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

History: This patient was a 80 year old G2 woman with an unknown transfusion history admitted to the hospital with a small bowel obstruction due to perforated diverticulitis and now scheduled for semi-urgent surgery.

	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	4+

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

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	4+	

Initial Plasma Panel

		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	0	+	+	+	0	+s	0	+		3	4+	
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	+	0	+		5	3+	
6	r"r	0	0	+	+	+	0	0	+	0	+	0	+	0	+	0	+	+	0	+	+	+	+	+	+	0	+		6	4+	
7	rr	0	0	0	+	+	0	+	+	0	+	0	+	0	+	0	+	0	0	+	0	+	+	+	0	0	+		7	3+	
8	rr	0	0	0	+	+	0	0	+	0	+	0	+	+	0	+	+	0	+	0	+	+	0	+	0	0	+		8	3+	
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	0	+		10	3+	
11	R1R1	+	+	0	0	+	0	+	+	0	+	0	+	0	+	0	+	+	0	+	+	+	+	0	+s	0	+		11	2+	
Patient																													AC	w+	

Second panel

		Rh system						Kell						Duffy		Kidd		Xg	Lewis		MNSs				P	Lutheren		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**	**+**	**0**	**+**	**0**	**+**	**+**	**0**	**+**	**C^w**	**1**	**3+**	
13	**rr**	**0**	**0**	**0**	**+**	**+**	**0**	**+**	**+**	**0**	**+**	**0**	**+**	**+**	**0**	**+**	**0**	**+**	**0**	**+**	**+**	**+**	**0**	**+**	**0**	**0**	**+**		**2**	**3+**	
14	**rr**	**0**	**0**	**0**	**+**	**+**	**0**	**0**	**+**	**0**	**+**	**0**	**+**	**+**	**+**	**0**	**+**	**+**	**0**	**+**	**+**	**0**	**+**	**0**	**+s**	**0**	**+**		**3**	**2+**	
15	**R2R2**	**+**	**0**	**+**	**+**	**0**	**0**	**0**	**+**	**0**	**+**	**0**	**+**	**+**	**+**	**0**	**+**	**+**	**+**	**0**	**+**	**+**	**+**	**0**	**0**	**0**	**+**		**4**	**4+**	
16	**R2R2**	**+**	**0**	**+**	**+**	**0**	**0**	**0**	**+**	**0**	**+**	**0**	**+**	**+**	**0**	**+**	**0**	**+**	**0**	**+**	**+**	**+**	**+**	**0**	**+**	**0**	**+**		**5**	**4+**	
17	**R2R2**	**+**	**0**	**+**	**+**	**0**	**0**	**0**	**+**	**0**	**+**	**0**	**+**	**0**	**+**	**+**	**+**	**0**	**0**	**+**	**+**	**+**	**+**	**0**	**+**	**0**	**+**		**6**	**4+**	
18	**R1R1**	**+**	**+**	**0**	**0**	**+**	**0**	**0**	**+**	**0**	**+**	**0**	**+**	**+**	**0**	**+**	**+**	**+**	**0**	**+**	**0**	**+**	**+**	**+**	**0**	**0**	**+**		**7**	**2+**	
19	**R1R1**	**+**	**+**	**0**	**0**	**+**	**0**	**+**	**+**	**0**	**+**	**0**	**+**	**0**	**+**	**0**	**+**	**+**	**0**	**+**	**+**	**0**	**+**	**+**	**+s**	**0**	**+**		**8**	**2+**	
20	**RZR1**	**+**	**+**	**+**	**0**	**+**	**0**	**0**	**+**	**0**	**+**	**0**	**+**	**+**	**0**	**+**	**0**	**+**	**0**	**+**	**+**	**0**	**+**	**+**	**+s**	**0**	**+**		**9**	**4+**	
21	**r'r**	**0**	**0**	**0**	**+**	**+**	**0**	**0**	**+**	**0**	**+**	**0**	**+**	**0**	**+**	**0**	**+**	**+**	**0**	**+**	**0**	**+**	**+**	**+**	**+**	**0**	**+**		**10**	**2+**	
22	**R2R2**	**+**	**0**	**+**	**+**	**0**	**0**	**+**	**0**	**0**	**+**	**0**	**+**	**+**	**0**	**+**	**+**	**0**	**0**	**+**	**+**	**+**	**+**	**0**	**+**	**0**	**+**		**11**	**4+**	
Patient																													**AC**	**w+**	

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**																											

An eluate from the patient's RBCs was non-reactive with the initial panel by the gel technique, as was the last wash test.

