“High” there!

Case Studies – NCABB 2019

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3450
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Wisconsin
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http://gees7.wordpress.com/2013/02/02/worlds-tallest-buildings-2013/

http://i.imgur.com/vOqhp4C.jpg

Case #1

Case 1

- 85 year old white male admitted with a 5.4g Hgb
- Diagnosis: anemia due GI bleed
- O positive
- Positive antibody screen - 3-4+ by Echo at hospital
- Transfusion history:
  - 2 units in 2002
  - 2 units in 2018
  - 5 units 1 month ago (2019) issued on negative antibody screen
- Current order for antibody ID and 2 units

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Case 1 - IRL Testing

- O positive, DAT positive

Poly AHG	Anti-IgG	Anti-C3
w+	w+	0V

- Unremarkable drug list

- Hallmark of a more difficult case – STAT and a short sample!

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Case 1 - Initial Panel

	D	C	c	E	e	Cw	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	Lua	Lub	IS	37C	LISS IAT
1	+	+	0	0	+	0	+	+	+	+	0	+	+	0	+	0	0	+	+	+	0	0	+	0	+	w+	1+	w+
2	+	+	0	0	+	0	0	+	0	+	0	+	0	+	0	+	0	+	+	0	+	0	+	0	+	w+	w+	1+
3	+	0	+	+	0	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	+	0	+	0	+	w+	1+	w+
4	+	0	+	0	+	0	0	+	0	+	0	+	0	0	+	0	0	0	+	0	+	0	+	0	+	w+	w+	1+
5	0	+	+	0	+	0	0	0	0	+	0	+	+	+	0	+	0	+	+	+	+	0	+	0	+	w+	1+	w+
6	0	0	+	+	+	0	0	+	0	+	0	+	+	0	0	+	0	+	0	0	+	+	+	0	+	w+	w+	w+
7	0	0	+	0	+	+	+	+	0	+	0	+	0	+	+	0	0	+	+	+	0	0	+	0	+	w+	2+	2+
8	0	0	+	0	+	0	0	+	0	+	0	+	+	0	+	+	+	0	+	+	+	0	+	0	+	w+	1+	2+
9	0	0	+	0	+	0	0	+	0	+	0	+	+	+	+	0	0	+	+	+	0	0	+	0	+	w+	w+	1+
10	+	0	+	0	0	0	0	+	0	+	0	+	+	0	+	+	+	0	0	+	+	+	0	0	+	w+	w+	1+
11	0	0	+	0	+	0	+	+	0	+	0	+	+	+	+	+	0	+	0	+	+	0	+	0	+	w+	w+	w+
AC																										0	0	0v

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Case 1 - Initial Panel

	D	C	c	E	e	Cw	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	Lua	Lub	IS	37C	LISS IAT
1	+	+	0	0	+	0	+	+	+	+	0	+	+	0	+	0	0	+	+	+	0	0	+	0	+	w+	1+	w+
2	+	+	0	0	+	0	0	+	0	+	0	+	0	+	0	+	0	+	+	0	+	0	+	0	+	w+	w+	1+
3	+	0	+	+	0	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	+	0	+	0	+	w+	1+	w+
4	+	0	+	0	+	0	0	+	0	+	0	+	0	0	+	0	0	0	+	0	+	0	+	0	+	w+	w+	1+
5	0	+	+	0	+	0	0	0	0	+	0	+	+	+	0	+	0	+	+	+	+	0	+	0	+	w+	1+	w+
6	0	0	+	+	+	0	0	+	0	+	0	+	+	0	0	+	0	+	0	0	+	+	+	0	+	w+	w+	w+
7	0	0	+	0	+	+	+	+	0	+	0	+	0	+	+	0	0	+	+	+	0	0	+	0	+	w+	2+	2+
8	0	0	+	0	+	0	0	+	0	+	0	+	+	0	+	+	+	0	+	+	+	0	+	0	+	w+	1+	2+
9	0	0	+	0	+	0	0	+	0	+	0	+	+	+	+	0	0	+	+	+	0	0	+	0	+	w+	w+	1+
10	+	0	+	0	0	0	0	+	0	+	0	+	+	0	+	+	+	0	0	+	+	+	0	0	+	w+	w+	1+
11	0	0	+	0	+	0	+	+	0	+	0	+	+	+	+	+	0	+	0	+	+	0	+	0	+	w+	w+	w+
AC																										0	0	0v

