

# Anti-Colton(a) and MMA

## *Learning Objectives*

1. To describe a transfusion-dependent case complicated by a typical alloantibody, a high-frequency alloantibody, and a warm autoantibody.
2. To apply the MMA to deduce the hemolytic significance of each antibody in a blend.
3. To navigate the sourcing and most judicious use of red cell units from the rare blood program.



# Our Facility:



- OSMH-Medium sized community hospital—200 beds
- Hospital staff—1200
- Core Laboratory staff—13 rotational MLT's
- Blood Bank/TM—2 staff during day 8-4, off shift is covered by core lab.
  - Type and Screen: 12/day (4300 annually)
  - Serological Crossmatches: 7/day (2600 annually)
  - Antibody Identification: 1/day (400 annually)



# What's on the Menu?



- ABO and Rh Group
- Antibody Screen
- Crossmatch
- Antibody identification
- Direct Antiglobulin Test
- Elution-- including Investigation of HDN
- Antibody Titration
- Phenotyping
- Transfusion Reaction Investigations



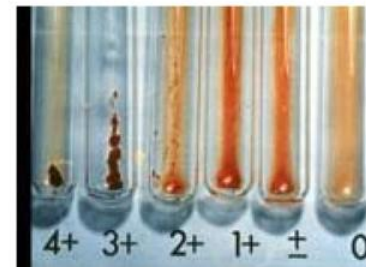
# Testing Platform(s)



- Solid phase (automated)—group, screen, initial panel investigation
- PEG/tube—all other work up/investigations including crossmatch.



