

CASE OF THE MONTH: JANUARY, 2019 (Case study by Jim Perkins)

History: This patient was a 28 year old woman admitted to labor and delivery.

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

Antibody Screen

Cell phenotype

Antibody Screen	
	Gel
SCI	1+
SCII	0

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

	Rh system	Kell										Duffy		Kidd		Xg		Lewis		MNSs				P		Lutheran		Cell	Gel		
		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	PI	Lu ^a	Lu ^b					
SC I	R1R1	+	+	0	0	+	0	+	0	+	0	+	0	+	0	+	+	0	0	+	0	+	+	0	+	+	0	+		SC I	1+
SC II	R2R2	+	0	+	+	0	+	+	0	+	/	+	+	0	0	+	+	0	+	+	+	+	0	+	+	0	+		SC II	0	

Initial Plasma Panel

VRA314		Rh system							Kell						Duffy		Kidd		Xg		Lewis		MNSs				P		Lutheran		Other Typings	Cell	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	PI	Lu ^a	Lu ^b						
1	R1wR1	+	+	0	0	+	0	0	+	0	+	0	+	+	0	0	+	+	0	+	0	+	+	0	0	0	0	+	+	HLA+	1	0	
2	R1R1	+	+	0	0	+	0	0	+	0	+	0	+	+	+	0	0	+	0	+	0	+	0	+	0	+	0	+		2	0		
3	R2R2	+	0	+	+	0	0	0	+	0	+	0	+	0	+	0	+	+	0	0	+	+	+	0	0	0	+	HLA+	3	0			
4	Ror	+	0	0	+	+	+	+	+	0	+	/	+	0	0	+	0	+	0	0	+	+	0	+	+	+	+		4	0			
5	r'r	0	+	0	+	+	0	0	+	0	+	/	+	+	+	+	+	0	0	+	+	0	+	0	+	0	+		5	0			
6	r''r	0	0	+	+	+	0	0	+	0	+	0	+	+	0	0	+	0	0	+	+	+	+	+	+	+	0	+		6	0		
7	rr	0	0	0	+	+	0	+	+	0	+	/	+	0	+	+	0	0	0	+	0	+	0	+	+	+	+	HLA+	7	0			
8	rr	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	+	+	+	+	+	HLA+	9	0			
10	rr	0	0	0	+	+	0	0	+	+	+	0	+	+	+	+	0	+	0	+	+	+	+	+	+	+	+		10	0			
11	R1R1	+	+	0	0	+	0	+	+	0	+	0	+	0	+	0	+	+	0	+	+	+	+	+	+	+	0	+		11	0		
Patient																													AC	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 A pos with a positive antibody screen. There are only two cells reactive among the 13 screening and antibody identification cells tested in gel, and on first pass "everything is ruled out" as we say. Possibilities include an antibody that is too weak to react consistently with its target antigen, an antibody against an antigen present on 2 of 13 cells but which is not listed in the antigram, an HLA antibody, and non-specific, possibly gel-dependent, reactivity.

The technologist elected to run a PEG/tube panel in the hope of finding additional reactive cells as well as to rule out gel-dependent reactivity. In addition, in order to investigate the possibility of an HLA antibody they adsorbed the patient's plasma with human platelet concentrate (HPC) and re-tested it against the previously-reactive RBC samples. The results are shown below.

Second panel; PEG tube technique

RA097	Rh system						Kell						Duffy		Kidd		Xg	Lewis			MNSs				P	Lutheran		Other	PEG	
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	PI	Lu ^a	Lu ^b	Typings	Cell	AHG
1	R1wR1	+	+	0	0	+	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	+	+	+	+s	0	+		1	0 ^v
2	R1R1	+	+	0	0	+	0	+	+	0	+	/	+	+	+	+	+	0	+	0	+	+	+	0	+s	0	+		2	1+
3	R2R2	+	0	+	+	0	0	0	+	0	+	0	+	0	+	0	+	+	0	+	+	+	+	+	0	0	+	HLA+	3	0 ^v
4	Ror	+	0	0	+	+	+	+	+	0	+	/	+	0	+	+	0	+	0	0	0	+	+	+	+	0	+	HLA+	4	0 ^v
5	r'r	0	+	0	+	+	0	0	+	0	+	/	+	+	0	+	+	+	0	+	0	+	+	+	+s	0	+		5	0 ^v
6	r"r	0	0	+	+	+	0	0	+	0	+	0	+	0	+	0	+	+	0	+	+	+	+	0	+s	0	+		6	0 ^v
7	rr	0	0	0	+	+	0	+	+	0	+	0	+	0	+	0	+	+	0	+	0	+	0	+	+s	0	+		7	0 ^v
8	rr	0	0	0	+	+	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	+	+	0	+	0	+		8	1+
9	rr	0	0	0	+	+	0	0	+	0	+	0	+	+	0	0	+	0	0	0	+	0	+	+	+	0	+	HLA+	9	0 ^v
10	rr	0	0	0	+	+	0	0	+	0	+	0	+	+	+	+	0	+	0	+	+	0	0	+	0	0	+		10	0 ^v
11	R1R1	+	+	0	0	+	0	0	0	0	+	0	+	0	+	0	+	+	0	+	+	0	+	+	+	0	+		11	0 ^v

Selected cells tested with HPC treated plasma.

