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

History: A 30 year old, G3P2002 woman was referred to a maternal fetal medicine specialist at 13 weeks gestation when she was found to have a positive blood group antibody screen due to anti-D plus anti-C (identified at another laboratory) on her first prenatal visit. Her first infant was born at 42 weeks gestation and was found to be severely anemic. The Kleihauer Betke test done at the time demonstrated an estimated fetal maternal hemorrhage of 1000cc fetal whole blood for which she is reported to have received 600 µg of intravenous Rh immune globulin every 8 hours for a total dose of 9000 µg.

ABO and Rh typing

<A	<B	A1 cells	B cells	6% alb	<D	<D/AHG	CCC	Interp
4+	0	0	4+		0	0	2+	

Antibody Screen

	Gel
SCI	See panels
SCII	

Direct Antiglobulin Test (tube method)

	Poly	IgG	<C3
AHG	0		
CCC	2+		

Intial Panel

Lot# VRA112		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	0	+	0	+	+	0	+	C^w	1	4+
2	R1R1	+	+	0	0	+	0	0	+	0	+	0	+	+	+	+	0	+	+	0	+	0	+	0	+s	0	+		2	4+
3	R2R2	+	0	+	+	0	0	0	+	0	+	0	+	0	+	0	+	+	0	+	+	+	+	0	+	0	+		3	3+
4	Ror	+	0	0	+	+	+	0	+	0	+	0	+	0	0	+	+	+	0	+	0	+	0	+	+	0	+		4	3+
5	r'r	0	+	0	+	+	0	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	+	+	0	+	0	+		6	0
7	rr	0	0	0	+	+	0	+	+	0	+	0	+	0	+	+	0	0	0	0	+	+	+	+	0	0	+		7	0
8	rr	0	0	0	+	+	0	0	+	0	+	0	+	0	+	0	+	+	+	0	+	0	+	0	0	0	+		8	0
9	rr	0	0	0	+	+	0	+	+	0	+	0	+	+	0	0	+	+	0	0	+	+	+	0	+	0	+		9	0
10	rr	0	0	0	+	+	0	0	+	0	+	0	+	0	+	+	0	+	0	+	0	+	0	+	+	+	+		10	0
11	R1R1	+	+	0	0	+	0	+	+	0	+	0	+	0	+	+	0	0	+	0	0	+	+	0	+	0	+		11	4+
Patient																													AC	

Additional cells

		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		Gel	
5207	r'r	0	+	0	+	+	0	0	+	0	+	0	+	+	0	+	0	+	0	+	+	+	0	+	+	0	+		3+	
8387	r'r	0	+	0	+	+	0	0	+	0	+	0	+	0	0	+	0	+	0	+	0	+	+	+	+	0	+		3+	

Titration (Tube IAT method using 2 drops plasma, 1 drop single dose RBCs, 30',37oC incubation, and AHG.)

ANTIBODY: Anti- C+D				EDC: 8/28/'08			Tech:		Date tested: 13 2/7 weeks			
	Sample dilution, Reaction strength at AHG phase:											
	1	2	4	8	16	32	64	128	256	512	1024	Titer
R1r (D+C+c+E-e+)	1+	vw+	0	0	0	0	0	0	0	0		1
Ror (D+C-c+E-e+)	0	0	0	0	0	0	0	0	0	0		<1
r'r (D-C+c+E-e+)	w+	0	0	0	0	0	0	0	0	0		1
Cell type: (see above)		Manufacturer:				Lot number:			Comment:			

Question:

1. The outside laboratory identified anti-D + anti-C. What are the titers of these two antibodies? Is this what we would expect? Is there any other possibility other than anti-D plus anti-C? If there are other possibilities how would you investigate?

   Three different titering cells were tested, an R1r cell (D+C+c+E-e+) that expresses D and C, an Ror cell (D+C-c+E-e+) that expresses D but not C, and a r'r cell (D-C+c+E-e+) that expresses C but not D. So if anti-D and anti-C were present, the R1r cell would give us a titer of 1 for the titer of both antibodies combined. The Ror cell would give us the anti-D titer as <1 (the titer is listed as <1 because it reacts in gel but not in the tube IAT method used for performing the titer). Finally the r'r cell would give us the anti-C titer as 1. This is an unexpected result however as we would expect the anti-D to be stronger than anti-C in a combination of the two antibodies.

