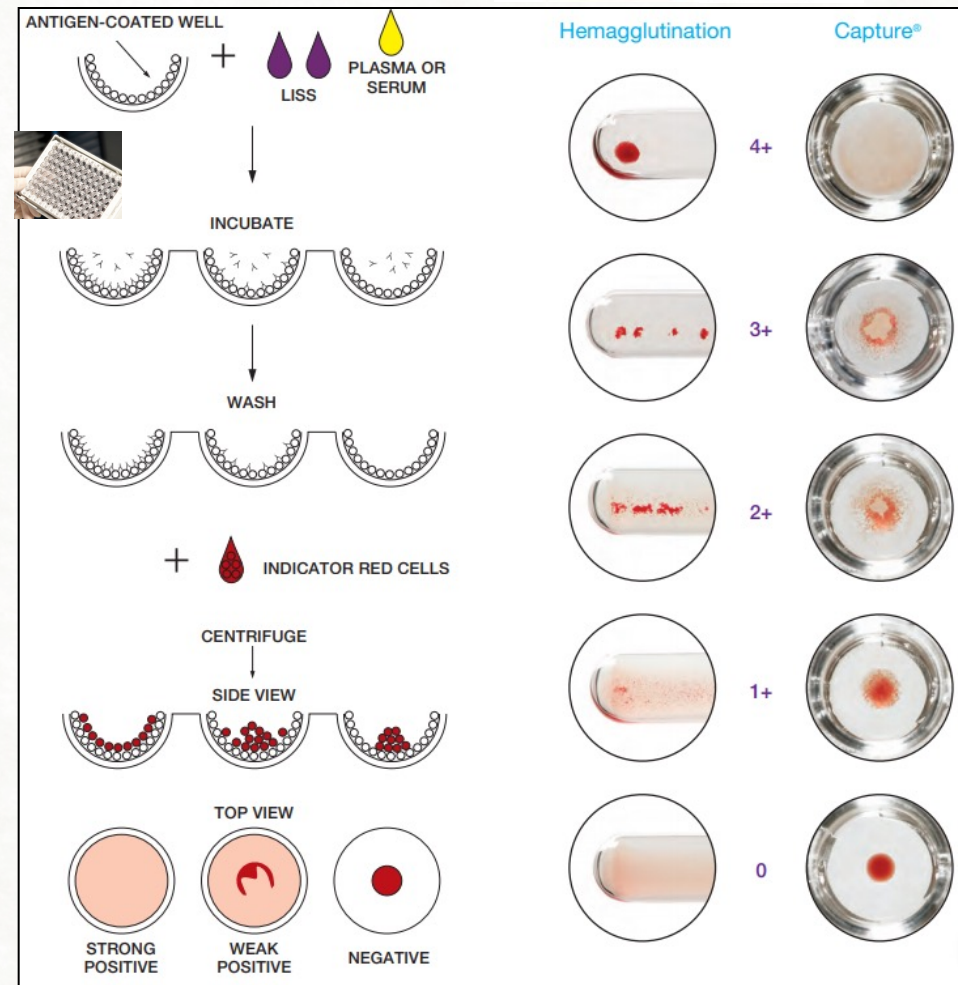
- Plasma and test cell interaction in tube
- Assess for agglutination

## Gel Testing



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

## Solid Phase

| Result | Hemagglutination             | Capture®            |
|--------|------------------------------|---------------------|
| 4+     | [Image: 4+ Hemagglutination] | [Image: 4+ Capture] |
| 3+     | [Image: 3+ Hemagglutination] | [Image: 3+ Capture] |
| 2+     | [Image: 2+ Hemagglutination] | [Image: 2+ Capture] |
| 1+     | [Image: 1+ Hemagglutination] | [Image: 1+ Capture] |
| 0      | [Image: 0 Hemagglutination]  | [Image: 0 Capture]  |

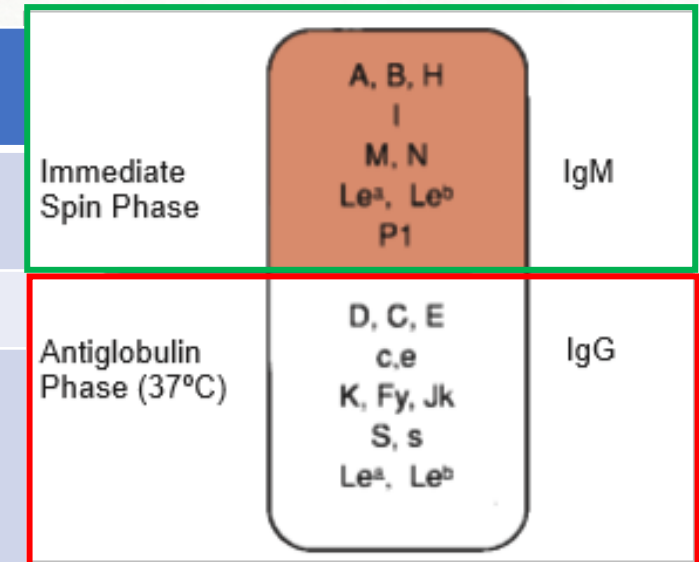


# Phases of Reaction

| Donor                         | Cell number | D | C | c | E | e | C <sup>w</sup> | K | k | Kp <sup>a</sup> | Kp <sup>b</sup> | Js <sup>a</sup> | Js <sup>b</sup> | Fy <sup>a</sup> | Fy <sup>b</sup> | Jk <sup>a</sup> | Jk <sup>b</sup> | Le <sup>a</sup> | Le <sup>b</sup> | P <sub>1</sub> | M | N | S | s | Lu <sup>a</sup> | Lu <sup>b</sup> | Xg <sup>a</sup> | IS | 37 | AHG | CC |
|-------------------------------|-------------|---|---|---|---|---|----------------|---|---|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|----------------|---|---|---|---|-----------------|-----------------|-----------------|----|----|-----|----|
| R <sub>1</sub> r              | 1           | + | + | + | 0 | + | 0              | 0 | + | 0               | +               | 0               | +               | +               | +               | +               | +               | 0               | +               | +              | + | + | + | + | 0               | +               | +               | 0  | 0  | 2+  |    |
| R <sub>1</sub> R <sub>1</sub> | 2           | + | + | 0 | 0 | + | +              | + | + | 0               | +               | 0               | +               | 0               | +               | 0               | 0               | 0               | 0               | +              | + | 0 | + | 0 | 0               | +               | +               | 0  | 0  | 0   | 3+ |
| R <sub>2</sub> R <sub>2</sub> | 3           | + | 0 | + | + | 0 | 0              | 0 | + | 0               | +               | 0               | +               | 0               | 0               | 0               | +               | +               | +               | 0              | + | 0 | + | 0 | +               | +               | 0               | 0  | 0  | 3+  |    |

