

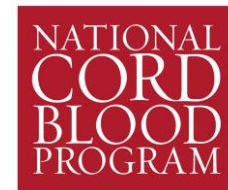
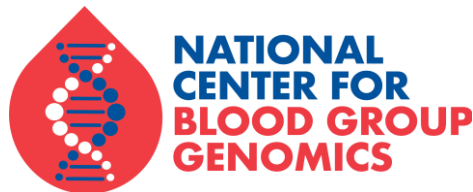


Danger on the Bunny Trail: The Many Paths to Antibody Identification

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Case Background



- 68 year old Caucasian female
- Diagnosis: Multiple Myeloma
- Hgb 6 g/dL
- Last transfused approximately 2 months (50 days) ago, at that time antibody screen was negative
- Submitting facility reports the patient currently types as A Pos & all cells tested in Gel are 2+
- Sample submitted to IRL for antibody identification & 1 unit for transfusion ASAP

ABO/Rh

ABO Group					Rh Type	
	Anti-A	Anti-B	A ₁ Cells	B Cells	Anti-D	Control
IS	4+ ^{mf}	0	0	1+ ^w	3+ ^{mf}	0

Causes of Mixed Field Reactivity:

- ABO Subgroup
- Recent Transfusion
- Transplantation
- Fetomaternal hemorrhage
- Chimerism



Submitting facility confirms transfusion of 2 O Pos, 2 O Neg, 1 A Neg RBCs

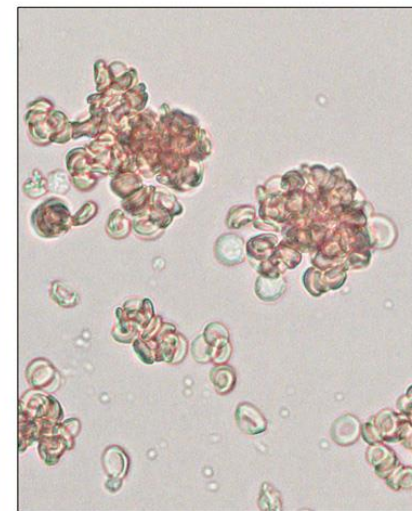
ABO Group							
	Anti-A	Anti-B	A ₁ Cells	A ₂ Cells	B Cells	O Cells	Auto
IS	4+ ^{mf}	0	0	0	1+ ^w	0	0
20'RT			r ^R	r ^R	4+ ^R	1+ ^{wR}	0 ^R
Saline Replacement			(0)	(0)	2+	(0)	(0)

Causes of Weak/missing serum reactivity:

- Age related (<4-6 months or elderly)
- ABO Subgroup
- Hypogammaglobulinemia
- Transplantation
- Prozone Effect
- Hemodilution



Rouleaux



Agglutination

wet mount
(undiluted)

<http://eclinpath.com/atlas/hematology/rbc-morphology/>

Direct Antiglobulin Test

Poly	IgG	C'	Saline
(+)	(+ ^w)	(0)✓	(0)

		Rh					Kell		Duffy		Kidd		MNS				Acid Eluate Results
		D	C	E	c	e	K	k	Fy ^a	Fy ^b	Jk ^a	Jk ^b	M	N	S	s	PEG IAT
1	R ₁ R ₁	+	+	0	0	+	+	+	+	+	+	0	+	0	+	0	3+
2	R ₂ R ₂	+	0	+	+	0	0	+	0	+	0	+	+	+	+	+	3+
3	rr	0	0	0	+	+	+	+	0	+	+	+	+	0	0	+	3+
4	rr	0	0	0	+	+	0	+	+	0	0	+	+	+	+	+	3+
5	rr	0	0	0	+	+	0	+	0	0	+	0	0	+	+	0	3+
6	R ₁ R ₁	+	+	0	0	+	0	+	0	+	0	+	0	0	0	+	3+
7	R ₂ R ₂	+	0	+	+	0	+	+	0	+	+	0	+	0	+	0	3+
8	rr	0	0	0	+	+	0	+	+	0	+	0	0	+	0	+	3+