   Instead the patient could have anti-G, or some other mixture of anti-G, anti-C and/or anti-D. G is an antigen on most individual's RHD protein as well as on most C-bearing RHCE proteins. Therefore anti-G produces reactions that look like anti-D plus anti-C in a panel. Anti-G typically reacts more strongly with D-neg/C-pos cells than it does with D-pos/C-neg cells. This is the tipoff that anti-G is present, and this is exactly what we see in these titration results. The distinction between anti-G and anti-D and/or anti-C as well as the possible mixtures can be made by performing alloadsorption procedures. Since G is present on most D-carrying proteins AND C-carrying proteins anti-G can be adsorbed by either D-pos/C-neg cells or D-neg/C-pos RBCs, in either case appearing to adsorb both anti-D and anti-C. An eluate from such adsorbing cells can also be shown to react with a D or C antigen that was NOT on the adsorbing cell. Again note that any combination of anti-G with anti-D or anti-C can exist, so careful interpretation of such tests is necessary.

A new specimen was received 6 weeks later, at which time the following tests were performed:

Raw serum panel

Lot# VRA113		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	+	+	+	0	0	0	+	C^w	1	
2	R1R1	+	+	0	0	+	0	+	+	0	+	0	+	0	+	0	+	+	0	+	0	+	0	+	0	0	+		2	
3	R2R2	+	0	+	+	0	0	0	+	0	+	0	+	+	+	+	+	+	0	0	+	+	+	+	0	+	+		3	2+
4	Ror	+	0	0	+	+	+	0	+	0	+	0	+	+	0	+	0	+	0	+	0	+	+	+	+	0	+		4	
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	+	0	+	0	+		6	0
7	rr	0	0	0	+	+	0	+	+	0	+	0	+	0	+	0	+	+	+	0	+	+	+	0	+s	0	+		7	0
8	rr	0	0	0	+	+	0	0	+	0	+	0	+	0	+	+	+	0	0	+	+	0	+	0	0	0	+		8	
9	rr	0	0	0	+	+	0	0	+	0	+	0	+	+	0	+	+	+	0	+	0	+	0	+	0	0	+		9	0
10	rr	0	0	0	+	+	0	0	+	0	+	0	+	0	+	+	0	0	+	0	+	+	+	0	+	0	+		10	
11	R1R1	+	+	0	0	+	0	0	+	+	+	0	+	0	+	+	+	+	0	0	0	+	+	0	+s	0	+		11	

Alloadsorbed serum panel; one aliquot adsorbed with Ror cells and one with r'r cells

Lot #05500		Rh system						Kell						Duffy		Kidd		Lewis		P	MNSs				Lutheran		Xg	Other Typings	Cell	Adsorbing cell phenotype	
																													Ror	r'r
Cell	Rh	D	C	c	E	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			AHG	AHG
1	R1R1	+	+	0	0	+	0	+	+	+	+	0	+	0	+	+	0	0	+	+	0	+	0	+	0	+	+		1	0	
2	R1wR1	+	+	0	0	+	0	0	+	0	+	0	+	0	+	+	0	0	0	+	+	0	+	0	0	+	+	C^w	2	0	0
3	R2R2	+	0	+	+	0	0	0	+	0	+	0	+	0	+	0	+	+	0	+	+	+	0	+	+	+	+		3	0	0
4	Ror	+	0	+	0	+	0	0	+	0	+	+	+	+	0	+	0	0	0	+	0	+	0	+	0	+	+		4	0	
5	r'wr	0	+	+	0	+	0	0	+	0	+	0	+	+	+	+	0	0	+	0	+	0	0	+	0	+	+		5	0	0
6	r"r	0	0	+	+	+	0	0	+	0	+	0	+	+	+	+	0	0	+	+	+	+	+	+	0	+	+	Co(b+)	6		
7	rr	0	0	+	0	+	0	+	+	0	+	0	+	0	+	+	+	+	0	0	0	+	0	+	0	+	+		7		
8	rr	0	0	+	0	+	0	0	+	+	+	0	+	+	0	0	+	0	+	+	+	+	0	+	+	+	+		8		
9	rr	0	0	+	0	+	0	0	+	0	+	0	+	0	0	+	+	+	0	+	0	+	0	0	0	+	+		9		
10	R1R1	+	+	0	0	+	0	+	+	+	+	0	+	+	0	0	+	0	+	+	+	+	0	+	0	+	+		10		
11	r"r	0	0	+	+	+	0	0	+	0	+	0	+	+	+	0	+	0	+	+	0	+	0	+	0	+	0	Co^b+, I-	11		