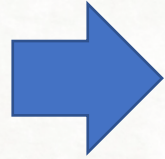


| Phase of Rxn   | Ab type detected | Mechanism   | Types of Abs Detected           |
|----------------|------------------|---|---------------------------------|
| IS             | IgM              | IgM react best at <i>lower</i> temp   | ABO, cold-reacting<br>Allo/Auto |
| 37 C           | IgG    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

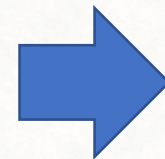


# Serologic Testing Overview

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 <sub>w</sub> | K    | k | Kp <sub>a</sub> | Kp <sub>b</sub> | Js <sub>a</sub> | Js <sub>b</sub> | Fy <sup>a</sup> | Fy <sup>b</sup> | Jk <sub>a</sub> | Jk <sub>b</sub> | Le <sup>a</sup> | Le <sup>b</sup> | P1  | M | N | S | s        | Lu <sup>a</sup> | Lu <sup>b</sup> | Xg <sub>a</sub> | IS | IAT |   |
| I   | R <sub>1</sub> R <sub>1</sub> | +  | + | 0 | 0 | + | 0 | 0 | 0              | 0    | + | 0               | +               | 0               | +               | +               | 0               | +               | 0               | +               | 0               | 0   | + | + | 0 | 0        | +               | 0               | +               | 0  | 0   | 0 |
| II  | R <sub>2</sub> R <sub>2</sub> | +  | 0 | + | + | 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  | 0   |   |

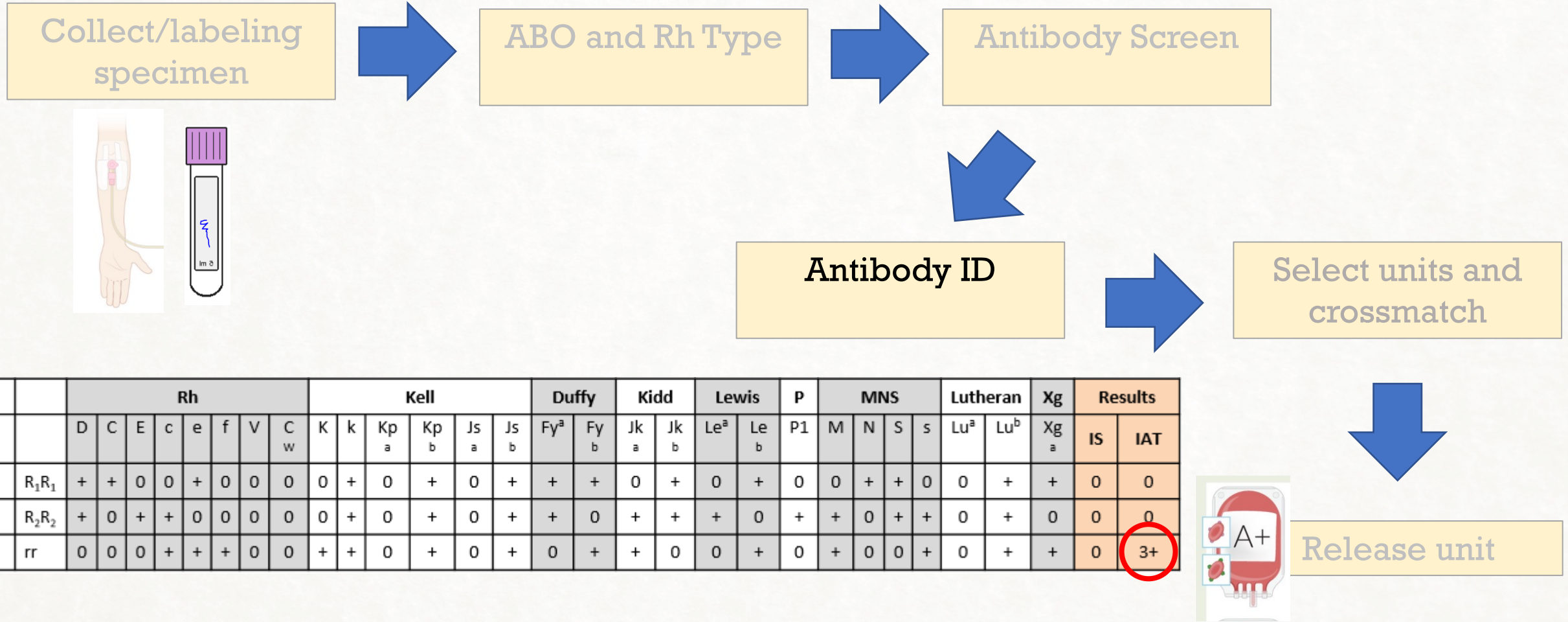
Select units and crossmatch



Release unit



# Serologic Testing Overview



# 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:*

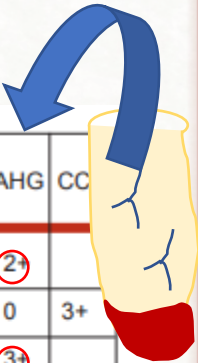
- Clinical significance varies even with antibodies of the same specificity
- Some antibodies are known to cause HDFN, while others may result in a (+) DAT in the fetus but no clinical HDFN
- Red Cell Antigen Expression can vary – reaction strength can vary and so reactivity of these antibodies can be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



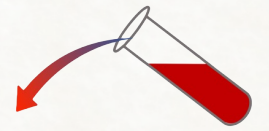
# Antibody Panel = extended antibody screen to determine AbID