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Case 1 - Initial Panel

	D	C	c	E	e	Cw	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	Lua	Lub	IS	37C	LISS IAT
1	+	+	0	0	+	0	+	+	+	+	0	+	+	0	+	0	0	+	+	+	0	0	+	0	+	w+	1+	w+
2	+	+	0	0	+	0	0	+	0	+	0	+	0	+	0	+	0	+	+	0	+	0	+	0	+	w+	w+	1+
3	+	0	+	+	0	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	+	0	+	0	+	w+	1+	w+
4	+	0	+	0	+	0	0	+	0	+	0	+	0	0	+	0	0	0	+	0	+	0	+	0	+	w+	w+	1+
5	0	+	+	0	+	0	0	0	0	+	0	+	+	+	0	+	0	+	+	+	+	0	+	0	+	w+	1+	w+
6	0	0	+	+	+	0	0	+	0	+	0	+	+	0	0	+	0	+	0	0	+	+	+	0	+	w+	w+	w+
7	0	0	+	0	+	+	+	+	0	+	0	+	0	+	+	0	0	+	+	+	0	0	+	0	+	w+	2+	2+
8	0	0	+	0	+	0	0	+	0	+	0	+	+	0	+	+	+	0	+	+	+	0	+	0	+	w+	1+	2+
9	0	0	+	0	+	0	0	+	0	+	0	+	+	+	+	0	0	+	+	+	0	0	+	0	+	w+	w+	1+
10	+	0	+	0	0	0	0	+	0	+	0	+	+	0	+	+	+	0	0	+	+	+	0	0	+	w+	w+	1+
11	0	0	+	0	+	0	+	+	0	+	0	+	+	+	+	+	0	+	0	+	+	0	+	0	+	w+	w+	w+
AC																										0	0	0v

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Case 1 - Ficin Panel

	D	C	c	E	e	Cw	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	Lua	Lub	Ficin IAT
1	+	+	0	0	+	0	+	+	+	+	0	+	+	0	+	0	0	+	+	+	0	0	+	0	+	0v
2	+	+	0	0	+	0	0	+	0	+	0	+	0	+	0	+	0	+	+	0	+	0	+	0	+	0v
3	+	0	+	+	0	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	+	0	+	0	+	0v
4	+	0	+	0	+	0	0	+	0	+	0	+	0	0	+	0	0	0	+	0	+	0	+	0	+	0v
5	0	+	+	0	+	0	0	0	0	+	0	+	+	+	0	+	0	+	+	+	+	0	+	0	+	0v
6	0	0	+	+	+	0	0	+	0	+	0	+	+	0	0	+	0	+	0	0	+	+	+	0	+	0v
7	0	0	+	0	+	+	+	+	0	+	0	+	0	+	+	0	0	+	+	+	0	0	+	0	+	0v
8	0	0	+	0	+	0	0	+	0	+	0	+	+	0	+	+	+	0	+	+	+	0	+	0	+	0v
9	0	0	+	0	+	0	0	+	0	+	0	+	+	+	+	0	0	+	+	+	0	0	+	0	+	0v
10	+	0	+	0	0	0	0	+	0	+	0	+	+	0	+	+	+	0	0	+	+	+	0	0	+	0v

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Case 1 - Phenotypically similar cell

C	c	E	e	K	Fya	Fyb	Jka	Jkb	S	s
+	+	0	+	0	0	+	+	+	0	+

D	C	c	E	e	Cw	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	Lua	Lub	IS	37C	LISS IAT
+	+	0	0	+	0	0	+	0	+	0	+	0	+	0	+	0	+	+	0	+	0	+	0	+	w+	w+	1+

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Case 1 - Cold screen

	IS	RT	15-18C	4C
SC1	w+	2+	2+	2+
SC2	w+	2+	2+	2+
SC3	w+	1+	1+	2+
Cord	0	2+	w+	1+
Auto*	0	w+	w+	w+
A1 cell				
B cell				

*unseparated cells

Cord	D	C	c	E	e	IS	37C	LISS IAT
	+	+	+	0	+	0	0	0V

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Case 1 - High Frequency Panel

Phenotype	LISS-IAT
k-	1+
U-	w+
Lu(a+b-)	0
Yt(a-)	1+
Vel-	2+
Kp(b-)	2+
Js(b-)	1+

Patient types Lu(b-) by commercial antisera

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Case 1 - DTT treated cells with last plasma of sample #1

	D	C	c	E	e	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	Lua	Lub	LISS-IAT
1	+	+	0	0	+	+	+	0	+	0	+	+	+	+	+	+	0	+	+	0	+	0	0	+	w+
2	+	0	+	+	0	0	+	0	+	0	+	+	0	+	0	0	+	+	0	+	0	+	0	+	w+
3	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	+	+	+	0	+	0	+	w+
4	+	+	0	0	+	0	+	0	+	0	+	0	+	+	0	0	+	+	+	0	+	0	0	+	w+
5	+	0	+	+	0	+	+	0	+	0	+	0	+	0	+	0	+	+	+	+	0	+	+	NT	0V
6	0	0	+	0	+	+	+	0	+	0	+	+	0	+	+	+	0	+	0	+	0	+	0	+	w+