Questions/discussion

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

The type-and-screen reveals the patient to be O-pos with a positive antibody screen. The DAT is weakly positive due to IgG on the patient's RBCs. The initial panel showed reactivity with all RBCs, and a second panel showed the same. An eluate, prepared per SOP for a positive DAT due to IgG on the cells, did not reveal RBC-bound allo or auto-antibody. An Rh phenotype, also done per SOP showed the patient to be R1R1.

When a patient's plasma reacts with all panel donor RBCs three scenarios should be considered, namely that the patient has an autoantibody, an alloantibody directed against a high frequency antigen, or a combination of multiple alloantibodies and/or autoantibodies. In this case the variation in the reaction strengths in the initial panel immediately suggested multiple alloantibodies, but the positive DAT was consistent with the presence of autoantibody as well. Luckily a warm autoantibody was ruled out when the eluate was non-reactive.
If there were at least one non-reactive donor cell sample one could probably start to narrow down the possible antibody specificities, which would then help us select further "rule-out cells." In the absence of any "rule-outs", it would be useful to determine the patient's extended phenotype using either

serologic or genetic methods. (The latter was not possible in this case given the relative urgency of the patient's surgery.) Finally, one could also guess that one or more antibodies with Rh system specificities are present. (Note that all of the 4+ reactive cells are E positive and all of the E-positive cels react 4+.) An old trick based on this logic is to eliminate Rh antibodies' potential contribution to the pattern of reactivity by testing cells with the same Rh phenotype as the patient. Both of the latter were approaches were adopted by the individuals handling this problem with the following results.

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	+	+	+	0								

Selected Rh specific cells (R1R1)

		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	
306017	**R1wR1**	+	+	0	0	+	0	0	+	0	+	0	+	+	+	0	+	+	0	+	+	+	+	0	+s	0	+	C^w, HLA+	1	2+	
316120	**R1R1**	+	+	0	0	+	0	+	+	0	+	0	+	+	+	+	+	+	+	0	0	+	+	+	0	0	+		2	2+	
302558	**R1R1**	+	+	0	0	+	0	0	+	0	+	0	+	0	+	0	+	0	0	+	+	0	+	+	+	+	+	HLA+	3	0	
306546	**R1R1**	+	+	0	0	+	0	0	+	0	+	0	+	0	+	0	+	0	+	0	0	+	0	+	+s	0	+		4	0	
318586	**R1wR1**	+	+	0	0	+	0	0	+	0	+	0	+	0	+	+	+	+	+	0	0	+	+	+	+	0	+	C^w	5	0	
316865	**R1R1**	+	+	0	0	+	0	+	+	0	+	0	+	+	+	0	+	+	0	0	+	+	+	0	+	0	+		6	2+	
321049	**R1R1**	+	+	0	0	+	0	0	+	0	+	0	+	0	+	+	0	+	0	+	+	0	+	0	+	0	+	C^w, HLA+	7	0	
118159	**R1wR1**	+	+	0	0	+	0	0	+	0	+	0	+	+	+	+	+	+	+	0	0	+	0	+	+	0	+		8	2+	
313887	**R1R1**	+	+	0	0	+	0	+	+	0	+	0	+	+	0	+	+	0	+	0	+	0	0	+	+	0	+		9	2+	
319654	**R1R1**	+	+	0	0	+	0	+	+	0	+	0	+	+	+	+	+	0	0	+	+	+	+	+	0	0	+		10	2+	
320747	**R1wR1**	+	+	0	0	+	0	0	+	0	+	0	+	0	+	+	0	+	0	+	+	+	+	+	+	0	+	C^w	11	0	
316707	**R1R1**	+	+	0	0	+	0	+	+	0	+	0	+	+	0	+	+	+	+	0	+	+	+	0	+	0	+	HLA+	12	2+	
302558	**R1R1**	+	+	0	0	+	0	0	+	0	+	0	+	0	+	0	+	0	0	+	+	0	+	+	+	+	+	HLA+	13	0	
317566	**R1wR1**	+	+	0	0	+	0	0	+	0	+	0	+	+	+	0	+	0	0	0	+	+	+	+	+	0	+		14	2+	
22884	**R1R1**	+	+	0	0	+	0	+	+	0	+	0	+	+	0	+	0	+	0	+	+	+	+	0	0	0	+		15	2+	
306000	**R1R1**	+	+	0	0	+	0	0	+	0	+	0	+	0	+	+	+	0	+	0	0	+	+	0	+	0	+		16	0	
309536	**R1R1**	+	+	0	0	+	0	+	+	0	+	0	+	0	+	+	0	+	+	0	0	+	0	+	+	0	+		17	2+	