- 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 | E | C | K | Kp <sup>a</sup> | Kp <sup>b</sup> | Js <sup>a</sup> | Js <sup>b</sup> | Fy <sup>a</sup> | Fy <sup>b</sup> | K <sup>a</sup> | K <sup>b</sup> | L <sup>s</sup> | L <sup>e</sup> | P <sup>1</sup> | M | N | S | s | Lu <sup>a</sup> | Lu <sup>b</sup> | Xg <sup>a</sup> | IS | 37 | AHG | CC |    |
|-------------------------------|---------------|---|---|---|---|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|----------------|----------------|----------------|----------------|----------------|---|---|---|---|-----------------|-----------------|-----------------|----|----|-----|----|----|
| R <sub>1</sub> r              | 1             | + | + | + | 0 | +               | 0               | 0               | +               | 0               | +               | 0              | +              | 0              | +              | +              | + | + | + | + | +               | 0               | +               | +  | 0  | 0   | 2+ |    |
| R <sub>1</sub> R <sub>1</sub> | 2             | / | / | 0 | 0 | /               | /               | /               | /               | 0               | /               | 0              | 0              | 0              | 0              | /              | / | 0 | / | 0 | 0               | +               | +               | 0  | 0  | 0   | 0  | 3+ |
| R <sub>2</sub> R <sub>2</sub> | 3             | + | 0 | + | + | 0               | 0               | +               | 0               | +               | 0               | +              | 0              | +              | +              | 0              | + | 0 | + | 0 | +               | +               | +               | 0  | 0  | 3+  |    |    |
| R <sub>0</sub> r              | 4             | + | 0 | + | 0 | +               | 0               | 0               | +               | 0               | +               | 0              | +              | 0              | 0              | 0              | + | 0 | + | 0 | +               | 0               | +               | 0  | 0  | 3+  |    |    |
| r <sub>1</sub> r              | 5             | 0 | + | + | 0 | +               | 0               | 0               | +               | 0               | +               | 0              | /              | 0              | /              | +              | + | 0 | + | + | 0               | +               | 0               | +  | 0  | 0   | 3+ |    |
| r <sup>o</sup> r              | 6             | 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  | 2+  |    |    |
| rr                            | 8             | 0 | 0 | / | 0 | +               | 0               | 0               | +               | 0               | +               | 0              | /              | 0              | +              | +              | + | + | 0 | + | +               | 0               | +               | 0  | 0  | 0   | 3+ |    |
| r <sup>o</sup> r              | 9             | 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  | 3+  |    |    |
| R <sub>1</sub> r              | 11            | + | + | + | 0 | +               | 0               | 0               | +               | 0               | +               | 0              | +              | 0              | +              | +              | + | + | + | + | 0               | +               | +               | 0  | 0  | 2+  |    |    |
|                               | Patient Cells |   |   |   |   |                 |                 |                 |                 |                 |                 |                |                |                |                |                |   |   |   |   |                 |                 |                 | 0  | 0  | 0   | 3+ |    |



Harmening, DM. Modern Blood Banking and Transfusion Practices 7<sup>th</sup> Ed.





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

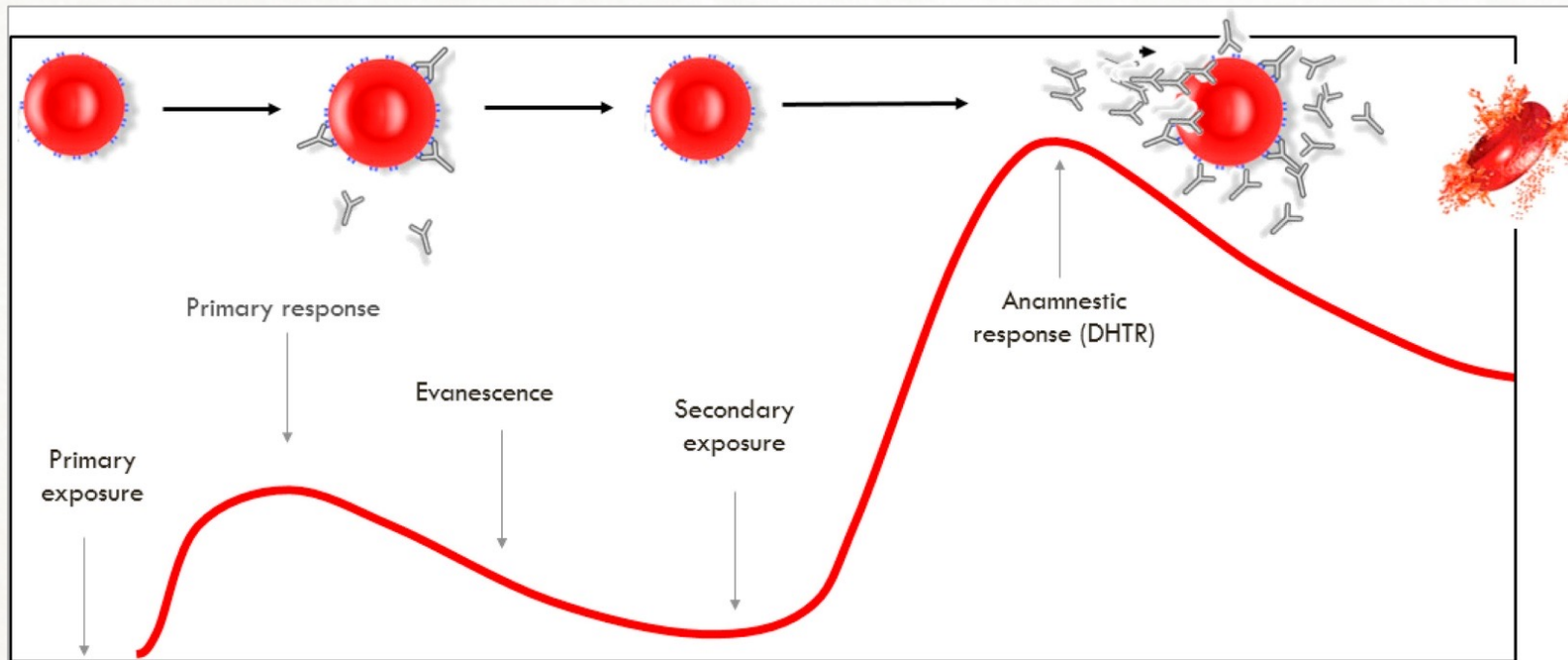
(+) Ab ID  $\neq$  clinically significant hemolysis