&





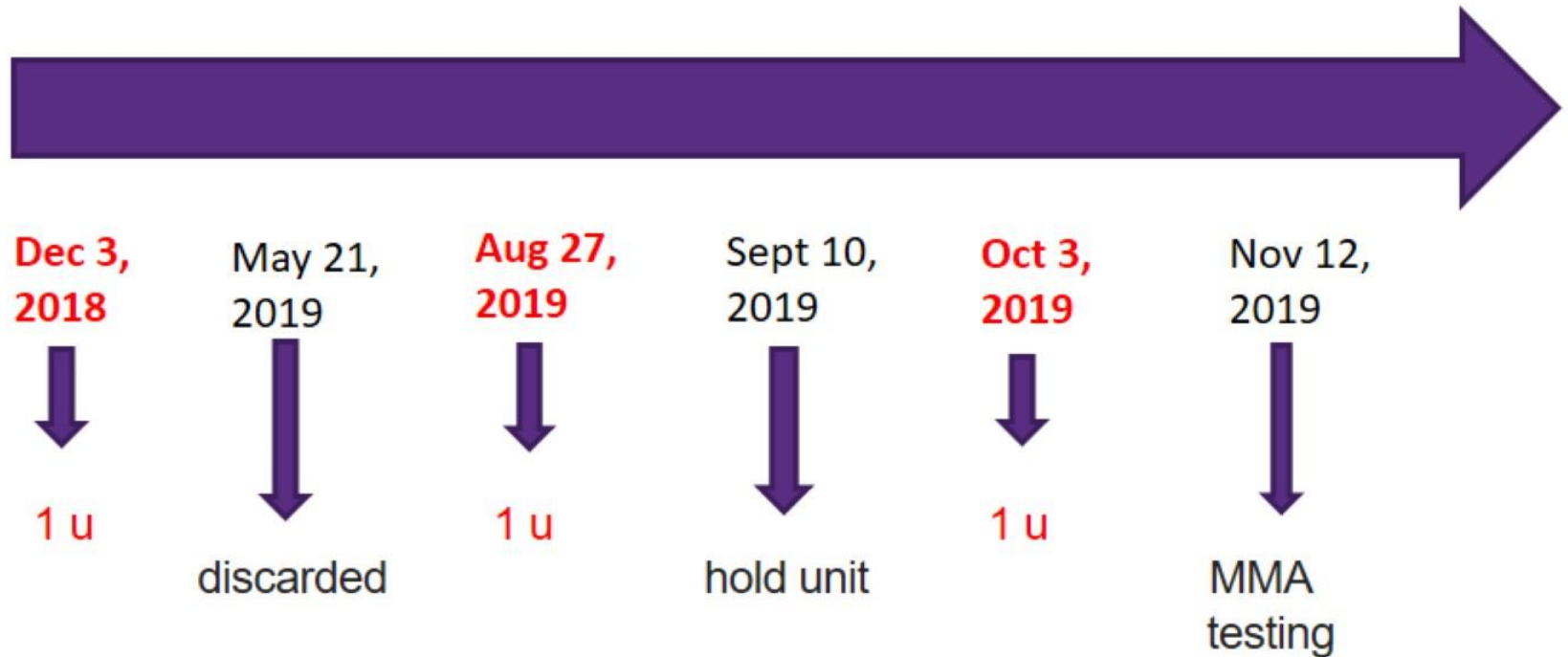
# The complicated patient



- Female in her 70's
- Presently: symptomatic, severe anemia;  
unresponsive to 25 weeks of ESA + IV Fe (>3000 mg)
- Medical History:
  - G2P2, no HDFN, no PPH
  - Diabetes
  - Wegener's granulomatosis with polyangiitis (1994) / +transfused
  - CKD on dialysis
  - Orthopedics: L TKA 2005, R TKA 2009 (+transfused)
  - Oncologic: endometrial carcinoma (2015, 2018) with combined modality treatments and radiation complications



# Timeline & Transfusions



# December 3 - Txn #1



- Outpatient Transfusion Clinic: CBC & 2 u XM (day-2)
- Serological findings:
  - B +, Ab screen & panel panreactive
  - DAT & Auto control negative
  - Hb=70, polychromasia
  - Patient knows of antibodies, difficult crossmatch, has card
  - Sample sent to CBS for identification, results received (D+3)




# Antibody Screen:



CAPTURE-R READY-SCREEN

CAPTURE-R READY-SCREEN

		CAPTURE-R READY-SCREEN (3)																				417-4													
		Master List																																	
		 R012																																	
		IMMUCOR, INC. Norcross, GA 30071 USA																																	
		US LICENSE NO: 886																																	
C E L		LOT NO: R012																																	
		EXPIRES: 2019/01/08																																	
	Donor	Rh - Hr								Kell				Duffy		Kidd		Lewis		P	MN				Luth- eran		X <sub>g</sub>								
		D	C	c	E	e	V*	C*	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 B8983	+	+	0	0	+	0	0	+	+	0	+	0	+	+	+	0	+	0	+	+	0	+	0	0	0	+	+	4						
II	R2R2 C6432	+	0	+	+	0	0	0	0	+	0	+	0	+	0	+	0	+	+	0	+	+	+	0	+	0	+	+	3						
III	rr G1731	0	0	+	0	+	0	0	+	+	0	+	0	+	+	0	+	0	0	+	+	0	+	+	+	0	+	+	4						
	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.





# Initial Panel Results:



## PANOCELL -10 Master List

IMMUCOR, INC. Norcross, GA 30071 USA  
US LICENSE NO: 886  
LOT NO: 42547  
EXPIRES: 2018/12/21

NAME \_\_\_\_\_  
NO. \_\_\_\_\_  
INSTITUTION \_\_\_\_\_  
BLOOD GROUP \_\_\_\_\_  
ANTIBODY IDENTITY \_\_\_\_\_  
TECH \_\_\_\_\_ DATE \_\_\_\_\_

LOT NO: 42547 EXPIRES: 2018/12/21			Rh - Hr								Kell								Duffy	Kidd	Lewis	P	MN					Luth- eran		Xg	PATIENTS SERUM TEST RESULTS TEST METHODS																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																							
VIAL	Special Type	Donor	D	C	c	E	e	V <sup>+</sup>	C <sup>+</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>	10																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																								



# Colton(a) Highlights:



- Occurrence—99.5% in all populations
- Alloanti-Co(a):
  - IgG with optimal reactivity at IAT
  - Can bind complement
  - Transfusion reaction;
    - No to moderate/delayed; immediate/hemolytic
- Fun Fact—Named in 1967, after the 1<sup>st</sup> producer, but misread on the tube (Calton!)