DTT treated control – Cell #1 vs. commercial anti-K = nonreactive

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Case 1 - Initial Eluate

	D	C	c	E	e	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	Lua	Lub	PEG-IAT
1	+	+	0	0	+	+	+	0	+	0	+	+	+	+	+	+	0	+	+	0	+	0	0	+	w+
2	+	0	+	+	0	0	+	0	+	0	+	+	0	+	0	0	+	+	0	+	0	+	0	+	w+
3	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	+	+	+	0	+	0	+	w+
4	+	+	0	0	+	0	+	0	+	0	+	0	+	+	0	0	+	+	+	0	+	0	0	+	w+
5	+	0	+	+	0	+	+	0	+	0	+	0	+	0	+	0	+	+	+	+	0	+	+	NT	w+
6	0	0	+	0	+	+	+	0	+	0	+	+	0	+	+	+	0	+	0	+	0	+	0	+	w+
Retics																									0√

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Looks like anti-Lu^{b}

- Except this antibody appears to be directed against an antigen that is ficin sensitive and DTT resistant…
- Possible false flag? We've been (temporarily) fooled before!

	1	2	4	8	16	32	64	128	256	512	1024
Cell	1+	1+	1+	1+	w+	w+	w+	w+	w+	w+	0V

- Pooled plasma neutralization unsuccessful

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New Sample

	Saline	LISS	PEG	Papain	Ficin	DTT
SC1	1+	1+	2+	1+	0V	2+
SC2	1+	1+	2+	1+	0V	2+
SC3	1+	1+	2+	w+	0V	1+

	LISS-IAT
Lub- #2	0V
Lub- #3	0V

DTT Effectiveness Re-check

		Patient plasma	Commercial Anti-K	Commercial Anti-Lu^{b}
Untreated	SC1 (K+)	2+	NT	2+
DTT treated cells	SC1 (K+)	2+	0	2+
	SC2	2+	NT	2+
	SC3	1+	NT	2+

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Conclusion

- Ignoring the ineffective DTT treatment, Anti-Lu^b confirmed
- Antibodies to common antigens ruled out
  - Lu(b-) cells or ficin treated cells
- Molecular confirms patient's predicted phenotype is Lu(a+b-)

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Anti-Lu^{b}

- Lu(a+b-) phenotype occurs in 0.2% of Caucasians
- Lu(a-b-) is less common, but often due to *In(Lu)* gene
- Cord cells have weak expression of Lutheran antigens
- “Mild to moderate” HTR (per The Blood Group Antigen FactsBook)
- Normally resistant to ficin, but sensitive to AET/DTT
  - May be weakened

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Case #2

Case 2

- 58 year old female with pancreatic cancer
- Routine presurgical sample
- Patient has been recently transfused

- Hospital reports patient is O positive with positive DAT and panreactive antibody on multiple solid phase and PEG-IAT panels – probable warm autoantibody

- Sample referred to IRL for alloadsorptions

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Case 2 - IRL Testing

- O positive, DAT positive

Poly AHG	Anti-IgG	Anti-C3
2+ / m+	2+	0v

- Unremarkable drug list

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Case 2 - IRL Workup – Initial Panel

	D	C	c	E	e	K	k	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	LISS-IAT
1	+	+	0	0	+	+	+	+	0	+	0	0	+	+	+	0	0	+	3+
2	+	+	0	0	+	0	+	0	+	0	+	0	+	+	0	+	0	+	3+
3	+	0	+	+	0	0	+	0	+	0	+	0	+	0	+	+	0	+	3+
4	+	0	+	0	+	0	+	0	0	+	0	0	0	+	0	+	0	+	3+
5	0	+	+	0	+	0	0	+	+	0	+	0	+	+	+	+	0	+	3+
6	0	0	+	+	+	0	+	+	0	0	+	0	+	0	0	+	+	+	3+
7	0	0	+	0	+	+	+	0	+	+	0	0	+	+	+	0	0	+	3+
8	0	0	+	0	+	0	+	+	0	+	+	+	0	+	+	+	0	+	3+
9	0	0	+	0	+	0	+	+	+	+	0	0	+	+	+	0	0	+	3+
10	+	0	+	0	0	0	+	+	0	+	+	+	0	0	+	+	+	0	3+
11	0	0	+	0	+	+	+	+	+	+	+	0	+	0	+	+	0	+	3+
AC																			2+

Case 2 – IRL Workup - Adsorptions

Cell	R1R1	R2R2	rr
1	0v	0v	0v
2	0v	0v	0v
3	3+	3+	3+

- All adsorbing cells K-
- Anti-K identified
- All other antibodies to common antigens ruled out