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

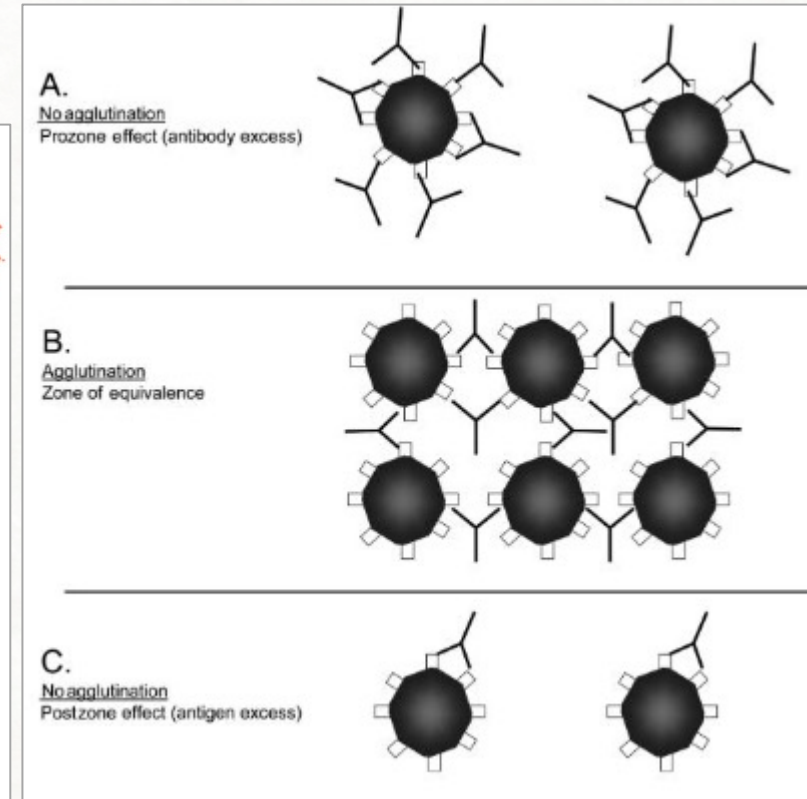


# Historical Antibodies

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



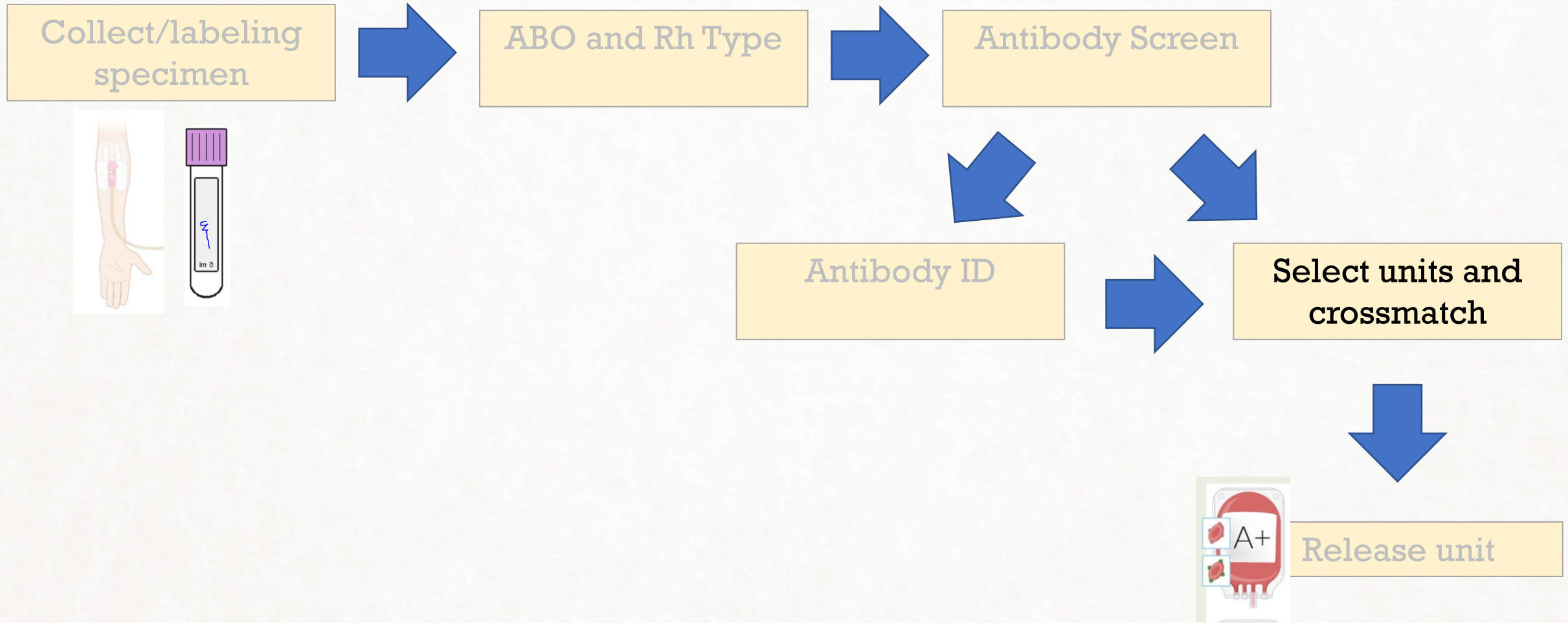
Fasano RM et al (2019) *Transfusion Clinique et Biologique*



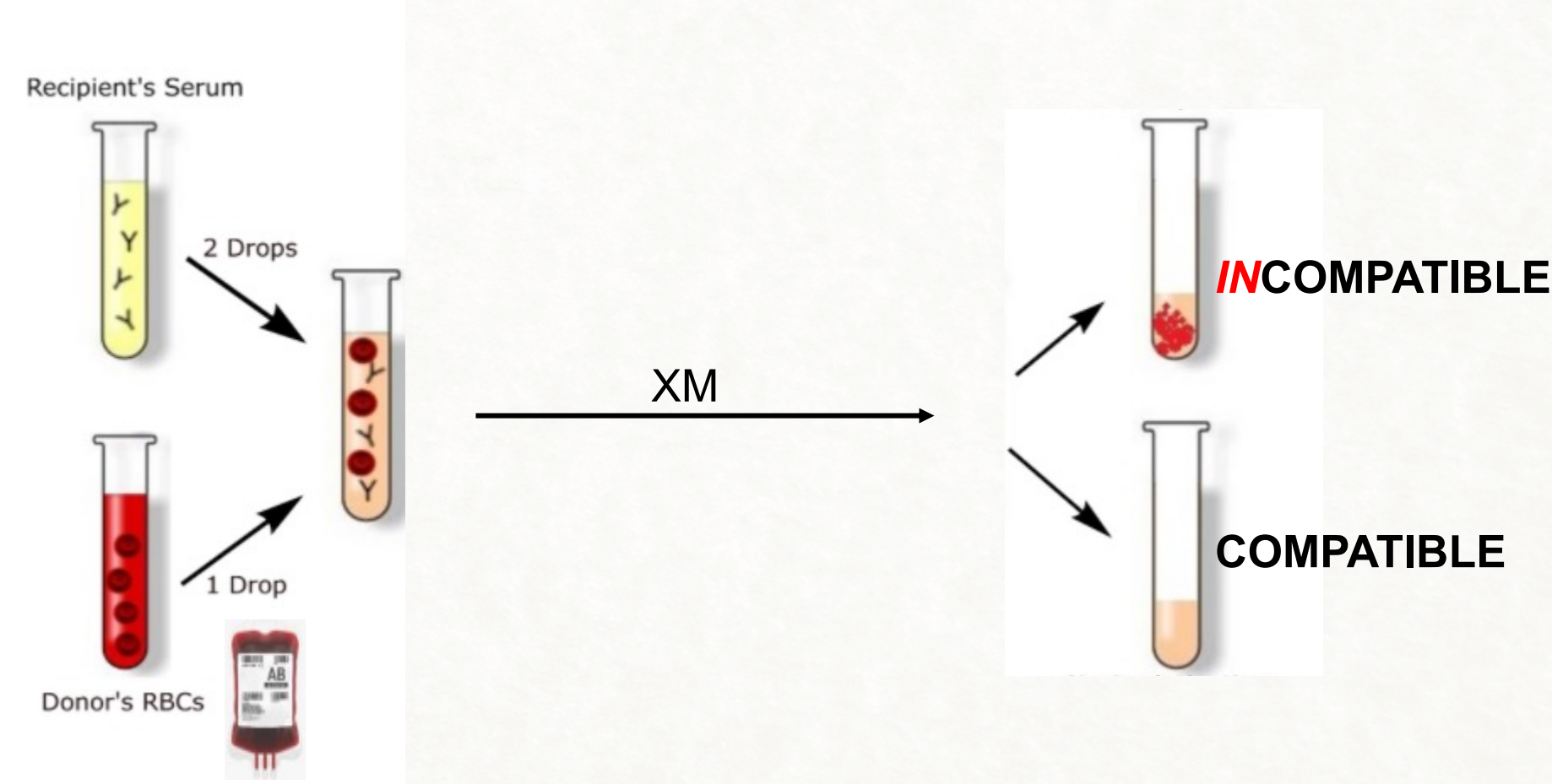
AABB Technical Manual 20<sup>th</sup> Ed