Suggestion: All of the patient's results have been given above. For greater educational impact we suggest you analyze the case on your own prior to turning to the next 3 pages.

Questions/discussion

Now what antibodies do you think are present? Are they all proven?

Standard crossing out technique allows one to rule out anti-D, -C, -e, -k, -Fy^{b}, -Jk$^{a\&b}$, -Le$^{a\&b}$, -S, -s, -M, -N, and -P1 (see below). The patient's phenotype allows her to form of all of the common alternate possibilities, i.e. anti-E, -c, -K, and -Fy^{a}, and we will analyze the hypothesis that all 4 of these antibodies are present So the next task is to find "rule-in" or "proof" cells for these specificities. Rule-in cells for anti-c and anti-E can be sought in the initial two panels; rule-in cells for anti-K and anti-Fy^{a} can be found most readily from the R1R1 cells in the first two panels or in the R1R1 selected cells. I find it easier if I use multiple highlighter pens as shown below.

Selected Rh specific cells (R1R1)

		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
306017	R1wR1	+	+	0	0	+	0	0	+	0	+	0	+	+	+	0	+	+	0	+	+	+	+	0	+s	0	+	C^{w}, HLA+	1	2+
316120	R1R1	+	+	0	0	+	0	+	+	0	+	0	+	+	+	+	+	+	+	0	0	+	+	+	0	0	+		2	2+
302558	R1R1			0	0		0	0		0	+	0	+	0		0		0	0			0	+	+	+	+	HLA+	3	0
306546	R1R1			0	0		0	0		0	+	0	+	0		0		0		0	0		0			0	+		4	0
318586	R1wR1			0	0		0	0		0	+	0	+	0		+	+			0	0		+	+	+	0	+	C^{w}	5	0
316865	R1R1	+	+	0	0	+	0	+	+	0	+	0	+	+	+	0	+	+	0	0	+	+	+	0	+	0	+		6	2+
321049	R1R1			0	0		0	0		0	+	0	+	0	+		0		0			0		0	+	0	+	C^{w}, HLA+	7	0
118159	R1wR1	+	+	0	0	+	0	0	+	0	+	0	+	+	+	+	+	+	+	0	0	+	0	+	+	0	+		8	2+
313887	R1R1	+	+	0	0	+	0	+	+	0	+	0	+	+	0	+	+	0	+	0	+	0	0	+	+	0	+		9	2+
319654	R1R1	+	+	0	0	+	0	+	+	0	+	0	+	+	+	+	+	0	0	+	+	+	+	+	0	0	+		10	2+
320747	R1wR1			0	0		0	0		0	+	0	+	0		+	0		0	+	+	+	+	+	+	0	+	C^{w}	11	0
316707	R1R1	+	+	0	0	+	0	+	+	0	+	0	+	+	0	+	+	+	+	0	+	+	+	0	+	0	+	HLA+	12	2+
302558	R1R1			0	0		0	0		0	+	0	+	0		0		0	0			0	+	+	+		+	HLA+	13	0
317566	R1wR1	+	+	0	0	+	0	0	+	0	+	0	+	+	+	0	+	0	0	0	+	+	+	+	+	0	+		14	2+
22884	R1R1	+	+	0	0	+	0	+	+	0	+	0	+	+	0	+	0	+	0	+	+	+	+	0	0	0	+		15	2+
306000	R1R1			0	0		0	0		0	+	0	+	0		+	+	0	+	0	0			0		0	+		16	0
309536	R1R1	+	+	0	0	+	0	+	+	0	+	0	+	0	+	+	0	+	+	0	0	+	0	+	+	0	+		17	2+

