

Fundamentals of pre-transfusion testing



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Disclosures

- No relevant disclosures



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



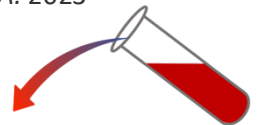
Blood Transfusion

- One of the most common medical procedures
- Can be lifesaving

In the US, annually transfused:

- ~ 11 million units of RBCs
- ~ 2.5 million units of platelets
- ~ 2.2 million units of plasma
- ~ 1.2 million units of cryoprecipitate

Cohn CS, Shaz BH. Blood and Its Components. *JAMA*. 2023



Why Blood Components?

- whole blood unit collected → transfusion to more than 1 individual
- targets patients' specific needs
- facilitates optimal storage of each blood component



Blood Components



TRANSFUSION TYPES

	VOLUME AND COMPOSITION	SHELF LIFE	TYPICAL INCREASE	COMPATIBILITY
Whole blood	Total volume: 400-550 mL Composition: Red blood cells (RBCs), plasma, and platelets	With citrate-phosphate-dextrose (CPD): 21 d With citrate-phosphate-dextrose-adenine (CPDA-1): 35 d	Equivalent to transfusion of 1 unit of RBCs plus 1 unit of plasma	Use group O with low titers of anti-A and anti-B antibodies
Plasma	Total volume: 200-250 mL Coagulation factors vary based on ABO group, storage conditions, processing, and product	Frozen: 1 y Plasma outdates 24 h after thawing, but may be relabeled as thawed plasma with 5 d of additional storage at 1-6 °C	10-20 mL/kg Increases factor levels by ≈30% Expected international normalized ratio change: -0.07 per unit	ABO compatibility required Rh compatibility not required
Cryoprecipitate	Total volume: 10-15 mL/unit (5 units usually pooled together) Factor VIII: 80-120 units Fibrinogen: 250-400 mg (1.3-2.0 g per 5-unit pool)	Frozen: 1 y Thawed/pooled in an open (nonsterile) system: 4 h Thawed/pooled in a closed (sterile) system: 6 h	Fibrinogen increase: ≈7 mg/dL/unit Expected fibrinogen increments 5-Unit pool: 40-50 mg/dL 10-Unit pool: 80-100 mg/dL	ABO and Rh compatibility not required
Platelets	Total volume: 200-300 mL per whole blood-derived platelet component or 200-400 mL per apheresis unit Composition: Platelets suspended in plasma or platelet additive solution	Room temperature: 5 or 7 d depending on bacterial mitigation measures taken Pooled in an open (nonsterile) system: 4 h Cold-stored platelets: 14 d	24 000-45 000/μL After 1 platelet dose (1 apheresis unit or 4-6 whole blood-derived platelet concentrates) Expected increment decreases ≤33% with pathogen-reduced platelets	ABO and Rh compatibility not required
Red blood cells	Total volume: 300-350 mL RBCs: 200 mL Plasma: 30-40 mL Anticoagulant/additive solution: 100-110 mL	With CPD: 21 d With CPDA-1: 35 d With additive solution: 42 d	Increase after transfusion of 1 unit Hemoglobin: ≈1 g/dL Hematocrit: ≈3%	ABO and Rh compatibility required

Cohn CS, Shaz BH. Blood and Its Components. JAMA. 2023



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

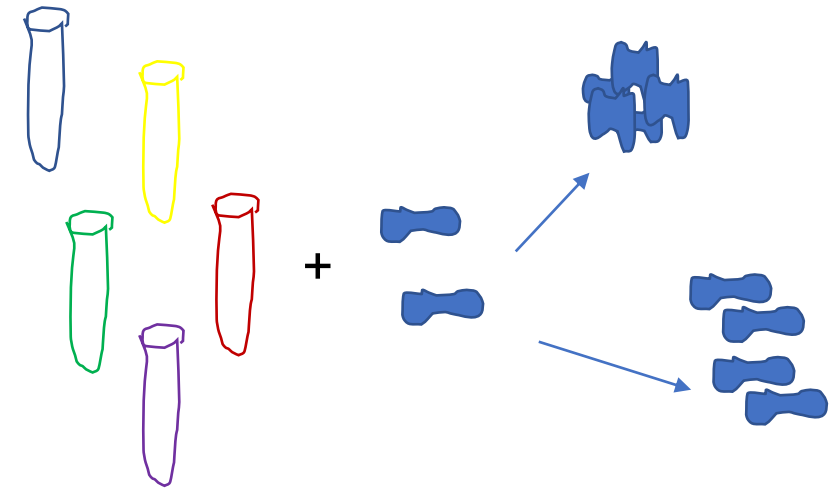
1901: Landsteiner’s Experiment

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

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

Blutkörperchen von:	Trautm.	Linsm.	Seil.	Freib.	Graupn.	Mittelb.
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Schwarz et al. (2003) *British Journal of Haematology*



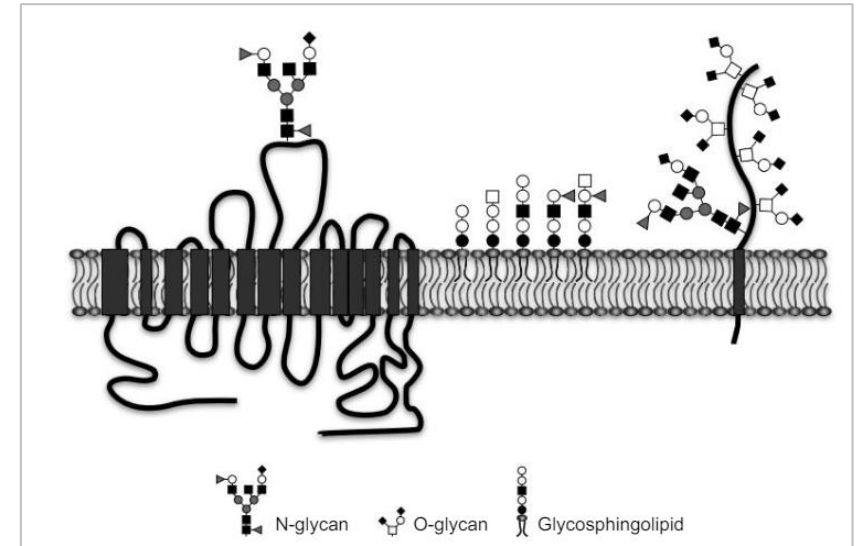
- Recognized a pattern of agglutination
- Blood can be divided into “groups”
- Marked the discovery of the ABO blood group system



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

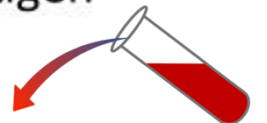
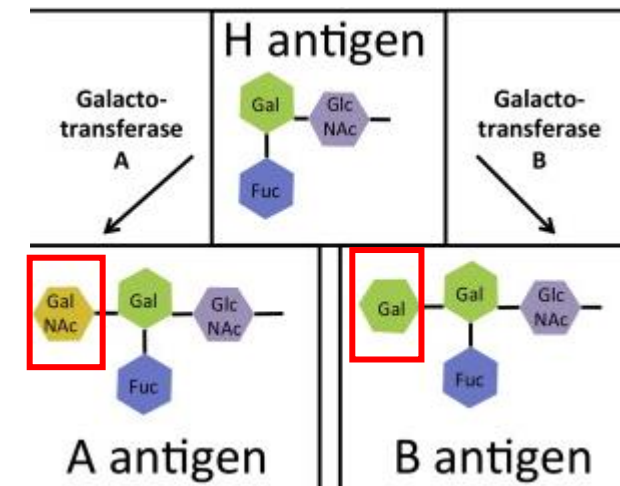


AABB Technical Manual 20th Ed

The ABO gene locus encodes glycosyltransferases

- 3 alleles/6 genotypes/4 phenotypes

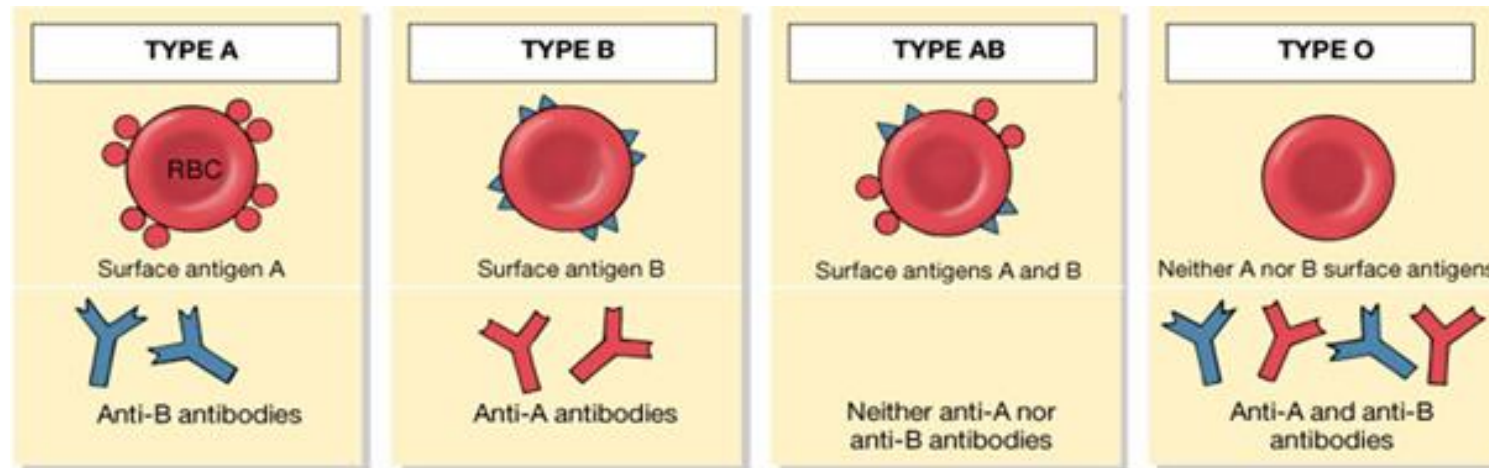
Physiological functions remain unknown



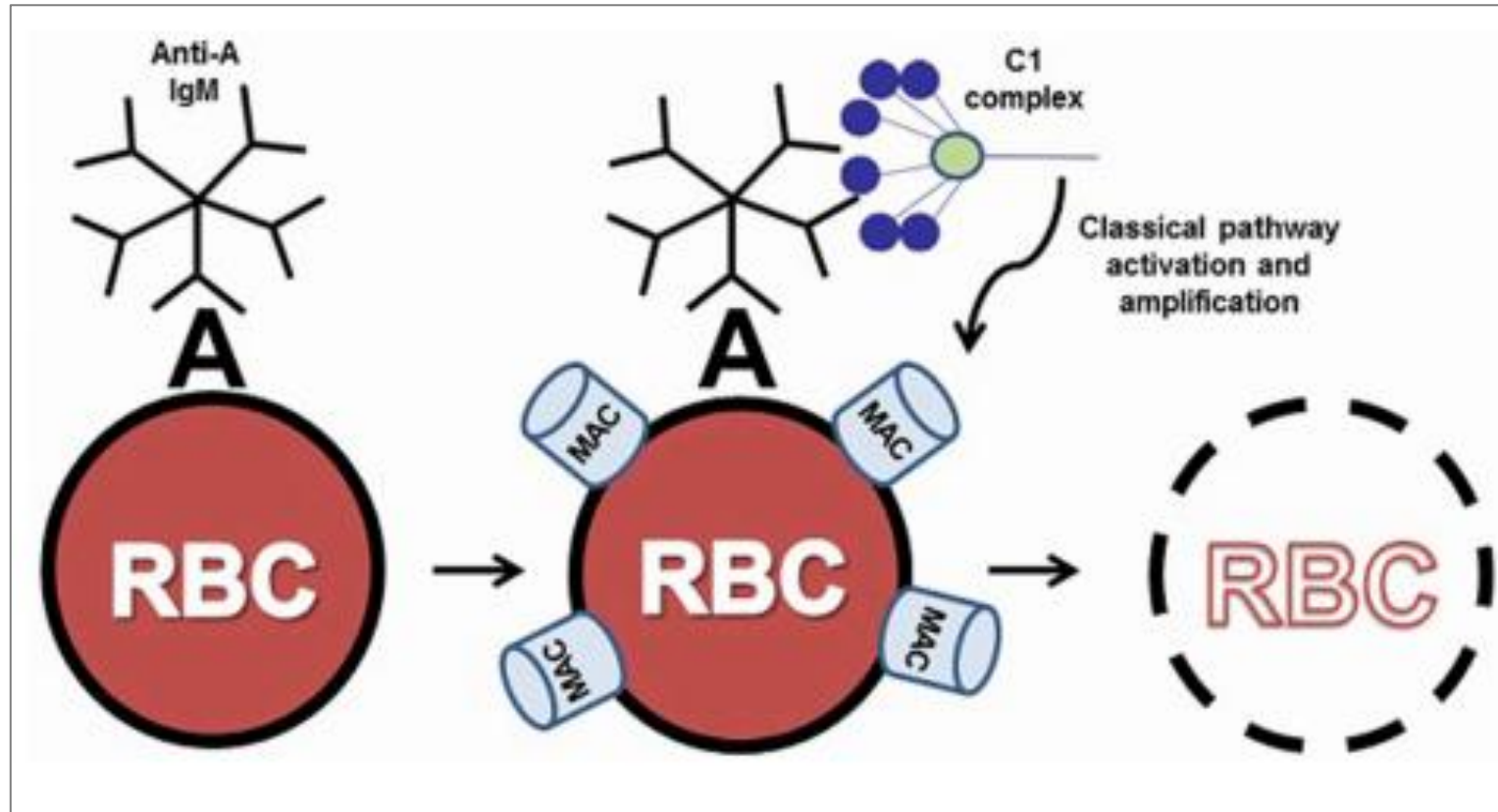
Antibodies to ABO antigens

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

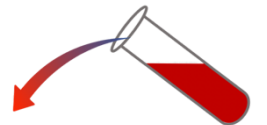
- “Naturally occurring”
- NOT in response to foreign RBC exposure (transfusion, pregnancy, etc.)
- exposure response to substances in the environment that resemble non-self RBC antigens
- Formed during the first years of life
- IgM (except anti-A,B, IgG); titers vary