# Serologic Testing Overview



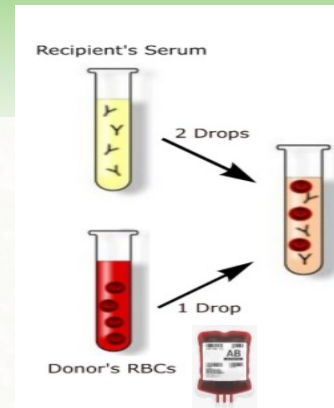
# IV. Crossmatch



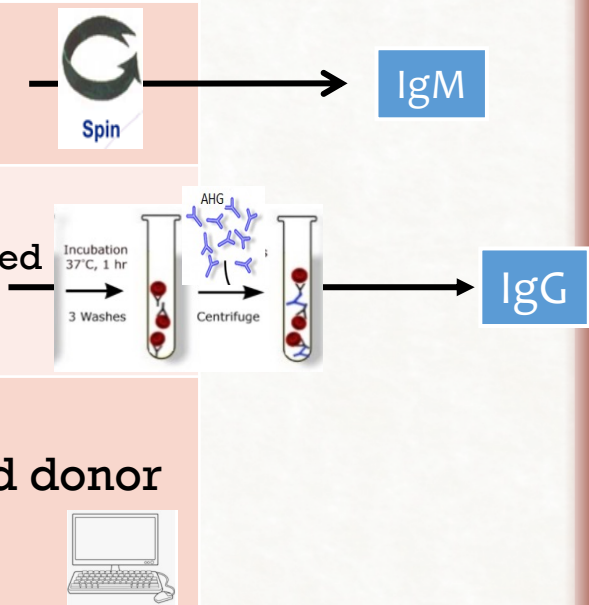
Modified from <https://laboratorytests.org/cross-matching/>



# 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 <ul style="list-style-type: none"> <li>• Agglutination seen at IS</li> <li>• Detects ABO incompatibility</li> </ul>      |
| IAT (“Full XM” or “Coombs crossmatch”) | (+) Ab screen<br>History of (+) screen   | Pt plasma + Donor RBC <ul style="list-style-type: none"> <li>• Incubate at 37 C, wash, AHG used (“bridging”)</li> <li>• Detects IgG</li> </ul> |
| 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 <ul style="list-style-type: none"> <li>• <i>No physical testing</i></li> </ul>          |

