CASE OF THE MONTH: DECEMBER, 2018 (Case study by Jim Perkins)

History: This patient was a 62 year old woman admitted with symptoms of dyspnea on exertion and lightheadedness, dark stools, and a hemoglobin level of 7.0. She had a history of chronic lower GI blood loss and had received 7 units of RBCs over a one week period about 5 weeks earlier. At that time her antibody screen was negative. Of note the patient also had coronary artery disease, status post bypass and cardiac valve surgery, a matter of concern given her significant anemia.

	Anti-A	Anti-B	A1 cells	B cells	6% alb	Anti-D	<D/AHG	Interp
IS	0	0	4+	4+		0		

Antibody Screen

Cell phenotype

Antibody Screen	
	Gel
SCI	2+
SCII	2+

Direct Antiglobulin Test (gel)			
	Polyspec.	Anti-IgG	Anti-C3
AHG	0		

		Rh system					Kell						Duffy		Kidd		Xg	Lewis		MNSs				P	Lutheran					
	Rh	D	C	E	c	e	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	Xg^a	Le^a	Le^b	S	s	M	N	P1	Lu^a	Lu^b		Cell	Gel	
SC I	R1R1	+	+	0	0	+	0	+	0	+	0	+	+	0	+	0	+	0	+	+	0	+	0	+	0	+		SC I	2+	
SC II	R2R2	+	0	+	+	0	+	+	0	+	0	+	+	+	0	+	+	+	0	0	+	+	+	+	0	+		SC II	2+	

Initial Plasma Panel

VRA302		Rh system						Kell						Duffy		Kidd		Xg	Lewis		MNSs				P	Lutheran		Other Typings			
Cell	Rh	D	C	E	c	e	V	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	Xg^a	Le^a	Le^b	S	s	M	N	P1	Lu^a	Lu^b		Cell	Gel	
1	R1wR1	+	+	0	0	+	0	0	+	0	+	0	+	+	+	+	+	+	0	+	+	+	+	+	+	0	+	C^w	1	2+	
2	R1R1	+	+	0	0	+	0	+	+	0	+	0	+	+	0	+	+	+	0	+	0	+	+	+	+	0	+		2	3+	
3	R2R2	+	0	+	+	0	0	0	+	0	+	0	+	0	+	+	0	+	0	0	+	+	+	+	+	0	+	HLA+	3	3+	
4	Ror	+	0	0	+	+	+	0	+	0	+	/	+	0	+	+	0	+	0	0	0	+	+	+	+s	0	+	HLA+	4	3+	
5	r'r	0	+	0	+	+	0	0	+	0	+	/	+	+	+	+	+	+	0	+	0	+	+	0	+	0	+		5	3+	
6	r"r	0	0	+	+	+	0	0	+	0	+	0	+	+	+	0	+	+	+	0	+	+	0	+	+	0	+		6	3+	
7	rr	0	0	0	+	+	0	+	+	0	+	/	+	0	+	+	+	+	0	+	+	0	+	0	0	0	+	HLA+	7	2+	
8	rr	0	0	0	+	+	0	0	+	0	+	/	+	+	0	0	+	0	+	0	+	+	+	+	0	0	+		8	w+	
9	rr	0	0	0	+	+	0	0	+	0	+	/	+	+	+	+	0	+	0	+	0	+	0	+	+	0	+	HLA+	9	3+	
10	rr	0	0	0	+	+	0	0	+	+	+	0	+	0	0	0	+	+	+	0	+	+	0	+	+	0	+		10	2+	
11	R1R1	+	+	0	0	+	0	0	+	0	+	0	+	0	+	0	+	+	0	+	+	0	+	+	+s	0	+		11	0	
Patient																													AC	0	

Rh Phenotype

	Rh system				Kell				Kidd		Duffy		Lewis		MNSs													
	C	E	c	e	K	k	Kp^a	Js^a	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	S	s	M	N	P1	I	H	A_1						
Patient	**0**	**0**	**+**																									

Questions/discussion

What do you think might be going on here? Based on your hypothesis(ies) how might we proceed?

The type-and-screen reveals the patient to be O-neg with a positive antibody screen. All cells but one on the initial panel are reactive, at varying strengths, and the autocontrol and DAT are negative. An Rh phenotype, done per routine, showed the patient's most likely genotype to be rr.

Given varying strength reactivity with most reagent cells and a negative autocontrol/DAT we might initially consider the possibility of multiple alloantibodies or possibly method-dependent reactivity. Certain antibodies are ruled out, but the pattern of the positive reactions doesn't suggest any particular antibody specificity(ies).