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



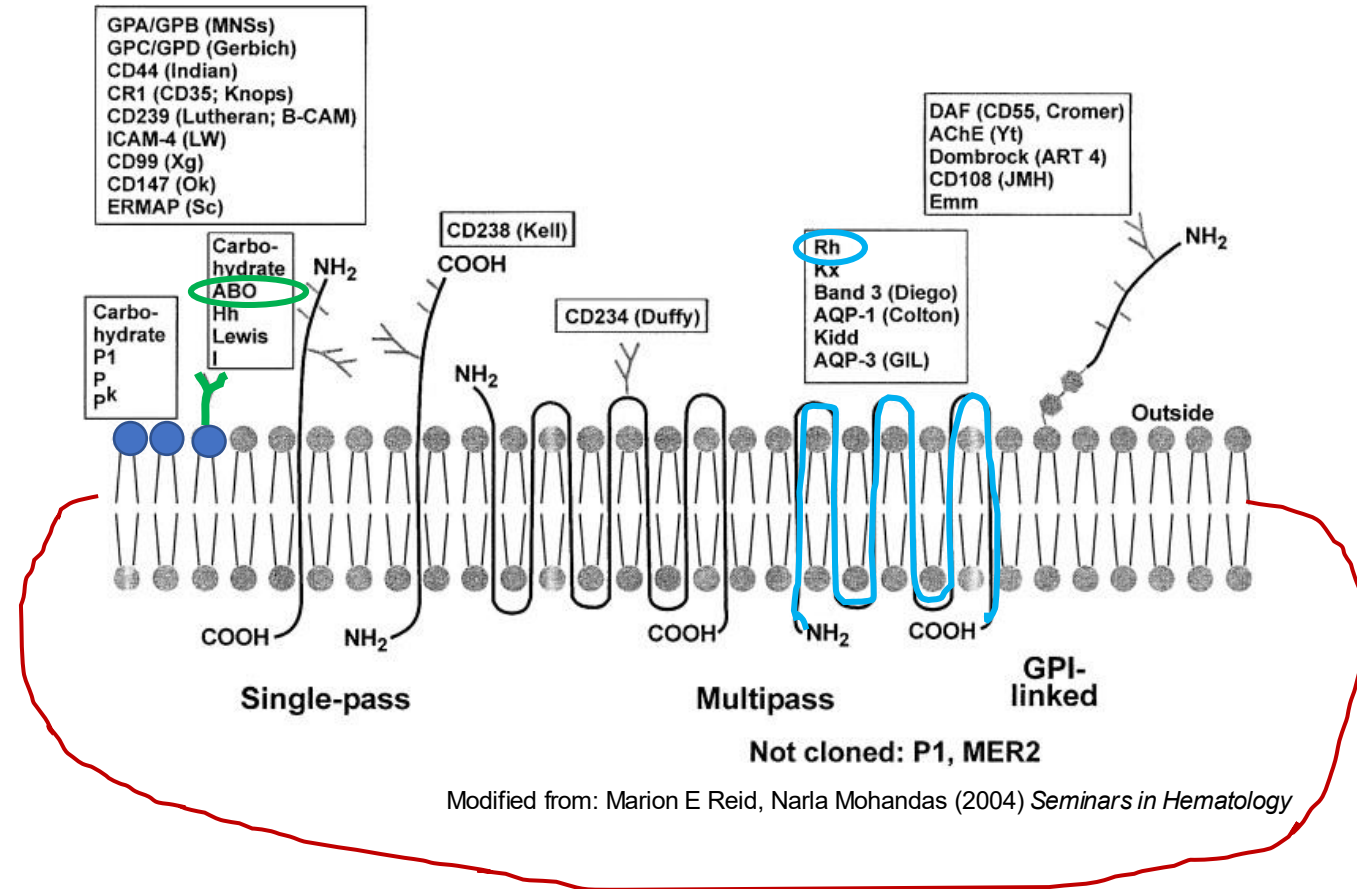
Sharp JA et al (2014) *Frontiers in Immunology*



Other Blood Group Antigen

Antigens:

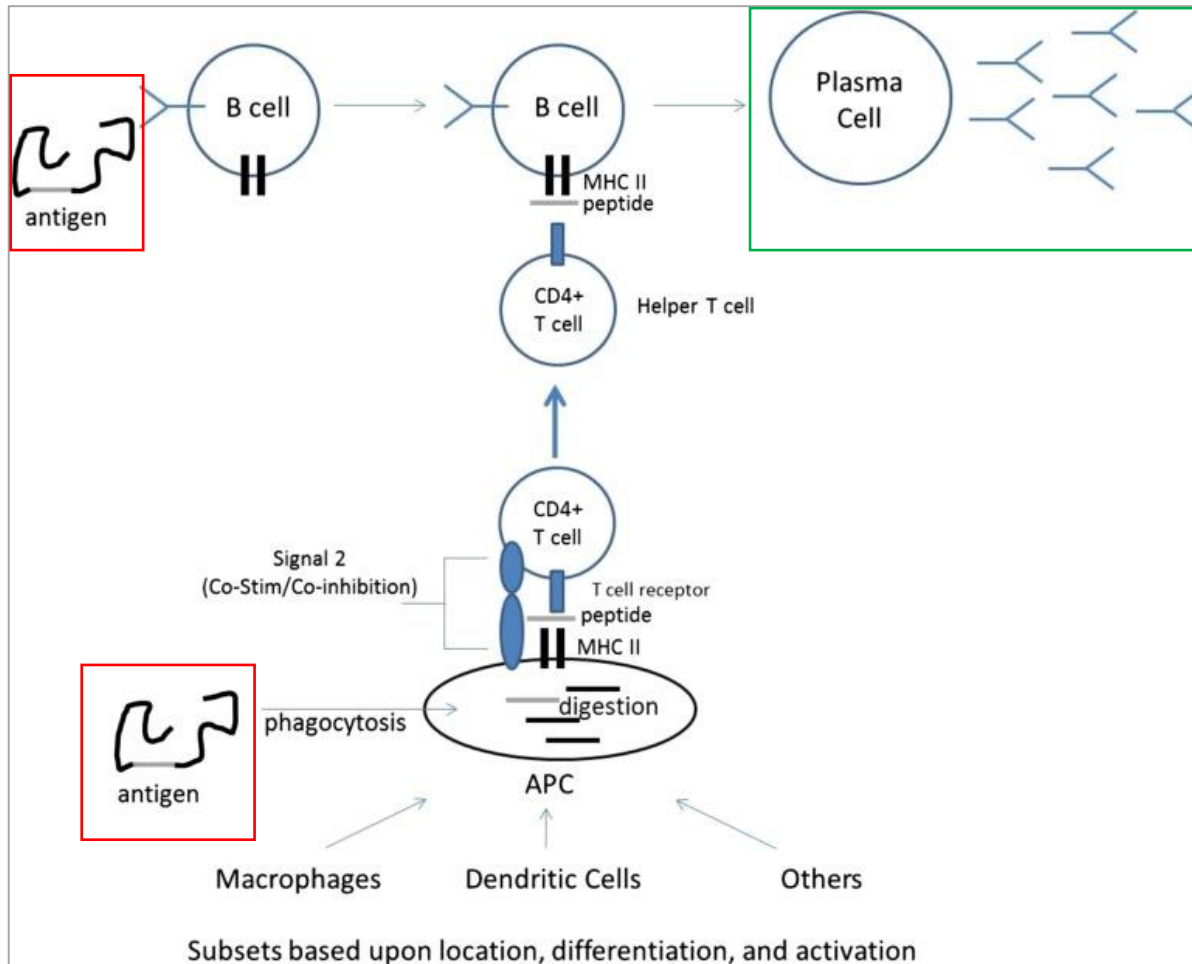
- >300 antigens (36 blood group systems) are known
- Polymorphic, inherited, carbohydrate or protein structures located on the extracellular surface of the RBC membrane.



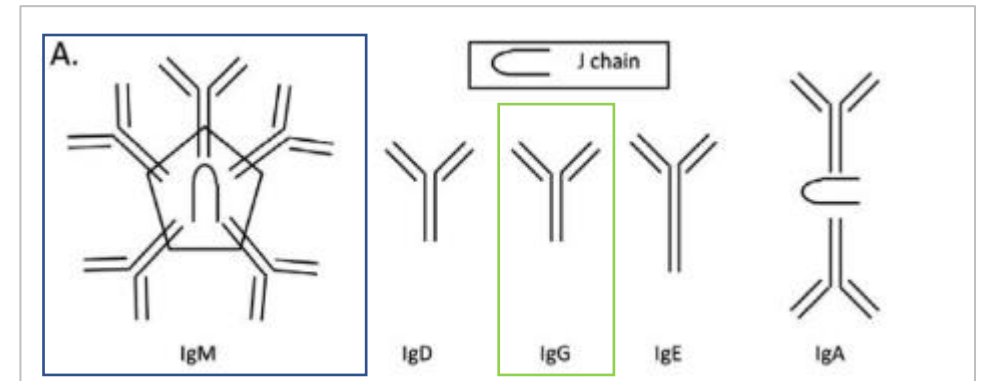
Modified from: Marion E Reid, Narla Mohandas (2004) *Seminars in Hematology*



Alloantibodies (“unexpected” antibodies)

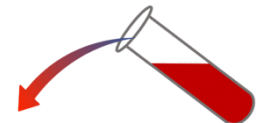


Zimring JC, Hudson KE (2016) *Hematology Am Soc Hematol Educ Program*.



AABB Technical Manual 20th Ed

- 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 (receiving multiple transfusions)
- ❑ 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

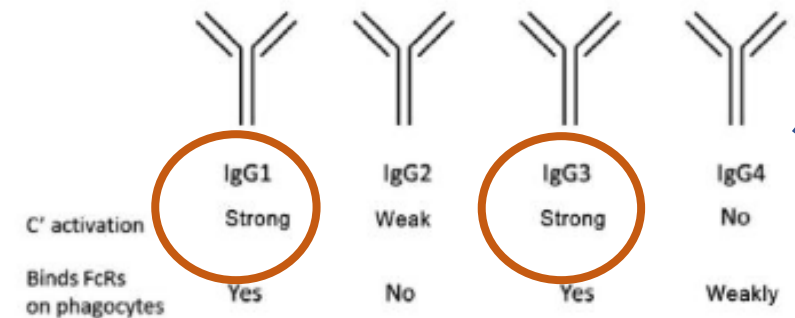
BLOOD GROUP ANTIGEN	BLOOD GROUP SYSTEM	IMMUNOGENICITY (%)*
D (Rh ₀)	Rh	50
K	Kell	5
c (hr')	Rh	2.05
E (rh'')	Rh	1.69
k	Kell	1.50
e (hr'')	Rh	0.56
Fy ^a	Duffy	0.23
C (rh')	Rh	0.11
Jk ^a	Kidd	0.07
S	MNSs	0.04
Jk ^b	Kidd	0.03
s	MNSs	0.03

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.

