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IRL Tech II

Nightmare on Main Street

Community Blood Center

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Objectives

- Demonstrate the importance of phenotypes/genotypes in patients with warm autoantibodies

- Understand the role of adsorptions in warm autoantibody identification

- Discuss transfusion options available for patients with warm autoantibodies

Then the phone rang....

- Received call from a local hospital about a male patient in the ER
  - 3.2 gram Hgb
  - Patient currently hemolyzing
  - DAT 4+, plasma positive with all cells tested, only history of transfusion from 2009
  - Needing worked up STAT
  - Need to transfuse at least 1 incompatible unit while work up is being done

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Once a sample is received...

Forward Typing			Reverse Typing	
Anti-A	Anti-B	Anti-D	A_1 Cells	B Cells
4+	0	0	0	4+

	Poly	IgG	C'	Saline
DAT	3+	3+^{s}	4+	0

- ABO/Rh: A Negative

- DAT: Positive with IgG and complement

Plasma testing

		Rh					MNSs				Kell		Duffy		Kidd		Results		
		D	C	E	c	e	M	N	S	s	K	k	Fy^a	Fy^b	Jk^a	Jk^b	5' RT	LISS 37C	IAT
1	R_1R_1	+	+	0	0	+	+	+	0	+	0	+	+	+	0	+	0	$1+^w$	3+
2	R_1R_1	+	+	0	0	+	+	0	+	+	0	+	+	0	+	0	0	1+	3+
3	R_2R_2	+	0	+	+	0	+	0	+	0	0	+	0	+	+	0	0	1+	3+
4	Ror	+	0	0	+	+	+	+	0	+	0	+	0	0	+	0	0	1+	3+
5	r'r	0	+	0	+	+	+	0	+	+	0	+	0	+	0	+	0	$1+^w$	3+
6	r"r"	0	0	+	+	0	+	0	0	+	0	+	+	+	+	+	0	$1+^w$	3+
7	rr	0	0	0	+	+	+	0	+	+	+	+	0	+	0	+	0	$1+^w$	3+
8	rr	0	0	0	+	+	0	+	+	0	0	+	0	+	+	0	0	1+	3+
9	rr	0	0	0	+	+	0	+	0	+	0	+	+	0	+	+	0	1+	3+
10	rr	0	0	0	+	+	+	+	+	0	0	+	0	+	+	0	0	1+	3+
11	R_1R_1	+	+	0	0	+	+	+	+	+	+	+	0	+	+	+	0	$1+^w$	3+
AC																	0	1+	3+

Eluate testing

|		Rh					MNSs				Kell		Duffy		Kidd		Results
		D	C	E	c	e	M	N	S	s	K	k	Fy^a	Fy^b	Jk^a	Jk^b	PEG IAT
1	R_1R_1	+	+	0	0	+	+	+	0	+	0	+	+	+	0	+	3+
2	R_1R_1	+	+	0	0	+	+	0	+	+	0	+	+	0	+	0	3+
3	R_2R_2	+	0	+	+	0	+	0	+	0	0	+	0	+	+	0	3+
4	Ror	+	0	0	+	+	+	+	0	+	0	+	0	0	+	0	3+
5	r'r	0	+	0	+	+	+	0	+	+	0	+	0	+	0	+	3+
6	r"r	0	0	+	+	+	+	0	0	+	0	+	+	+	+	+	3+
7	rr	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	+	+	3+
10	rr	0	0	0	+	+	+	+	+	0	0	+	0	+	+	0	3+
11	R_1R_1	+	+	0	0	+	+	+	+	+	+	+	0	+	+	+	3+
EGA AC																	3+

Time for adsorptions

- Since all panel cells are positive, we need to get rid of the warm autoantibody reactivity

- To make adsorptions easier, a phenotype is needed

	E	c	C	e	K	Fy^a	Fy^b	Jk^a	Jk^b	S	s
RBC's	0 $^{(mf)}$	4+	1+^{mf}	4+	1+^{mf}	3+^{s} *	2+^{mf} *	4+	2+^{mf}	4+	3+^{s} *

- Phenotype revealed mixed field in almost all antigen typings

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* Tested with EGA treated RBC's

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Reticulocyte cell separation