The technologist elected to run a second gel panel in the hope of finding additional "rule-out cell's", as well as running a panel by the LISS/tube method to rule out gel-dependent reactivity.

Second panel

		Rh system						Kell						Duffy		Kidd		Xg	Lewis		MNSs				P	Lutheran		Other Typings		Plasma	
Cell	Rh	D	C	E	c	e	V	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	Xg^a	Le^a	Le^b	S	s	M	N	P1	Lu^a	Lu^b		Cell	Gel	
12	**rr**	**0**	**0**	**0**	**+**	**+**	**0**	**0**	**+**	**0**	**+**	**/**	**+**	**+**	**+**	**0**	**+**	**+**	**+**	**0**	**+**	**+**	**0**	**+**	**+**	**0**	**+**		**12**	**2+**	
13	**rr**	**0**	**0**	**0**	**+**	**+**	**0**	**+**	**+**	**0**	**+**	**0**	**+**	**0**	**+**	**+**	**+**	**0**	**0**	**+**	**0**	**+**	**+**	**+**	**+**	**0**	**+**	**HLA+**	**13**	**3+**	
14	**rr**	**0**	**0**	**0**	**+**	**+**	**0**	**0**	**+**	**0**	**+**	**/**	**+**	**+**	**+**	**+**	**0**	**+**	**0**	**+**	**+**	**0**	**+**	**0**	**+**	**0**	**+**		**14**	**3+**	
15	**R2R2**	**+**	**0**	**+**	**+**	**0**	**0**	**0**	**+**	**0**	**+**	**/**	**+**	**+**	**0**	**+**	**0**	**+**	**0**	**0**	**0**	**+**	**+**	**0**	**+**	**0**	**+**		**15**	**3+**	
16	**R2R2**	**+**	**0**	**+**	**+**	**0**	**0**	**0**	**+**	**0**	**+**	**/**	**+**	**+**	**0**	**+**	**0**	**0**	**0**	**+**	**+**	**+**	**+**	**0**	**0**	**0**	**+**		**16**	**1+**	
17	**R2R2**	**+**	**0**	**+**	**+**	**0**	**0**	**0**	**+**	**0**	**+**	**/**	**+**	**+**	**+**	**0**	**+**	**+**	**0**	**0**	**+**	**0**	**+**	**+**	**+**	**+**	**+**		**17**	**2+**	
18	**R1R1**	**+**	**+**	**0**	**0**	**+**	**0**	**0**	**+**	**0**	**+**	**/**	**+**	**+**	**0**	**0**	**+**	**+**	**0**	**+**	**+**	**+**	**+**	**+**	**+**	**0**	**+**		**18**	**1+**	
19	**R1R1**	**+**	**+**	**0**	**0**	**+**	**0**	**0**	**+**	**0**	**+**	**0**	**+**	**0**	**+**	**+**	**0**	**+**	**+**	**0**	**0**	**+**	**0**	**+**	**+s**	**0**	**+**	**HLA+**	**19**	**1+**	
20	**RZR1**	**+**	**+**	**+**	**0**	**+**	**0**	**0**	**+**	**0**	**+**	**0**	**+**	**+**	**+**	**+**	**0**	**+**	**0**	**+**	**+**	**0**	**+**	**+**	**+**	**0**	**+**	**HLA+**	**20**	**2+**	
21	**r'r**	**0**	**+**	**0**	**+**	**+**	**0**	**0**	**+**	**0**	**+**	**/**	**+**	**+**	**0**	**0**	**+**	**+**	**0**	**+**	**+**	**+**	**+**	**+**	**+**	**0**	**+**	**HLA+**	**21**	**2+**	
22	**rr**	**0**	**0**	**0**	**+**	**+**	**0**	**+**	**0**	**0**	**+**	**/**	**+**	**+**	**+**	**+**	**+**	**0**	**0**	**+**	**+**	**+**	**+**	**+**	**+**	**0**	**+**		**22**	**2+**	

