

Immunohematology Reference Laboratory Case Studies

*Connecting our Donors to Patients
since 1974*

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Objectives

- 1) Describe the technical steps involved in case #1 workup
- 2) Apply the basic principles of identifying a complex alloantibody in your own laboratory
- 3) List the benefits of molecular typing for unique patient populations with complex antibody issues

Case Study #1:

Patient Demographics:

- 37 year old female
- African American
- Transfusion History: Unknown
- Diagnosis: GI Bleed, Hypertension, Vomiting
- Patient is now unresponsive



Case Study #1:

Blood Type: O Negative

Antibody Screen: Positive

DAT: Positive



Case Study #1

Antibody Screen:																								PeG							
Cell	Rh Type	Rh-hr						Kell						Duffy		Lutheran		Kidd		MNS				P	Lewis		Xg	IS	Ig G	CC	
		D	C	E	c	e	Cw	V	K	k	Kp a	Kp b	Js a	Js b	Fya	Fyb	Lu a	Lu b	Jk a	Jk b	M	N	S	s	P1	Le a	Le b				Xg a
1	R1 R1	+	+	0	0	+	+	0	0	+	0	+	0	+	+	0	+	+	0	+	0	+	0	+	0	+	+	+	1	0	2+
2	R2 R2	+	0	+	+	0	0	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	+	0	+	+	2	0	3+
3	rr	0	0	0	+	+	0	+	+	+	0	+	0	+	0	+	+	0	+	+	+	+	+	+	+	0	0	3	0	2+	
Patient Cells																												A C	0	3+	

DAT Results:

Poly AHG	Anti-IgG	Anti-C3'
2+	2+	Negative

Case Study #1

Neat Plasma / Neat Panel

	D	C	E	c	e	f	M	N	S	s	P1	Le ^a	Le ^b	K	k	Kp ^b	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	(PeG) IgG	CC	
1	0	+	0	+	+	+	+	+	+	0	+	0	0	0	+	+	+	0	0	+	0	0	+	
2	+	+	0	0	+	0	+	+	+	+	0	0	+	0	+	+	+	0	+	+	+	+	4+	
3	+	+	0	0	+	0	+	+	0	+	+	+	0	0	+	+	+	+	+	+	+	+	4+	
4	+	0	+	+	0	0	+	+	0	+	+	0	+	+	+	+	+	+	+	+	+	+	2+	
5	0	0	+	+	+	+	0	+	0	0	0	0	+	0	+	+	+	0	+	+	+	+	3+	
6	0	0	0	+	+	+	0	+	+	+	+	0	+	0	+	+	+	0	0	+	0	0	0	+
7	0	0	0	+	+	+	0	+	0	+	+	0	+	+	0	+	+	0	+	0	+	0	4+	
8	0	0	0	+	+	+	+	0	+	0	+	+	0	0	+	+	+	0	+	+	+	0	4+	
9	0	0	0	+	+	+	+	0	+	+	+	+	0	0	+	+	+	+	0	0	0	+	4+	
10	0	0	0	+	+	+	+	+	+	+	+	+	0	+	+	+	+	0	+	0	0	+	4+	
AC																							3+	

Case Study #1

Neat Plasma / Neat Panel

	D	C	E	c	e	f	M	N	S	s	PI	Le ^a	Le ^b	K	k	Kp ^b	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	(PeG) IgG	CC	
1	+	0	0	+	+	+	+	+	+	0	+	0	0	0	+	+	+	0	0	+	0	0	+	
2	+	+	0	0	+	0	+	+	+	+	0	0	+	0	+	+	+	0	+	+	+	+	4+	
3	+	+	0	0	+	0	+	+	0	+	+	+	0	0	+	+	+	+	+	+	+	+	4+	
4	+	0	+	+	0	0	+	+	0	+	+	0	+	+	+	+	+	+	+	+	+	+	2+	
5	0	0	+	+	+	+	0	+	0	0	0	0	+	0	+	+	+	0	+	+	+	+	3+	
6	0	0	0	+	+	+	0	+	+	+	+	0	+	0	+	+	+	0	0	+	0	0	0	+
7	0	0	0	+	+	+	0	+	0	+	+	0	+	+	0	+	+	0	+	0	0	+	4+	
8	0	0	0	+	+	+	+	0	+	0	+	+	0	0	+	+	+	0	+	+	0	0	4+	
9	0	0	0	+	+	+	+	0	+	+	+	+	0	0	+	+	+	+	0	0	0	+	4+	
10	0	0	0	+	+	+	+	+	+	+	+	+	0	+	+	+	+	0	+	0	0	+	4+	
AC																							3+	