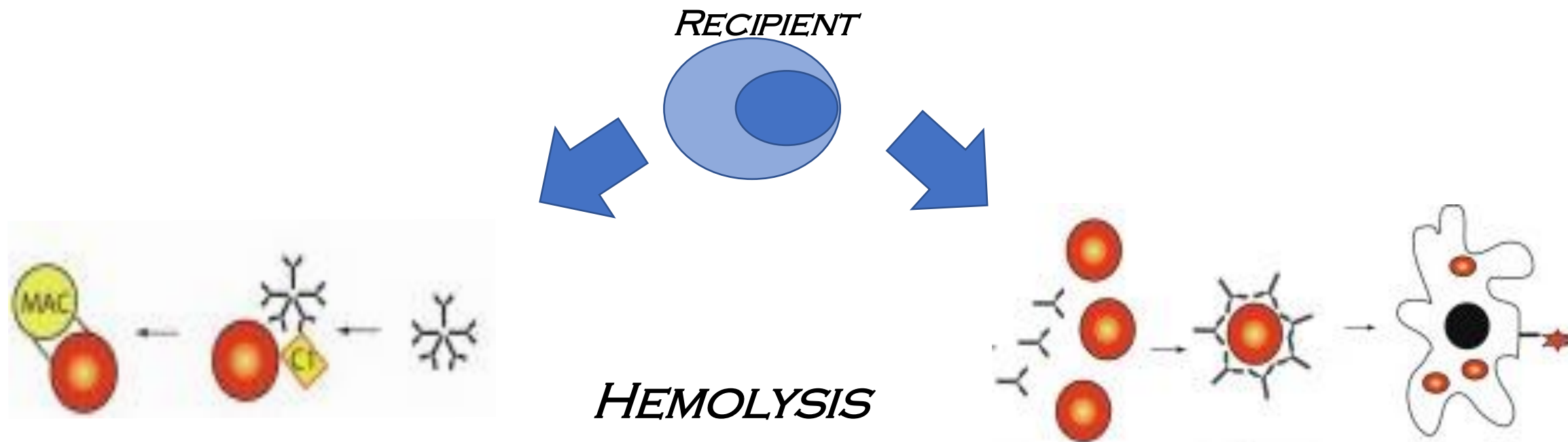
Type of response

	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

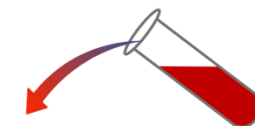
**exception: ABO



Intravascular and extravascular hemolysis



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

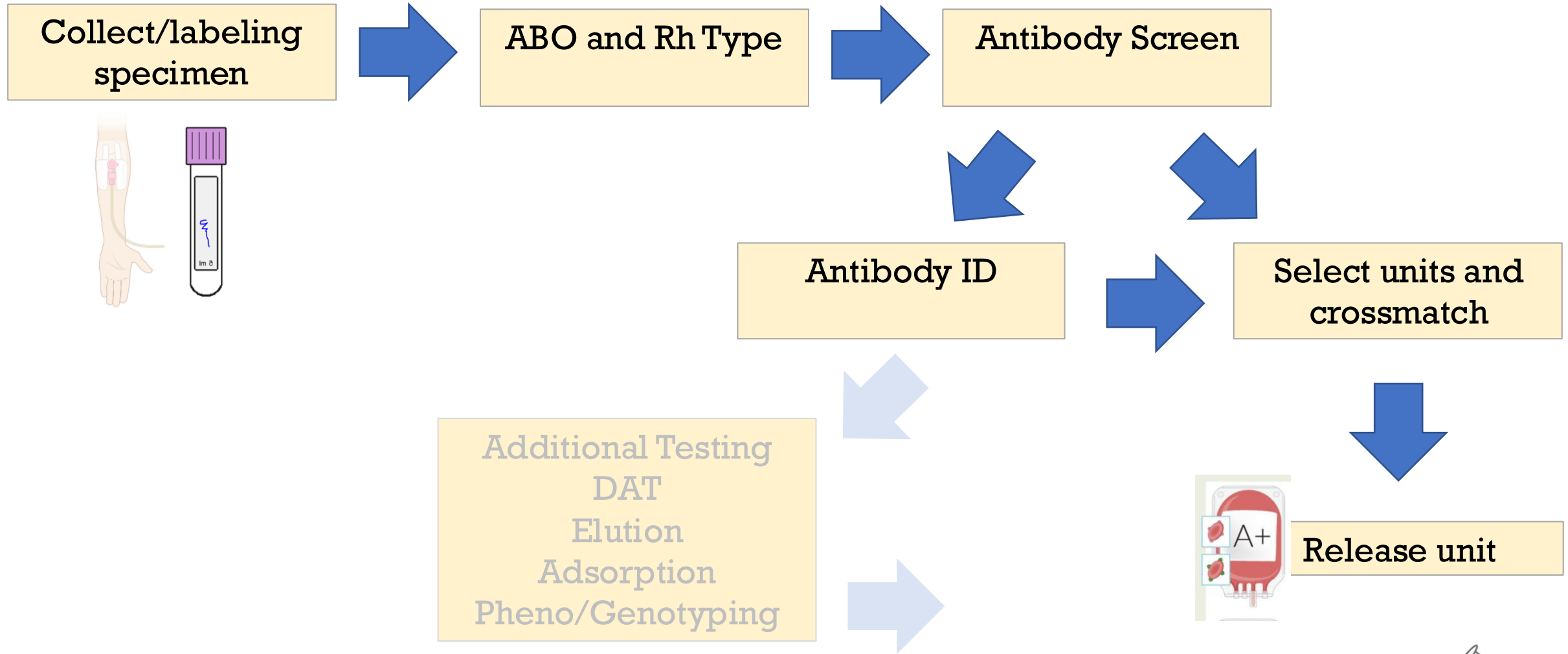


Why do we need to perform serologic pre-transfusion testing?

*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*

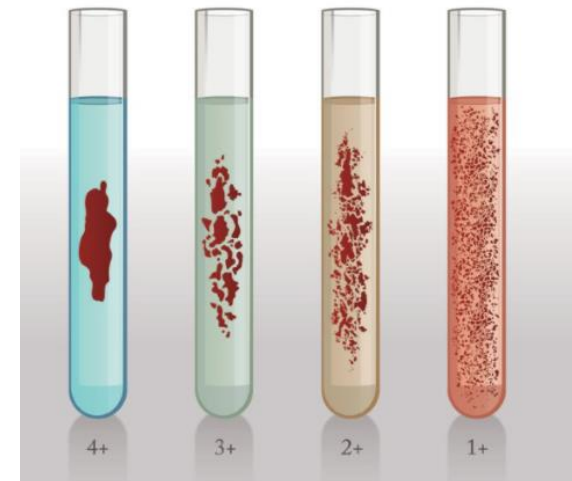
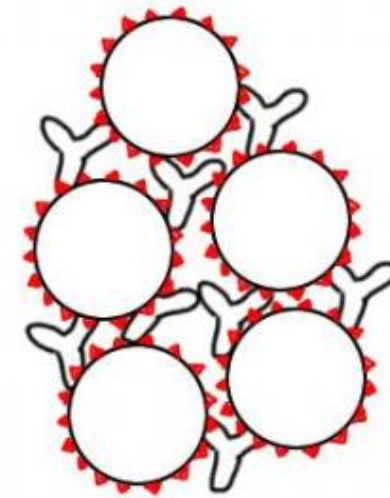


Serologic Testing Overview

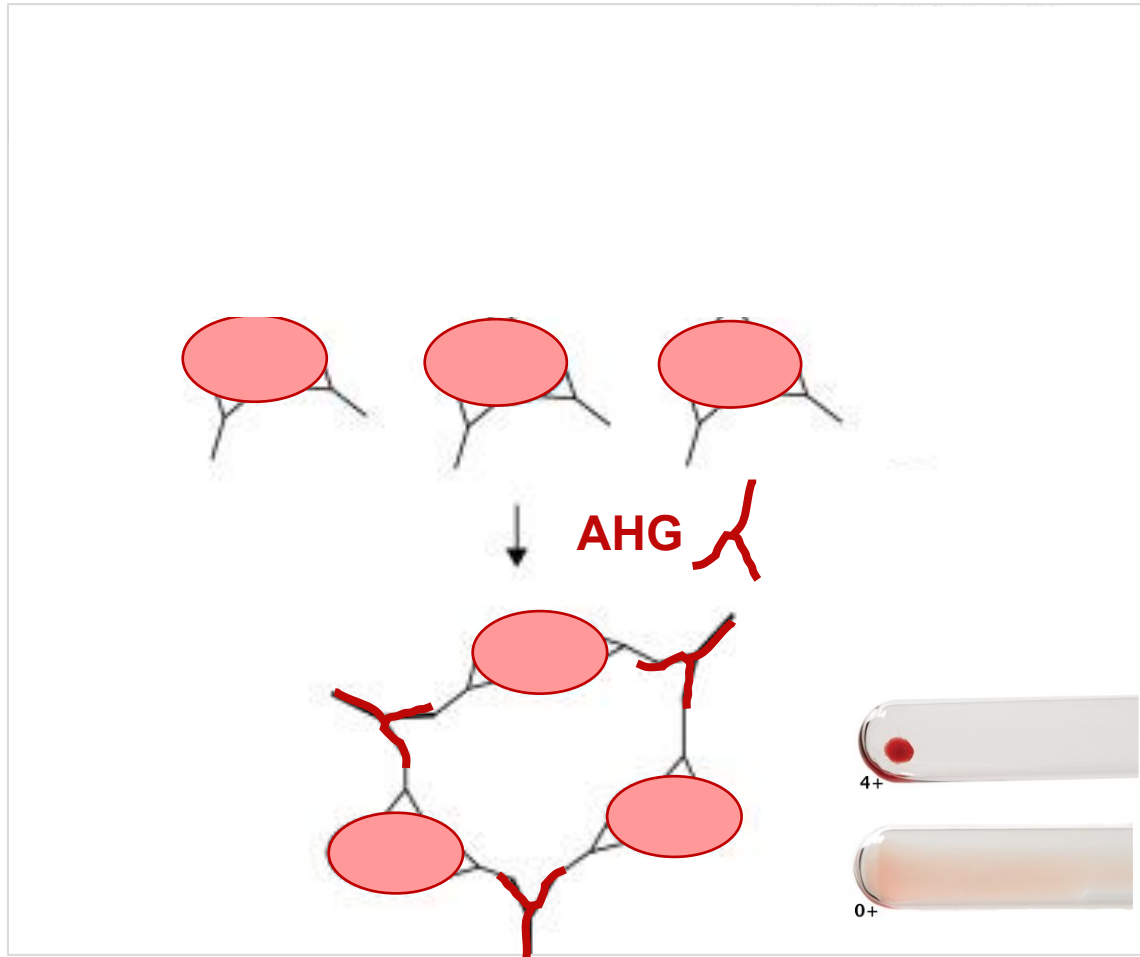


Hemagglutination is the basis for all BB testing

- 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

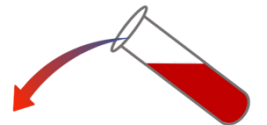


Direct Antiglobulin Test (DAT) = Coombs

- 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

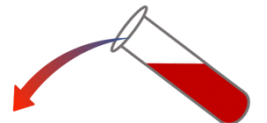
Are there *unexpected* antibodies in the patient's serum that may react with other antigens on the donor RBC?

III. Antibody Identification

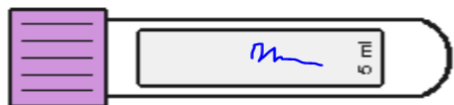
What are those unexpected antibodies – Ab ID?
Performed only if screen is positive

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

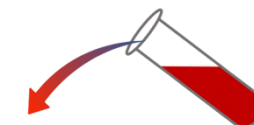
Inpatient:

- 3 days

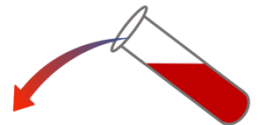
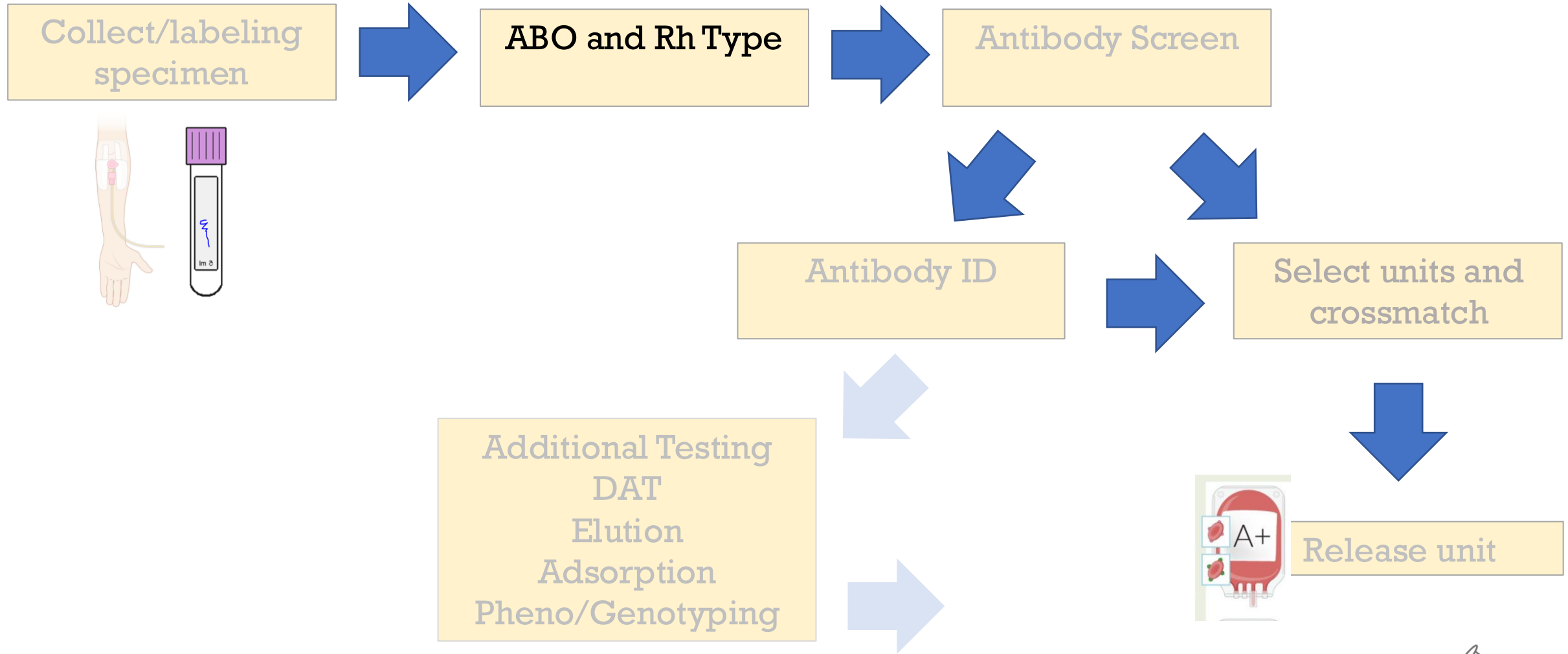
Neonates (<4 mo):

- 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)

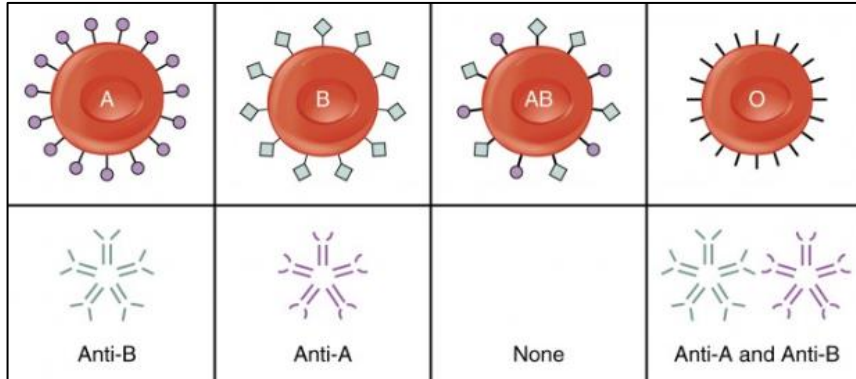
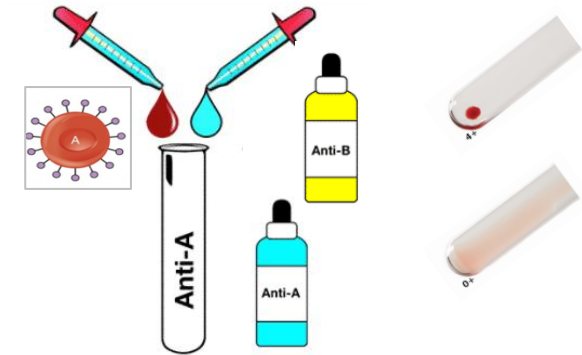


TABLE 2. Distribution (%)* of ABO phenotypes by race/ethnicity

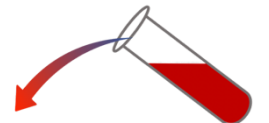
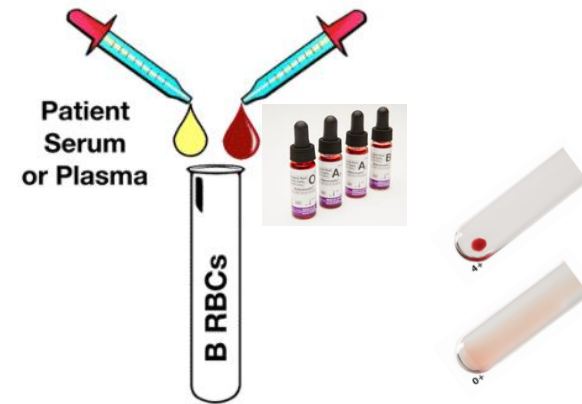
Race or ethnicity	Number	Phenotype			
		O	A	B	AB
White non-Hispanic	2,215,623	45.2	39.7	10.9	4.1
Hispanic†	259,233	56.5	31.1	9.9	2.5
Black non-Hispanic	236,050	50.2	25.8	19.7	4.3
Asian‡	126,780	39.8	27.8	25.4	7.1
North American Indian	19,664	54.6	35.0	7.9	2.5
All donors	3,086,215	46.6	37.1	12.2	4.1

Garratty G et al. (2004) *Transfusion*

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



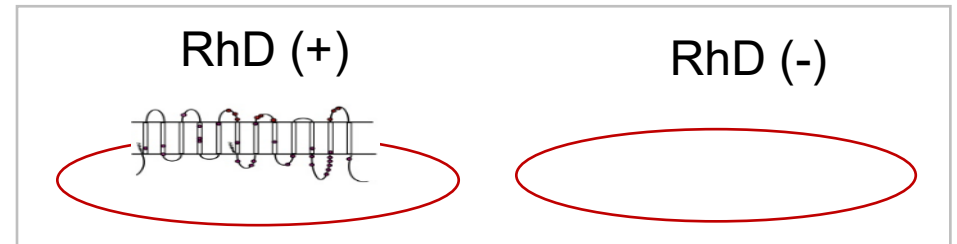
2. **REVERSE TYPE:** Is Anti-A or Anti-B present in the patient's plasma?



