Antibody Screen Negative?
Don't be Fooled!

Understanding When the Antibody Screen Does Not Detect Alloantibodies

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Objectives

- Describe at least two situations where alloantibody is present in plasma but the antibody detection test is negative.
- Describe factors that may impact differences in antibody detection and crossmatch results.
- Outline additional tests to be performed when an antibody is suspected after a negative Ab screen.

Case 1

- AS, a 50-year old female
- Pre-operative type and screen prior to hysterectomy
- Previously transfused 3 units of RBCs for low hemoglobin due to menorrhagia
- Patient record review reveals the patient is an A Rh positive with a previous negative antibody detection test (screen)

Case 1: ABO/Rh type (tube)

Forward			Reverse	
Anti-A	Anti-B	Anti-D	A1 cells	B cells
4+	0	3+	3+	4+

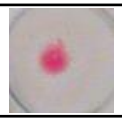
Is the forward type or reverse type most likely the correct blood type?

What is likely the problem?

A. Forward type: Extra antibody in reverse
B. Forward type: Missing antibody in reverse
C. Reverse type: Extra antigen in forward
D. Reverse type: Missing antigen in forward

Case 1: Antibody screen

	Rh						MNS				P	Lewis		Kell		Duffy		Kidd		SP
	D	C	E	c	e	f	M	N	S	s	P_1	Le^a	Le^b	K	k	Fy^a	Fy^b	Jk^a	Jk^b	
1	+	+	0	0	+	0	+	+	+	+	0	+	0	0	+	0	+	0	+	
2	+	0	+	+	0	0	0	+	0	+	+	0	+	0	+	+	+	+	0	
3	0	0	0	+	+	+	+	0	+	0	+	0	+	+	+	+	+	+	0	
Positive Control																				

Lot R564	Screen 1	Screen 2	Screen 3	Pos Ctrl
AS Plasma				
Interpretation	0	0	0	4+

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Why is the antibody detection test negative when extra antibody is detected in the ABO reverse/back type?

A. IgM antibody is usually negative in SP

B. IgG antibody is usually negative in SP

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Case 1: ABO/Rh type (tube)

Forward				Reverse		
Anti-A	Anti-B	Anti-A,B	Anti-A1 lectin	A1 cells	A2 cells	B cells
4+	0	4+	4+	3+	2+	4+

What testing would be helpful to explain the ABO discrepancy?

A. Test a second SP panel

B. Test a saline panel at IAT/AHG only

C. Test an enzyme panel at IAT/AHG only

D. Test a saline panel at IS, 37C and IAT/AHG

Antibody Identification Panel

	Rh						MNS				Lu		P	Lewis		Kell		Duffy		Kidd		Saline		
	D	C	E	c	e	f	M	N	S	s	Lu^a	Lu^b	P_1	Le^a	Le^b	K	k	Fy^a	Fy^b	Jk^a	Jk^b	IS	37C	IAT
1)R_1R_1	+	+	0	0	+	0	0	+	+	+	0	+	0	+	0	+	0	0	+	0	+	0	0	0
2) rr	0	0	0	+	+	+	+	0	+	0	0	+	+	0	+	0	+	+	+	+	0	3+	2+	1+
3) r'r	0	+	0	+	+	+	+	+	0	+	0	+	+	0	+	+	+	+	0	0	+	2+	1+	0
4) R_1r	+	+	0	+	+	+	0	+	+	0	0	+	0	0	0	0	+	+	+	+	+	0	0	0
5) r''r	0	0	+	+	+	+	+	+	0	+	0	+	0	0	+	0	+	0	+	0	+	2+	1+	0
6) r'r'	0	+	0	0	+	0	+	+	0	+	0	+	+	0	+	0	+	+	+	+	+	2+	1+	0
7) R_1R_1	+	+	0	0	+	0	+	0	0	+	0	+	0	0	+	0	+	0	+	+	0	3+	2+	1+
8) rr	0	0	0	+	+	+	+	0	+	0	0	+	0	0	+	0	+	+	0	0	+	3+	2+	1+
9) R_1r	+	+	0	+	+	+	0	+	0	+	0	+	+	+	0	+	+	+	+	+	+	0	0	0
10) R_2R_2	+	0	+	+	0	0	0	+	+	+	0	+	+	0	+	0	+	+	0	+	0	0	0	0
11) R_0r	+	0	0	+	+	+	+	+	+	0	0	+	+	0	+	0	+	0	0	+	+	2+	1+	0
Auto																						0	0	0