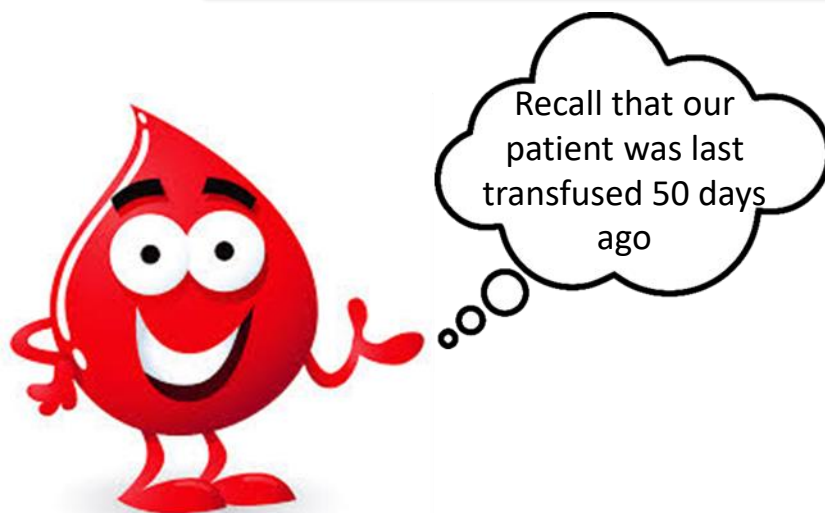
Eluate Interpretation



Causes of panreactive eluate:

- Autoantibody
- Antibody to a high-prevalence antigen

Need to test our patient's autologous cells against eluate...



Obtaining Autologous RBCs



How to separate transfused cells from autologous cells:

- Microhematocrit centrifugation
 - ≥ 3 days since last transfusion
 - Sample < 24 hours old
 - Drawn in EDTA
- Hypotonic saline wash
 - Sick cells containing hemoglobin SS are resistant to lysis

Unfortunately...

- Limited sample submitted for workup
- Only serum tube remains after eluate preparation
- Submitting hospital is 2 hours away
- Outpatient returning for transfusion the next day
- 6 g/dL hemoglobin



So, what's in the plasma?

		Rh					Kell		Duffy		Kidd		MNS				Plasma Results		
		D	C	E	c	e	K	k	Fy ^a	Fy ^b	Jk ^a	Jk ^b	M	N	S	s	5' RT	Sal Rep	PEG IAT
1	R ₁ R ₁	+	+	0	0	+	+	+	+	+	+	0	+	0	+	0	R	(0)	2+
2	R ₂ R ₂	+	0	+	+	0	0	+	0	+	0	+	+	+	+	+	R	(0)	1+
3	rr	0	0	0	+	+	+	+	0	+	+	+	+	0	0	+	1+ ^R	(0)	2+
4	rr	0	0	0	+	+	0	+	+	0	0	+	+	+	+	+	R	(0)	2+
5	rr	0	0	0	+	+	0	+	0	0	+	0	0	+	+	0	R	(0)	2+
6	R ₁ R ₁	+	+	0	0	+	0	+	0	+	0	+	0	0	0	+	1+ ^R	(0)	1+
7	R ₂ R ₂	+	0	+	+	0	+	+	0	+	+	0	+	0	+	0	1+ ^R	(0)	3+
8	rr	0	0	0	+	+	0	+	+	0	+	0	0	+	0	+	1+ ^R	(0)	2+
Pt. Cell																	R	(0)	2+

Plasma Interpretation



Causes of panreactive plasma:

- Warm autoantibody
- Monoclonal antibody
- Multiple antibodies
- Antibody to high prevalence antigen



Plasma Bunny Trail #1: Warm Autoantibody?

Have no way of obtaining autologous cells to PROVE warm auto...

	Rh					Kell		Duffy		Kidd		MNS				Acid Eluate Results
	D	C	E	c	e	K	k	Fy ^a	Fy ^b	Jk ^a	Jk ^b	M	N	S	s	PEG IAT
EGAx1 Pt. Cells																1+

EGA treated to remove bound IgG (not retics)



All other cells tested against eluate were 3+...



Plasma Bunny Trail #2: Monoclonal Antibody Interference?



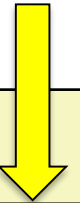
- Patient's diagnosis is Multiple Myeloma.
- Submitting facility states there is no record of anti-CD38 (Daratumumab) therapy.
- Perhaps the patient had treatment at a different facility???