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?

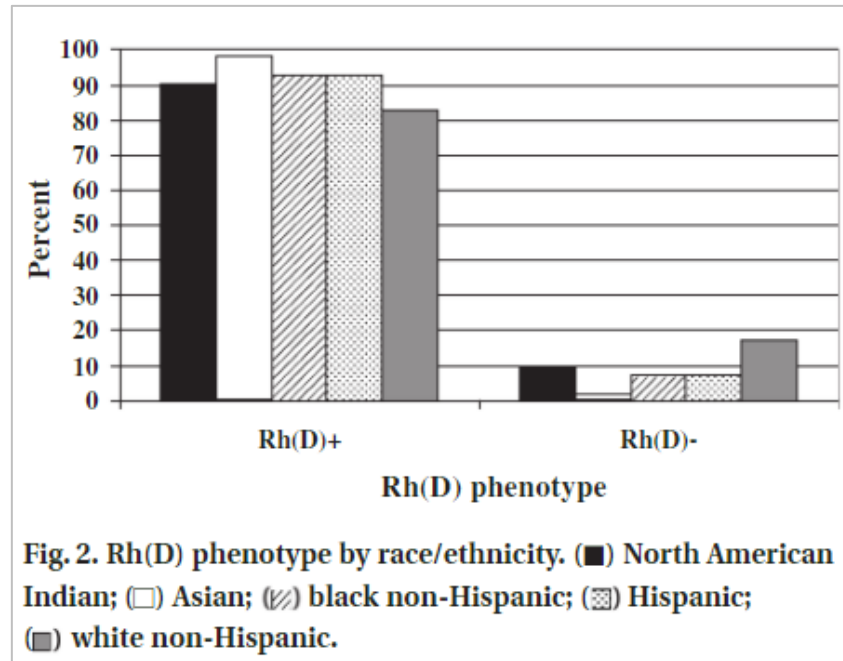
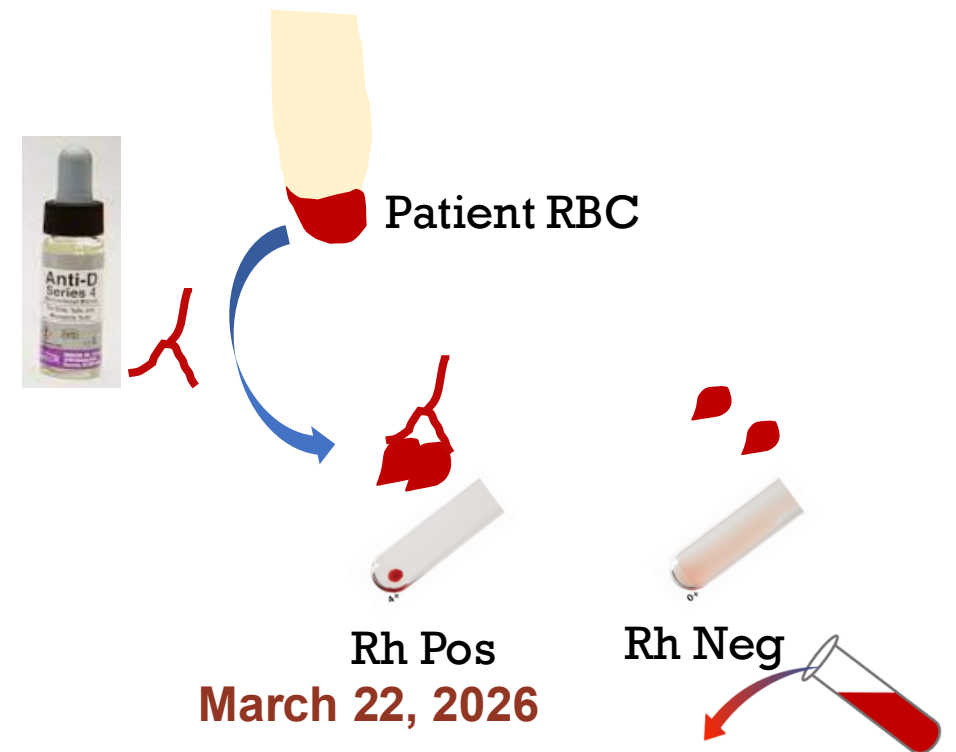


Fig. 2. Rh(D) phenotype by race/ethnicity. (■) North American Indian; (□) Asian; (▨) black non-Hispanic; (▩) Hispanic; (■) white non-Hispanic.

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

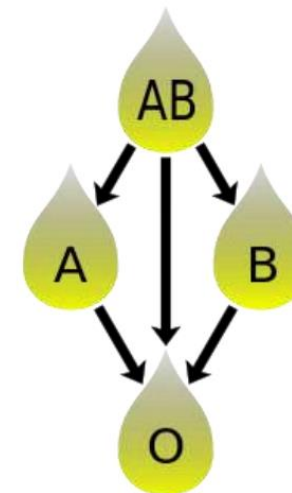
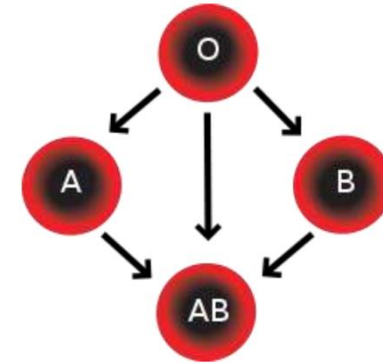
Per AABB Standards, if **> than 2 mL of RBCs** are present in any product, those RBCs must be **compatible with the recipient's plasma** antibodies.

Plasma-Containing blood products:

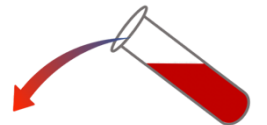
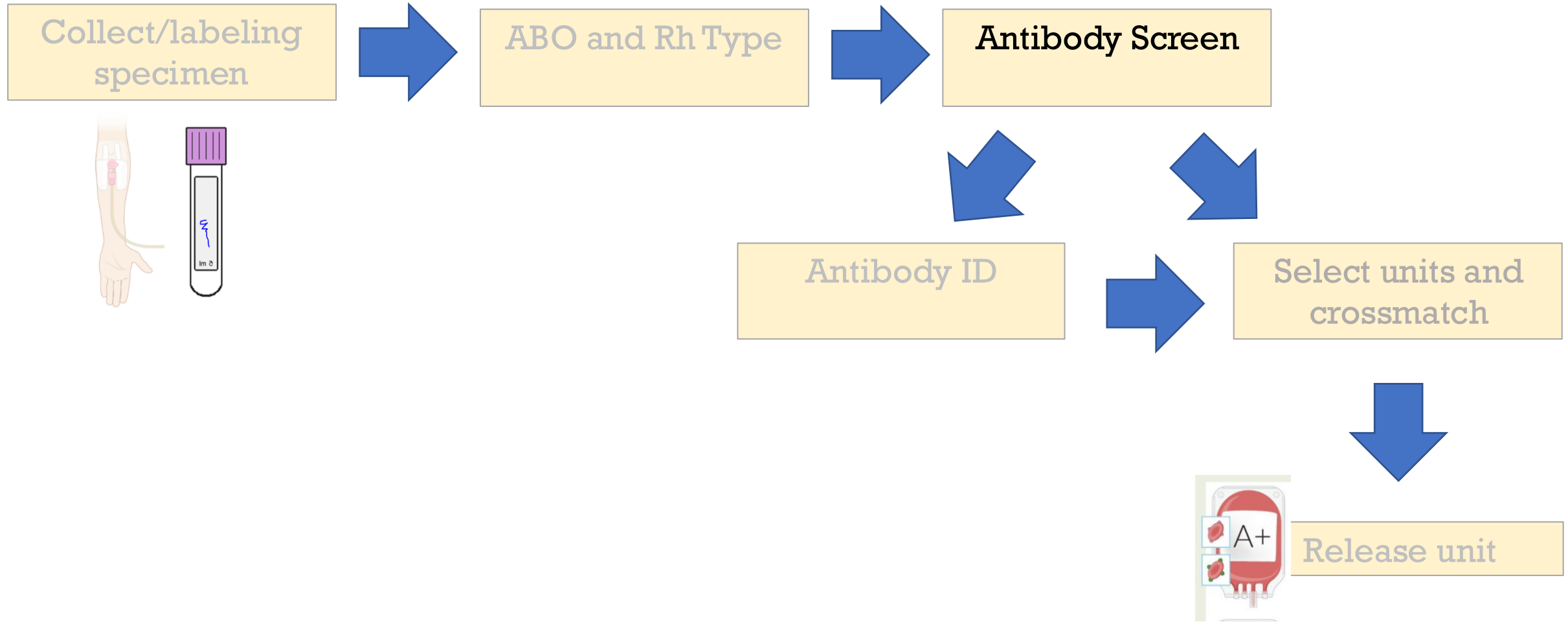
FFP – ABO matched/compatible with recipient RBCs

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”

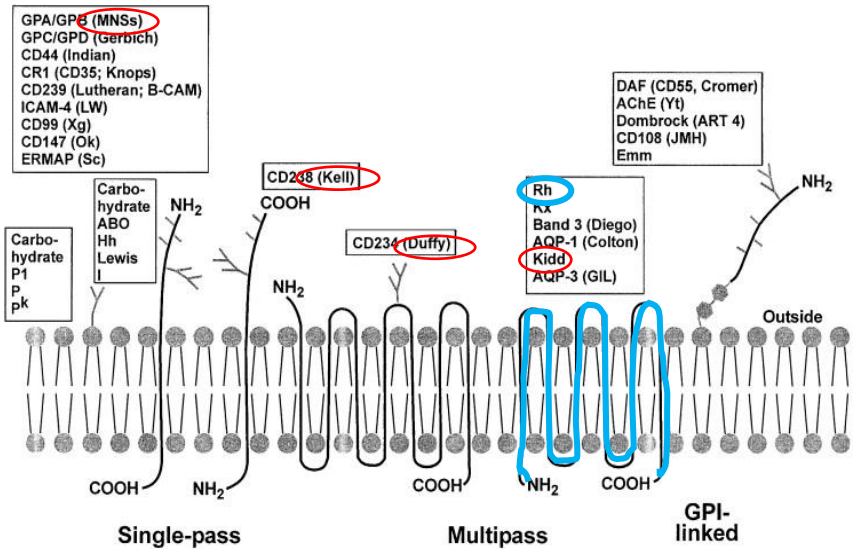
*Allo*antibodies

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

*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, Passenger Lymphocytes in transplanted organs, HPCs



Not cloned: P1, MER2

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



II. Antibody screen

Screening Cells:

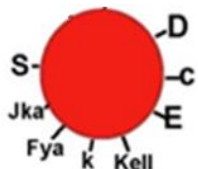
2 cells; reagent RBCs

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



CAPTURE-R READY-SCREEN (3)
Master List

06/06/2014 4174

IMMUCOR, INC. Norcross, GA 30071 USA
US LICENSE NO: 886
LOT NO: R407
EXPIRES: 2014/07/01

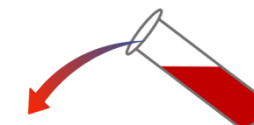
CELL	Donor	Rh - Hr		Kell					Duffy		Kidd		Lewis		P		MN		Lutheran		Xg							
		D	C	c	E	e	V	C ^a	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	P ₁	M	N	S	s	Lu ^a	Lu ^b	Xg ^a
I	R1R1 B8314	+	+	0	0	+	0	0	+	+	0	+	0	+	+	+	+	+	0	0	0	0	+	0	+	0	+	0
II	R2R2 C4205	+	0	+	+	0	0	0	0	+	0	+	0	+	+	0	0	+	+	0	+	0	+	0	+	0	+	0
III	rr H555	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