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Antibody Identification Panel

	Rh						MNS				Lu		P	Lewis		Kell		Duffy		Kidd		Saline		
	D	C	E	c	e	f	M	N	S	s	Lu^a	Lu^b	P_1	Le^a	Le^b	K	k	Fy^a	Fy^b	Jk^a	Jk^b	IS	37C	IAT
1)R_1R_1	+	+	0	0	+	0	0	+	+	+	0	+	0	+	0	+	0	0	+	0	+	**0**	**0**	**0**
2) rr	0	0	0	+	+	+	+	0	+	0	0	+	+	0	+	0	+	+	+	+	0	**3+**	**2+**	**1+**
3) r'r	0	+	0	+	+	+	+	+	0	+	0	+	+	0	+	+	+	+	0	0	+	**2+**	**1+**	**0**
4) R_1r	+	+	0	+	+	+	0	+	+	0	0	+	0	0	0	0	+	+	+	+	+	**0**	**0**	**0**
5) r''r	0	0	+	+	+	+	+	+	0	+	0	+	0	0	+	0	+	0	+	0	+	**2+**	**1+**	**0**
6) r'r'	0	+	0	0	+	0	+	+	0	+	0	+	+	0	+	0	+	+	+	+	+	**2+**	**1+**	**0**
7) R_1R_1	+	+	0	0	+	0	+	0	0	+	0	+	0	0	+	0	+	0	+	+	0	**3+**	**2+**	**1+**
8) rr	0	0	0	+	+	+	+	0	+	0	0	+	0	0	+	0	+	+	0	0	+	**3+**	**2+**	**1+**
9) R_1r	+	+	0	+	+	+	0	+	0	+	0	+	+	+	0	+	+	+	+	+	+	**0**	**0**	**0**
10) R_2R_2	+	0	+	+	0	0	0	+	+	+	0	+	+	0	+	0	+	+	0	+	0	**0**	**0**	**0**
11) R_0r	+	0	0	+	+	+	+	+	+	0	0	+	+	0	+	0	+	0	0	+	+	**2+**	**1+**	**0**
Auto																						**0**	**0**	**0**

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What antibody is identified?

A. Anti-K

B. Anti-Jk^a

C. Anti-Fy^a

D. Anti-M

Antibody Identification Panel

	Rh						MNS				Lu		P	Lewis		Kell		Duffy		Kidd		Saline		
	D	C	E	c	e	f	M	N	S	s	Lu^a	Lu^b	P_1	Le^a	Le^b	K	k	Fy^a	Fy^b	Jk^a	Jk^b	IS	37C	IAT
1)R_1R_1	+	+	0	0	+	0	0	+	+	+	0	+	0	+	0	+	0	0	+	0	+	0	0	0
2) rr	0	0	0	+	+	+	+	0	+	0	0	+	+	0	+	0	+	+	+	+	0	3+	2+	1+
3) r'r	0	+	0	+	+	+	+	+	0	+	0	+	+	0	+	+	+	+	0	0	+	2+	1+	0
4) R_1r	+	+	0	+	+	+	0	+	+	0	0	+	0	0	0	0	+	+	+	+	+	0	0	0
5) r''r	0	0	+	+	+	+	+	+	0	+	0	+	0	0	+	0	+	0	+	0	+	2+	1+	0
6) r'r'	0	+	0	0	+	0	+	+	0	+	0	+	+	0	+	0	+	+	+	+	+	2+	1+	0
7) R_1R_1	+	+	0	0	+	0	+	0	0	+	0	+	0	0	+	0	+	0	+	+	0	3+	2+	1+
8) rr	0	0	0	+	+	+	+	0	+	0	0	+	0	0	+	0	+	+	0	0	+	3+	2+	1+
9) R_1r	+	+	0	+	+	+	0	+	0	+	0	+	+	+	0	+	+	+	+	+	+	0	0	0
10) R_2R_2	+	0	+	+	0	0	0	+	+	+	0	+	+	0	+	0	+	+	0	+	0	0	0	0
11) R_0r	+	0	0	+	+	+	+	+	+	0	0	+	+	0	+	0	+	0	0	+	+	2+	1+	0
Auto																						0	0	0