# Duffy(a) Highlights:



- Occurrence—66% Caucasians, 10% Blacks, 99% Asians, 97% Thai
- Alloanti-Fy(a):
  - IgG with optimal reactivity at IAT
  - Rare complement binding
  - Transfusion reactions;
    - Mild to severe, immediate/delayed



# Rare Blood Reality:



- Patient was informed about the rare blood program when initially identified in 2009
- Family screening was performed at that time, no compatible family member found
- Blood conservation and bone marrow stimulation became essential as transfusion support would not be sustainable.





# CBS, the Lifeline



- Report: anti-Fy(a) & anti-Co(a), nil else
- Patient must receive Co(a-), Fy(a-) red cells
- Patient phenotype:
  - Positive: C,E,c,e,s,k,Kpb,Lub,Fyb,Jka,Jkb
  - Negative: Fya, Coa, S, K
- CBS sent 1 B+, Coa-, Fya- RBC\* unit on Dec. 21.
- Serologically compatible, uneventful transfusion



# \* Rare Units from Kansas



# May 21:



- Dialysis labs sent with 2 unit RBC request
- Hemoglobin 65
- DAT=weak +, Auto Control=weak +
- Multiple discussions with Nephrologists
- (ordering nephrologist ≠ on-site nephrologist!)



# Antibody Screen:



CAPTURE-R READY-SCREEN

		CAPTURE-R READY-SCREEN (3) Master List																				417-5											
		Rh - Hr						Kell					Duffy		Kidd		Lewis		P	MN				Lutheran		Xg							
CELL	Donor	D	C	c	E	e	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 B10052	+	+	0	0	+	0	0	+	0	+	0	+	+	0	+	+	0	+	0	+	0	+	0	0	+	0	4					
II	R2R2 C6432	+	0	+	+	0	0	0	+	0	+	0	+	0	+	0	+	+	0	+	+	+	0	+	0	+	3						
III	rr G1731	0	0	+	0	+	0	+	+	0	+	0	+	+	0	+	0	0	+	+	0	+	+	+	0	+	4						
	Positive Control																																

CAPTURE-R READY-SCREEN

\* 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.



# Antibody Panel:



## CAPTURE-R READY-ID Master List

NAME \_\_\_\_\_  
NO. \_\_\_\_\_  
INSTITUTION \_\_\_\_\_  
BLOOD GROUP \_\_\_\_\_  
ANTIBODY IDENTITY \_\_\_\_\_  
TECH \_\_\_\_\_ DATE \_\_\_\_\_