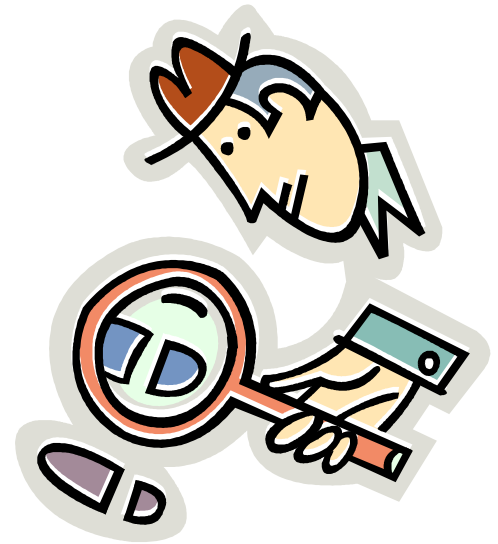
Case Study #1

Neat Plasma / Ficin-Treated Panel:

	D	C	E	c	e	f	M	N	S	s	P1	Le ^a	Le ^b	K	k	Kp ^b	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	(PeG) IgG	CC
1	0	+	0	+	+	+	+	+	+	0	+	0	0	0	+	+	+	0	0	+	0	0	+
2	+	+	0	0	+	0	+	+	+	+	0	0	+	0	+	+	+	0	+	+	+	+	4+
3	+	+	0	0	+	0	+	+	0	+	+	+	0	0	+	+	+	+	+	+	+	+	4+
4	+	0	+	+	0	0	+	+	0	+	+	0	+	+	+	+	+	+	+	+	+	+	2+
5	0	0	+	+	+	+	0	+	0	0	0	0	+	0	+	+	+	0	+	+	+	+	3+
6	0	0	0	+	+	+	0	+	+	+	+	0	+	0	+	+	+	0	0	+	0	0	0
7	0	0	0	+	+	+	0	+	0	+	+	0	+	+	0	+	+	0	+	0	+	+	4+
8	0	0	0	+	+	+	+	0	+	0	+	+	0	0	+	+	+	0	+	+	0	+	4+
9	0	0	0	+	+	+	+	0	+	+	+	+	0	0	+	+	+	+	0	0	+	+	4+
10	0	0	0	+	+	+	+	+	+	+	+	+	0	+	+	+	+	0	+	0	+	+	4+
AC																							3+

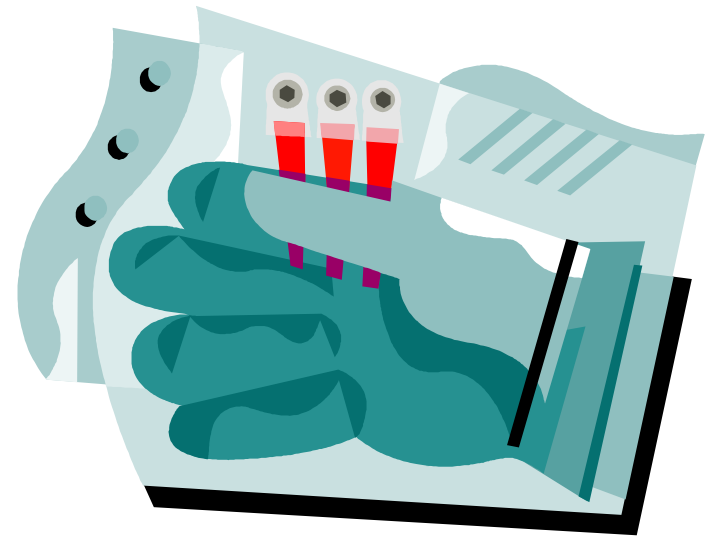
Further Investigation

- Transfusion History?
 - Transfusion history is still unknown, patient unresponsive, no family members
- HgB S status?
 - Patient is negative for Hemoglobin S