Questions:

2. What antibody specificities are suggested by the gel reaction strengths and adsorption results shown above? Explain your answer.

Again the panel suggests that both anti-D and anti-C are present, but the anti-C reaction strength appears slightly stronger consistent with anti-G.

The adsorption results are consistent with anti-G without any admixture of anti-D or anti-C. The reactions of various mixtures of these 3 antibodies after adsorption are compared to those of anti-G in the following table.

Phenotype of adsorbing cell	*Reactions of plasmas which have been adsorbed by cells listed in the left column*							
	anti-G		*anti-D plus anti-C*		*anti-G plus anti-D*		*anti-G plus anti-C*	
	Ror	*r'r*	*Ror*	*r'r*	*Ror*	*r'r*	*Ror*	*r'r*
Ror	*0*	*0*	*0*	+	*0*	*0*	*0*	+
r'r	*0*	*0*	+	*0*	+	*0*	*0*	*0*

3. Is any further workup needed to prove it? Are there any problems with the way in which the tests were performed?

Unfortunately appropriate controls were not performed with these tests. Adsorption inevitably causes some level of dilution of the serum, and controls are needed to demonstrate that the loss of activity is not due to dilution alone. This could have been done by performing an adsorption with rr (D-neg, C-neg) RBCs in addition to the Ror and r'r cells. After adsorption with such cells one would expect the anti-G (apparent anti-D + anti-C) reactivity to persist. Nonetheless, the gel reactivity was sufficiently strong that we would not expect the antibody to be diluted away.

An alternative, very elegant procedure developed by Vos (Vox Sang., 1960) is to perform the following sequence:

1. *Adsorb the serum with dCe/dce (r'r) cells.*
2. *Prepare an eluate from the adsorbing cells.*
3. *Adsorb the eluate with Dce/dce (Ror) cells.*
4. *Prepare an eluate from the second adsorbing cells.*
5. *Test the adsorbed serum, the adsorbed eluate (first adsorption supernatant), and both eluates with r'r and Ror cells.*

Reactions of the adsorbed serum and eluates in the case of the possible antibody combinations are shown in the table below.

Possible combinations of anti-G, anti-D, and anti-C	*Reactions with Ror and r'r cells*							
	Serum ads. with r'r cells		*Eluate 1*		*Eluate 1 ads. with Ror cells*		*Eluate 2*	
	Ror	*r'r*	*Ror*	*r'r*	*Ror*	*r'r*	*Ror*	*r'r*
Anti-G	*0*	*0*	+	+	*0*	*0*	+	+
Anti-D + anti-C	+	*0*	*0*	+	*0*	+	*0*	*0*
Anti-D + anti-G	+	*0*	+	+	*0*	*0*	+	+
Anti-C + anti-G	*0*	*0*	+	+	*0*	+	+	+
Anti-D + anti-C + anti-G	+	*0*	+	+	*0*	+	+	+

Rare cells exist which have G in the absence of D or C antigens, and which have D but not G. Consistent reactions with such cells demonstrate anti-G specificity. Anti-G specificity is demonstrated in the above sequence without access to such rare cells, but it is technically challenging, particularly if the antibodies are weak.

4. Is this patient a candidate for antenatal Rh immune globulin? Does it appear that the Rh immune globulin (RhIG) given after the first pregnancy prevented the mother from being immunized to anti-D.

   If anti-G is her only antibody she is not immunized against the D antigen, so she remains at risk for forming anti-D in addition to her anti-G. Therefore, she is still an RhIG candidate. Determination of RhIG candidacy is the reason to make these complicated distinctions, They are only academic with respect to transfusion for patients other than women of childbearing age, since we would give D-neg, C-neg RBCs in any case.