		Rh system					Kell						Duffy		Kidd		Xg	Lewis			MNSs				P	Lutheran			HPC	
	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	PI	Lu ^a	Lu ^b		Cell	Gel	
SC I	R1R1	+	+	0	0	+	0	+	0	+	0	+	0	+	+	0	+	+	0	0	+	0	+	+	+s	0	+		SC I	0
8	rr	0	0	0	+	+	0	+	0	+	/	+	+	0	0	+	+	+	0	0	+	+	0	+	0	+	+		8	0

Questions/discussion

What is your impression now? Is any additional work-up required?

On the basis of the ability of HPC to neutralize the reactivity, and the fact that other blood group antibodies had been ruled out, a diagnosis of anti-HLA antibody was made and the workup was submitted for review.

The reviewer noted that although anti-Le^a had been ruled out on a single cell from the initial panel, all of the reactive cells were Le^a positive. They elected to have additional selected cells tested as follows:

Selected cell panel; manual gel technique

		Rh system						Kell						Duffy		Kidd		Xg	Lewis			MNSs				P	Lutheran		Other			
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	PI	Lu ^a	Lu ^b	Typings	Cell	Gel		
1	R1R1	+	+	0	0	+	0	0	+	0	+	/	+	+	+	0	+	+	0	0	+	+	0	0	0	0	+		1	1+		
2	R2R2	+	0	+	+	0	0	0	+	0	+	/	+	0	+	+	+	+	+	0	+	+	+	+	0	0	+		2	1+		
3	rr	0	0	0	+	+	0	0	+	0	+	/	+	+	+	0	+	+	+	0	0	+	0	+	+	0	+		3	2+		
4	R2R2	+	0	+	+	0	0	0	+	0	+	/	+	+	+	0	+	0	+	0	+	+	+	+	0	0	+		4	1+		
5	rr	0	0	0	+	+	0	+	+	0	+	/	+	0	+	+	+	+	+	0	0	+	+	+	+	0	+		5	1+		
6	rr	0	0	0	+	+	0	0	+	0	+	0	+	0	+	+	0	0	+	0	+	+	+	0	+s	0	+		6	1+		

Questions/discussion

What is your interpretation now? What was the problem with the initial workup as presented above? What other approaches can be used to identify these antibodies?

In this case there was an initial error in identification of the patient's antibody as anti-HLA; on review the antibody was correctly identified as anti-Le^a. and additional testing was requested, confirming the anti-Le^a. Another minor issue was that no autocontrol was performed with the PEG panel; when a different testing method is used, the autocontrol should be repeated.

HPC is a preparation of membranes (stroma) of pooled donor platelets that can be prepared in-house or obtained commercially in lyophilized form. Since platelets strongly express class I HLA, HPC will adsorb HLA antibodies from plasma. Therefore, if otherwise non-specific plasma reactivity is removed by incubation with HPC it can be inferred that the reactivity was due to anti-HLA. HLA antigenicity is destroyed by chloroquine and EDTA-glycine, so elimination of reactivity with a particular cell(s) by these agents allows one to make the same inference.

HPC treatment is simple and fast, requiring only that patient plasma be added to the vial of lyophilized material and incubated for 15 minutes at room temperature, transferred to a test tube, and centrifuged. However, an important caveat must be kept in mind, namely that other RBC-reactive antibodies may be adsorbed by HPC as well, as listed in the manufacturer's product insert. These include anti-A, -B, -H, -I, -Le^a, -Le^b, and -PP1P^k (Tj^a). Rh, Kell, Kidd, Duffy, MNSs and Lutheran system antibodies, and anti-PI, and -Xg^a are not removed. Therefore, antibody identification panel cells that were previously reactive but which are non-reactive with HPC-adsorbed plasma should, if possible, not be used as "rule out" cells, and because it adsorbs anti-A and -B, HPC-adsorbed plasma cannot be used for

crossmatching. Analogous considerations apply to the use of Rabbit Erythrocyte Stroma (R.E.S.T.TM), just as the pattern of antigen destruction must be considered in using enzyme-, DTT-, ZZAP-, or chloroquine-treated RBCs for ruling out or adsorption. In this case HPC appears to have adsorbed the anti-Le^a, which was a "red herring" misleading the technologist into an incorrect interpretation.

HLA antibodies, or so-called "anti-Bg", reactive with RBCs are common, particularly in pregnant and heavily transfused patients. Although generally regarded as clinically insignificant, rare cases of significant hemolysis are reported (see Daniels, "Human blood Groups", chapter 32), so an antiglobulin crossmatch should be performed when they are detected with the current specimen.

Strong anti-Le^a can cause hemolysis of transfused Le^a-positive RBCs so crossmatch-compatible RBCs should be selected. However, there is no need to select Le^a-negative RBCs if this criterion is met.

Perhaps the most important insight illustrated by this case regards the reviewer's thought process that prompted reevaluation and identification of anti-Le^a. On the first panel anti-Le^a appeared to be ruled out on the basis of a single non-reactive Le^a positive cell, but the reviewer noted that all of the reactive cells were Le^a positive. It's important to look for this feature whenever reactions are weak and inconsistent. Many cases of weak anti-Jk^a have been identified by noting that all reactive cells were Jk^a positive, in spite of the fact that anti-Jk^a had been "ruled-out" on one or more cells. For this reason some laboratories require that two non-reactive, double dose cells are required to rule out an antibody.

Take home points

Use of HPC adsorption to identify HLA antibodies and its limitations

Other methods for identifying the presence of HLA antibodies which complicate blood group antibody identification

Inspection of reaction patterns of weak antibodies for antigens present on all reactive cells even when the corresponding specificity appeared ruled out.