Anti-M identified!

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Causes of unexpected reactivity in ABO reverse typing

- Rouleaux
- A subgroup with an Anti-A1
- Cold-reactive autoantibodies (Autoanti-I or –IH)
- Cold-reactive alloantibodies

  (Anti-M, Anti-P1, Anti-Le^a, Anti-Le^b)
- Passively acquired Anti-A or Anti-B

Case 1 : Resolve reverse type

Prewarm

A_1 Cells	B Cells	Interpretation
0	4+	A

Test antigen negative A1/B cells

A_1 Cells, M-	B Cells, M-	Interpretation
0	4+	A

Case 1: Antigen type

Anti-M Lot 123	Positive Control	Negative Control	Patient	Interpretation
	#3	#4		
	4+	0	0	M negative

Anti-M initially detected in ABO reverse type
Anti-M identified in a saline tube test method.

Don’t be fooled…

- Antibody screen method may not be optimal for the antibody.
  - IgM antibodies less reactive in solid phase
    - anti-M, Lewis Ab, anti-P1
  - Anti-Jk^a best detected/ only detectable in solid phase

- Retain the ability to test with >1 method

Case 2 History

- 72 year old male
- Admitted to ED on 9-23-16
- Chief complaint: red urine

- 9-24-16: Hemoglobin 8.1 gm/dl
- Order 2 units RBC

Case 2 9-24-16 plasma

Type and screen results

ABO/Rh	Antibody detection I Gel	Antibody detection II Gel
O Pos	0	0

Plasma appearance

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What would you do next?

A. Collect new sample to exclude traumatic phlebotomy.

B. Crossmatch additional units for transfusion.

C. Request review of IV solutions used since admission.

D. Obtain transfusion history, diagnosis and medications.

Patient History

- Admitted 9-14-16. Hgb 11.6 gm
- Autoimmune hepatitis with liver cirrhosis
- Experienced significant GI bleed during admission

- 9-15-16: Hgb 7.7
- Received 6 units RBC 9-15 to 9-18-16
- Antibody screens and XM: all non-reactive
- Discharged 9-21-16. Hgb 9.4

Blood Bank Medical Director initiates a transfusion reaction investigation.

Clerical check of all records: No discrepancies

Sample date	Hemolysis present?	DAT
9-18-16	No	Negative
9-24-16	Yes	1+w

9-18-16

9-24-16

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What testing would be most informative?

A. Repeat ABO/Rh/Ab screen 9-18-16 sample
B. Re-crossmatch units with 9-18-16 and 9-24-16 plasma.
C. Crossmatch more units with 9-24-16 plasma.
D. Test antibody identification panel using 9-24-16 plasma.

Repeat testing:

Ab Screen and XM

	9-18-16 Plasma Gel	9-24-16 Plasma Gel
Ab Screen I	0	0
Ab Screen II	0	0
Unit #1	0	1+w
Unit #2	0	1+w
Unit #3	0	2+
Unit #4	0	0
Unit #5	0	2+
Unit #6	0	1+w

Most likely explanation?