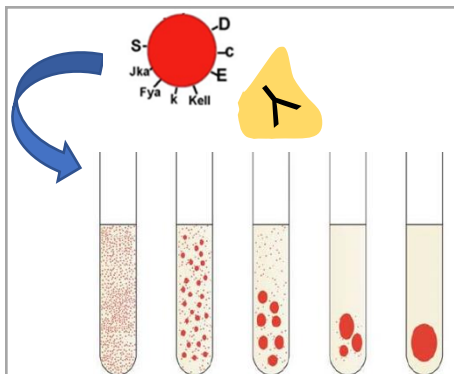


Reactivity



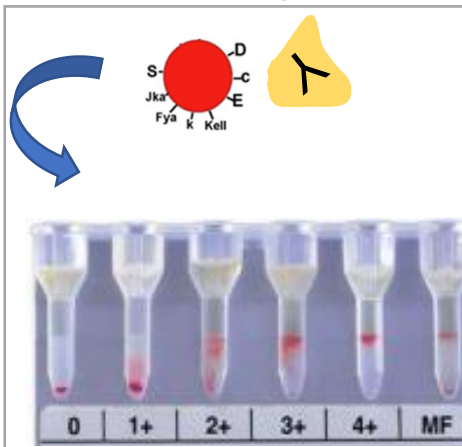
Antibody detection methodology

Tube Testing



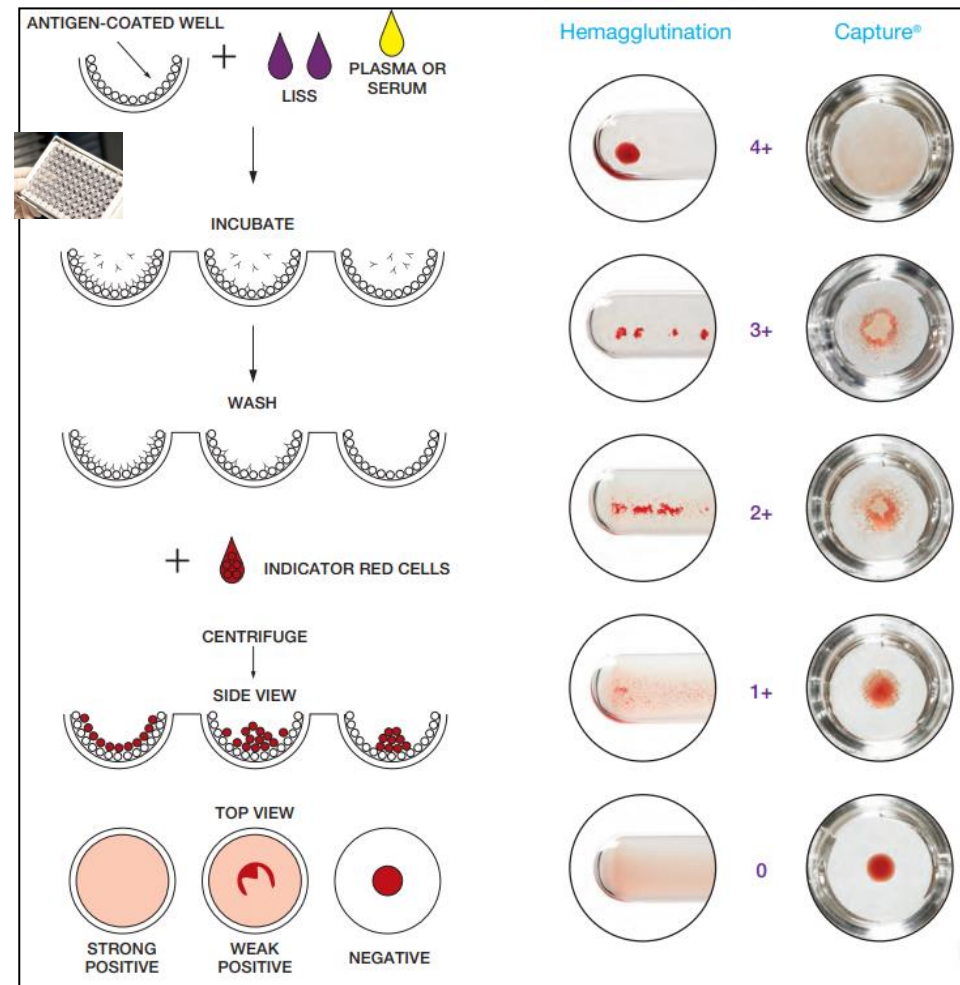
- Plasma and test cell interaction in tube
- Assess for agglutination

Gel Testing

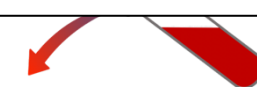


- Plasma and test cell interaction in chamber
- Centrifugation of RBCs through gel column
- Agglutinates remain on top

Solid Phase

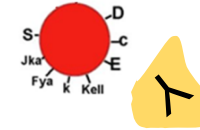



Hemagglutination	Capture®
4+	
3+	
2+	
1+	
0	



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 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	0	3+
R ₂ R ₂	3	+	0	+	+	0	0	0	+	0	+	0	+	0	0	0	+	+	+	0	+	0	+	0	+	+	0	0	0	3+	



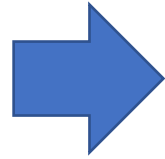
Phase of Rxn	Ab type detected	Mechanism	Types of Abs Detected
IS	IgM	IgM react best at <i>lower</i> temp	ABO, cold-reacting Allo/Auto
37 C	IgG	IgG react best at <i>warm</i> temp	Warm-reacting abs
AHG/37 C (IAT)	IgG	AHG displays specificity for the Fc portion of the heavy chain of IgG or complement "bridges"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

Immediate Spin Phase	A, B, H I M, N Le ^a , Le ^b P1	IgM
Antiglobulin Phase (37°C)	D, C, E c, e K, Fy, Jk S, s Le ^a , Le ^b	IgG

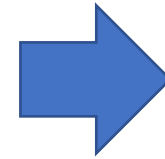


Serologic Testing Overview

Collect/labeling specimen



ABO and Rh Type



Antibody Screen

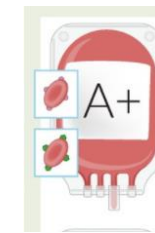


		Rh								Kell					Duffy		Kidd		Lewis		P	MNS				Lutheran		Xg	Results			
		D	C	E	c	e	f	V	C _w	K	k	Kp _a	Kp _b	Js _a	Js _b	Fy ^a	Fy ^b	Jk _a	Jk _b	Le ^a	Le ^b	P1	M	N	S	s	Lu ^a	Lu ^b	Xg _a	IS	IAT	
I	R ₁ R ₁	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	0	+	0	+	0	0	+	+	0	0	+	+	0	0	0	0
II	R ₂ R ₂	+	0	+	+	0	0	0	0	0	+	0	+	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		

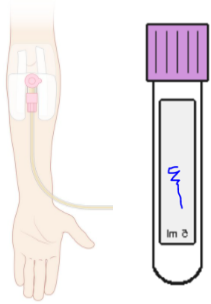
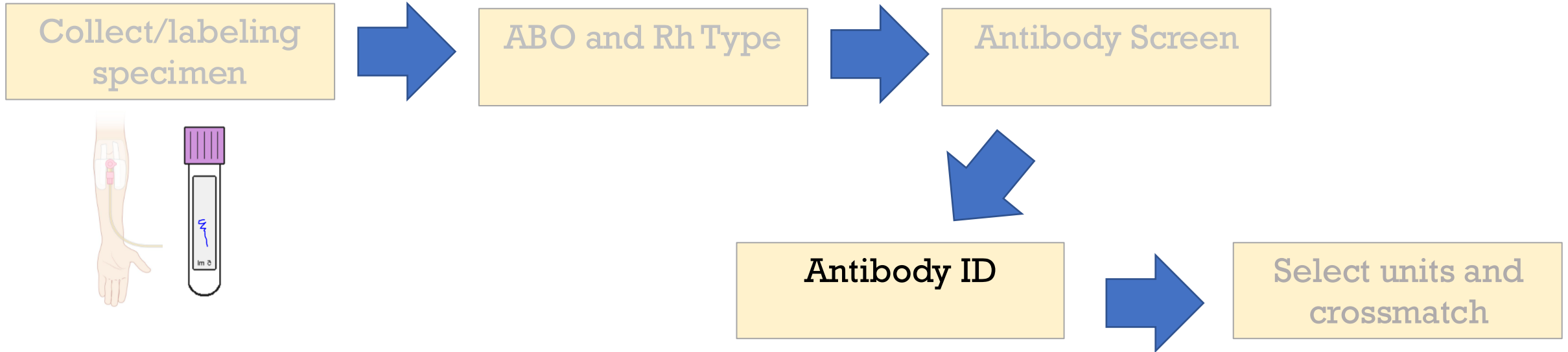
Select units and crossmatch



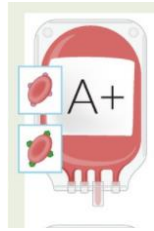
Release unit



Serologic Testing Overview



		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 ₁ R ₁	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	0	+	0	+	0	0	+	+	0	0	0	+	+	0	0
II	R ₂ R 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	3+	



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:

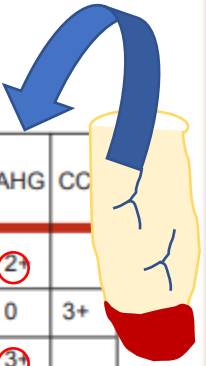
- Clinical significance varies even with antibodies of the same specificity
- Some antibodies only appear to be an issue in vitro, not in vivo (i.e., may result in a (+) DAT in the fetus but no clinical HDFN)



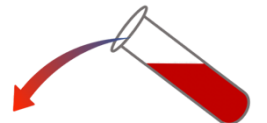
Antibody Panel = extended antibody screen to determine AbID

- 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	E	C ^c	K	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	K ^a	K ^b	L ^s ^a	L ^s ^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	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	3+	
R ₀ r	4	+	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	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	3+
r'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	0	0	3+	
R ₁ r	11	+	+	+	0	+	0	0	+	0	+	+	0	0	+	+	+	+	+	+	+	0	+	+	0	0	0	2+	
	Patient Cells																									0	0	0	3+



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



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

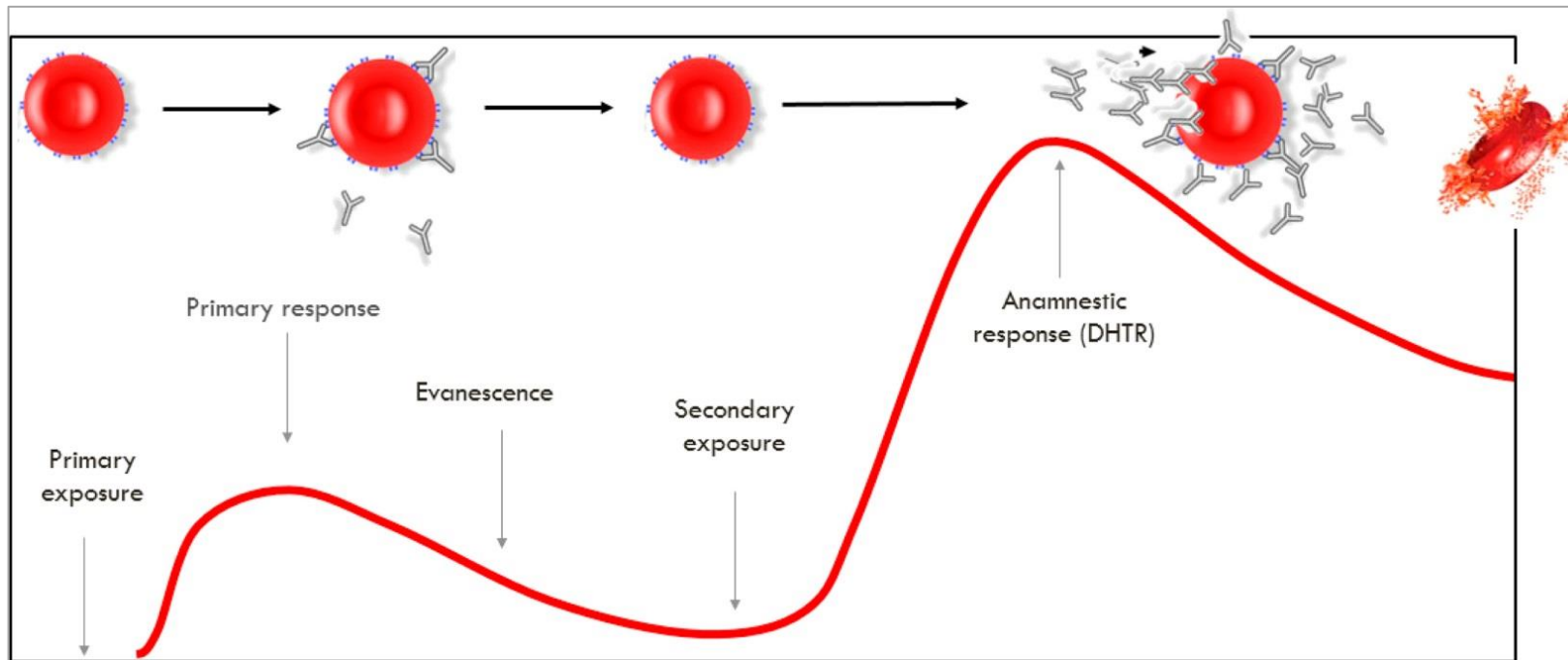
(+) Ab ID \neq clinically significant hemolysis