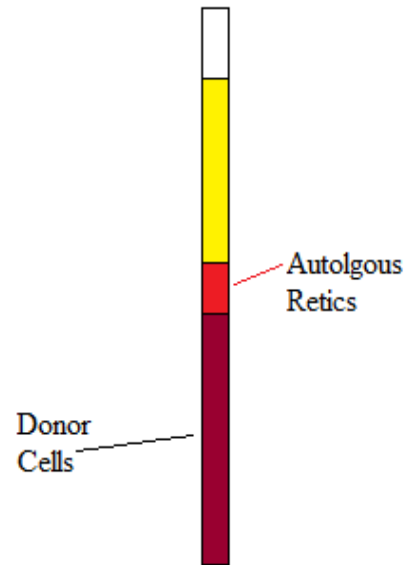
Phenotype Patient RBCs

- Microhematocrit centrifugation performed
 - Harvest patient autologous reticulocytes
 - Separate out the donor red cells

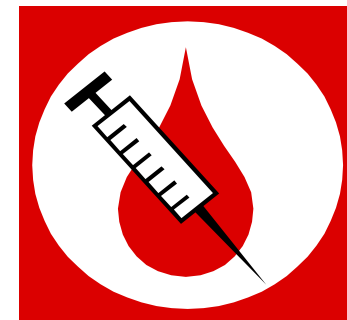


Microhematocrit Separation

- Autologous Retics have less weight than donor cells
- Usually constitute the first 5 mm of the column
- Heavily transfused patients can be less than 3mm



Phenotyping



- Post-Harvest Autologous Retics
- DAT Results: Negative

Poly AHG 0	Anti-IgG 0
Negative	Negative

Serological Phenotype Results

C	E	c	e	K1	Fy ^a	Fy ^b	Jk ^a	Jk ^b	S	s	M	N
0	0	+	+	0	0	0	+	+	+	+	0	+

- Patient is rr
- Negative for K1, both Fy^a and Fy^b, and M
- 68% of Blacks phenotype Fy(a-b-)