9-24-16 plasma: Negative antibody screen but positive XM

A. The plasma contains Ab to a low prevalence antigen.

B. The reactive units have a positive DAT.

C. The units carry an antigen not on the Ab Scrn cells.

D. The reactive units are ABO incompatible.

What testing should be performed next?

A. Antibdy ID panel using 9-24 plasma

B. Repeat Ab screen with another lot of cells

C. Perform DAT on donor units

D. Perform DAT on 9-24 RBC using monospecific AHG.

9-24-16 plasma (IRL testing)

		Rh					Kell		Duffy		Kidd		Lewis		P	MN				PEG
Cell		D	C	c	E	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P_1	M	N	S	s	Anti-IgG
1	R1R1	+	+	0	0	+	+	+	+	+	0	+	+	0	+	+	+	0	+	0√
2	R1R1	+	+	0	0	+	0	+	+	0	+	0	0	+	0	+	+	0	+	0√
3	R2R2	+	0	+	+	0	0	+	0	+	0	+	0	+	+	0	+	0	+	0√
4	Ror	+	0	+	0	+	0	+	0	+	0	+	0	+	+	+	+	+	+	3+
5	r'r	0	+	+	0	+	0	+	+	+	+	+	0	+	+	+	0	0	+	2+
6	r''r	0	0	+	+	+	0	+	+	+	+	+	+	0	+	0	+	0	+	2+
7	rr	0	0	+	0	+	+	+	0	+	+	0	0	+	+	+	0	+	0	3+
8	rr	0	0	+	0	+	0	+	+	0	+	+	+	0	0	0	+	+	+	3+
9	rr	0	0	+	0	+	0	+	0	+	0	+	0	+	+w	0	+	0	+	3+
10	R1R1	+	+	0	0	+	0	+	0	+	+	+	0	+	+	+	0	+	0	0√
TC	Ror	+	0	+	0	+	0	+	0	0	+	0	+	0	+	0	+	0	0	3+
WB																				1+w

9-24-16 plasma (IRL testing)

		Rh					Kell		Duffy		Kidd		Lewis		P	MN				PEG
Cell		D	C	c	E	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P_1	M	N	S	s	Anti-IgG
1	R1R1	+	+	0	0	+	+	+	+	+	0	+	+	0	+	+	+	0	+	0√
2	R1R1	+	+	0	0	+	0	+	+	0	+	0	0	+	0	+	+	0	+	0√
3	R2R2	+	0	+	+	0	0	+	0	+	0	+	0	+	+	0	+	0	+	0√
4	Ror	+	0	+	0	+	0	+	0	+	0	+	0	+	+	+	+	+	+	3+
5	r'r	0	+	+	0	+	0	+	+	+	+	+	0	+	+	+	0	0	+	2+
6	r''r	0	0	+	+	+	0	+	+	+	+	+	+	0	+	0	+	0	+	2+
7	rr	0	0	+	0	+	+	+	0	+	+	0	0	+	+	+	0	+	0	3+
8	rr	0	0	+	0	+	0	+	+	0	+	+	+	0	0	0	+	+	+	3+
9	rr	0	0	+	0	+	0	+	0	+	0	+	0	+	+w	0	+	0	+	3+
10	R1R1	+	+	0	0	+	0	+	0	+	+	+	0	+	+	+	0	+	0	0√
TC	Ror	+	0	+	0	+	0	+	0	0	+	0	+	0	+	0	+	0	0	3+
WB																				1+w

What do the reactive cells have in common?