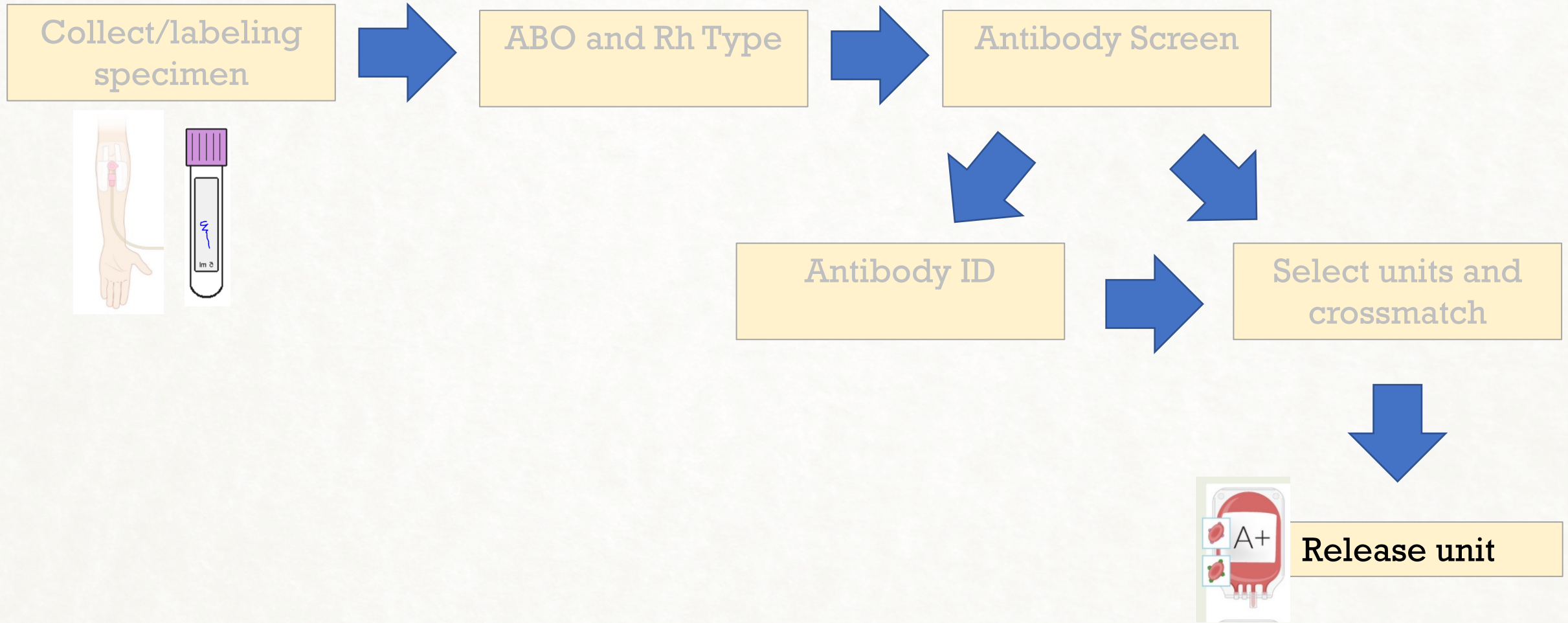


# Timeline for RBC availability

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



# Serologic Testing Overview



# Pan-reactive antibody panel

| Donor | Cell number   | D | C | c | E | e | C <sup>w</sup> | K | k | Kp <sup>a</sup> | Kp <sup>b</sup> | Js <sup>a</sup> | Js <sup>b</sup> | Fy <sup>a</sup> | Fy <sup>b</sup> | Jk <sup>a</sup> | Jk <sup>b</sup> | Le <sup>a</sup> | Le <sup>b</sup> | P <sub>1</sub> | M | N | S | s | Lu <sup>a</sup> | Lu <sup>b</sup> | Xg <sup>a</sup> | 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   | 11            | + | 0 | + | 0 | + | 0              | 0 | + | 0               | +               | 0               | +               | +               | +               | 0               | +               | 0               | +               | +              | 0 | + | 0 | + | 0               | +               | +               | 2+       |
|       | Patient Cells |   |   |   |   |   |                |   |   |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                |   |   |   |   |                 |                 |                 | 2+       |

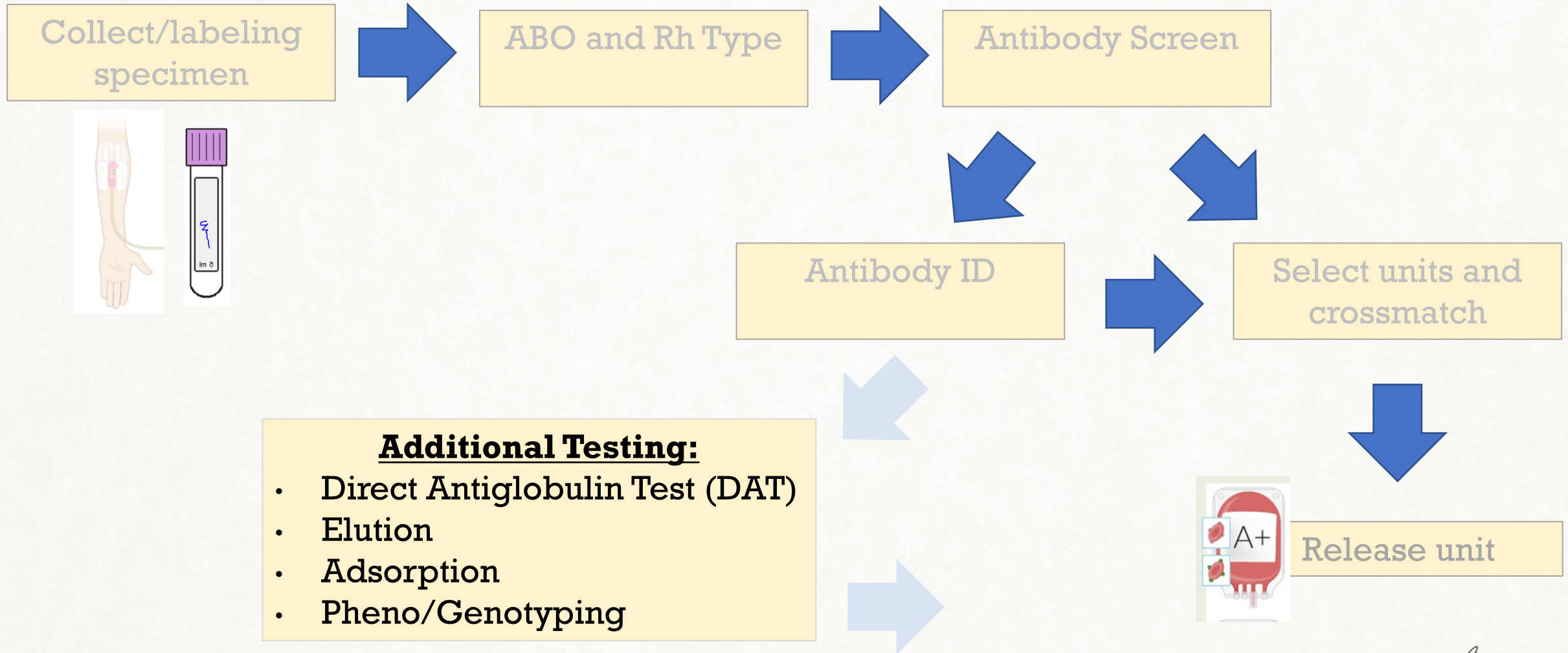
- Alloantibody to High Frequency Antigen
- **Autoantibody**
- Multiple Alloantibodies
- Daratumumab (anti-CD38)

Harmening, DM. Modern Blood Banking and Transfusion Practices 7<sup>th</sup> Ed.

