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

History: This patient was a 46 year old Caucasian, G5P5 woman presenting to the Emergency Department with lower abdominal pain who had received 2 units of RBCs 8 years earlier. At that time she bled 2 weeks after delivery due to retained products of conception and required a hysterectomy to stop the bleeding. Now her CT scan suggested a hemorrhagic right Ovarian cyst.

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

Antibody Screen	
	Gel
SCI	2+
SCII	2+

Direct Antiglobulin Test				
	Gel		IS	5' Incub.
Polyspec. AHG	0	Anti-C3		
Anti-IgG				

Antibody Screen Cell phenotype

		Rh system					Kell						Duffy		Kidd		Xg	Lewis		MNSs				P	Lutheren					
	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		
SC I	R1R1	+	+	0	0	+	+	+	0	+	0	+	0	+	+	0	+	0	+	0	+	0	+	+	0	+		SC I	2+	
SC II	R2R2	+	0	+	+	0	0	+	0	+	0	+	+	0	+	+	+	+	0	+	+	+	0	0	0	+		SC II	2+	

Initial Plasma Panel

VRA302		Rh system						Kell						Duffy		Kidd		Xg	Lewis		MNSs				P	Lutheren		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	+	0	+	+	+	0	0	+	C^w	1	2+	
2	R1R1	+	+	0	0	+	0	0	+	0	+	/	+	+	+	+	0	0	0	+	+	0	+	0	+s	0	+		2	2+	
3	R2R2	+	0	+	+	0	0	+	+	0	+	/	+	+	+	+	0	+	0	0	+	+	+	0	+s	0	+		3	2+	
4	Ror	+	0	0	+	+	+	0	+	0	+	0	+	0	0	+	+	+	0	0	0	+	+	+	+s	0	+		4	2+	
5	r'r	0	+	0	+	+	0	0	+	0	+	/	+	+	0	+	+	+	+	0	0	+	+	0	+	0	+		5	2+	
6	r"r	0	0	+	+	+	0	0	+	0	+	/	+	0	+	0	+	+	0	+	+	+	+	+	+	0	+		6	2+	
7	Rr	0	0	0	+	+	0	+	+	0	+	0	+	0	+	0	+	0	0	+	0	+	+	+	0	0	+		7	2+	
8	Rr	0	0	0	+	+	0	0	+	0	+	0	+	+	0	+	+	0	+	0	+	+	0	+	0	0	+		8	2+	
9	Rr	0	0	0	+	+	0	0	+	0	+	0	+	+	+	0	+	+	0	+	+	0	+	0	+	0	+		9	2+	
10	Rr	0	0	0	+	+	0	0	+	0	+	/	+	+	0	+	0	+	+	0	0	+	0	+	0	0	+		10	2+	
11	R1R1	+	+	0	0	+	0	+	+	0	+	/	+	0	+	0	+	+	0	+	+	+	+	0	+s	0	+		11	2+	
Patient																													AC	$0^{\surd}$	

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**	+	+	+																								

Questions/discussion

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

The type-and-screen reveals the patient to be O-pos with a positive antibody screen. The initial panel showed reactivity with all RBCs, and the autocontrol and DAT are negative. An Rh phenotype, done per routine, showed the patient to be R2r.

As discussed in last month's case, when a patient's plasma reacts with all panel donor RBCs there are classically three scenarios to be considered, namely an autoantibody, an alloantibody directed against a high frequency antigen, or multiple alloantibodies with or without concomitant autoantibodies. Today however, one might add a fourth scenario, namely method-dependent pan-agglutination when initial testing is by column agglutination ("gel") or solid phase technique. In this case the reaction strength is the same with all panel cells, and the autocontrol and DAT are negative suggesting that she has an alloantibody against a high frequency antigen or a method-dependent antibody.

In working up a possible alloantibody against a high frequency antigen one of the first considerations is the patient's ethnicity. Since the patient is Caucasian anti-k is one of the most common antibodies she might make that would react with all cells on many panels, as about 2 in 1,000 Caucasians are k-negative, and Kell system antigens are relatively immunogenic. Although most panels prepared for routine or initial antibody identification would not have a K+k- cell, secondary panels from most manufacturer's will have at least one, so such a cell could be tested. Demonstration that the patient lacks a high frequency antigen is strong evidence that the corresponding antibody is present, and commercial typing sera allow phenotyping for multiple high frequency antigens including k, Fy3 (Fy^{a-b-}), Jk3 (Jk^{a-b-}), U (S-s-), and En^a (M-N-). Other relatively common antibodies against high frequency antigens show a "High-Titer, Low-Avidity" (HTLA) type of reactivity, demonstrated by testing serial dilutions of the patient's plasma. Another approach many reference laboratories pursue relatively early in their workup when confronted with an apparent antibody against a high-frequency antigen is to test enzyme- or DTT-treated cells. Finally, method-dependent antibodies can be ruled out by showing that the plasma fails to react in a different test system such as PEG/tube.

In this case the technologists' first hypothesis was that she might be dealing with method-dependent reactivity, so she performed the following.