A. D+ or C+

B. c+ or e+

C. c+ and e+

D. D-

9-24-16 plasma (IRL testing)

		Rh					Kell		Duffy		Kidd		Lewis		P	MN				PEG
Cell		D	C	c	E	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P_1	M	N	S	s	Anti-IgG
1	R1R1	+	+	0	0	+	+	+	+	+	0	+	+	0	+	+	+	0	+	0√
2	R1R1	+	+	0	0	+	0	+	+	0	+	0	0	+	0	+	+	0	+	0√
3	R2R2	+	0	+	+	0	0	+	0	+	0	+	0	+	+	0	+	0	+	0√
4	Ror	+	0	+	0	+	0	+	0	+	0	+	0	+	+	+	+	+	+	3+
5	r'r	0	+	+	0	+	0	+	+	+	+	+	0	+	+	+	0	0	+	2+
6	r''r	0	0	+	+	+	0	+	+	+	+	+	+	0	+	0	+	0	+	2+
7	rr	0	0	+	0	+	+	+	0	+	+	0	0	+	+	+	0	+	0	3+
8	rr	0	0	+	0	+	0	+	+	0	+	+	+	0	0	0	+	+	+	3+
9	rr	0	0	+	0	+	0	+	0	+	0	+	0	+	+w	0	+	0	+	3+
10	R1R1	+	+	0	0	+	0	+	0	+	+	+	0	+	+	+	0	+	0	0√
TC	Ror	+	0	+	0	+	0	+	0	0	+	0	+	0	+	0	+	0	0	3+
WB																				1+w

The strongly reactive c+e+ cells are suggestive of what specificity?

A. anti-G

B. anti-Rh_i

C. anti-f

D. I could pick A, B, or C but I would be guessing

f antigen (RH6)

- f antigen is produced by whenever c and e antigens are expressed on same protein: *RHCE*ce* allele

  Note: f expression can be altered in variant *RHCE*ce* alleles

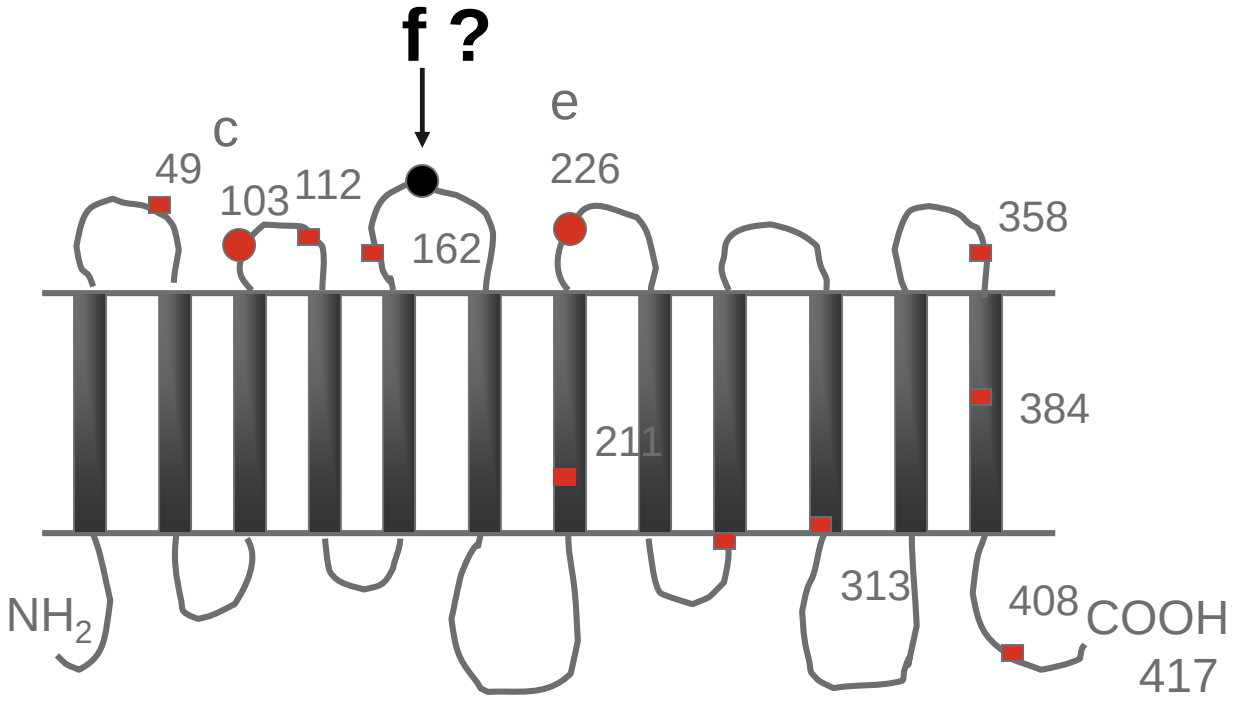
- Anti-f acts like other Rh antibodies:

  mostly IgG Ab; reactive in ficin

- Antigen status not marked on most panel antigrams

  Must infer from Rh phenotype/most probable genotype (mpg)

RHce protein



Requirements for f antigen expression not well understood

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Which are f+?

	f+	f-
R1R2 (DCe/DcE)		X
R1r (DCe/dce)	X	
R1R1 (DCe/DCe)		X
rr (dce/dce)	X	
Ror (Dce/dce)	X	
R2R2 (DcE/DcE)		X
RzR1 (DCE/DCe)		X
RzR2 (DCE/DcE)		X

→ D+C+E+c+e+
Pos for all common Rh Ag:
"can't make Rh Ab"

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For completeness…

1) D+C+E+c+e+ mpg = R_1R_2

Could also be…

R_1r'' (DCe/dcE)	R_or (Dce/dce) f+
R_2r' (DcE/dCe)	R_oR_z (Dce/DCE) f+
R_zr (DCE/dce) f+	R_oR_y (DCE/dCE) f+

2) R_1R_1 and R_2R_2 individuals CAN make anti-f but generally make anti-c or anti-e. Anti-f may be hidden.

9-24-16 plasma: Ab exclusion

		Rh					Kell		Duffy		Kidd		Lewis		P	MN				PEG
Cell		D	C	c	E	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P_1	M	N	S	s	Anti-IgG
1	R1R1	~~+~~	~~+~~	0	0	~~+~~	~~+~~	+	+	+	0	~~+~~	~~+~~	0	~~+~~	+	+	0	~~+~~	0
2	R1R1	~~+~~	~~+~~	0	0	~~+~~	0	~~+~~	~~+~~	0	~~+~~	0	0	~~+~~	0	+	+	0	~~+~~	0
3	R2R2	~~+~~	0	~~+~~	~~+~~	0	0	~~+~~	0	~~+~~	0	~~+~~	0	~~+~~	~~+~~	0	~~+~~	0	~~+~~	0
4	Ror	+	0	+	0	+	0	+	0	+	0	+	0	+	+	+	+	+	+	3+
5	r'r	0	+	+	0	+	0	+	+	+	+	+	0	+	+	+	0	0	+	2+
6	r''r	0	0	+	+	+	0	+	+	+	+	+	+	0	+	0	+	0	+	2+
7	rr	0	0	+	0	+	+	+	0	+	+	0	0	+	+	+	0	+	0	3+
8	rr	0	0	+	0	+	0	+	+	0	+	+	+	0	0	0	+	+	+	3+
9	rr	0	0	+	0	+	0	+	0	+	0	+	0	+	+w	0	+	0	+	3+
10	R1R1	~~+~~	~~+~~	0	0	~~+~~	0	~~+~~	0	~~+~~	+	+	0	~~+~~	~~+~~	~~+~~	0	~~+~~	0	0
TC	Ror	+	0	+	0	+	0	+	0	0	+	0	+	0	+	0	+	0	0	3+
WB																				1+w

What additional testing is needed to complete the investigation?