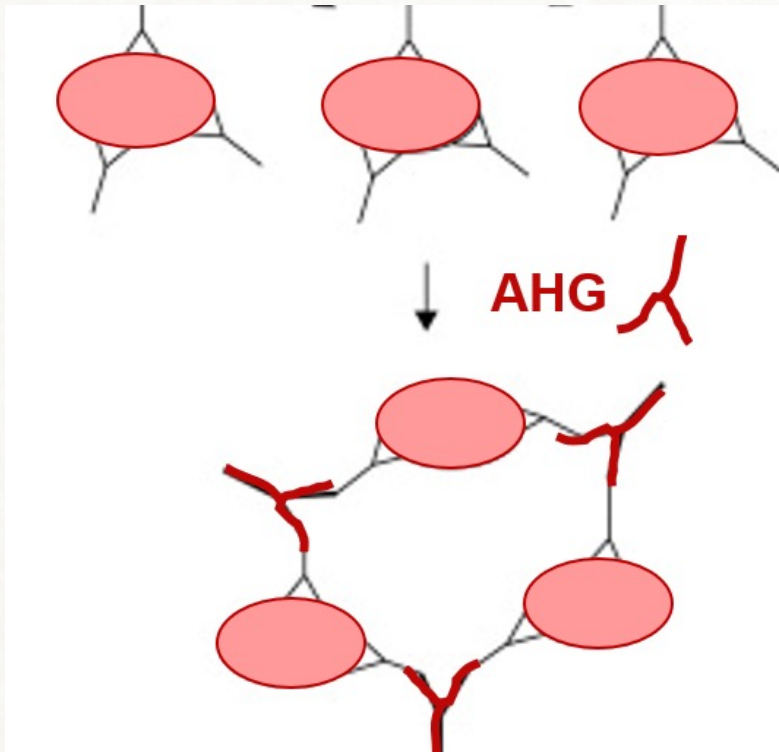




# Additional techniques in the toolbox



# Positive autocontrol: autoantibody?



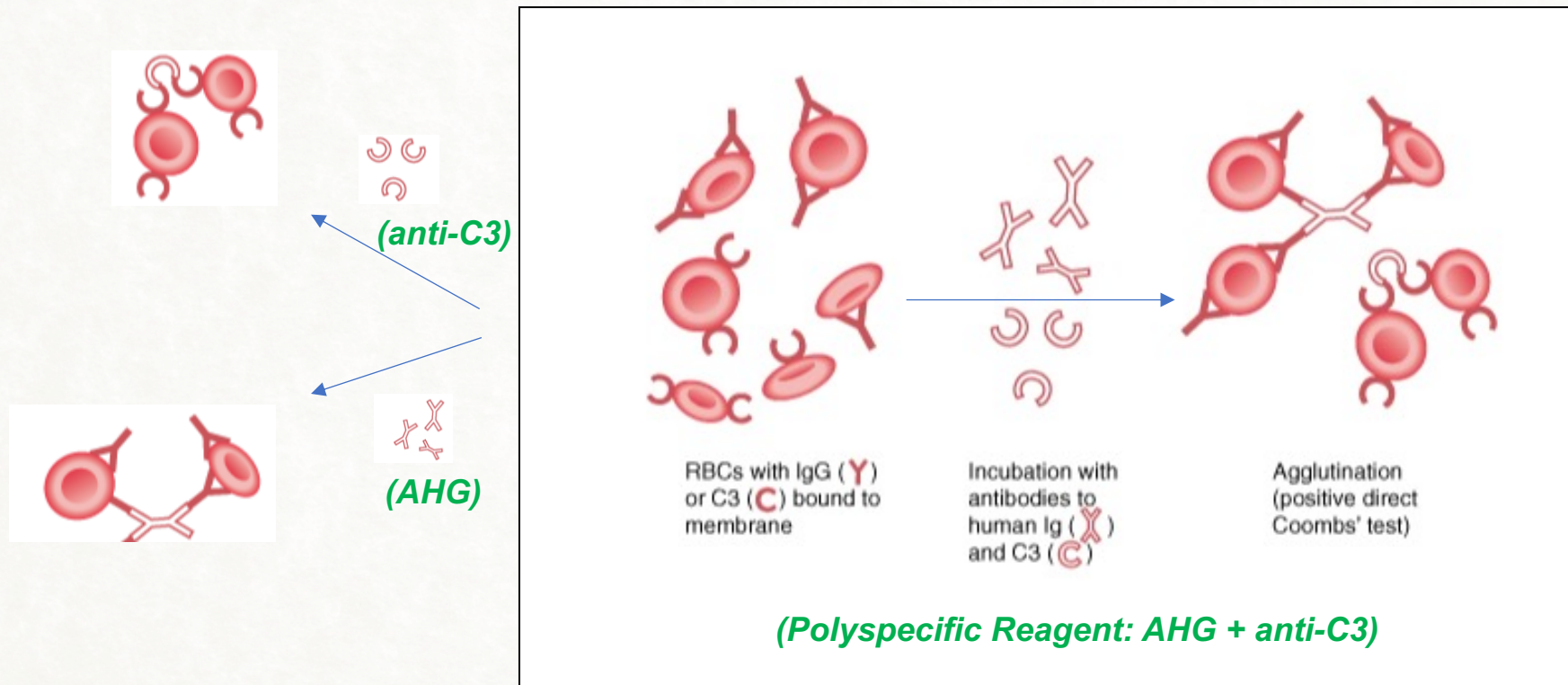
| Donor         | Cell number | D | C | c | E | e | C <sup>w</sup> | K | k | Kp <sup>a</sup> | Kp <sup>b</sup> | Js <sup>a</sup> | Js <sup>b</sup> | Fy <sup>a</sup> | Fy <sup>b</sup> | Jk <sup>a</sup> | Jk <sup>b</sup> | Le <sup>a</sup> | Le <sup>b</sup> | P <sub>1</sub> | M | N | S | s | Lu <sup>a</sup> | Lu <sup>b</sup> | Xg <sup>a</sup> | 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           | 11          | + | 0 | + | 0 | + | 0              | 0 | + | 0               | +               | 0               | +               | +               | +               | 0               | +               | 0               | +               | +              | 0 | + | 0 | + | 0               | +               | +               | 2+      |
| Patient Cells |             |   |   |   |   |   |                |   |   |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                |   |   |   |   |                 |                 |                 | 2+      |



# DAT: what is coating the RBCs?

*In-Vivo* antibody coating

Positive DAT = IgG or Complement (C3) is present on the patient's RBCs



IgG reactivity often correlating with a warm-reactive antibody/extravascular hemolysis

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



# (+) DAT

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

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

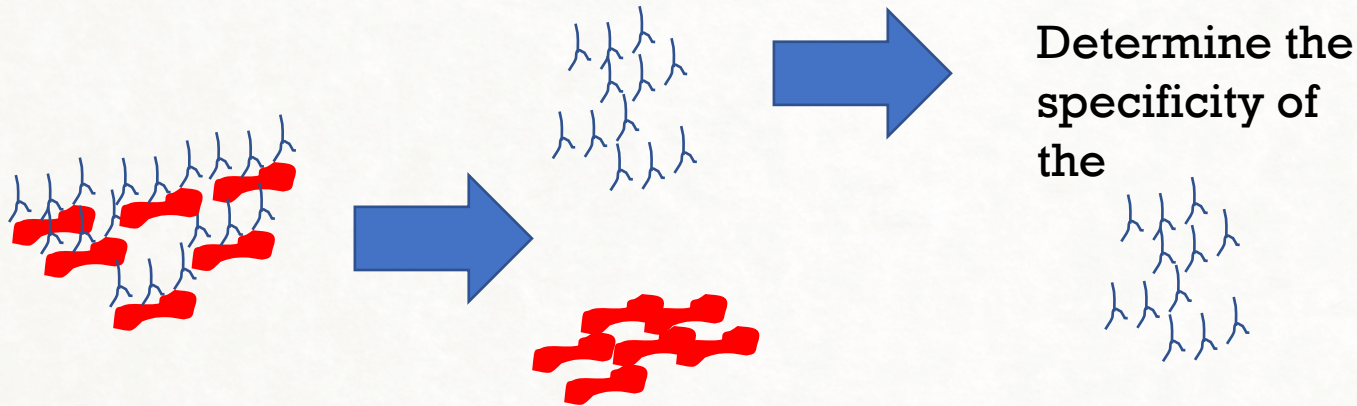
## Other causes of a + DAT

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



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

Run on test

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

*Note: 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

|                            | <b>WAIHA</b>                                      | <b>CAS</b>   | <b>Mixed-type AIHA</b>   | <b>PCH</b>  |
|----------------------------|---|--|--|---|
| <b>DAT</b><br>(routine)    | IgG<br>IgG + C3<br>C3                             | C3 only  | IgG + C3<br>C3   | C3 only   |
| <b>Immunoglobulin type</b> | IgG   | IgM  | IgG, IgM   | IgG   |
| <b>Eluate</b>              | IgG antibody                                      | Nonreactive  | IgG antibody   | Nonreactive   |
| <b>Serum</b>               | IAT; 35% agglutinate untreated red cells at 20 C  | IgM agglutinating antibody; titer $\geq 1000$ (60%) at 4 C; react at $\geq 30$ C | IgG IAT-reactive antibody plus IgM agglutinating antibody react at $\geq 30$ C | Routine IAT negative; IgG biphasic hemolysin in Donath-Landsteiner test |
| <b>Specificity</b>         | 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.