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

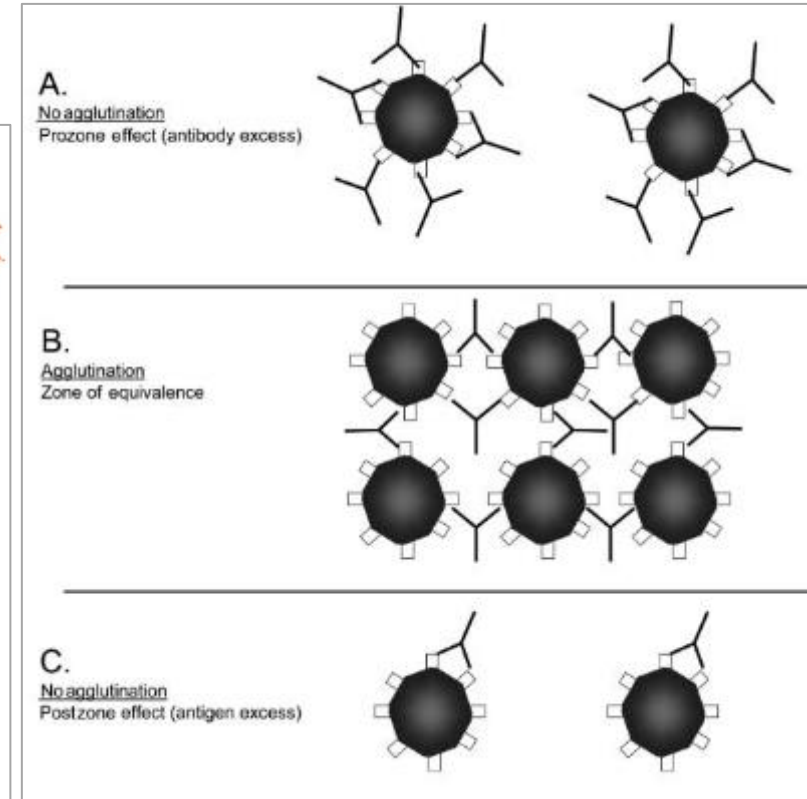


Historical Antibodies

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



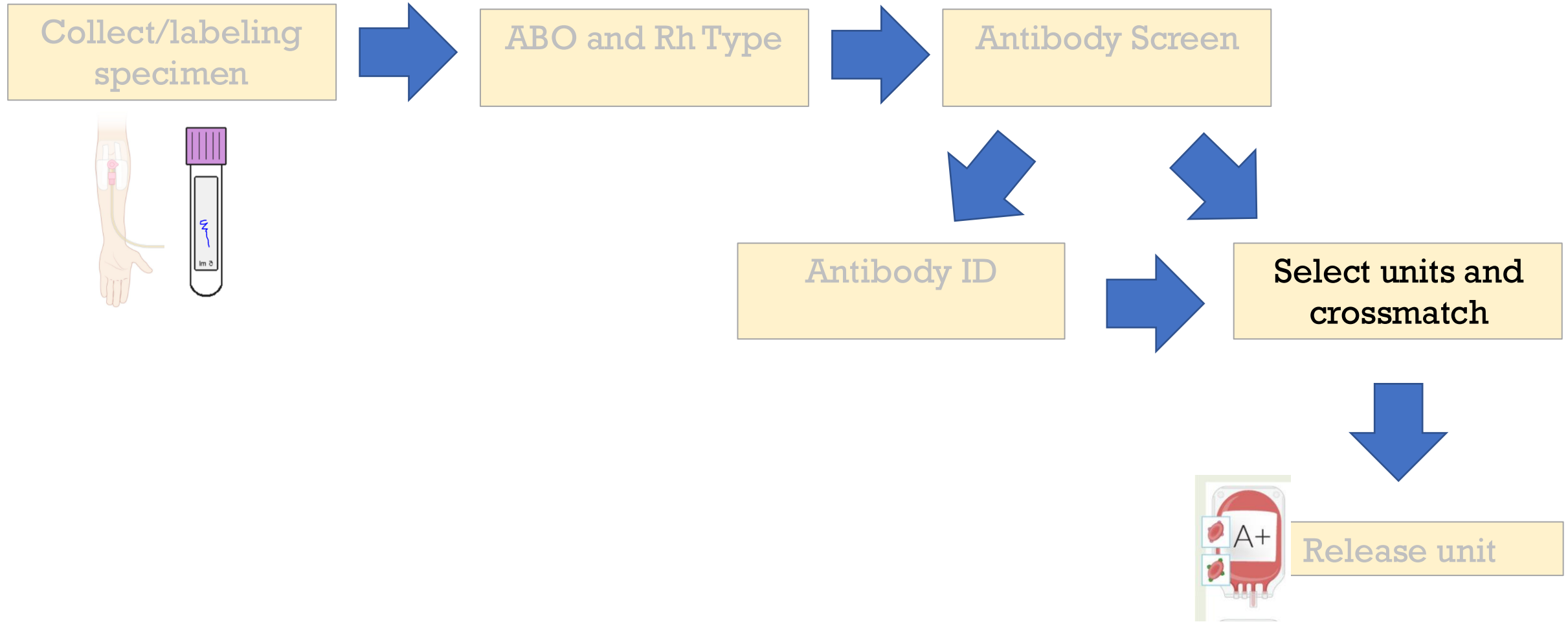
Fasano RM et al (2019) *Transfusion Clinique et Biologique*



AABB Technical Manual 20th Ed



Serologic Testing Overview



How difficult is it to obtain antigen negative units?

Donor units needed to screen = # of units ordered/Frequency of the antigen neg unit

The physician has requested 2 units of RBCs for patient who has an anti-X and anti-Y. The frequency of antigen X is **45%**, and the frequency of antigen Y is **70%** in the donor population.

Approximately how many units will need to be antigen-typed for X and Y to find compatible units? $2/(0.55 * 0.3) = 12$

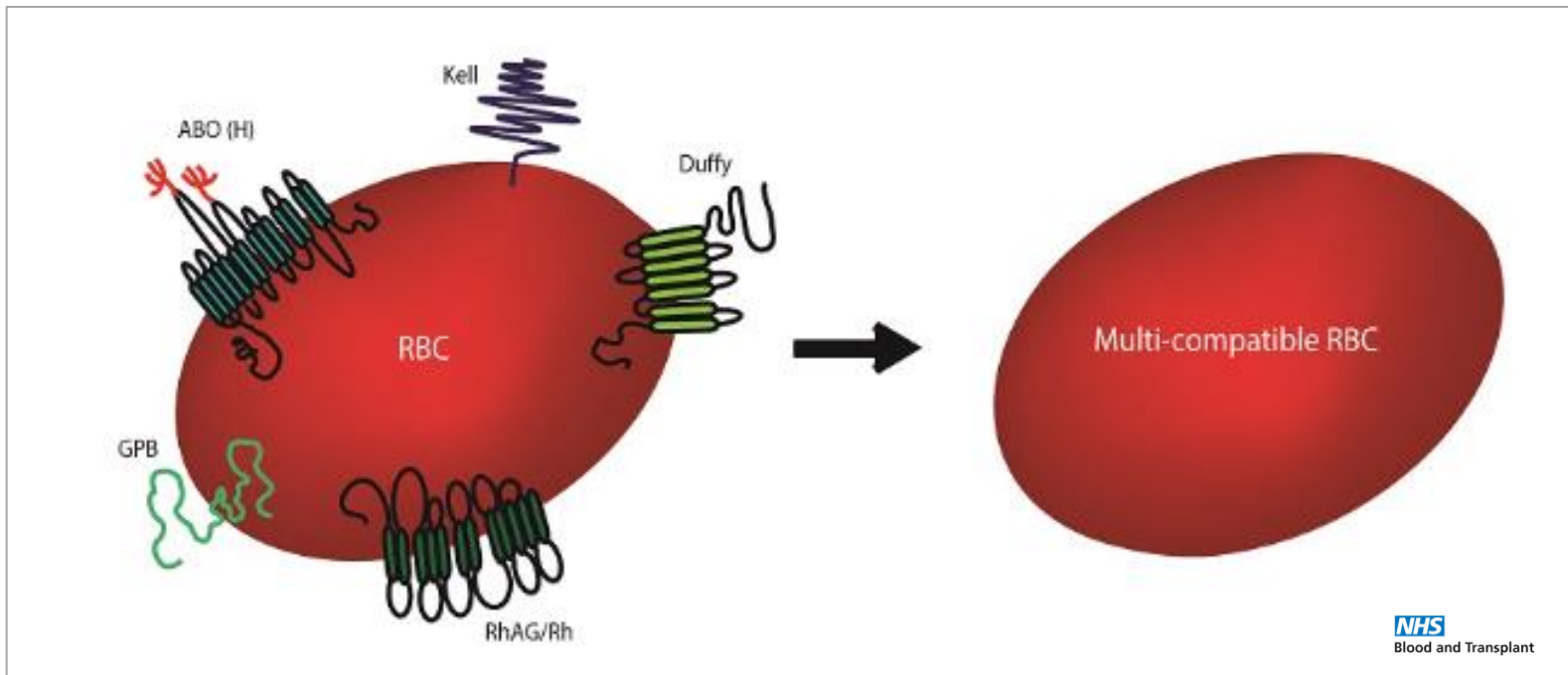
- a. 8
- b. 12**
- c. 2
- d. 7



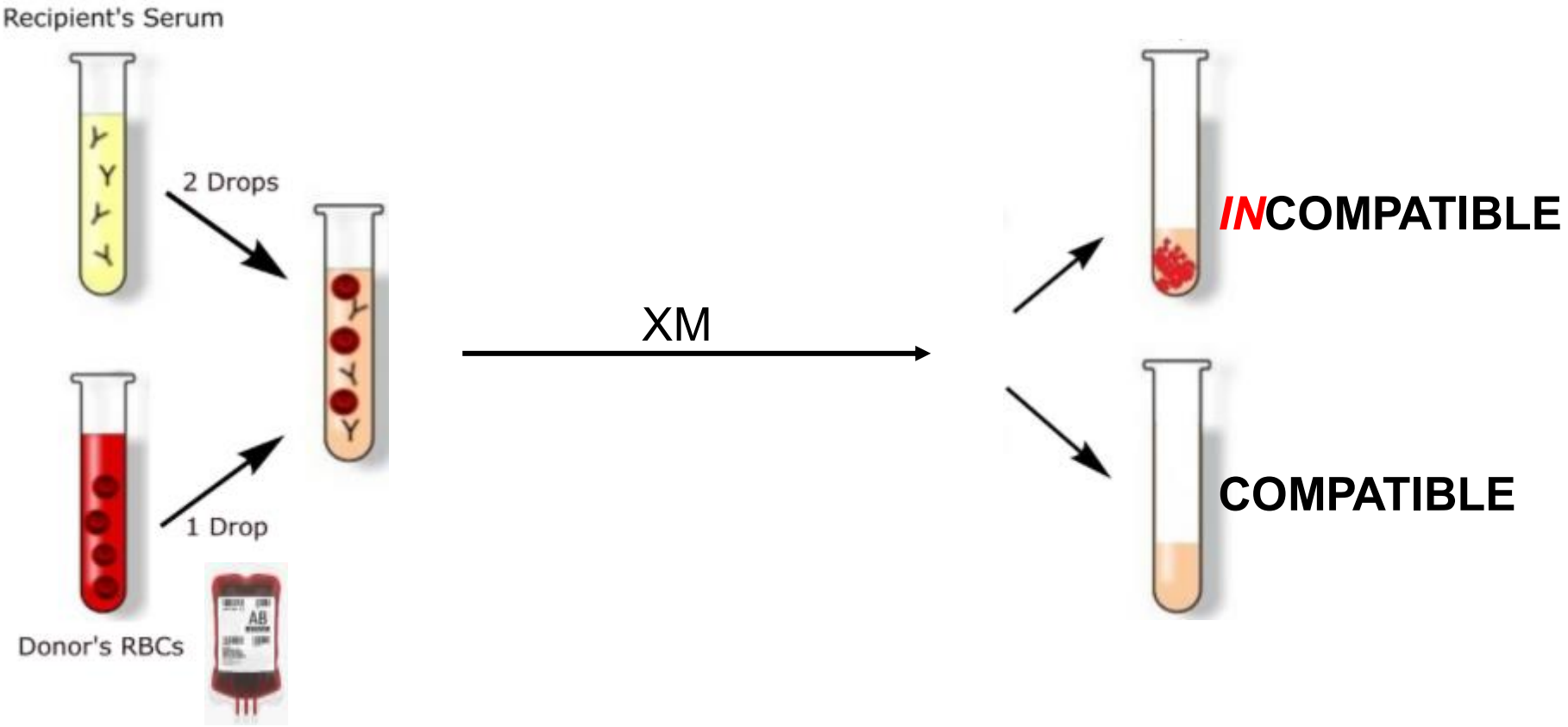
Enhancement of red blood cell transfusion compatibility using CRISPR-mediated erythroblast gene editing

Joseph Hawksworth^{1,3,†}, Timothy J Satchwell^{1,2,3,†}, Marjolein Meinders¹, Deborah E Daniels^{1,2}, Fiona Regan^{4,5}, Nicole M Thornton⁶, Marieangela C Wilson¹, Johannes GG Dobbe⁷, Geert J Streekstra⁷, Kongtana Trakarnsanga⁸, Kate J Heesom¹, David J Anstee^{1,2,3}, Jan Frayne^{1,2} & Ashley M Toye^{1,2,3,*} 

Antigen Negative RBCs?



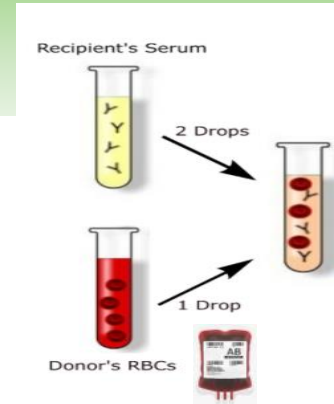
IV. Crossmatch



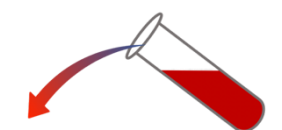
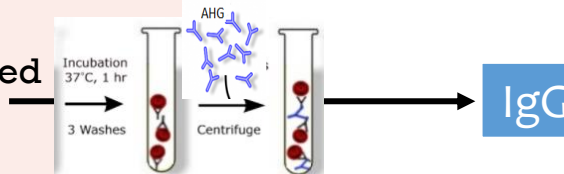
Modified from <https://laboratorytests.org/cross-matching/>



Types of Crossmatch



Type of XM	When is it used?	Description
Immediate Spin (IS)	(-) Ab screen No history of prior (+) screen	Pt plasma + Donor RBC <ul style="list-style-type: none"> • Agglutination seen at IS • Detects ABO incompatibility
IAT (“Full XM” or “Coombs crossmatch”)	(+) Ab screen History of (+) screen	Pt plasma + Donor RBC <ul style="list-style-type: none"> • Incubate at 37 C, wash, AHG used (“bridging”) • Detects IgG
Electronic XM	(-) Ab screen No history of prior (+) screen 2 ABO/Rh types on record Computer software validated	Computer checks ABO compatibility of patient and donor <ul style="list-style-type: none"> • <i>No physical testing</i>