Case 2 – IRL Workup - Eluate

	D	C	c	E	e	Cw	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	Lua	Lub	IAT
1	+	+	0	0	+	0	+	+	+	+	0	+	+	0	+	0	0	+	+	+	0	0	+	0	+	3+
2	+	+	0	0	+	0	0	+	0	+	0	+	0	+	0	+	0	+	+	0	+	0	+	0	+	3+
3	+	0	+	+	0	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	+	0	+	0	+	3+
4	+	0	+	0	+	0	0	+	0	+	0	+	0	0	+	0	0	0	+	0	+	0	+	0	+	3+
5	0	+	+	0	+	0	0	0	0	+	0	+	+	+	0	+	0	+	+	+	+	0	+	0	+	3+
6	0	0	+	+	+	0	0	+	0	+	0	+	+	0	0	+	0	+	0	0	+	+	+	0	+	3+
7	0	0	+	0	+	+	+	+	0	+	0	+	0	+	+	0	0	+	+	+	0	0	+	0	+	3+
8	0	0	+	0	+	0	0	+	0	+	0	+	+	0	+	+	+	0	+	+	+	0	+	0	+	3+
9	0	0	+	0	+	0	0	+	0	+	0	+	+	+	+	0	0	+	+	+	0	0	+	0	+	3+
10	+	0	+	0	0	0	0	+	0	+	0	+	+	0	+	+	+	0	0	+	+	+	0	0	+	3+
11	0	0	+	0	+	0	+	+	0	+	0	+	+	+	+	+	0	+	0	+	+	0	+	0	+	3+
AC																										0v

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Case 2 – IRL Workup

- Eluate autocontrol was prepared by treating retics with EDTA glycine acid (EGA)
  - DAT negative
  - Known to denature Kell system and Er^a antigens
- Eluate autocontrol was repeated following treatment with chloroquine diphosphate (CPD)

Autocontrol Preparation	Eluate
No treatment - unseparated	NT
EGA treated – retic separated	0ν
Chloroquine treated – retic separated	2+

Case 2 – IRL Workup - Phenotype

Retic separated cells

C	c	E	e	K	k	Kp^a	Kp^b
+	+	0	+	0	+	+	0

- k, Kp^a, and Kp^b typings performed using CDP treated cells

Additional Eluate Testing

	Eluate IAT
Untreated, Kp(b+)	3+
Untreated, Kp(b-)	1+
DTT treated, Kp(b+)	2+

*Informational only

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Revisit Plasma Testing

Autocontrol Preparation	Plasma	Eluate
No treatment - unseparated	2+	NT
EGA treated – retic separated	0v	0v
Chloroquine treated – retic separated	0v	2+

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Case 2 – Repeat Plasma Testing

Selected cell panel – DTT treated cells vs. patient plasma

Cell #	IAT
1	0v
2	0v
3	0v
4	0v

Untreated rare cell vs. patient plasma

Cell	Plasma
Kp(b-)	0v

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Case 2 – Autoanti-Kp^b?

Autoimmune hemolytic anemia and a further example of autoanti-Kp^b

E. Lee, G. Burgess, and N. Win

A 65-year-old Caucasian man with myelodysplasia was admitted with autoimmune hemolytic anemia and a Hb of 5.6 g/dL. The patient's serum contained anti-K; the DAT on the patient's RBCs reacted 3+ with anti-IgG and 3+ with anti-C3d. K– RBC units were transfused, but there was no sustained increase in Hb level. The samples were referred to the reference laboratory of the National Blood Service. The DAT results remained the same, with anti-K detected only in the serum. An eluate prepared from the patient's DAT-positive RBCs revealed anti-Kp^b specificity. This study reports an unusual case of autoanti-Kp^b, which is different from previously published cases in that no free anti-Kp^b was detectable in the serum. *Immunohematology* 2005;21:119–121.

there was no sustained response in Hb
samples were submitted to the Reference La
the National Blood Service (NBS), North L
investigation.

Materials and Methods

Antigen typing for ABO, Rh, and K were
by standard tube technique. Anti-A and anti
were from Diagnostic Scotland, Edinburgh
anti-D reagents (RUM1 and BS226) were

THE JOURNAL OF AABB

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TRANSFUSION

Auto-Anti-Kp^b Associated with Weakened Antigenicity in the Kell Blood Group System: A Second Example

M. L. Beck, W. L. Marsh, S. R. Pierce, J. DiNapoli, R. ØYen, M. E. Nichols

First published: March-April 1979 | https://doi.org/10.1046/j.1537-2995.1979.19279160294.x |

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Blood Group	Antigen	Result
Rh	c	+
	C	+
	e	+
	E	0
	V	0
	VS	0
Kell	K	0
	k	+
	Kp^a	+
	Kp^b	0
	Js^a	0
	Js^b	+
Duffy	Fy^a	+
	Fy^b	+
Kidd	Jk^a	+
	Jk^b	+
MNS	M	0
	N	+
	S	0
	s	+
	U	+
Lutheran	Lu^a	0
	Lu^b	+

Summary

- Likely an alloanti-Kp^b in the plasma… plus the anti-K
- Warm autoantibody in the eluate

- Kp^b is a high prevalence antigen in the Kell system
- >99.9% of the population is Kp^b positive
- Antigen negative blood may be required for transfusion
  - "No to moderate" transfusion reactions per Blood Group Antigens FactsBook

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Case #3

Case #3

- 59 year old female with diabetes
- Routine type and screen order
- One previous pregnancy ended in miscarriage at 12 weeks gestation
- Patient states she has never been transfused, but was once told there would be “no compatible blood for her.”