IMMUCOR, INC. Norcross, GA 30071 USA

US LICENSE NO: 886

LOT NO: ID376

EXPIRES: 2019/05/28

REFERENCE NO: 000

LOT NO: ID376

EXPIRES: 2019/05/28

			Rh - Hr					Kell					Duffy		Kidd	Lewis		P	MN				Lutheran		Xg	CELL	PATIENT'S TEST RESULTS							
CELL	Special Type	Donor	D	C	c	E	e	C+	K	k	Kp+	Kp-	Js+	Js-	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>					
1	M(a+), GP.Mur	RzR1 A4485	+	+	0	+	+	0	0	+	0	+	0	+	+	0	0	+	+	W	+	+	0	0	+	0	+	+	1	4				
2		R1wR1 B9604	+	+	0	0	+	+	0	+	0	+	0	+	0	+	0	0	+	+	0	+	0	+	0	+	0	+	2	4				
3	Bg(a+)	R2R2 C4638	+	0	+	+	0	0	+	+	0	+	0	+	0	+	0	+	0	+	0	+	0	0	+	0	+	+	3	3				
4	V+, VS+	Ror D691	+	0	+	0	+	0	0	+	0	+	0	+	0	+	+	+	0	+	+	+	0	+	0	0	+	+	4	4				
5		r'r E294	0	+	+	0	+	0	0	+	0	+	0	+	0	+	+	+	0	+	0	0	+	0	+	0	+	+	5	3				
6		r'r F566	0	0	+	+	+	0	0	+	0	+	0	+	+	0	0	+	+	0	0	+	+	+	+	0	+	+	6	4				
7		rr N4251	0	0	+	0	+	0	0	+	0	+	0	+	0	+	0	+	0	+	+	+	+	0	+	0	+	+	7	3				
8		rr G1729	0	0	+	0	+	0	+	0	0	+	0	+	0	+	0	+	0	0	0	0	+	+	+	0	+	0	8	4				
9		rr H1393	0	0	+	0	+	0	0	+	0	+	0	+	+	0	+	0	0	+	0	+	0	+	0	0	+	+	9	4				
10		rr G964	0	0	+	0	+	0	+	+	0	+	0	+	+	+	0	0	+	+	+	+	0	+	+	+	+	+	10	4				
11		rr N4634	0	0	+	0	+	0	0	+	+	+	0	+	0	+	+	+	0	+	0	0	+	0	+	0	+	+	11	4				
12		rr N4386	0	0	+	0	+	0	0	+	0	+	0	+	+	W	0	+	0	+	+	0	+	0	+	0	+	0	12	4				
13		rr N4000	0	0	+	0	+	0	0	+	0	+	+	+	0	0	+	0	+	0	+	+	+	0	+	0	+	0	13	4				
14	D(a+)	R1R1 B9565	+	+	0	0	+	0	0	+	0	+	0	+	+	0	0	+	0	0	+	+	0	0	+	0	+	+	14	4				
15		POSITIVE CONTROL	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	PC	4					
16		NEGATIVE CONTROL	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	NC	0					



# Incompatible Crossmatch



- Sample resent to CBS
- Confirmed DAT + (with IgG), eluate negative
- 1 B+, Fya-, Coa- RBC\* unit sent
- Serological crossmatch with PEG/tube and Saline IAT weak positive
- Unit tested on site for DAT: negative

# Unit Discarded:



- Current Nephrologist disagrees with order
- Unit returned, no longer viable, but retested
- CBS found unit was compatible by MTS and Saline IAT
- Started IV iron and increased EPO



shutterstock.com • 63932485

# August 27



- 1 unit RBC requested
- Hemoglobin 64
- Antibody screen and panel (solid phase)= 3-4+
- DAT & Auto Control=1<sub>w</sub>
- Elution: variable reactivity 0-1+
- Extended antigen match requested due to autoantibody
- Coa-, Fya-, K-, S-



# Transfusion # 2:



- Liquid unit received
- MD signed for incompatible crossmatch
- Transfusion was uneventful, but post Hb only 69
- \*\*\*OSMH requested a guidance letter from CBS/rare blood program to forward to Nephrologists/Dialysis department



# Details of Guidance letter:



- Group B +, with anti-Coa, anti-Fya, autoantibody
- Autoantibody: unlikely to cause hemolysis, but affects compatibility testing
- CBS procured the solitary unit in database from the American rare donor program
- Reiterated that transfusion support could not be maintained

# Suggestions:



1. Hematology consult
2. Limiting blood loss
3. Ensure all aware of blood scarcity

\*\*\*Unit was kept 'on hold' at CBS until the need was considered greatest

# October 3



- 1 unit RBC requested for Hb 54
- Patient aware of unit and its limitations (NOT phenotype specific/unable to phenotype)
- Antibody reactivity unchanged
- RBC\* unit given 2 days later
- Uneventful transfusion with good long lasting increment (Oct 24 Hb 73!)