A. Determine if the patient can make anti-f

B. Investigate the positive DAT

C. Perform Rh phenotype on transfused units

D. All of the above

Patient RBC antigen typing

Sample date	C	E	c	e	Jk^a	Jk^b
9-24-16	4+	4+ mf	4+	4+	3+	1+w, mf
9-18-16	4+ mf	4+ mf	4+	4+	3+	2+ mf
9-14-16 (pre)	4+	4+	4+	4+	4+	0

f -

Eluate from 9-24-16 RBC

		Rh					Kell		Duffy		Kidd		Lewis		P	MN				PEG
Cell		D	C	c	E	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P_1	M	N	S	s	Anti-IgG
1	R1R1	~~+~~	~~+~~	0	0	~~+~~	~~+~~	+	+	+	0	~~+~~	~~+~~	0	~~+~~	+	+	0	~~+~~	0√
2	R1R1	~~+~~	~~+~~	0	0	~~+~~	0	~~+~~	~~+~~	0	~~+~~	0	0	~~+~~	0	+	+	0	~~+~~	0√
3	R2R2	~~+~~	0	~~+~~	~~+~~	0	0	~~+~~	0	~~+~~	0	~~+~~	0	~~+~~	~~+~~	0	~~+~~	0	~~+~~	0√
4	Ror	+	0	+	0	+	0	+	0	+	0	+	0	+	+	+	+	+	+	1+w
5	r'r	0	+	+	0	+	0	+	+	+	+	+	0	+	+	+	0	0	+	1+w
6	r''r	0	0	+	+	+	0	+	+	+	+	+	+	0	+	0	+	0	+	1+w
7	rr	0	0	+	0	+	+	+	0	+	+	0	0	+	+	+	0	+	0	1+w
8	rr	0	0	+	0	+	0	+	+	0	+	+	+	0	0	0	+	+	+	1+w
9	rr	0	0	+	0	+	0	+	0	+	0	+	0	+	+w	0	+	0	+	1+w
10	R1R1	~~+~~	~~+~~	0	0	~~+~~	0	~~+~~	0	~~+~~	+	+	0	~~+~~	~~+~~	~~+~~	0	~~+~~	0	0√
TC	Ror	+	0	+	0	+	0	+	0	0	+	0	+	0	+	0	+	0	0	1+w

RBC Phenotyping

	9-24-16 Plasma Gel	C	E	c	e	MPG
Unit #1 - O Pos	1+w	+	0	+	+	R_1r
Unit #2 - O Pos	1+w	+	0	+	+	R_1r
Unit #3 - O Neg	2+	0	0	+	+	rr
Unit #4 – O Pos	0	+	+	+	+	R_1R_2
Unit #5 – O Pos	2+	+	0	+	+	R_1r
Unit #6 – O Pos	1+w	+	0	+	+	R_1r

Additional information

- Tested additional R_1R_1 and R_2R_2 cells with 9-24 plasma to insure other alloantibodies were well excluded.
- Tested 9-24 plasma against ficin-treated RBC: no additional Ab
- Confirmed negative Ab screen in 9-14 and 9-18 plasmas

- Patient received 4 additional f- RBC (c-): 9-25, 9-26,10-2
- Discharged 10-12-16. Hgb 8.4 gm

Hospital testing → How to interpret?

Screen Cell	Rh mgp	Gel	Solid phase
I	R_1R_1	0	0
II	R_2R_2	0	0
III	rr		3+

Solid phase panel I – routine
Solid phase panel 2 – R_1R_1/R_2R_2

Positive and negative reactions
All negative ??

All clues to anti-f !!

Don’t be fooled…

- Antibody screen configuration may affect detection of antibodies: 2 cells vs 3 cells
  - anti-f will not be detected
  - non-Rh antigens may be present in single dose expression. An antibody showing dosage may not be reactive.

D+C+c-E-e+ D+C-c+E+e-

Fy(a+b+) vs Fy(a+b-)

Case 3

- 32 year old female admitted in active labor
- G3P2
- Received routine prenatal care
- O Positive
- Admission antibody screen: Negative

Case 3: Newborn

- 7 lb 4 oz male
- Apgar scores-normal
- Routine cord blood studies
  - Group A Positive (RBC only)
  - DAT 3+

What is the most likely cause of the infant's positive DAT?