		Rh					Kell		Duffy		Kidd		MNS				Plasma Results	
		D	C	E	c	e	K	k	Fy ^a	Fy ^b	Jk ^a	Jk ^b	M	N	S	s	PEG IAT	DTT-Tx PEG IAT
1	R ₁ R ₁	+	+	0	0	+	+	+	+	+	+	0	+	0	+	0	2+	(0) ✓
2	R ₂ R ₂	+	0	+	+	0	0	+	0	+	0	+	+	+	+	+	1+	(0) ✓
3	rr	0	0	0	+	+	+	+	0	+	+	+	+	0	0	+	2+	NT
4	rr	0	0	0	+	+	0	+	+	0	0	+	+	+	+	+	2+	(0) ✓
5	rr	0	0	0	+	+	0	+	0	0	+	0	0	+	+	0	2+	(0) ✓
6	R ₁ R ₁	+	+	0	0	+	0	+	0	+	0	+	0	0	0	+	1+	(0) ✓
7	R ₂ R ₂	+	0	+	+	0	+	+	0	+	+	0	+	0	+	0	3+	(0) ✓
8	rr	0	0	0	+	+	0	+	+	0	+	0	0	+	0	+	2+	(0) ✓



Plasma Bunny Trail #2: Monoclonal Antibody Interference?

- Daratumumab (anti-CD38) reacts with CD38 on RBCs resulting in positive AHG tests.
- DTT & Trypsin destroy CD38 – can use treated cells to mitigate antibody & perform rule outs.



		Rh					Kell		Duffy		Kidd		MNS				Plasma Results		
		D	C	E	c	e	K	k	Fy ^a	Fy ^b	Jk ^a	Jk ^b	M	N	S	s	PEG IAT	DTT-Tx PEG IAT	Trypsin-Tx PEG IAT
1	R ₁ R ₁	+	+	0	0	+	+	+	+	+	+	0	+	0	+	0	2+	(0) ✓	1+
2	R ₂ R ₂	+	0	+	+	0	0	+	0	+	0	+	+	+	+	+	1+	(0) ✓	NT
3	rr	0	0	0	+	+	+	+	0	+	+	+	+	0	0	+	2+	NT	NT
4	rr	0	0	0	+	+	0	+	+	0	0	+	+	+	+	+	2+	(0) ✓	NT
5	rr	0	0	0	+	+	0	+	0	0	+	0	0	+	+	0	2+	(0) ✓	NT
6	R ₁ R ₁	+	+	0	0	+	0	+	0	+	0	+	0	0	0	+	1+	(0) ✓	1+
7	R ₂ R ₂	+	0	+	+	0	+	+	0	+	+	0	+	0	+	0	3+	(0) ✓	1+
8	rr	0	0	0	+	+	0	+	+	0	+	0	0	+	0	+	2+	(0) ✓	1+



Plasma Bunny Trail #3: Antibody to High Prevalence Antigen?

		Rh					Kell		Duffy		Kidd		MNS				Plasma Results		
		D	C	E	c	e	K	k	Fy ^a	Fy ^b	Jk ^a	Jk ^b	M	N	S	s	PEG IAT	DTT-Tx PEG IAT	Trypsin-Tx PEG IAT
		X	X	X	X	X			X	X	X	X	X	X	X	X			
1	R ₁ R ₁	+	+	0	0	+	+	+	+	+	+	0	+	0	+	0	2+	(0) ✓	1+
2	R ₂ R ₂	+	0	+	+	0	0	+	0	+	0	+	+	+	+	+	1+	(0) ✓	NT
3	rr	0	0	0	+	+	+	+	0	+	+	+	+	0	0	+	2+	NT	NT
4	rr	0	0	0	+	+	0	+	+	0	0	+	+	+	+	+	2+	(0) ✓	NT
5	rr	0	0	0	+	+	0	+	0	0	+	0	0	+	+	0	2+	(0) ✓	NT
6	R ₁ R ₁	+	+	0	0	+	0	+	0	+	0	+	0	0	0	+	1+	(0) ✓	1+
7	R ₂ R ₂	+	0	+	+	0	+	+	0	+	+	0	+	0	+	0	3+	(0) ✓	1+
8	rr	0	0	0	+	+	0	+	+	0	+	0	0	+	0	+	2+	(0) ✓	1+

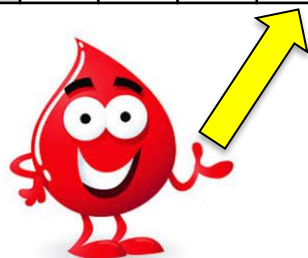
Ficin/Papain	Trypsin	alpha-Chymotrypsin	DTT/AET	Possible specificity
Positive	Positive	Positive	Negative	KEL

Adapted from: Reid ME, Lomas-Francis, C, Olsson ML. The Blood Group Antigen FactsBook. 3rd ed.