Hospital work

- B positive
- Plasma panreactive with all cells tested by solid phase and PEG-IAT
- DAT negative
- Autocontrol negative

Initial IRL Work

	D	C	c	E	e	K	k	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	IS	37C	LISS-IAT
1	+	+	0	0	+	+	+	+	0	+	0	0	+	+	+	0	0	+	1+	1+	4+
2	+	+	0	0	+	0	+	0	+	0	+	0	+	+	0	+	0	+	w+	w+	3+
3	+	0	+	+	0	0	+	0	+	0	+	0	+	0	+	+	0	+	w+	w+	3+
4	+	0	+	0	+	0	+	0	0	+	0	0	0	+	0	+	0	+	w+	w+	3+
5	0	+	+	0	+	0	0	+	+	0	+	0	+	+	+	+	0	+	w+	w+	3+
6	0	0	+	+	+	0	+	+	0	0	+	0	+	0	0	+	+	+	w+	w+	3+
7	0	0	+	0	+	+	+	0	+	+	0	0	+	+	+	0	0	+	w+	w+	3+
8	0	0	+	0	+	0	+	+	0	+	+	+	0	+	+	+	0	+	w+	w+	3+
9	0	0	+	0	+	0	+	+	+	+	0	0	+	+	+	0	0	+	w+	w+	3+
10	+	0	+	0	0	0	+	+	0	+	+	+	0	0	+	+	+	0	w+	w+	3+
11	0	0	+	0	+	+	+	+	+	+	+	0	+	0	+	+	0	+	w+	w+	3+
AC																			0	0	0V

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Patient Phenotype

C	c	E	e	K	Fya	Fyb
4+	4+	0	4+	0	3+	0

M	N	S	s	Lea	Leb
0	??	0	4+	3+	4+

- N typing “scratchy”
- Patient cells type M- N- using human source antisera
- Hospital has commercial genotyping platform

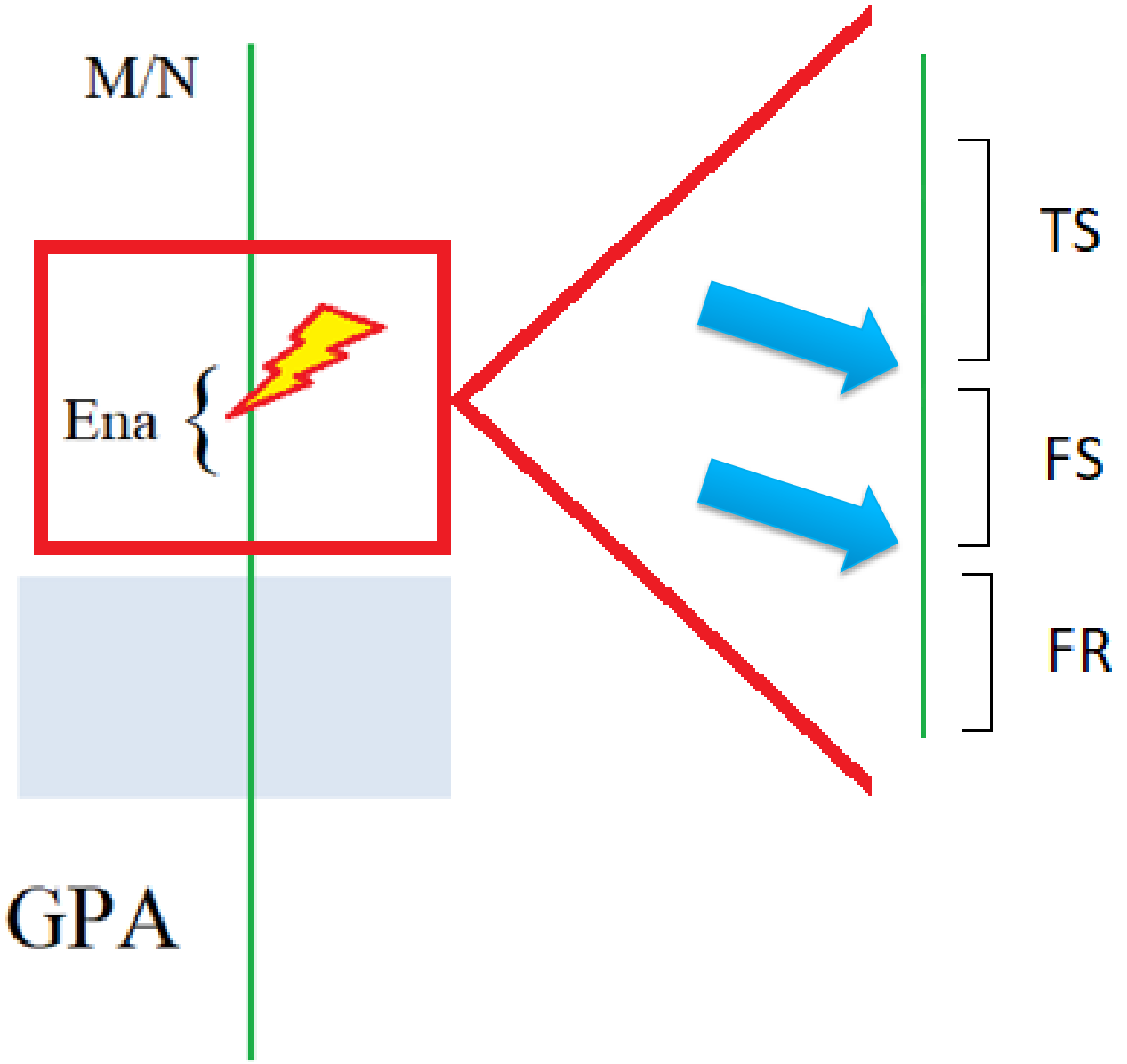
En(a-)?