# Toronto Consultation



- B+ D+ C+e+ c+E+ K- Jk(a+b+) Fy(<sup>33%</sup>a-b+) S- Coa-<sup>0.2%</sup>  
R<sub>1</sub>R<sub>2</sub>, ie- f-  
↓ ↓  
Anti-Fya & Anti-Coa since 2009  
?WAA (iXM despite Fya- Coa- RBCs)

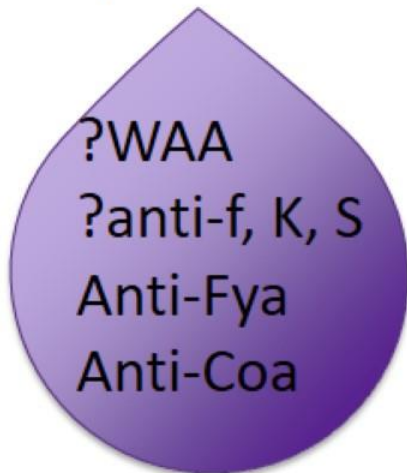
## Questions:

1. Have the other alloantibody possibilities (anti-f, anti-K, anti-S) been ruled out? *(Some Fya-Coa- units performed worse than others...)*
2. How hemolytic is the WAA? *(Does it warrant treating for PBM sake?)*
3. How hemolytic are the alloantibodies? *(Can Fya-/Coa- be waived?)*

# Interrogation Plan



Patient serum  
containing a blend  
of antibodies of  
unknown clinical  
importance



X

5 Informative RBC surfaces



Self RBCs  
(autocontrol)



Positive (ant-D R<sub>2</sub>R<sub>2</sub>)  
Control



Fya+ Coa-



Fya- Coa+



Fya- Coa- \*

X

“ Phagocytosis  
Indicators ”:

A

Monocyte  
Monolayer  
Assay...