   It initially appeared that the massive dose of RhIG given after the first pregnancy, complicated by chronic fetal maternal hemorrhage, was successful. However, but anti-D immunization may only become evident during a subsequent at-risk pregnancy.

5. Is this patient at risk for a hemolytic transfusion reaction? HDFN?

   Anti-G can cause HTRs, particularly delayed reactions. Anti-G causes HDFN, but it is less severe than that due to anti-D, so it is still important to prevent anti-D if possible.

Serial titration yielded the following results:

Titration using the same R1r cell target in each case

Sample day by gestation	**Sample dilution; Reaction strength at AHG phase:**												
	1	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**512**	**1024**	**2048**	**Titer**
13w2d	**1+**	**vw+**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**			**1**
19w4d	**w+**	**vw+**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**			**1**
24w	**vw+**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**			**<1**
27w6d	**w+**	**w+**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**			**2**
29w6d	**3+**	**2+**	**w+**	**0**	**0**	**0**	**0**	**0**	**0**	**0**			**4**
31w6d	**4+**	**4+**	**3+**	**2+**	**1+**	**w+**	**0**	**0**	**0**	**0**			**32**
33w2d	**4+**	**4+**	**4+**	**4+**	**3+**	**2+**	**1+**	**w+**	**vw+**	**0**			**128**

Alloadsorbed serum panel, specimen from 29 weeks & 6 days adsorbed with r'r RBCs.

		Rh system						Kell						Duffy		Kidd		Lewis		P	MNSs				Lutheran		Xg		LISS, AHG	
Cell	Rh	D	C	c	E	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	Cell	Raw plasma	Adsorbed plasma
3	R2R2	+	0	+	+	0	0	0	+	0	+	0	+	+	0	+	0	0	+	+	+	0	0	+	0	+	+	3	3+	3+
4	Ror	+	0	+	0	+	+	0	+	0	+	0	+	+	0	+	+	0	+	+	+	+	0	0	0	+	0	4	3+	3+
5	r'r	0	+	+	0	+	0	0	+	0	+	0	+	+	+	0	+	+	0	0	+	+	+	+	0	+	+	5	2+	0
11	r'r	0	+	+	0	+	0	0	+	0	+	0	+	+	+	+	0	0	+	+	+	+	0	+	0	+	+	11	1+	0

Questions:

6. What antibody specificity(ies) is demonstrated now? What do you note about the titer?

   The patient now is making anti-D as well as the anti-G since an r'r cell (D-C+) is no longer able to remove the anti-D-like activity. Anti-C is not ruled out.

   Note that the titer tested against the R1r cell (D+C+c+E-e+) that was being used to follow the titer has only increased from 2 to 4 based on the endpoint of the dilution. However, the reaction strengths with undiluted serum and with the 1:2 dilution have increased markedly. If the titration score is calculated for the 27w6d specimen versus the 29w6d, the titration score has increased from 4 to 20 (see AABB Technical Manual Methods section for antibody titration). An increase in score of 10 regarded as a significant increase.

7. What would you advise the patient's physician?

   The patient is no longer a candidate for Rh immune globulin and should be treated as any other patient with anti-D. Although our institution regards 128 as the critical titer for anti-D, we begin non-invasive monitoring of the fetus with middle cerebral artery blood velocity studies when the titer reaches 32. In this case early delivery was performed as soon as there was evidence of fetal compromise and the infant received phototherapy and one exchange transfusion.

CASE FOLLOWUP:

Middle cerebral artery velocity was determined by doppler sonography at 32w3d, 33w2d, and 34w4d. It was normal on the first two occasions but was elevated on the third exam (Peak systolic velocity (PSV)=0.82 m/s, Upper limit of normal (ULN) = 0.731 m/s). Amniocentesis at 34w4d also demonstrated a slightly elevated ΔOD_{450} = 0.5 (ULN 0.4) and fetal lung maturity, so the patient was referred for delivery at her local hospital the next day. The newborn infant did well on "bili lights" but was given one exchange transfusion.