- En(a-) cells type Wr(a-b-)
- En(a-) cells have reduced sialic acid levels compared to normal RBCs
  - Glycine soja lectin (used as control for enzyme treatment)

Anti-Wr^b	Glycine soja lectin
0	++

En(a-)??

- Patient may be En(a-)
- One En(a-) cell is nonreactive with patient plasma.
- No other En(a-) cells available



IRL Work

	D	C	c	E	e	K	k	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	LISS-IAT	Ficin IAT
1	+	+	0	0	+	+	+	+	0	+	0	0	+	+	+	0	0	+	4+	3+
2	+	+	0	0	+	0	+	0	+	0	+	0	+	+	0	+	0	+	3+	3+
3	+	0	+	+	0	0	+	0	+	0	+	0	+	0	+	+	0	+	3+	3
4	+	0	+	0	+	0	+	0	0	+	0	0	0	+	0	+	0	+	3+	4+
5	0	+	+	0	+	0	0	+	+	0	+	0	+	+	+	+	0	+	3+	3+
6	0	0	+	+	+	0	+	+	0	0	+	0	+	0	0	+	+	+	3+	4+
7	0	0	+	0	+	+	+	0	+	+	0	0	+	+	+	0	0	+	3+	3+
8	0	0	+	0	+	0	+	+	0	+	+	+	0	+	+	+	0	+	3+	3+
9	0	0	+	0	+	0	+	+	+	+	0	0	+	+	+	0	0	+	3+	3+
10	+	0	+	0	0	0	+	+	0	+	+	+	0	0	+	+	+	0	3+	3+
11	0	0	+	0	+	+	+	+	+	+	+	0	+	0	+	+	0	+	3+	3+
AC																			0V	0V