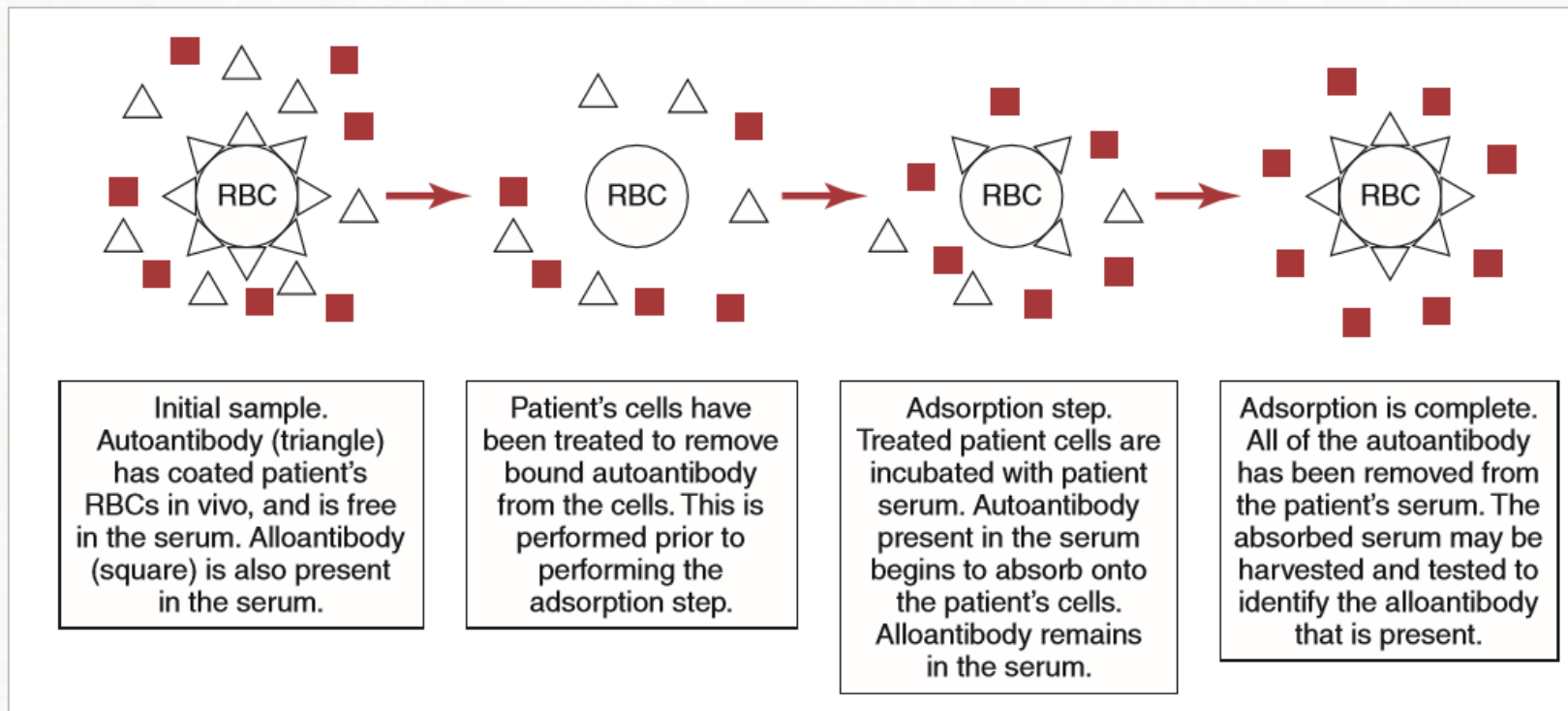
AABB Technical Manual 18th Edition



# Adsorptions: removing *auto*-antibodies to detect underlying *allo*-antibodies

## Autoadsorption

- uses patient's own RBCs to adsorb out the autoantibody in the serum
- 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 <sup>w</sup> | K | k | Kp <sup>a</sup> | Kp <sup>b</sup> | Js <sup>a</sup> | Js <sup>b</sup> | Fy <sup>a</sup> | Fy <sup>b</sup> | Jk <sup>a</sup> | Jk <sup>b</sup> | Le <sup>a</sup> | Le <sup>b</sup> | P <sub>1</sub> | M | N | S | s | Lu <sup>a</sup> | Lu <sup>b</sup> | Xg <sup>a</sup> | 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+ |
| r̄r   | 5             | 0 | + | + | 0 | + | 0              | 0 | + | 0               | +               | 0               | +               | +               | 0               | +               | 0               | 0               | 0               | 0              | 0 | + | 0 | + | 0               | +               | 0               | 2+      |    | 0              | 3+ |
| r̄r   | 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   | 11            | + | 0 | + | 0 | + | 0              | 0 | + | 0               | +               | 0               | +               | +               | +               | 0               | +               | 0               | +               | +              | 0 | + | 0 | + | 0               | +               | +               | 2+      |    | 0              | 3+ |
|       | Patient Cells |   |   |   |   |   |                |   |   |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                |   |   |   |   |                 |                 |                 | 2+      |    |                |    |

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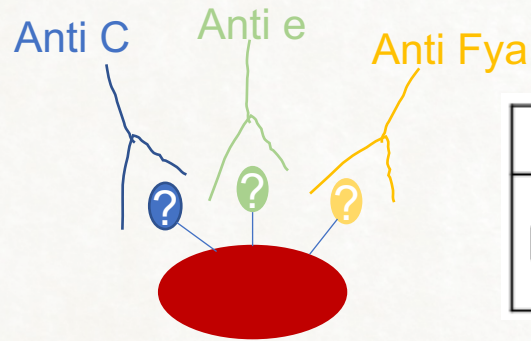


# Phenotyping patient RBCs

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 <sup>a</sup> | Fy <sup>b</sup> | Jk <sup>a</sup> | Jk <sup>b</sup> | 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