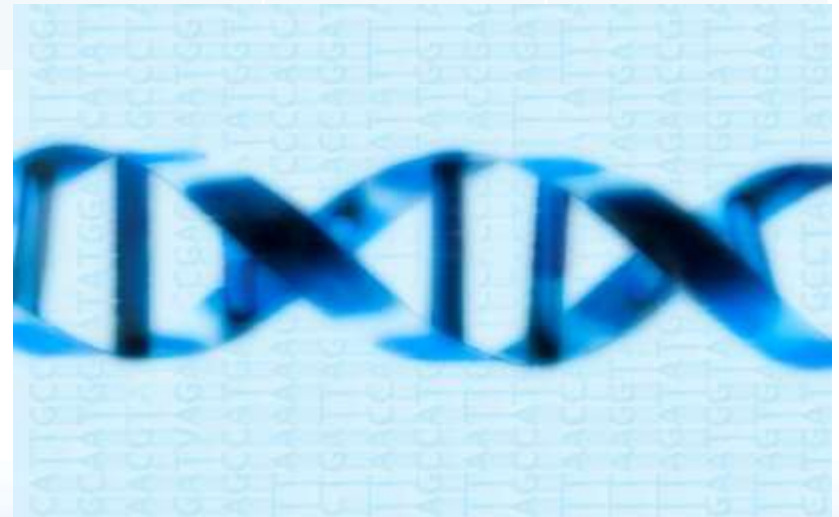
Genotype Results

Molecular Testing by Bioarray

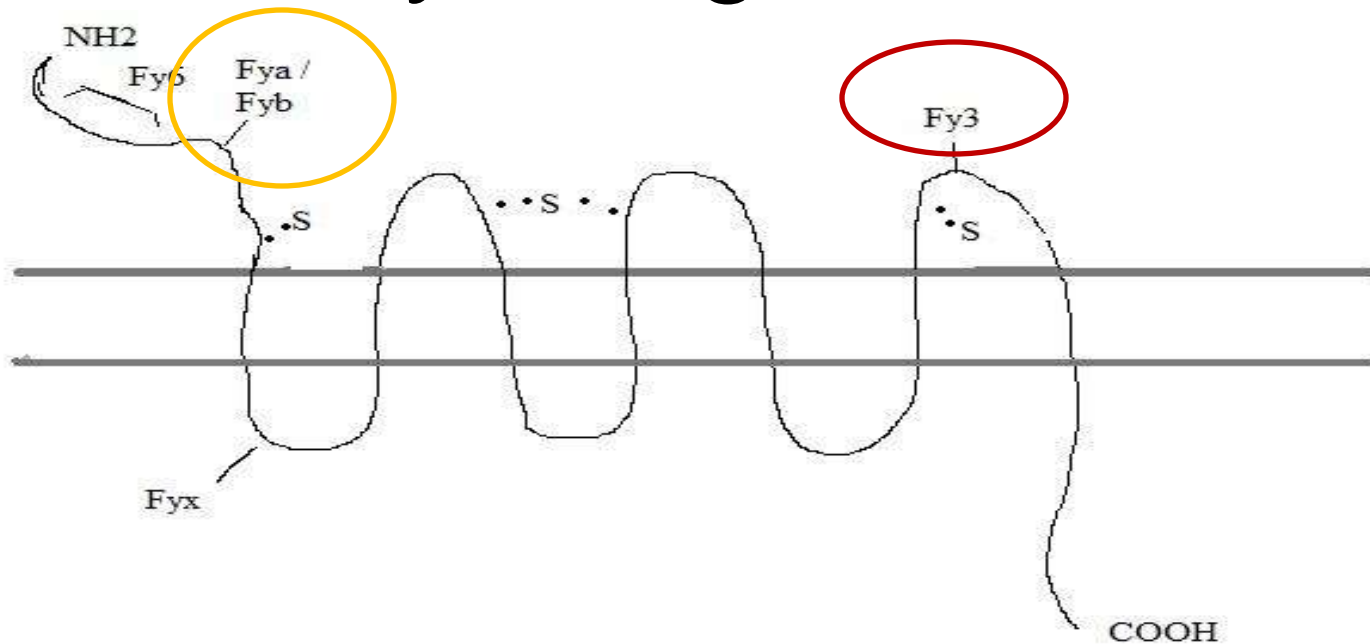
- Genotype vs. Phenotype results
- **Fy^a negative** *genetically and serologically*
- **Fy^b positive** *genetically*
- **Fy^b negative** **serologically**

Genetically Fy^b Positive

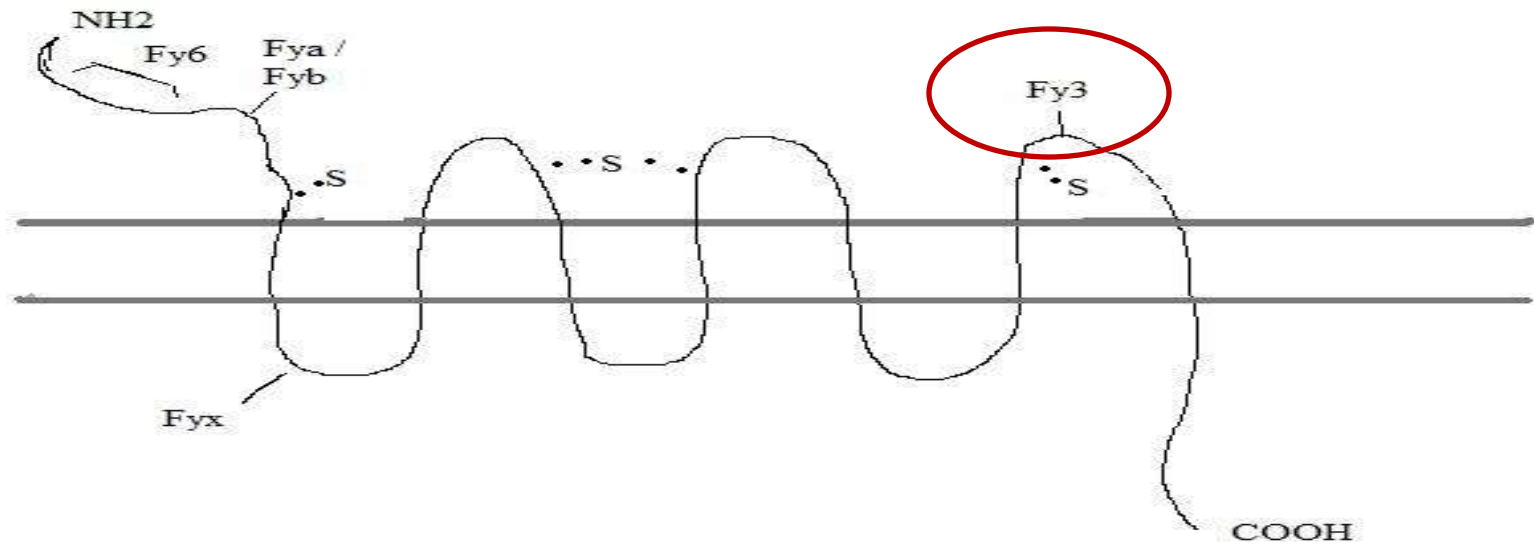
Fy	GATA	
BB	BB	



Duffy Antigen Structure



- Action of enzymes removes Fy^a or Fy^b from surface of red cells.
- Fy3 antigen is separate protein, resistant to enzyme treatment.



