

HDN ABID CASE # 4 Case study by Jim Perkins, M.D. (© 2010)

History: A 37 year old woman in her first pregnancy had a blood group antibody detected on routine prenatal testing at another facility. She was referred to a maternal-fetal medicine specialist at our hospital at approximately 16 weeks gestation. She denied previous transfusion.

ABO and Rh Typing

<A	<B	A1 cells	B cells	6% alb	<D	<D/AHG	CCC	Interp
4+	0	3+	4+		4+			A pos

Repeat ABO and Rh typing with ficin treated RBCs

A1 cells	B cells	Pt. cells
0	4+	0

Antibody Screen

	Gel
OI	4+
OII	3+

Direct Antiglobulin Test

	Poly	IgG	<C3
AHG	0		
CCC	2+		

Intial panel

Lot# 8RA206	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	P1	Lu ^a	Lu ^b	Typings	Cell	Gel		
1	R1wR1	+	+	0	0	+	0	0	+	0	+	0	+	+	0	+	+	0	0	+	0	+	+	0	+s	0	+	C ^w	1	4+		
2	R1R1	+	+	0	0	+	0	+	+	0	+	0	+	+	+	+	+	0	+	0	0	+	+	+	0	0	+		2	4+		
3	R2R2	+	0	+	+	0	0	0	+	0	+	0	+	+	+	0	+	0	0	0	+	+	0	+	0	0	+		3	0		
4	Ror	+	0	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	+	+s	0	+		5	0		
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	+		7	3+		
8	rr	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	+	+	+	+	0	+		9	3+		
10	rr	0	0	0	+	+	0	0	+	0	+	0	+	0	+	+	0	+	0	0	0	+	+	+	0	+	0	+	10	4+		
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						
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	Typings	Cell	Gel		
1		+	w	+	+	w	0	0	+	0	+	0	+	0	0	+	0	+	0	+	+	0	0	+	+	0	+		1	0		
2		0	0	0	+	+	0	+	+	0	+	0	+	0	+	0	+	0	0	+	0	+	0	+	+	0	+		2	0		
3		+	+	0	0	+	0	0	+	0	+	0	+	+	0	0	+	0	0	+	+	+	0	+	+	0	+		3	0		
Patient																													AC			

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“Tube test” panel

Lot #06776		Rh system					Kell						Duffy		Kidd		Lewis		P	MNSs				Lutheran		Xg	Other	Saline-susp. RBCs, 4 drops plasma						
Cell	Rh	D	C	c	E	e	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	Typings	Cell	IS	RT	37°	AHG	cc	
1	R1R1	+	+	0	0	+	+	+	0	+	0	+	+	+	+	0	0	+	+	+	+	0	+	0	+	0		1						
2	R1wR1	+	+	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	+	+	+	0	+	0	+	+	Co ^{b+} , C ^w	2						
3	R2R2	+	0	+	+	0	0	+	0	+	0	+	+	0	0	+	0	+	+	+	0	0	+	0	+	0		3						
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	+	0	+	0	+	+		5	0	0	0	0	0	2+
6	r''r	0	0	+	+	+	0	+	0	+	0	+	0	0	+	0	0	+	+	+	+	0	+	+	+	+		6	1+	w+	0	0	0	2+
7	rr	0	0	+	0	+	+	+	0	+	0	+	0	+	0	+	0	+	+	+	0	+	+	0	+	+		7	3+	3+	0	0	0	2+
8	rr	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						
10	R1R1	+	+	0	0	+	0	+	0	+	0	+	+	0	0	+	0	+	+	0	+	+	+	0	+	0		10						
11	R1R1	+	+	0	0	+	0	+	0	+	0	+	+	0	+	+	+	0	0	+	+	0	+	0	+	+	I-, Yt ^{b+}	11						
Patient																											AC							

Antigen phenotype

	Rh system				Kell				Duffy		Kidd		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	4+	4+	0	4+													0	4+																		
Pos control	4+	4+	4+	4+													4+	4+																		
Neg Control	0	0	0	0													0	0																		

Titration (IAT test with 2 drops plasma, 1 drop saline-suspended target RBCs, 30' incubation at 37°C, then addition of AHG)

ANTIBODY: Anti-M				EDC: ((day 280)				Tech:				Date tested: (Day 89)			
Sample dilution, Reaction strength at AHG phase:															
Sample date:	1	2	4	8	16	32	64	128	256	512	1024	2048	4096	8192	Titer
New: (day 89)	0	0	w+	1+	2+	1+	w+								
New: (repeat)	0	0	vw+	2+	2+	1+	w+	vw+	0	0	0				
Cell type: R1R1, M+N+				Manufacturer:				Lot number:				Comment:			

HDN ABID CASE # 4, pg 2: QUESTIONS

1. What is the probable identity of this antibody? Is any further workup needed to prove it? Why were the additional 3 gel test panel cells run after the initial 11 cell panel?
2. What was the cause of the ABO discrepancy? How was this resolved?
3. What is the indirect antiglobulin test (IAT) titer of this antibody? Why was the titer repeated?
4. The tube IAT was initially negative (see tube test panel #06776), but in the titration the antibody reacted in the IAT? Can you explain this discrepancy?
5. Are you concerned that this pregnancy might be affected by hemolytic disease of the fetus and newborn? If so, what would you do to investigate this possibility?