Selected 3% cell panel tested by LISS/tube technique and by gel technique after resuspension to 0.8% with raw patient plasma

		Rh system						Kell						Duffy		Kidd		Xg	Lewis		MNSs				P	Lutheren		Other Typings	Gel*	LISS		
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			IS	37	AHG
315177	**R1R1**	+	+	**0**	**0**	+	**0**	+	+	**0**	+	/	+	+	**0**	**0**	+	+	**0**	**0**	+	+	+	+	+	**0**	+	**HLA+**	2+	0	0	2+
315088	**R2R2**	+	**0**	+	+	**0**	**0**	**0**	+	**0**	+	/	+	0	+	+	**0**	+	**0**	+	+	+	+	+	**0**	+	+		2+	0	0	2+
302800	**r"r**	**0**	**0**	+	+	+	**0**	**0**	+	+	+	**0**	+	+	+	**0**	+	+	**0**	+	**0**	+	**0**	+	+	**0**	+		2+	0	0	2+
111895	**rr**	**0**	**0**	**0**	+	+	**0**	**0**	+	**0**	+	**0**	+	+	+	+	**0**	0	**0**	+	+	**0**	+	**0**	+	**0**	+	**HLA+**	2+	0	0	2+
315417	**R1R1**	+	+	**0**	**0**	+	**0**	**0**	+	**0**	+	/	+	0	+	+	**0**	+	+	**0**	+	**0**	+	+	**+s**	**0**	+	**HLA+**	2+	0	0	2+
Patient																											**AC**		**0**	**0**	**0**√

***Cells for gel testing were washed and resuspended to 0.8% in MTS diluent.**

Questions/discussion

What is your conclusion regarding the possibility of a method-dependent antibody? Given your answer what might you do next?

The above testing rules out a gel-dependent antibody as the pan-agglutinin is still evident by a tube IAT with LISS enhancement. Many gel-dependent antibodies can also be eliminated by diluting the commercial panel cells in MTS buffer™ which does not have the same enhancement agents present in the diluent that the panel cells come in, and which seem to be mediating the gel-dependent agglutination..

When that hypothesis was discarded the technologist pursued the possibility of an anti-k, typing the patient's cells for k and testing multiple k-negative cells from current and out-of-date panels, as shown below.

Extended 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		+	0			0	+	+	+			0	+											

Selected cell panel tested against raw patient plasma by gel technique

		Rh system						Kell						Duffy		Kidd		Xg	Lewis		MNSs				P	Lutheren		Other Typings	Gel
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		
316992	**R2R2**	+	**0**	+	+	**0**	**0**	+	**0**	**0**	+	/	+	+	**0**	+	+	**0**	**0**	+	+	+	+	**0**	+	**0**	+		0
109192	**R1R2**	+	+	+	+	+	**0**	+	**0**	**0**	+	**0**	+	+	**0**	**0**	+	+	**0**	+	+	+	+	**0**	+	**0**	+		0
313510	**R2r**	+	**0**	+	+	+	**0**	+	**0**	**0**	+	/	+	**0**	+	+	+	+	**0**	**0**	**0**	+	+	+	+	**0**	+		0
320910	**R1R1**	+	+	**0**	**0**	+	**0**	+	**0**	**0**	+	/	+	+	+	+	**0**	+	+	**0**	**0**	+	+	**0**	**0**	**0**	+		0
317519	**R1r**	+	+	**0**	+	+	**0**	+	**0**	**0**	+	/	+	+	+	+	**0**	+	**0**	+	**0**	+	**0**	+	**0**	**0**	+		0
Patient																												**AC**	

Questions/discussion

Is the technologist's hypothesis proven? Is any more work needed?

The presence of anti-k is essentially proven (3 reactive k-positive cells, 3 nonreactive k-negative cells, patient is k-negative), but the workup is incomplete as anti-S is not ruled out. Presumably the technologist tested all of the k-negative cells she had access to, so an anti-S rule-out cell is not available. In general, the problem of ruling out other, underlying antibodies is shared by all forms of panagglutination.

Of note, had testing with chemically-treated RBCs (typically ficin/papain and DTT/AET) been pursued as a first step to identify the pan-agglutinin, RBCs treated with the sulfhydryl reducing agent DTT would have been expected to be non-reactive with anti-k since the Kell protein depends on di-sulfide bonds for its overall configuration. This would have been a clue that the patient's antibody was directed against one of the high-frequency antigens in the Kell system.

How might one rule out anti-S?

A second technologist then ran the following tests:

Antibody Screen Cells with and without DTT treatment tested against raw patient plasma by saline/tube technique

		Rh system					Kell						Duffy		Kidd		Xg	Lewis		MNSs				P	Lutheren			Un-tx'd		DTT tx'd	
	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	IS	AHG	IS	AHG
SC I	R1R1	+	+	0	0	+	0	+	0	+	/	+	0	+	0	+	+	0	+	+	0	+	+	+	0	+	SC I	0	1+	0	$0^{\surd}$
SC II	R2R2	+	0	+	+	0	+	+	0	+	0	+	+	0	+	0	0	+	0	0	+	+	+	+	0	+	SC II	0	2+	0	$0^{\surd}$

Is anti-S ruled out?

Yes! Treatment of screening cell I with DTT renders it k antigen negative as discussed above. The S antigen is NOT destroyed by DTT, so it is an S+s- rule-out cell for anti-S. So the final criterion for proof that the patient has anti-k is met.

Take home points

The immunohematologic differential diagnosis for a patient plasma reacting with all cells on the initial panel(s).
An approach to ruling out gel-dependent antibodies.
Approaches to identifying an antibody directed against a high frequency antigen.
The need to rule out antibodies underlying an antibody reacting with most RBCs by use of multiple techniques including chemical inactivation of antigens.