- The main alleles FYA and FYB differ by a single nucleotide at position 125 (G and A, respectively)
 - Encodes Fy^a and Fy^b antigens that differ by a single amino acid at residue 42 (glycine and aspartic acid, respectively)
- Most common mutation causing a Duffy negative phenotype is a point mutation in the erythroid specific promoter (a T -> C mutation at position -33 in the **GATA box**)
 - African Americans (70%) and West Africans (approaching 100%)

Fy^b GATA Mutation

Inheritance Patterns

- Found in majority of patients with Sickle Cell Disease

Action of mutation on gene promoter

- Prevents the Fy^b antigen from expression on surface of Red Blood Cells
- Patients have Fy^b antigen present on other tissues
- Patients cannot develop Alloanti- Fy^b

Case Study #1

What was the cause of positive results in the original DAT?

What is coating donor cells?



****Perform **Eluate** to conclude investigation****

Case Study #1

Eluate
Screen:

																											Elution					
Cell	Rh Type	Rh-hr							Kell						Duffy		Lutheran		Kidd		MNS				P	Lewis		Xg	Elu	LW IgG	LW IgG	LW C C
		D	C	E	c	e	Cw	V	K	k	Kp a	Kp b	Js a	Js b	Fy a	Fy b	Lu a	Lu b	Jk a	Jk b	M	N	S	s	P 1	Le a	Le b	Xg a				
1	R1R1	+	+	0	0	+	+	0	0	+	0	+	0	+	+	0	+	+	0	0	+	0	+	0	+	0	+	+	1	4+	0	+
2	R2R2	+	0	+	+	0	0	0	0	+	0	+	0	0	+	0	+	0	+	0	+	0	+	+	0	+	+	2	4+	0	+	
3	rr	0	0	0	+	+	0	+	+	+	0	+	0	+	0	+	+	0	+	+	+	+	+	+	+	0	0	3	4+	0	+	

Case Study #1

Eluate Panel:

	D	C	E	c	e	f	M	N	S	s	P1	Le ^a	Le ^b	K	k	Kp ^b	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	IgG	CC	
1	0	+	0	+	+	+	+	+	+	0	+	0	0	0	+	+	+	0	0	+	0	0	+	
2	+	+	0	0	+	0	+	+	+	+	0	0	+	0	+	+	+	0	+	+	+	+	4+	
3	+	+	0	0	+	0	+	+	0	+	+	+	0	0	+	+	+	+	+	+	+	+	4+	
4	+	0	+	+	0	0	+	+	0	+	+	0	+	+	+	+	+	+	+	+	+	+	4+	
5	0	0	+	+	+	+	0	+	0	0	0	0	+	0	+	+	+	0	+	+	+	+	4+	
6	0	0	0	+	+	+	0	+	+	+	+	0	+	0	+	+	+	0	0	+	0	0	0	+
7	0	0	0	+	+	+	0	+	0	+	+	0	+	+	0	+	+	0	+	0	0	+	4+	
8	0	0	0	+	+	+	+	0	+	0	+	+	0	0	+	+	+	0	+	+	+	0	4+	
9	0	0	0	+	+	+	+	0	+	+	+	+	0	0	+	+	+	+	0	0	0	+	4+	
10	0	0	0	+	+	+	+	+	+	+	+	+	0	+	+	+	+	0	+	0	0	+	4+	
AC																							NT	

Autoantibody Reattachment? NO!

	Eluate	Neat Plasma
Autologous Reticulocytes	Negative	Negative

EXCLUDES presence of Warm autoantibody!