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Additional specimen was obtained, and the plasma was incubated with an equal volume of 0.01 M dithiothreitol (DTT). Titration was then repeated with DTT-treated plasma, as well as with a typical, cold-reactive anti-M serum for a control.

Titration with DTT treated serum: (AHG test with 2 drops plasma, 1 drop saline-suspended target RBCs, 30' incubation at 37°C)

ANTIBODY: Anti-M	EDC: ((day 280)			Tech:				Date tested: (Day 90)					
	Sample dilution, Reaction strength at AHG phase:												
	1	2	4	8	16	32	64	128	256	512	1024	2048	Titer
Patient serum tx'd with DTT	0	0	0	0	0	0	0	0					0
Dilution control: (serum + PBS)	vw+	vw+	w+	w+	w+	vw+	0	0					32
Control study with anti-M from a blood donor													
Donor serum tx'd with DTT	0	0	0										0
Dilution control: (serum + PBS)	2+	w+	0										4
Cell type: R1R1, M+N+	Manufacturer:				Lot number:				Comment:				

QUESTIONS

6. What is your conclusion regarding the likelihood of this antibody to produce HDFN? Why is the titer of the dilution control interpreted as "32" rather than "16"?
7. Would you recommend any other testing related to this problem during this pregnancy?

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The mother reported that her first child did not have jaundice or other problems at birth. She became pregnant again 2 years later. Her titer, determined at another laboratory, increased from 32 on her first visit to 128 over several months. Samples were submitted to our laboratory for study at 27 weeks, 4 days gestation. The serologic findings are shown below.

ABO and Rh Typing

<A	<B	A1 cells	B cells	6% alb	<D	<D/AHG	CCC	Interp
4+	0	1+	4+		4+			A pos

Repeat ABO and Rh typing with ficin treated RBCs

A1 cells	B cells	Pt. cells
0	4+	0

Antibody Screen

	Gel
OI	NT
OII	NT

Direct Antiglobulin Test

	Poly	IgG	<C3
AHG	0		
CCC	2+		

Selected cell panel

Cell	Rh	Rh system						Kell						Duffy		Kidd		Xg		Lewis		MNSs				P		Lutheran		Other Typings	Cell	Gel
		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					
1	R1wR1	+	+	0	0	+	0	0	+	0	+	0	+	0	+	+	0	+	0	0	+	0	+	+	0	+	+	0	+	C ^w , Lan ⁺ , JMH+	1	0
2	R1R1	+	+	0	0	+	0	0	+	0	+	0	+	0	0	+	0	+	0	+	+	0	+	+	0	0	+	Bg+	2	4+		
3	R1wR1	+	+	0	0	+	0	0	+	0	+	0	+	0	+	+	0	+	0	+	0	+	0	+	0	0	+	C ^w	3	0		
4	R2R2	+	0	+	+	0	0	0	+	0	+	0	+	0	+	+	0	+	+	0	+	+	+	+	0	+		4	4+			
5	R2R2	+	0	+	+	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	+	+	+	0	+		6	0			
7	rr	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	+		8	0			
Patient																												AC				

Selected cell panel, pre-warmed testing (4 drops plasma, saline suspended RBCs; warmed to 37°C before addition)

Lot #51877	Cell	Rh	Rh system						Kell						Duffy		Kidd		Lewis		MNSs				Lutheran		Xg	Other Typings	4 drops/saline		
			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
	1	R1wR1	+	+	0	0	+	0	0	+	0	+	0	+	+	+	+	0	+	+	+	0	+	0	+	+	+	C ^w	1	1+	1+
	2	R1R1	+	+	0	0	+	0	0	+	0	+	+	+	0	0	+	+	+	0	0	+	0	+	0	+		2	4+	4+	
	3	R1R1	+	+	0	0	+	0	0	+	0	+	0	+	+	0	0	0	+	0	+	0	+	0	+	0	+		3	0	0
	5	RzR1	+	+	0	+	+	0	0	+	0	+	0	+	+	0	+	+	+	0	0	+	0	+	+	+		5	4+	4+	
	6	R2R2	+	0	+	+	0	0	0	+	0	+	0	+	+	+	0	+	+	0	+	0	+	0	+	+		6	0	0	

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Titration with DTT treated serum: (AHG test with 2 drops plasma, 1 drop saline-suspended target RBCs, 30' incubation at 37°C)

ANTIBODY: Anti-M		EDC: ((day 280)			Tech:				Date tested: (Day 90)				
Sample dilution, Reaction strength at AHG phase:													
	1	2	4	8	16	32	64	128	256	512	1024	2048	Titer
Patient serum tx'd with DTT	0	0	0	0	0	0	0	0	0				0
Dilution control: (serum + PBS)	3+	3+	3+	2+	2+	1+	w+	vw+	0				128
Cell type: R1R1, M+N+	Manufacturer:				Lot number:				Comment:				

DTT control studies	Immediate spin testing			
	Group A, M-neg donor RBCs	M-neg	Group B, M-neg donor RBCs	M-neg
DTT-treated patient serum	0		0	
Patient serum + saline	0		4+	

QUESTIONS

8. What do the DTT control studies demonstrate?

9. Would you make any other recommendations regarding testing or management during this pregnancy?

FOLLOW-UP AND COMMENT:

The second child also did well, without jaundice or anemia in the newborn period.

In pregnant women with anti-M our laboratory performs a pre-warmed test as shown above. DTT treatment is only performed if the patient's anti-M reacts at 37°C or in the IAT.