A. Warm autoantibody

B. Maternal anti-A

C. Maternal anti-c

D. Ab to low prevalence Ag

Elution studies on cord blood

	Freeze-Thaw Eluate	
	IS	PEG-IgG
Group A	0	0√
Group B	0	0√
Group O	0	0√

ABO antibodies

	Acid Eluate	Last Wash
	PEG-IgG	PEG-IgG
Screen I	0√	0√
Screen II	0√	0√
Screen III	0√	0√

Non-ABO antibodies

Given the elution results, what has not been eliminated as a cause of the infant's positive DAT?

A. Warm autoantibody

B. Ab to low prevalence antigen

C. Maternal anti-A

D. Common red cell alloantibody

Low prevalence Ag+ test cells

Found on current panels

	Cord Eluate PEG-IgG
C^w+	0√
V+	0√
Kp(a+)	0√
Co(b+)	0√
Js(a+)	0√
Lu(a+)	0√

What is the next step your lab would take?

A. Prepare another eluate to confirm lack of reactivity.

B. Report “antibody to unidentified low prevalence antigen”

C. Ask for a paternal blood sample

D. Send sample to a Reference Laboratory

Why is the paternal sample tested?

A. The plasma will be a good source of antibody.

B. There are insufficient cord cells for testing.

C. The red cells will carry the low prevalence antigen.

D. The paternal cells can predict the ABO zygosity of the neonate.

Paternal RBC testing

	Cord Eluate PEG-IgG
Grp A paternal RBC	4+

What is the BEST source of the antibody for additional testing?

A. Eluate from additional aliquot of cord cells
B. Eluate prepared from a neonatal blood sample
C. Neonatal plasma
D. Maternal plasma

IRL investigation

- Treat paternal cells with ficin and dithiotreitol (DTT) and retest.

	Eluate PEG-IgG	6% alb PEG-IgG
Paternal RBC – ficin treated	4+	0√
Paternal RBC – 0.2M DTT treated	4+	0√

Eliminates low prevalence antigens in KEL, MNS, YT, DO, IN, KN blood group systems.

IRL investigation

Maternal and paternal samples are ABO incompatible!

- Test maternal plasma with additional low-prevalence antigen-positive RBC.
- Test paternal RBC for low-prevalence antigens.
- Depends entirely on IRL inventory.

IRL investigation

- Paternal cells: 4+ with anti-Rd
- Maternal plasma testing

	Maternal plasma* Peg-IgG	Cord eluate PEG-IgG
Grp A Rd+ RBC	3+	4+

*Adsorbed to remove anti-A and anti-B

Presumptive ID: anti-Rd

Rd antigen (Sc4)

- In Scianna blood group system
  - 2 high prevalence Ag: Sc1, Sc3
  - 2 low prevalence Ag: Sc2, Rd (Sc4)
- <0.01% incidence in any population
- Well expressed on cord red cells
- Most HDFN in Rd+ infants is mild to moderate
- Does not cause transfusion reactions

If the newborn does require transfusion, what is the best source of red cells?

A. Directed donation from mother

B. Directed donation from father

C. Crossmatch compatible RBC from inventory

D. Choices A, B and C are equally acceptable.

Don’t be fooled…

- Antibody screening cells don’t carry all antigens.
- Antigen in question may be only on neonate or paternal red cells.
- Antigen may be only on a donor’s red cells when a single XM is incompatible.
- Identifying antibodies to antigens of low prevalence is dependent on available resources.

Objectives

- ✓ Antibodies may be directed at antigens not on Ab screen cells: HDFN, unexpected incompatible XM.
- ✓ Antigen may be present but test system will not adequately detect: test method or difference in antigen dosage between Ab screen cells and crossmatch.
- ✓ Resolution may involve testing plasma by various test methods, testing a panel even when the Ab screen is negative, testing low-prevalence Ag-positive cells / assessing impact of chemicals on antigen.