Conclusion

- Patient has developed Alloanti-Fy3 and is experiencing a delayed HTR
 - **Very Rare to have the erythroid mutation and make anti-Fy3**
 - **Fy3:** 100% Caucasians, 32% Blacks, 99.9% Asians
 - Anti-Fy3 typically a moderate, delayed transfusion reaction and are uncommon causes of mild HDN.



Case Study #1

Neat Plasma / Neat Panel

	D	C	E	c	e	f	M	N	S	s	P1	Le ^a	Le ^b	K	k	Kp ^b	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	(PeG) IgG	CC	
1	0	+	0	+	+	+	+	+	+	0	+	0	0	0	+	+	+	0	0	+	0	0	+	
2	+	+	0	0	+	0	+	+	+	+	0	0	+	0	+	+	+	0	+	+	+	+	4+	
3	+	+	0	0	+	0	+	+	0	+	+	+	0	0	+	+	+	+	+	+	+	+	4+	
4	+	0	+	+	0	0	+	+	0	+	+	0	+	+	+	+	+	+	+	+	+	+	2+	
5	0	0	+	+	+	+	0	+	0	0	0	0	+	0	+	+	+	0	+	+	+	+	3+	
6	0	0	0	+	+	+	0	+	+	+	+	0	+	0	+	+	+	0	0	+	0	0	0	+
7	0	0	0	+	+	+	0	+	0	+	+	0	+	+	0	+	+	0	+	0	+	+	4+	
8	0	0	0	+	+	+	+	0	+	0	+	+	0	0	+	+	+	0	+	+	0	0	4+	
9	0	0	0	+	+	+	+	0	+	+	+	+	0	0	+	+	+	+	0	0	+	+	4+	
10	0	0	0	+	+	+	+	+	+	+	+	+	0	+	+	+	+	0	+	0	+	+	4+	
AC																							3+	

CASE #2



Case Study #2:

Patient Demographics:

- 16 year old female
- African American
- Transfusion History: Multiple units
- Diagnosis: Sickle Cell Anemia
- Patient is undergoing RBC exchange almost every 6 weeks, with increasing frequency



Case Study #2

Blood Type: B Negative

Antibody Screen: Positive

DAT: Positive

Ab History: WAA, Anti-E, Anti-S



Case Study #2

Antibody Screen:																								PeG								
Cell	Rh Type	Rh-hr						Kell						Duffy		Lutheran		Kidd		MNS				P	Lewis		Xg		IS	Ig G	CC	
		D	C	E	c	e	C w	V	K	k	Kp a	Kp b	Js a	Js b	Fya	Fyb	Lu a	Lu b	Jk a	Jk b	M	N	S	s	P1	Le a	Le b	Xg a				
1	R1 R1	+	+	0	0	+	+	0	0	+	0	+	0	+	+	0	+	+	0	+	0	+	0	+	0	+	0	+	+	1	0	3+
2	R2 R2	+	0	+	+	0	0	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	+	0	+	+	2	0	W+	
3	rr	0	0	0	+	+	0	+	+	+	0	+	0	+	0	0	+	+	0	+	+	+	+	+	+	0	0	3	0	W+		
Patient Cells																												A C	0	3+		

DAT Results:

Poly AHG	Anti-IgG	Anti-C3'
2+	2+	Negative

Case Study #2

Neat Plasma / Neat Panel

	D	C	E	c	e	f	M	N	S	s	PI	Le ^a	Le ^b	K	k	Kp ^b	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	(PeG) IgG	CC	
1	0	+	0	+	+	+	+	+	+	0	+	0	0	0	+	+	+	0	0	+	0	3+		
2	+	+	0	0	+	0	+	+	+	+	0	0	+	0	+	+	+	0	+	+	+	+	4+	
3	+	+	0	0	+	0	+	+	0	+	+	+	0	0	+	+	+	+	+	+	+	+	4+	
4	+	+	+	+	0	0	+	+	0	+	+	0	+	+	+	+	+	+	+	+	+	+	3+	
5	+	+	+	+	+	0	0	+	0	0	0	0	+	0	+	+	+	0	+	+	+	+	3+	
6	0	0	0	+	+	+	0	+	+	+	+	0	+	0	+	+	+	0	0	+	0	W+		
7	0	0	0	+	+	+	0	+	0	+	+	0	+	+	0	+	+	0	+	0	+	0	+	+
8	0	0	0	+	+	+	+	0	+	0	+	+	0	0	+	+	+	0	+	+	0	W+		
9	0	0	0	+	+	+	+	0	+	+	+	+	0	0	+	+	+	+	0	0	+	W+		
10	0	0	0	+	+	+	+	+	+	+	+	+	0	+	+	+	+	0	+	0	+	W+		
AC																							3+	