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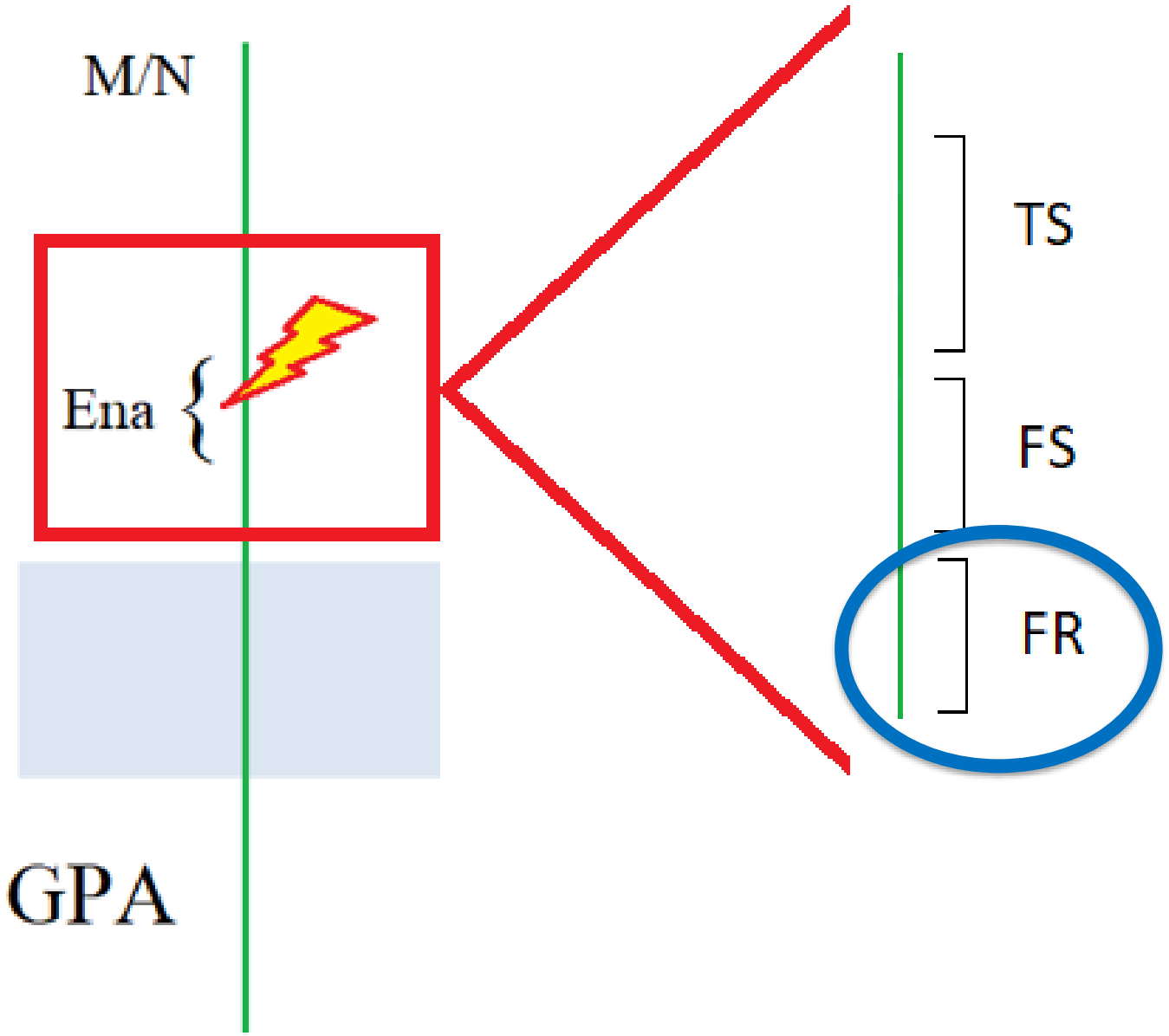
M/N
Ena
GPA
TS
FS
FR

Hospital Molecular + Additional Testing

- Patient's Predicted Phenotype… M+N-
- Two more En(a-) cells are nonreactive
- Probable anti-En^{a}FR, but molecular testing is discrepant
- What to do next? Refer for sequencing

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Sequencing at NYBC

Long range PCR and sequencing of *GYPA* exons 2 to 7

"Exon 2: 59C/C (20Ser), 71 G/G and 72 T/T (24 Gly), consistent with *GYPA*M/M* background"

No changes identified in Exons 3, 4, 6, and 7

"Exon 5: Homozygous for nucleotide G insertion that causes a premature stop codon; c.314_315insG (p.Thr106Asnfs*19)"

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Summary

Patient is predicted to be M-N-

Patient consultation:

- Patient was born in Pakistan
- Parents are distant cousins
- 4 siblings – all living in Pakistan
- Reports she was told at age 14 about issues with blood compatibility
  - Naturally occurring antibody?

En(a)

- Anti-En^a is extremely rare – only handful of case reports
- Some reports of shortened RBC survival (1987) and HTR (1973)
- Two cases of En(a-) patients typing Le(a+b+)
  - Our case + one report from UK

- En(a-) or M^kM^k blood for transfusion
- Our patient did not require blood
- One autologous unit collected!

Case 4

- 81 year old female of African descent
- Unspecified anemia – 6.2g Hgb
- Recently transfused 2 units of RBC
- Unknown pregnancy history
- Unknown medication history
- Hospital reports an anti-Fy^a identified in 2006

Case #4

Case 4 – Hospital Report

- Patient types O positive
- DAT negative
- Hospital reports positive antibody screen by gel – all cells positive
- Autocontrol negative

Case 4 - IRL Testing

Anti-A	Anti-B	Anti-D	A1 Cells	B cells	Interp
0	0	4+	4+	4+	O Pos

Poly AHG
0v

- After tech starts initial panel - additional history located at another facility
- Anti-C and anti-E added to history

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Case 4 – IRL Work

	D	C	c	E	e	Cw	K	k	Kpb	Jsb	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	Lub	IS	37C	LISS IAT
1	+	+	0	0	+	0	+	+	+	+	+	0	+	0	0	+	+	+	0	0	+	+	0	0	3+
2	+	+	0	0	+	0	0	+	+	+	0	+	0	+	0	+	+	0	+	0	+	+	0	0	3+
3	+	0	+	+	0	0	0	+	+	+	0	+	0	+	0	+	0	+	+	0	+	+	0	0	3+
4	+	0	+	0	+	0	0	+	+	+	0	0	+	0	0	0	+	0	+	0	+	+	0	0	3+
5	0	+	+	0	+	0	0	0	+	+	+	+	0	+	0	+	+	+	+	0	+	+	0	0	3+
6	0	0	+	+	+	0	0	+	+	+	+	0	0	+	0	+	0	0	+	+	+	+	0	0	3+
7	0	0	+	0	+	+	+	+	+	+	0	+	+	0	0	+	+	+	0	0	+	+	0	0	3+
8	0	0	+	0	+	0	0	+	+	+	+	0	+	+	+	0	+	+	+	0	+	+	0	0	3+
9	0	0	+	0	+	0	0	+	+	+	0	+	+	0	0	+	+	+	0	0	+	+	0	0	3+
10	+	0	+	0	0	0	0	+	+	+	+	0	+	+	+	0	0	+	+	+	0	+	0	0	3+
11	0	0	+	0	+	0	+	+	+	+	+	+	+	+	0	+	0	+	+	0	+	+	0	0	3+
AC																							0	0	0V