3% cell panel tested by LISS/tube technique with raw patient plasma

		Rh system						Kell						Duffy		Kidd		Lewis		P	MNSs					Lutheran		Xg	Other Typings	LISS		
Cell	**Rh**	**D**	**C**	**E**	**c**	**e**	**V**	**K**	**k**	**Kp^a**	**Xg^a**	**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**	**37°**	**AHG**
1	**R1R1**	+	+	**0**	**0**	+	**0**	**0**	+	**0**	+	**0**	+	**0**	+	**0**	+	**0**	+	**0**	+	+	+	+	**0**	+	+		**0**	**0**	**1+**
2	**R1wR1**	+	+	**0**	**0**	+	**0**	**0**	+	**0**		**0**	+	+	**0**	+	**0**	**0**	+	**0**	+	+	**0**	+	**0**	+	+		**0**	**0**	**1+**
3	**R2R2**	+	**0**	+	+	**0**	**0**	**0**	+	**0**	+	**0**	+	+	**0**	+	+	+	**0**	+	+	+	+	+	**0**	+	+		**0**	**0**	**w+**
4	**Ror**	+	**0**	**0**	+	+	+	**0**	+	**0**		**0**	+	**0**	**0**	+	**0**	**0**	+	+	**0**	+	**0**	**0**	**0**	+	+		**0**	**0**	**3+**
5	**r'r**	**0**	+	**0**	+	+	**0**	**0**	+	**0**		**0**	+	+	**0**	+	**0**	**0**	+	+	+	+	**0**	+	**0**	+	+	**Co(b+)**	**0**	**0**	**1+**
6	**r"r**	**0**	**0**	+	+	+	**0**	**0**	+	**0**	+	**0**	+	+	**0**	**0**	+	**0**	+	+	+	+	**0**	+	**0**	+	+		**0**	**0**	**2+**
7	**rr**	**0**	**0**	**0**	+	+	**0**	+	+	**0**	0	**0**	+	**0**	+	+	**0**	**0**	+	**0**	**0**	+	+	+	**0**	+	+		**0**	**0**	**0$^{\surd}$**
8	**rr**	**0**	**0**	**0**	+	+	**0**	**0**	+	**0**		**0**	+	+	**0**	**0**	+	**0**	+	+	+	**0**	+	+	**0**	+	+		**0**	**0**	**1+**
9	**rr**	**0**	**0**	**0**	+	+	**0**	**0**	+	**0**		**0**	+	**0**	+	+	**0**	+	**0**	+	+	**0**	**0**	+	**0**	+	+	**Co(b+)**	**0**	**0**	**w+**
10	**R1R1**	+	+	**0**	**0**	+	**0**	+	+	**0**	+	**0**	+	+	+	+	+	+	**0**	**0**	+	**0**	+	**0**	**0**	+	+		**0**	**0**	**2+**
TC	**R1r**	+	+	**0**	+	+	**0**	**0**	+	**0**		**0**	+	**0**	**0**	+	**0**	**0**	+	+	+	+	**0**	+	**0**	+	+	**Go(a+)**			
Patient																															

Questions/discussion

What is your impression now? What might you try next?

The second gel panel is consistent with multiple alloantibodies, but it doesn't really help us as there are no more non-reactive cells. The LISS/tube panel rules out gel-dependent reactivity and shows that we are dealing with a "real antibody(ies)". Also there is a second non-reactive cell to use in ruling-out. Of the usual antibodies we are left with the possibilities of anti-E, anti-Le^a, and anti-s, but none of these, and no combination of them, will explain all of the reactivity. So a less common answer must be considered.

The technologist noted that the reactions were weak and involved most donor RBCs. This suggested the possibility of a so-called high-titer, low-avidity (HTLA) antibody, so she decided to determine the antibody titer. Also, two to three hours had passed and the service was anxious to transfuse, so she elected to concurrently attempt to find crossmatch compatible RBCs using the saline/tube technique with an increased serum:cell ratio, the crossmatch procedure then in use in the laboratory. Finally, reasoning that the latter technique might allow her to rule out additional antibodies, she elected to run additional RBCs with the crossmatches. The results were as follows:

Plasma dilutions tested by saline/tube test at AHG phase only

Dilution	**1:1 (Neet)**	**1:2**	**1:4**	**1:8**	**1:16**	**1:32**	**1:64**	**1:128**	**1:256**	**1:512**	**1:1024**
SCI	**2+**	**2+**	**2+**	**1+^{s}**	**1+**	**vw+***	**vw+***	**0$^{\surd}$**	**0$^{\surd}$**		

***Microscopic reactions only**

3% selected cell panel tested by saline/tube technique at a 4:1 serum/cell ratio with raw patient plasma read at AHG phase only after 37º, 30" incubation

		Rh system						Kell						Duffy		Kidd		Lewis		P	MNSs				Lutheran		Xg	Other Typings	4:1
Cell	Rh	D	C	E	c	e	V	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		IgG
SCI	R1R1	+	+	0	0	+	0	+	+	0	+	0	+	+	+	+	0	0	+	0	+	+	0	+	0	+	+		2+
SCII	R2R2	+	0	+	+	0	0	0	+	0	+	0	+	+	0	+	+	+	0	+	+	0	+	0	0	+	+		1+
	R1wR1	+	+	0	0	+	0	0	+	0		0	+	0	+	0	+	+	0	0	+	+	+	+	0	+	+		0√
	R2R2	+	0	+	+	0	0	0	+	0	+	0	+	+	0	+	0	0	+	+	0	+	0	+	0	+	+	Co(b+), Yt(b+)	0√
	r'r	0	+	0	+	+	0	+	+	0		0	+	0	+	+	0	0	+	+	+	0	+	0	0	+	+		0√
	rr	0	0	0	+	+	0	0	+	0	0	+	+	+	0	+	+	+	0	0	+	+	0	+	0	+	0		0√
Donor #1																													3+
Donor #2																													1+
Donor #3																													w+
Donor #4																													3+

Questions/discussion

Now what do you think, and what would you do next?