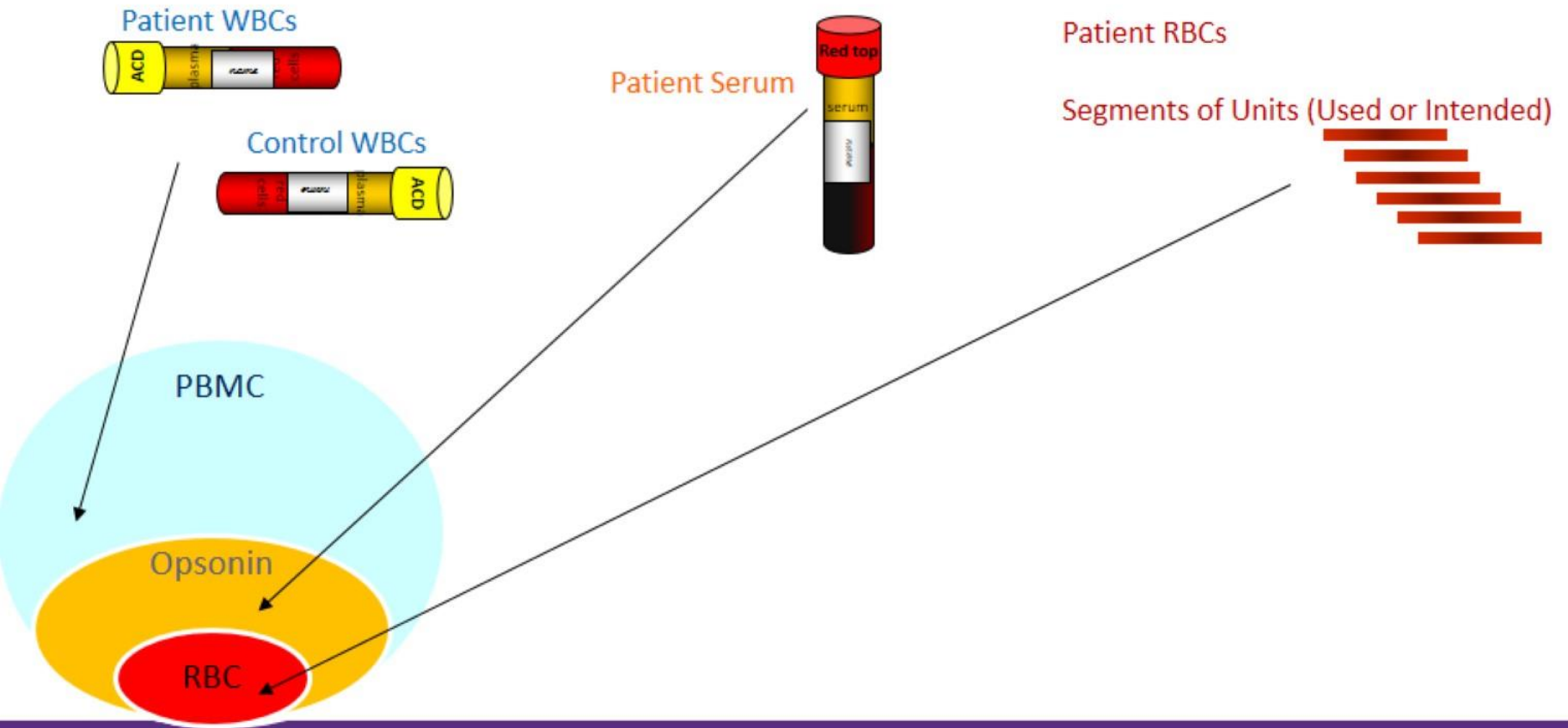


Normal control  
monocytes  
(relaxed)



Patient  
monocytes  
(?activated?)

# MMA Materials: Red Stuff, Yellow Stuff, White Stuff

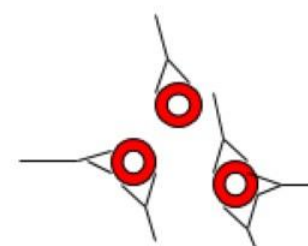
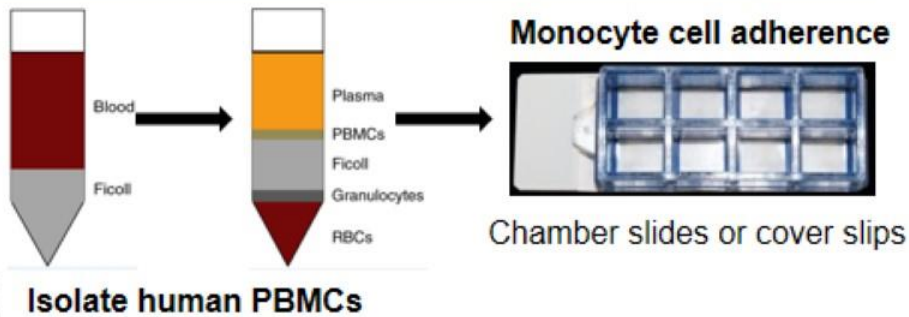