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Selected Cell Panel

	D	C	c	E	e	K	k	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	LISS IAT
1	0	0	+	0	+	0	+	0	0	+	0	+	0	+	+	0	+	0	3+
2	0	0	+	0	+	0	+	0	+	0	+	+	0	0	+	+	+	+	3+
3	+	0	+	0	+	+	+	0	0	+	0	0	+	+	+	+	+	0	3+
4	0	0	+	0	+	0	+	0	0	+	+	+	0	+	+	+	+	+	3+

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High Prevalence Panel

Cell Type	LISS-IAT
k-	4+
Kp(a+b-)	4+
Js(a+b-)	4+
U-	0v
Lu(a+b-)	4+
Yt(a-)	4+
Vel-	4+

Selected U- Panel

	D	C	c	E	e	K	k	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	U	LISS IAT
1	0	0	+	0	+	0	+	0	0	+	0	+	0	+	+	0	0	0	0	0V
2	0	0	+	0	+	0	+	0	+	0	+	+	0	0	+	+	0	0	0	3+
3	+	0	+	0	+	0	+	0	0	+	0	0	+	+	+	+	0	0	0	0V
4	0	0	+	0	+	0	+	0	0	+	+	+	0	+	+	+	0	0	0	0V

- Probable anti-U
- Possible antibody to low prevalence antigen?
  - V, VS, Js^a, etc?

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Patient phenotype

D	C	E	c	e
+	0	0	+	+

K	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s	U
0	0	0	+	+	+	+	0	+	+

U- Panel: A Closer Look

	D	C	c	E	e	K	k	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	U	LISS IAT
1	0	0	+	0	+	0	+	0	0	+	0	+	0	+	+	0	0	0	0	0V
2	0	0	+	0	+	0	+	0	+	0	+	+	0	0	+	+	0	0	0	3+
3	+	0	+	0	+	0	+	0	0	+	0	0	+	+	+	+	0	0	0	0V
4	0	0	+	0	+	0	+	0	0	+	+	+	0	+	+	+	0	0	0	0V
AC																				0V

- The U- cells selected were mostly Fy(a-b-), except cell #2
- Possible anti-Fy3?

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Selected Fy(a-b-) Panel

	D	C	c	E	e	K	k	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	LISS IAT
1	+	0	+	0	+	0	+	0	0	+	+	0	+	+	+	+	+	0	3+
2	+	0	+	0	+	0	+	0	0	+	0	0	+	+	0	+	0	+	0v
3	+	0	+	0	+	0	+	0	0	0	+	0	+	0	+	+	0	+	0v
4	+	0	+	0	+	0	+	0	0	0	+	0	+	0	0	+	0	+	0v

- Anti-U is excluded
- Is *this* the antibody to a low prevalence antigen?

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Finally Solved!

	D	C	c	E	e	K	k	Fya	Fyb	Jka	Jkb	M	N	S	s	LISS-IAT	Ficin IAT
1	+	+	+	0	+	0	+	0	0	+	0	+	0	0	+	1+	NT
2	+	0	+	+	+	0	+	0	0	0	+	0	+	0	+	1+	NT
3	0	0	+	0	0	0	+	+	0	0	+	+	+	0	+	3+	3+
4	+	0	+	0	+	0	+	0	+	+	0	0	+	0	+	3+	3+
5	+	0	+	0	+	0	0	0	0	0	+	+	+	+	+	3+	0V
6	+	0	+	0	+	0	+	0	0	0	+	0	+	0	+	0V	0V

- Adsorptions performed to exclude anti-K

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Summary

- Final antibody identification: Anti-C, -E, -Fy3, -S
- Anti-Fy^{a} reported in patient history was not evaluated due to presence of anti-Fy3
  - Anti-Fy5 was not evaluated
- Anti-Fy3 is considered a clinically significant red cell alloantibody and has been implicated in hemolytic transfusion reactions
- This patient should receive C-, E-, Fy(a-b-), S- red blood cells if transfusion is required

Many thanks!

Versiti IRL teams in Illinois, Wisconsin, Indiana, and Michigan for their hard work on these cases

Dr. Glenn Ramsey and NYBC for their collaboration on some of these cases

NCABB for inviting me here today!