The titration shows that the patient's antibody has a high titer (64) in spite of the relatively weak reactivity of the undiluted plasma, consistent with an HTLA antibody. Although results using the saline/tube technique with a serum/cell ration of 4:1 are somewhat anomalous in that the two screening cells tested were reactive and the four selected panel cells are non-reactive, in spite of being tested by the same technologist. Nonetheless, to the extent that HTLA's can be regarded as clinically insignificant, the results are reassuring that none of the common, clinically-significant alloantibodies are present.

Although antibodies which display an HTLA type of reactivity are generally regarded as benign, this is not entirely the case, and identifying their specificity is preferred. Testing with cells treated with ficin/papain and a sulfhydryl reducing agent can help determine the specificity of antibodies directed against high frequency antigens in general, including HTLA antibodies. At this point a new technologist took over the problem and performed the following testing.

3% cell panel treated with ficin and AET as shown. Ficin-treated cells were tested with a 2:1 saline/tube technique. AET-treated cells were tested by LISS/tube technique.

		Rh system						Kell						Duffy		Kidd		Lewis		P	MNSs				Lutheran		Xg	Other Typings	Ficin tx'd cells			AET
Cell	Rh	D	C	E	c	e	V	K	k	Kp^a	Xg^a	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	37º	IgG	IgG
1	R1R1	+	+	0	0	+	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	+	+	+	0	+	+		0	0	0√	2+
2	R1wR1	+	+	0	0	+	0	0	+	0		0	+	+	0	+	0	0	+	0	+	+	0	+	0	+	+		0	0	0√	w+
3	R2R2	+	0	+	+	0	0	0	+	0	+	0	+	+	0	+	+	+	0	+	+	+	+	+	0	+	+		0	0	0√	1+
4	Ror	+	0	0	+	+	+	0	+	0		0	+	0	0	+	0	0	+	+	0	+	0	0	0	+	+		0	0	0√	1+
10	R1R1	+	+	0	0	+	0	+	+	0	+	0	+	+	+	+	+	+	0	0	+	0	+	0	0	+	+		0	0	0√	2+*
Patient																																

***AET-treated cell non-reactive with anti-k**

Questions/discussion

What is your interpretation? How would you proceed?

The most recent results demonstrate that the patient's antibody is directed against an antigen that is ficin sensitive and AET resistant. Inspection of a table of chemical effects on blood group antigens shows that this is consistent with anti-Chido/Rodgers (anti-Ch/Rg), which identifies high frequency antigens carried on C'4 molecules that bind to and coat RBCs. Anti-Ch/Rg typically behaves as an HTLA. Since C'4 is present in normal plasma anti-Ch/Rg can be neutralized by pooled plasma, and a plasma inhibition test was performed.

Note that the results with ficin treated RBCs do not rule out antibodies directed against other ficin-sensitive antigens such as Duffy.

Plasma inhibition test

	Neutralized	Saline control
	Gel	Gel
SCI	0	1+
SCII	0	1+

Questions/discussion

What is the interpretation? Are any clinical problems in recipients associated with this antibody? Do you see any problems with the workup as presented above?

These results prove the antibody to be anti-Ch/Rg, and taken together they rule out other blood group alloantibodies.

Patients with anti-Ch/Rg have been reported to experience severe allergic reactions to plasma-containing components, and the author has seen one such case. This is one of the few situations in which we know the identity of the antigen mediating an allergic reaction. Presumably a patient with a history of such a reaction could be safely treated with washed RBCs.

Regarding "problems with the workup" note that an autocontrol was only performed with the initial gel panel. It's probably acceptable that it was not performed with the less sensitive LISS/tube and saline/tube panels, but an autocontrol should have been done with the ficin and AET panels.

Take home points

The immunohematologic differential diagnosis for a patient plasma reacting with all or most cells on the initial panel(s).
The importance of ruling out method-dependent reactivity.
Approaches to identifying an antibody directed against a high frequency antigen, in particular the use of chemical inactivation of antigens.
The behavior of anti-Ch/Rg.