# The Mechanics of the MMA

The White Stuff

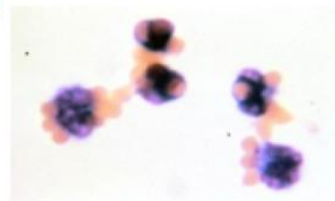
The Yellow Stuff

The Red Stuff



Count phagocytosed RBCs

#RBC phagocytosed/  
100 monocytes

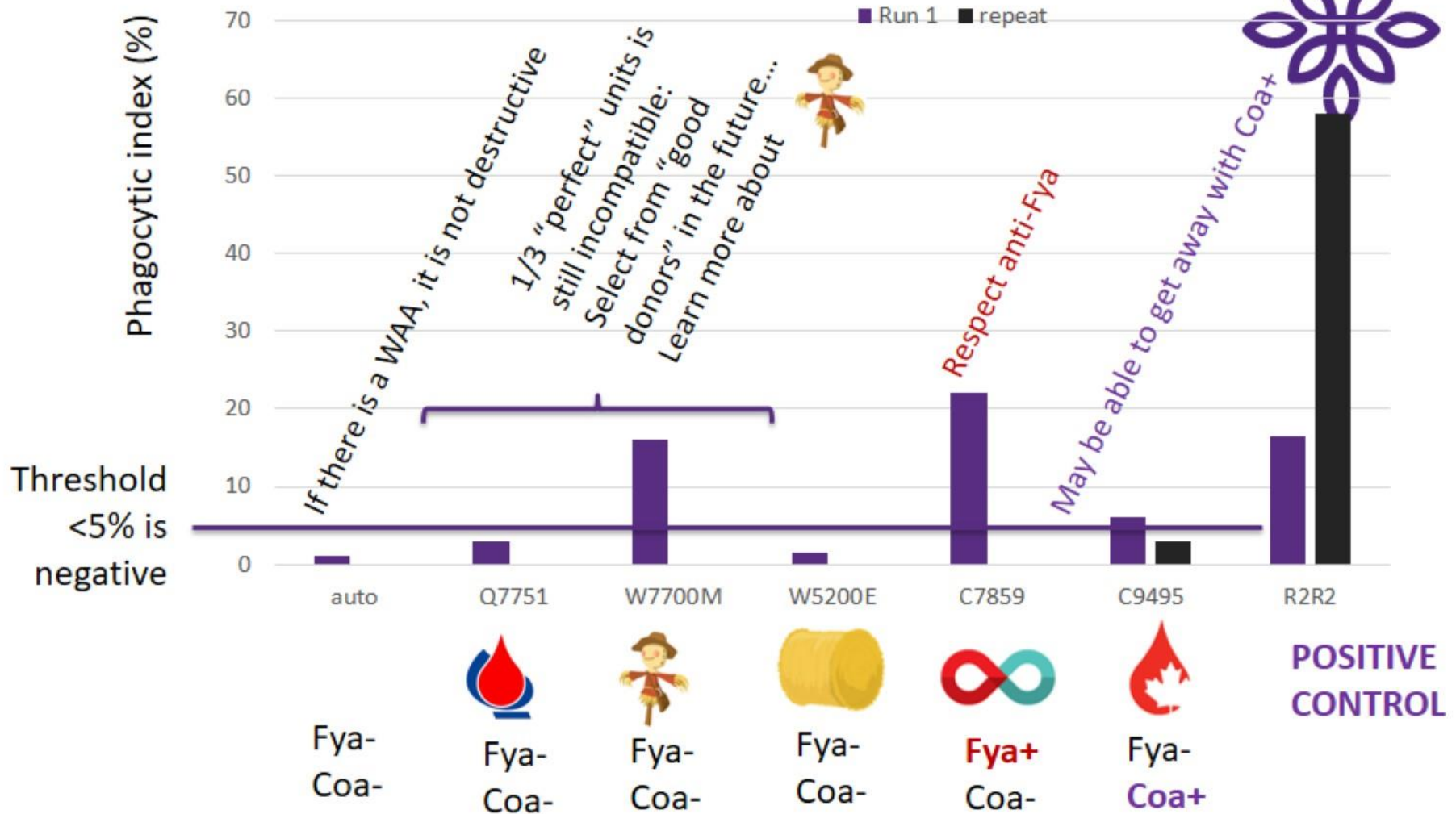


Adapted from Dr Don Branch





# What Was Tested & Seen



# Questions



1. Had Scarecrow units ever been transfused /  
Was the increment poor?  
Was Scarecrow positive for K, f, or S?



2. Were the HQ or haybale units ever transfused /  
Were the increments good?  
Were these donors all K- f- S-?



3. Was Coa+ blood ever transfused /  
Was it well-tolerated?

# In Summary



- Three RBC txns over 10 months
- Communication with care providers
- Tireless efforts by CBS/rare blood program
- Implications of MMA results for future txn's?

# Questions ???????