Initial Plasma Panel

		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	0	+	+	+	0	+s	0	+		3	4+	
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	+	0	+		5	3+	
6	r"r	0	0	+	+	+	0	0	+	0	+	0	+	0	+	0	+	+	0	+	+	+	+	+	+	0	+		6	4+	
7	rr	0	0	0	+	+	0	+	+	0	+	0	+	0	+	0	+	0	0	+	0	+	+	+	0	0	+		7	3+	
8	rr	0	0	0	+	+	0	0	+	0	+	0	+	+	0	+	+	0	+	0	+	+	0	+	0	0	+		8	3+	
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	0	+		10	3+	
11	R1R1	+	+	0	0	+	0	+	+	0	+	0	+	0	+	0	+	+	0	+	+	+	+	0	+s	0	+		11	2+	
Patient																													AC	w+	

Second panel

		Rh system						Kell						Duffy		Kidd		Xg	Lewis		MNSs				P	Lutheren		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	+	0	+	0	+	+	0	+	C^w	1	3+	
13	rr	0	0	0	+	+	0	+	+	0	+	0	+	+	0	+	0	+	0	+	+	+	0	+	0	0	+		2	3+	
14	rr	0	0	0	+	+	0	0	+	0	+	0	+	+	+	0	+	+	0	+	+	0	+	0	+s	0	+		3	2+	
15	R2R2	+	0	+	+	0	0	0	+	0	+	0	+	+	+	0	+	+	+	0	+	+	+	0	0	0	+		4	4+	
16	R2R2	+	0	+	+	0	0	0	+	0	+	0	+	+	0	+	0	+	0	+	+	+	+	0	+	0	+		5	4+	
17	R2R2	+	0	+	+	0	0	0	+	0	+	0	+	0	+	+	+	0	0	+	+	+	+	0	+	0	+		6	4+	
18	R1R1	+	+	0	0	+	0	0	+	0	+	0	+	+	0	+	+	+	0	+	0	+	+	+	0	0	+		7	2+	
19	R1R1	+	+	0	0	+	0	+	+	0	+	0	+	0	+	0	+	+	0	+	+	0	+	+	+s	0	+		8	2+	
20	RZR1	+	+	+	0	+	0	0	+	0	+	0	+	+	0	+	0	+	0	+	+	0	+	+	+s	0	+		9	4+	
21	r'r	0	0	0	+	+	0	0	+	0	+	0	+	0	+	0	+	+	0	+	0	+	+	+	+	0	+		10	2+	
22	R2R2	+	0	+	+	0	0	+	0	0	+	0	+	+	0	+	+	0	0	+	+	+	+	0	+	0	+		11	4+	
Patient																													AC	w+	

Having done that analysis, there are 7 non-reactive cells lacking all 4 of antigens corresponding to the patient's postulated antibodies. Also note that there are 6 anti-Fy^{a} rule-in cells that express Fy^{a} but lack the other 3 putative antigens, so the "rule of 3's" (3 reactive cells with the antigen, 3 non-reactive cells lacking the antigen) is met, indicating that there is a likelihood of 95% or greater that anti-Fy^{a} is present. The criterion is also met for anti-K. There are only 2 anti-c rule-in cells, but the combination of 2 reactive antigen positive cells and 5 non-reactive antigen negative cells also provides us with 95% confidence that anti-c is present. Because of the rarity of RzR1 cells, there are no E+, c-, Fy $^{a-}$, K- rule-in cells for anti-E. Nonetheless, as mentioned above the fact that the 4+ reactive cells follow E specificity perfectly indicates that this antibody is almost certainly present. And when it comes to selecting compatible RBCs for this patient we have to provide c-negative RBCs and we know the vast majority of these will be E-negative as well. (The donor cells should still be tested to insure that they are indeed E-negative.)

Note that the positive DAT with a negative eluate was a "red herring" in this case. This phenomenon may be most-commonly due to high levels of IgG (polyclonal hypergammaglobulinemia) in the patient's plasma. This can be seen in many patients' routine lab results as a widened gap between their total protein and albumin levels. Inspection of their problem list may show some form of chronic immune stimulation such as unresolved infection or bed sores, or renal failure.

Take home points

The differential diagnosis when all cells on the initial panel(s) are reactive
The approach to the problem by testing Rh (and other) phenotype-identical RBCs
Analysis of panel results