Obtain Phenotype

- Next Logical Step
- Hypotonic Wash
- Post Separation DAT:

IgG
Negative

Serological Phenotype

- Serological Phenotype results:

C	E	c	e	K1	k	Fy ^a	Fy ^b	Jk ^a	Jk ^b	S	s	M	N
+	0	+	+	+	+	+	0	+	+	0	+	+	+

Case Study #2

Neat Plasma / Selected Cell Panel

	D	C	E	c	e	f	M	N	S	s	P1	Le ^a	Le ^b	K	k	Kp ^b	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	(PeG) IgG	CC	
1	+	0	0	+	+	+	+	+	0	0	+	0	0	0	+	+	+	0	0	+	0	0	+	
2	+	+	0	0	+	0	+	+	0	+	0	0	+	0	+	+	+	0	+	+	+	+	4+	
3	+	+	0	0	+	0	+	+	0	+	+	+	0	0	+	+	+	+	+	+	+	+	4+	
4	+	0	+	+	0	0	+	+	0	+	+	0	+	+	+	+	+	+	+	+	+	+	0	+
5	0	+	+	+	+	+	0	+	0	0	0	0	+	0	+	+	+	0	+	+	+	+	3+	
6	0	+	0	+	+	+	0	+	+	0	+	0	+	0	+	+	+	0	0	+	0	+	2+	
7	0	0	0	+	+	+	0	+	0	+	+	0	+	+	0	+	+	0	+	0	+	+	0	+
8	0	0	0	+	+	+	+	0	+	0	+	+	0	0	+	+	+	0	+	+	0	+	2+	
9	0	0	0	+	+	+	+	0	0	+	+	+	0	0	+	+	+	+	0	0	+	+	0	+
10	0	0	0	+	+	+	+	+	0	+	+	+	0	+	+	+	+	0	+	0	+	+	0	+
Retics																							0	+

- The autologous control with retics is negative
- Alloanti-E is not present in current sample
- Alloanti-S is still reactive
- WAA is not demonstrating

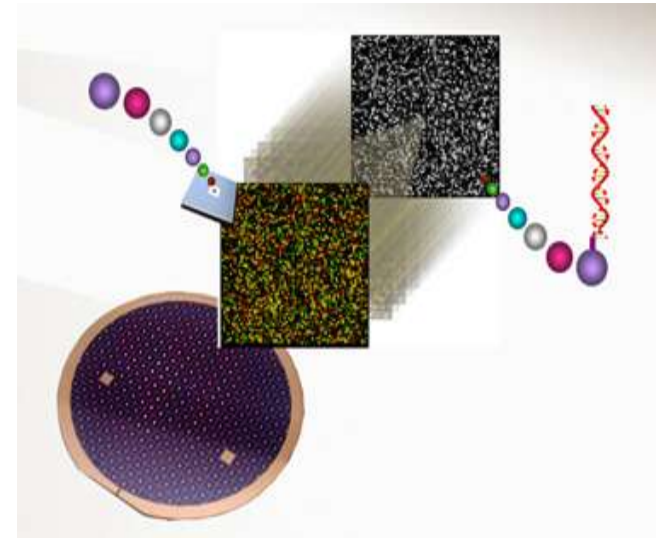
Autoanti-C?

	D	C	E	c	e	f	M	N	S	s	P1	Le ^a	Le ^b	K	k	Kp ^b	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	(PeG) IgG	CC	
1	+	0	0	+	+	+	+	+	0	0	+	0	0	0	+	+	+	0	0	+	0	0	+	
2	+	+	0	0	+	0	+	+	0	+	0	0	+	0	+	+	+	0	+	+	+	+	4+	
3	+	+	0	0	+	0	+	+	0	+	+	+	0	0	+	+	+	+	+	+	+	+	4+	
4	+	0	+	+	0	0	+	+	0	+	+	0	+	+	+	+	+	+	+	+	+	+	0	+
5	0	+	+	+	+	+	0	+	0	0	0	0	+	0	+	+	+	0	+	+	+	+	3+	
6	0	+	0	+	+	+	0	+	+	0	+	0	+	0	+	+	+	0	0	+	0	0	2+	
7	0	0	0	+	+	+	0	+	0	+	+	0	+	+	0	+	+	0	+	0	+	+	0	+
8	0	0	0	+	+	+	+	0	+	0	+	+	0	0	+	+	+	0	+	+	0	0	2+	
9	0	0	0	+	+	+	+	0	0	+	+	+	0	0	+	+	+	+	0	0	0	+	0	+
10	0	0	0	+	+	+	+	+	0	+	+	+	0	+	+	+	+	0	+	0	0	+	0	+
Retics																							0	+

- Patient appears to have developed Anti-C
- Auto or allo?