Plasma Bunny Trail #3: Antibody to High Prevalence Antigen?

	Rh					Kell						Duffy		Kidd		MNS				Plasma Results
	D	C	E	c	e	K	k	Kp^a	Kp ^b	Js ^a	Js^b	Fy ^a	Fy^b	Jk^a	Jk ^b	M	N	S	s	PEG IAT
R ₂ r	+	0	+	+	+	+	0	0	+	0	+	+	0	0	+	+	+	0	+	1+
rr	0	0	0	+	+	0	+	+	0	0	+	0	+	+	0	0	+	0	+	(0) ✓



	Rh					Kell						Duffy		Kidd		MNS				Plasma Results	Acid Eluate Results
	D	C	E	c	e	K	k	Kp^a	Kp ^b	Js ^a	Js^b	Fy ^a	Fy^b	Jk ^a	Jk ^b	M	N	S	s	PEG IAT	PEG IAT
rr	0	0	0	+	+	0	+	+	0	0	+	0	+	+	+	+	+	+	+	(0) ✓	1+
R ₁ R ₁	+	+	0	0	+	0	+	+	0	0	+	0	+	+	+	0	+	+	+	(0) ✓	1+



Remaining Questions...

Other antibodies to common RBC antigens excluded?

- Anti-K **NOT** excluded
- No K+, Kp(b-) cells available

Warm autoantibody?

- Eluate reactive with all cells tested including Kp(b-)
- Need retics to confirm

Anti-Kp^b: allo or autoantibody?

- Reported cases of autoanti-Kp^b
- Need retics for serological typing...or even better, a genotype!



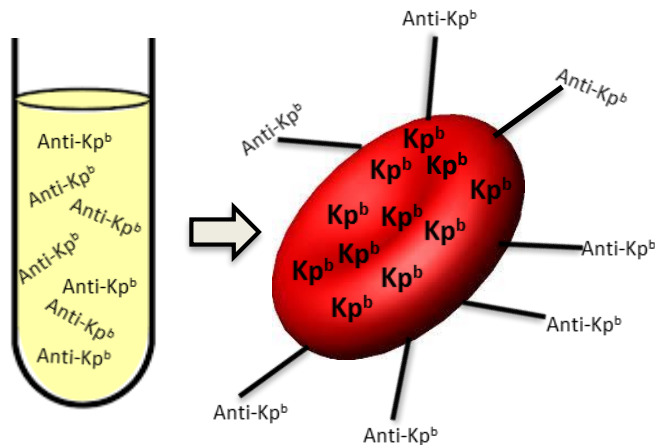
Workup Conclusions

ABO Rh	A Pos
DAT	Pos IgG
Eluate	Probable warm autoantibody
Plasma	Anti-Kp ^b Rouleaux
Transfusion Recommendation	K-, Kp(b-)
Units Provided	1 K-negative, Kp(b-) deglycerolized unit nonreactive with patient's plasma PEG IAT



Danger of Bunny Trail #1: Warm Autoantibody

What if we had performed adsorptions?



< 0.1% of population is Kp(b-)

	Rh					Kell		Duffy		Kidd		MNS				Alloads Plasma Results
	D	C	E	c	e	K	k	Fy ^a	Fy ^b	Jk ^a	Jk ^b	M	N	S	s	LISS IAT
1	+	+	0	0	+	+	+	+	+	+	0	+	0	+	0	(0) ✓
2	+	0	+	+	0	0	+	0	+	0	+	+	+	+	+	(0) ✓
3	0	0	0	+	+	+	+	0	+	+	+	+	0	0	+	(0) ✓
4	0	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) ✓
7	+	0	+	+	0	+	+	0	+	+	0	+	0	+	0	(0) ✓
8	0	0	0	+	+	0	+	+	0	+	0	0	+	0	+	(0) ✓

Adsorption studies cannot exclude the presence of all potential alloantibodies, especially antibodies against antigens of high incidence.

References

Fung MK, Eder AF, Spitalnik SL, Westhoff CM. Technical Manual. 19th ed. Bethesda, Maryland: AABB; 2017.

Reid ME, Lomas-Francis, C, Olsson ML. The Blood Group Antigen FactsBook. 3rd ed. Elsevier Ltd; 2012.

QUESTIONS?