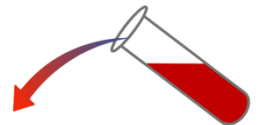
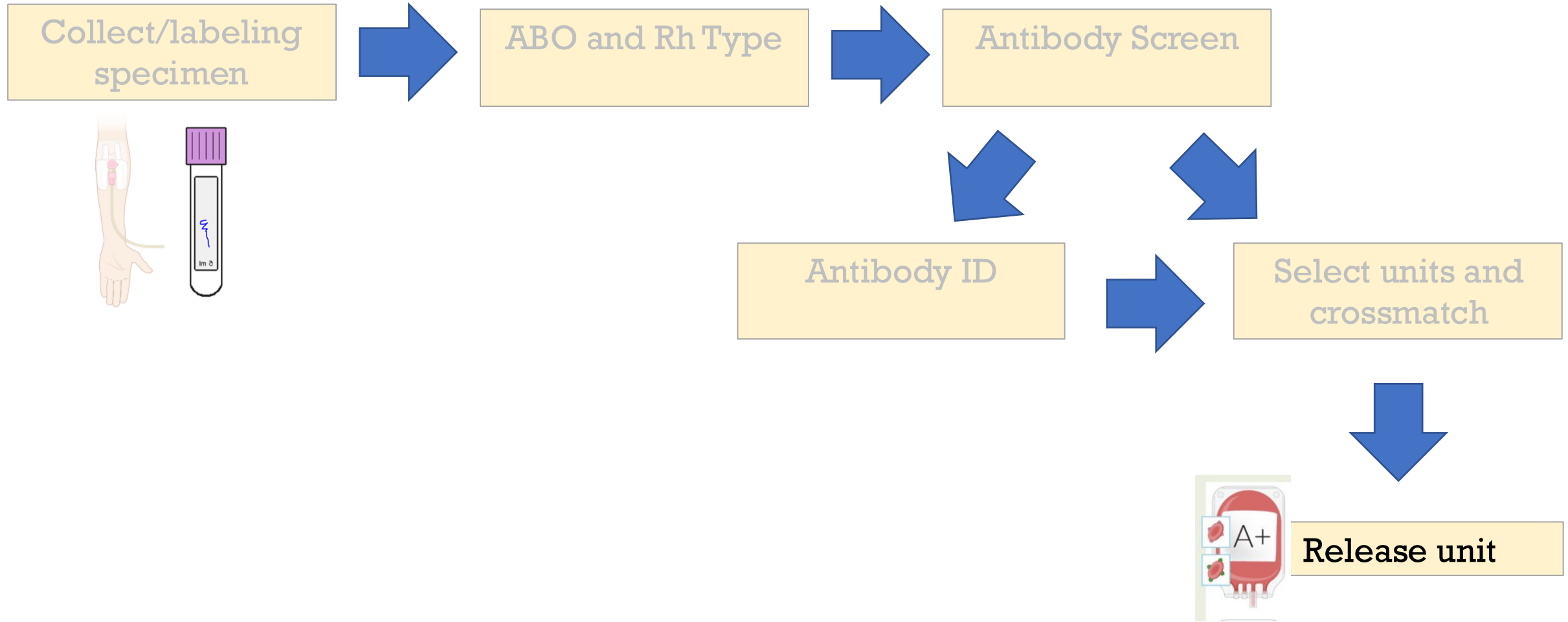


Timeline for RBC availability

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, <u>high risk – EMERGENCY RELEASE.</u> Please clearly communicate urgency w/ BB!



Serologic Testing Overview



Workups can get more complicated...



Pan-reactive antibody panel

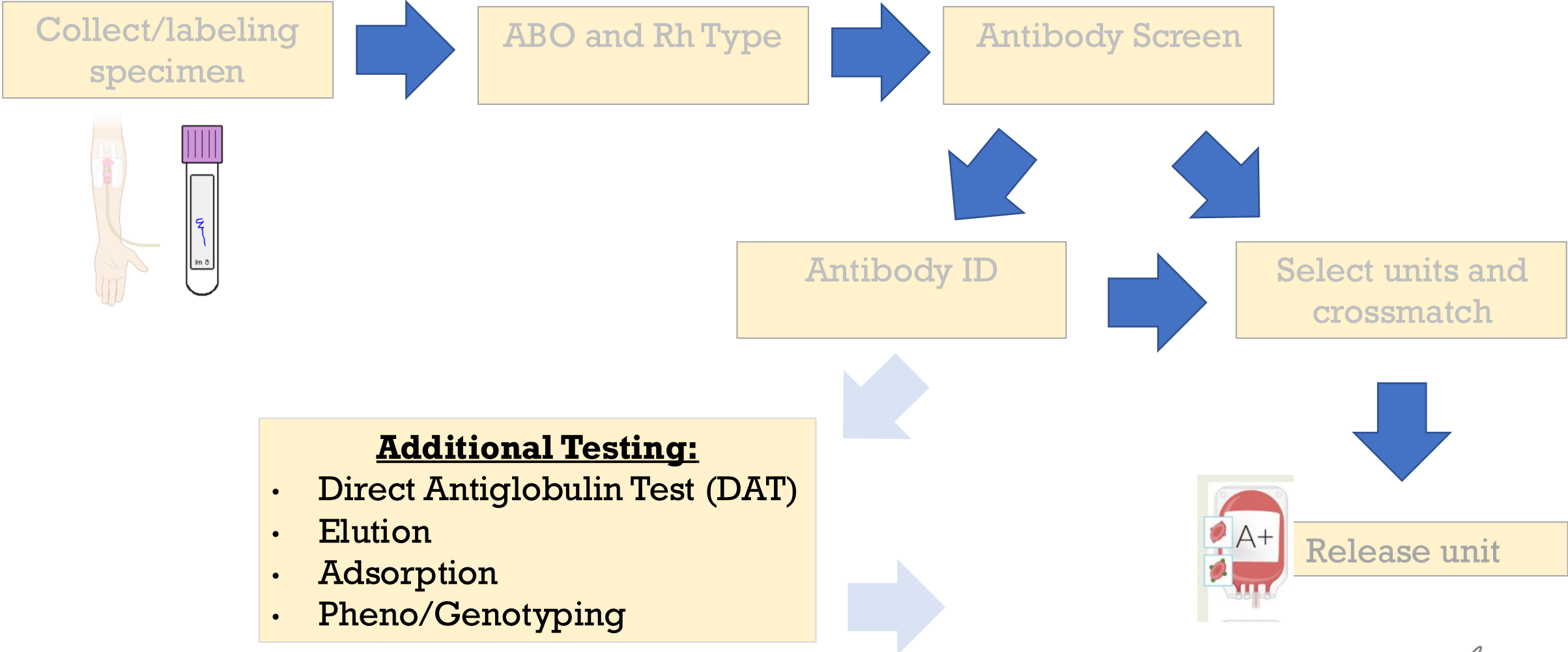
Donor	Cell number	D	C	c	E	e	C ^w	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	P ₁	M	N	S	s	Lu ^a	Lu ^b	Xg ^a	Peg/ IgG
R1R1	1	+	+	0	0	+	0	0	+	0	+	0	+	+	0	+	+	+	0	+	+	+	+	+	0	+	+	2+
R1wR1	2	+	+	0	0	+	+	+	+	0	+	0	+	+	+	0	+	0	+	+	+	0	+	+	0	+	+	3+
R2R2	3	+	0	+	+	0	0	0	+	0	+	0	+	0	+	0	+	0	+	+	+	+	+	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'Y	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)

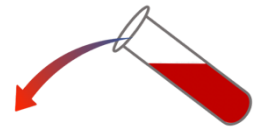
Hamening, DM. Modern Blood Banking and Transfusion Practices 7th Ed.



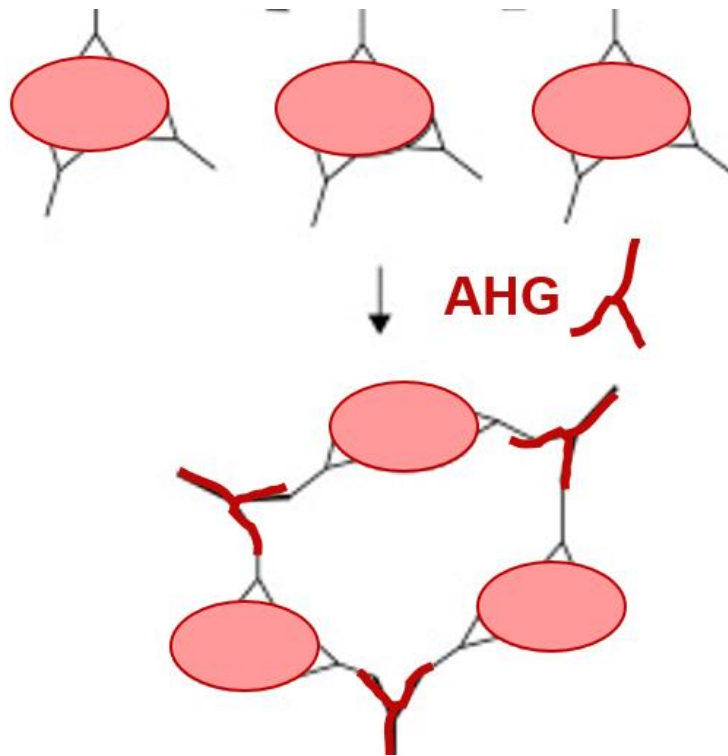
Additional techniques in the toolbox



- Additional Testing:**
- Direct Antiglobulin Test (DAT)
 - Elution
 - Adsorption
 - Pheno/Genotyping



Positive autocontrol: autoantibody?



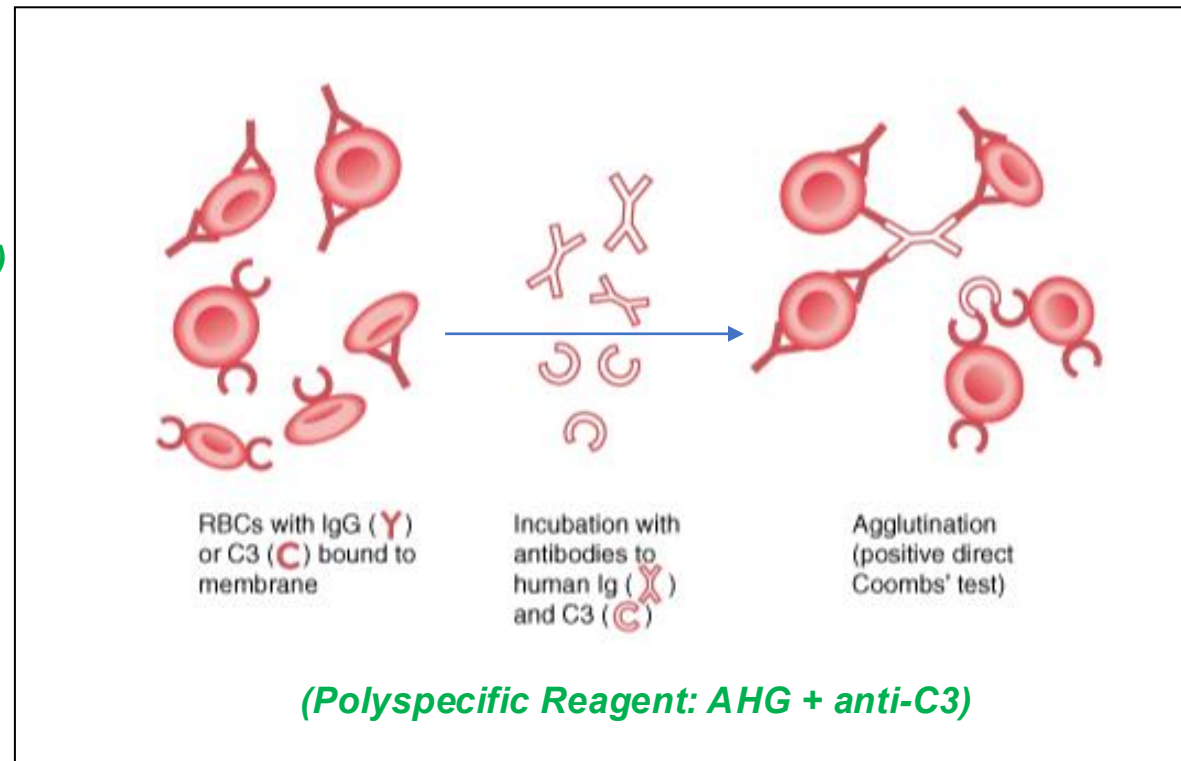
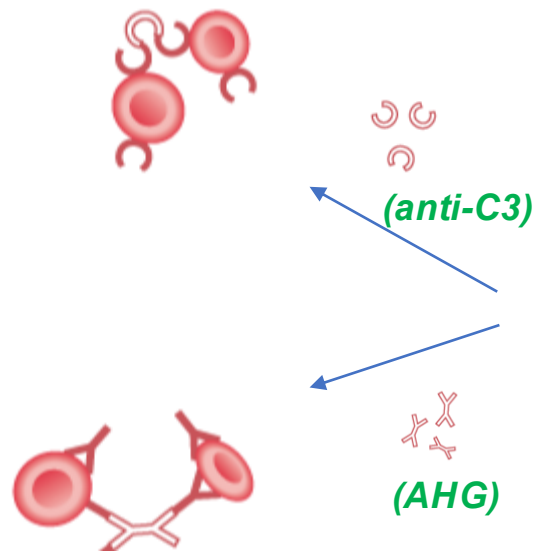
Donor	Cell number	D	C	c	E	e	C ^w	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	P ₁	M	N	S	s	Lu ^a	Lu ^b	Xg ^a	Peg/IgG	
R1R1	1	+	+	0	0	+	0	0	+	0	+	0	+	+	0	+	+	+	0	+	+	+	+	+	0	+	+	+	2+
R1wR1	2	+	+	0	0	+	+	+	+	0	+	0	+	+	+	0	+	0	+	+	+	0	+	+	0	+	+	+	3+
R2R2	3	+	0	+	+	0	0	0	+	0	+	0	+	0	+	0	+	0	+	+	+	+	+	0	0	+	+	+	2+
R0r	4	+	0	+	0	+	0	0	+	0	+	0	+	0	0	+	+	0	+	+	+	0	0	0	0	+	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	0	+	+	+	2+
	Patient Cells																											2+	



DAT: what is coating the RBCs?

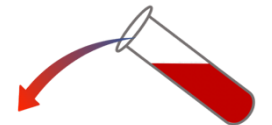
In-Vivo antibody coating

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 \neq Hemolysis
 \neq RBC antibodies
 \neq Immune cause

Anemia?
Reticulocytosis?
Laboratory evidence of hemolysis?