Molecular HEA typing

- Patient DNA extracted for HEA typing
- More Accurate
- Reflects genotype versus phenotype
- Helpful in detection of gene silencers in MNSs, Duffy Systems
- May also detect partial inheritance of some blood groups



HEA phenotype

- C antigen typing shows patient is r^s
- Patient is genetically C “negative”
- Phenotypes serologically as C positive

- Patient is Genetically Fy^b positive
- Patient is positive for GATA mutation for Fy^b promoter

r's

- Gene is rare in Caucasians but not infrequent in Blacks
- Hybrid gene, with Rh C exons inserted into the D gene, Causing Weak Expression
- Patient has inherited the ability to make a form of C antigen
- Does not possess the complete antigen
- C antigen in r's is detectable with modern monoclonal Anti-C reagents (results ranging from w+ to 2+)
- Can make alloantibodies to components of C antigen

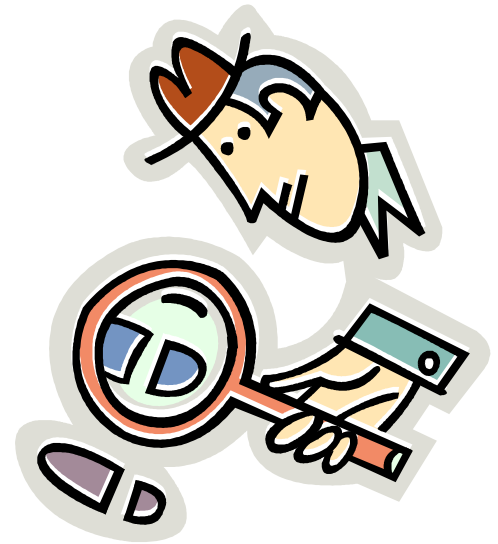
Case Study #2

Eluate Panel:

	D	C	E	c	e	f	M	N	S	s	Pl	Le ^a	Le ^b	K	k	Kp ^b	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	(PeG) IgG	CC	
1	0	+	0	+	+	+	+	+	+	0	+	0	0	0	+	+	+	0	0	+	0	4+		
2	+	+	0	0	+	0	+	+	+	+	0	0	+	0	+	+	+	0	+	+	+	+	4+	
3	+	+	0	0	+	0	+	+	0	+	+	+	0	0	+	+	+	+	+	+	+	+	4+	
4	+	0	+	+	0	0	+	+	0	+	+	0	+	+	+	+	+	+	+	+	+	+	0	+
5	0	0	+	+	+	+	0	+	0	0	0	0	+	0	+	+	+	0	+	+	+	+	0	+
6	0	0	0	+	+	+	0	+	+	+	+	0	+	0	+	+	+	0	0	+	0	0	0	+
7	0	0	0	+	+	+	0	+	0	+	+	0	+	+	0	+	+	0	+	0	+	0	0	+
8	0	0	0	+	+	+	+	0	+	0	+	+	0	0	+	+	+	0	+	+	0	0	0	+
9	0	0	0	+	+	+	+	0	+	+	+	+	0	0	+	+	+	+	0	0	+	0	0	+
10	0	0	0	+	+	+	+	+	+	+	+	+	0	+	+	+	+	0	+	0	+	0	0	+
AC																								

Further Investigation

- Transfusion History?
 - Patient was being transfused with “matched units”
 - Some units were C positive
 - Explains lack of response to units post-exchange
 - Patient experiencing delayed HTR after RBC exchange
 - Original presence of WAA is questionable



Transfusion Recommendations

- Transfuse C negative Units
- Patient can receive Fy^b positive units without fear of developing Alloanti-Fy^b
- GATA positive
- Fy^a positive, so no risk of developing Anti-Fy³

Questions?



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