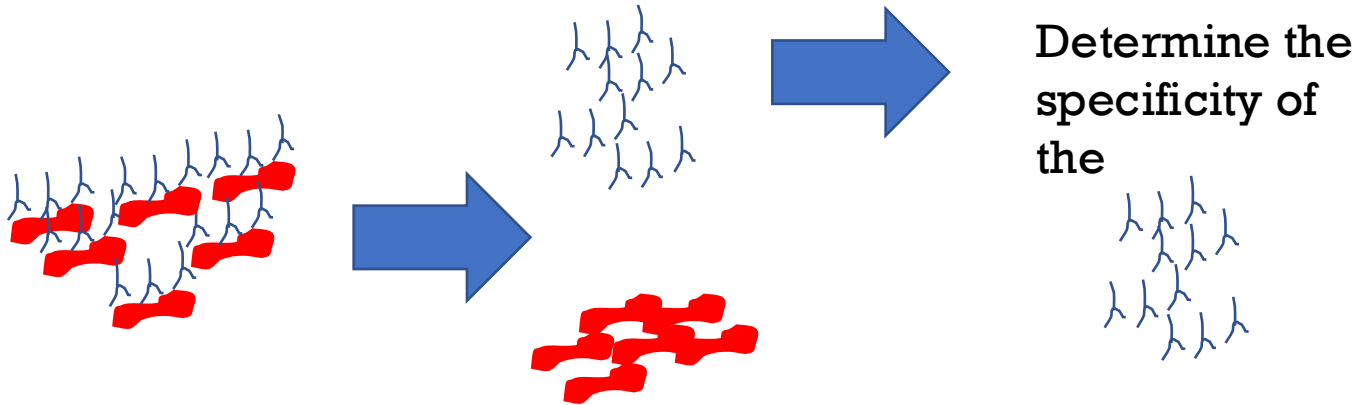
Other causes of a + DAT

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?

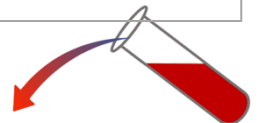


Donor	Cell number	D	C	c	E	e	C ^w	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	P ₁	M	N	S	s	Lu ^a	Lu ^b	Xg ^a	IS	37	AHG	CC	
R ₁ r	1	+	+	+	0	+	0	+	0	+	0	+	+	+	+	+	+	0	+	+	+	+	+	+	0	+	+					
R ₁ R ₁	2	+	+	0	0	+	+	+	+	0	+	+	+	+	+	+	+	0	0	+	+	+	+	0	0	+	+					
R ₂ R ₂	3	+	0	+	+	0	0	+	0	+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

	WAIHA	CAS	Mixed-type AIHA	PCH
DAT (routine)	IgG IgG + C3 C3	C3 only	IgG + C3 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 hemolysis 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.

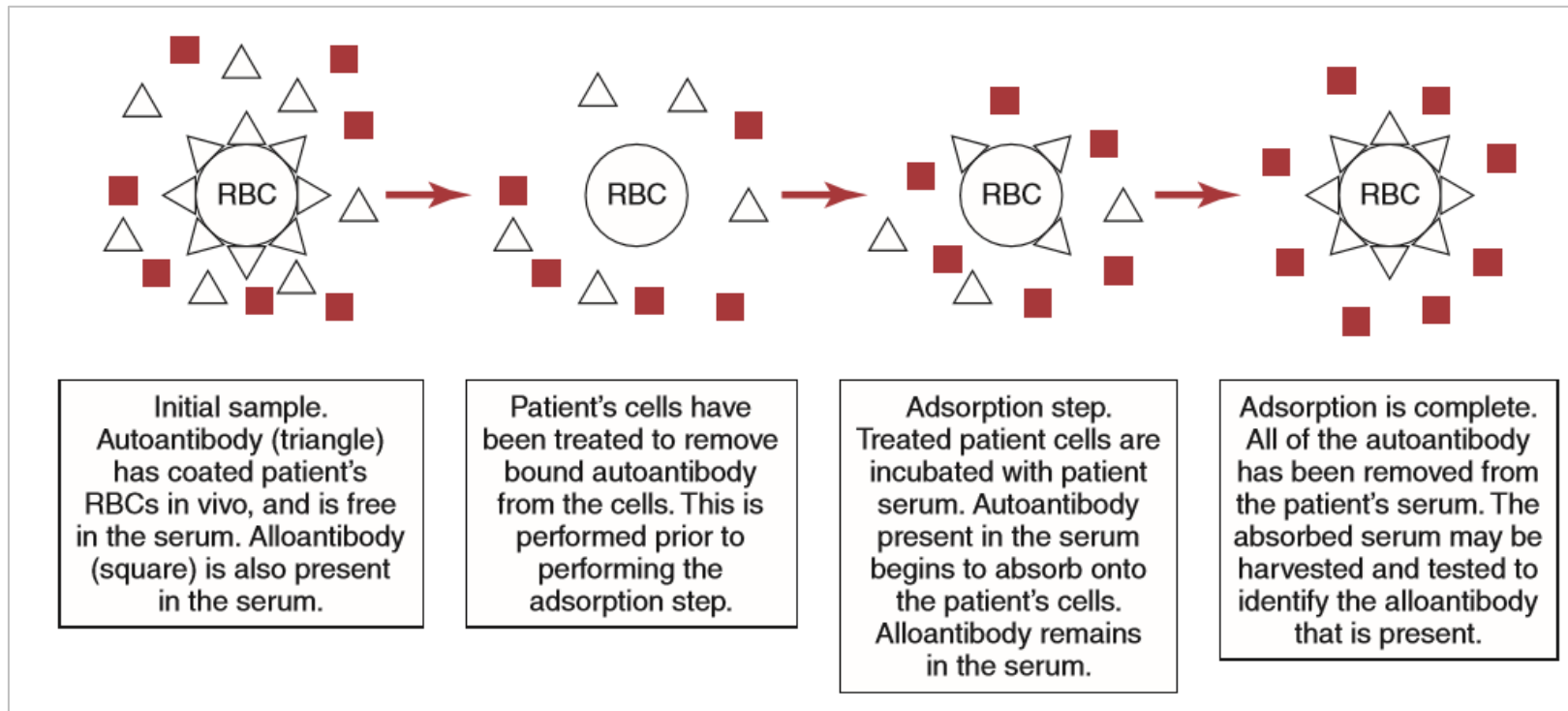
AABB Technical Manual 18th Edition



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



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

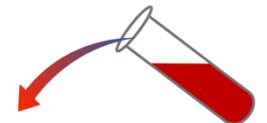
~30% patients with autos will also have allos (Branch Dr and Petz LD. Transfusion 1999)



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	11	+	0	+	0	+	0	0	+	0	+	0	+	+	+	0	+	0	+	+	0	+	0	+	0	+	+	2+		0	3+
	Patient Cells																											2+			

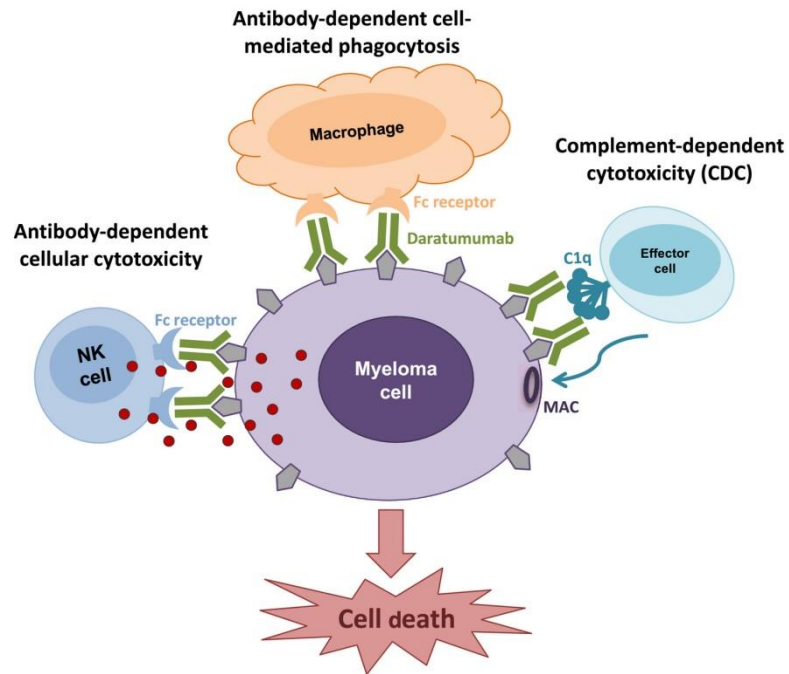
Hamening, DM. Modern Blood Banking and Transfusion Practices 7th Ed.



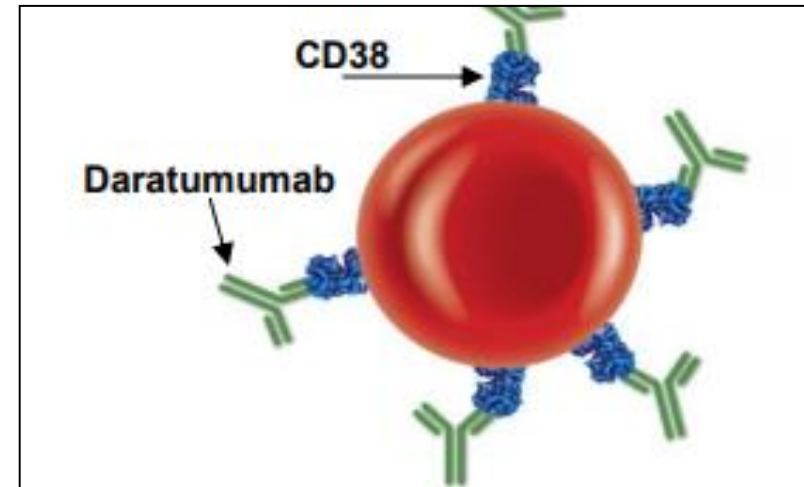
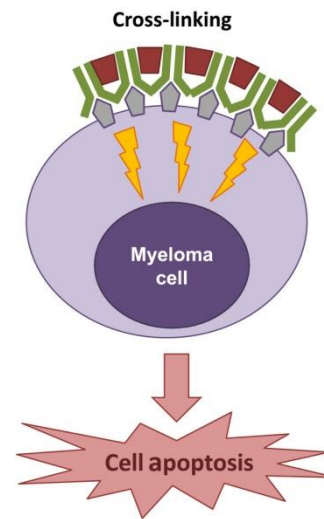
Daratumumab interference in BB testing

Daratumumab Mechanisms of Action

Indirect effects



Direct effects



British Journal of Haematology, (2018) Volume: 181, Issue: 4, Pages: 447-459,

Recommended to obtain ABORh, AB Screen, DAT, phenotype/genotype of patient prior to initiation of therapy (especially Kell antigen typing)

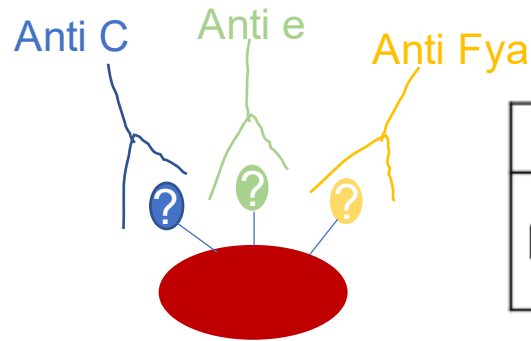


Phenotyping patient RBCs

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)

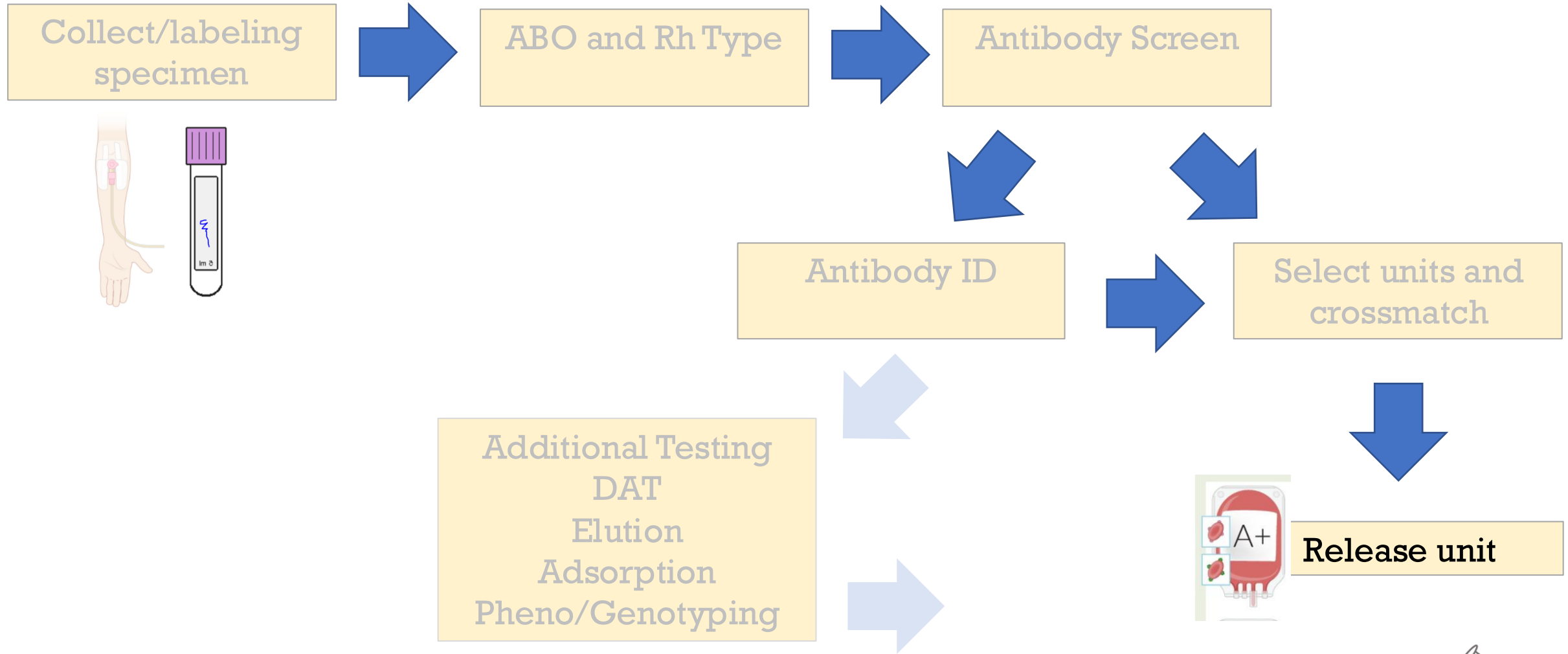
Genotyping:

- 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



Remember, Blood Bank is part of the clinical team!

Thank you for your attention!