- Typically, when mixed field is observed, retics are acquired and tested…

- Due to the 3 g hgb, that wasn't an option
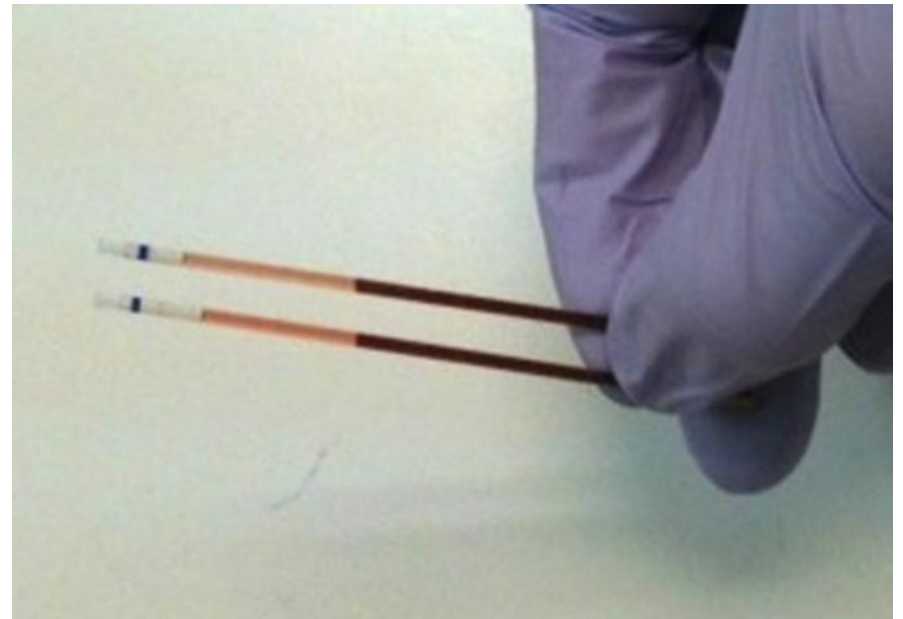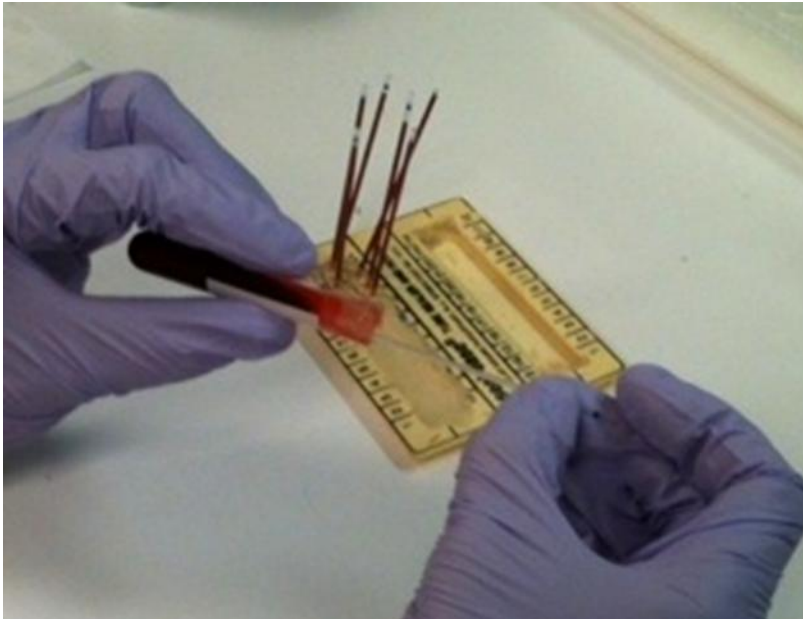
What's the need for a phenotype?

- Typically we adsorb with phenotypically matched, ficin treated cells

- Without a phenotype, adsorptions will be much more difficult and time consuming

- If transfusion is urgently needed, IRL will recommend giving phenotypically matched RBC's while workup is being done...

No phenotype, no problem

- Since a phenotype was unavailable, we can still do adsorptions
  - R1R1
  - R2R2
  - rr
- Number of Absorptions = Strength of reactivity + 1, for minimum of 10 min at 37C

- Run each adsorbing set in tandem, and test separately

Alloadsorbed plasma

|		Rh					MNSs				Kell		Duffy		Kidd		R1R1	R2R2	rr
		D	C	E	c	e	M	N	S	s	K	k	Fy^a	Fy^b	Jk^a	Jk^b	LISS IAT	LISS IAT	LISS IAT
1	R$_1$R$_1$	+	+	0	0	+	+	+	0	+	0	+	+	+	0	+	3+	3+	3+
2	R$_1$R$_1$	+	+	0	0	+	+	0	+	+	0	+	+	0	+	0	3+	3+	3+
3	R$_2$R$_2$	+	0	+	+	0	+	0	+	0	0	+	0	+	+	0	3+	3+	3+
4	Ror	+	0	0	+	+	+	+	0	+	0	+	0	0	+	0	3+	3+	3+
5	r'r	0	+	0	+	+	+	0	+	+	0	+	0	+	0	+	3+	3+	3+
6	r"r	0	0	+	+	+	+	0	0	+	0	+	+	+	+	+	3+	3+	3+
7	rr	0	0	0	+	+	+	0	+	+	+	+	0	+	0	+	3+	3+	3+
8	rr	0	0	0	+	+	0	+	+	0	0	+	0	+	+	0	3+	3+	3+
9	rr	0	0	0	+	+	0	+	0	+	0	+	+	0	+	+	3+	3+	3+
10	rr	0	0	0	+	+	+	+	+	0	0	+	0	+	+	0	3+	3+	3+
11	R$_1$R$_1$	+	+	0	0	+	+	+	+	+	+	+	0	+	+	+	3+	3+	3+

What went wrong??

- Ficin treated cells usually enhance antibody uptake, but some antibodies are not removed

- Our next option is to repeat the adsorbing sets, but using untreated RBC's

  - This requires more antigen matching, and more time…

Alloadsorbed plasma with untreated cells

		Rh					MNSs				Kell		Duffy		Kidd		R1R1	R2R2	rr
		D	C	E	c	e	M	N	S	s	K	k	Fy^a	Fy^b	Jk^a	Jk^b	LISS IAT	LISS IAT	LISS IAT
1	R_1R_1	+	+	0	0	+	+	+	0	+	0	+	+	+	0	+	0	0	0
2	R_1R_1	+	+	0	0	+	+	0	+	+	0	+	+	0	+	0	0	0	0
3	R_2R_2	+	0	+	+	0	+	0	+	0	0	+	0	+	+	0	0	0	0
4	Ror	+	0	0	+	+	+	+	0	+	0	+	0	0	+	0	0	0	0
5	r'r	0	+	0	+	+	+	0	+	+	0	+	0	+	0	+	0	0	0
6	r"r"	0	0	+	+	0	+	0	0	+	0	+	+	+	+	+	0	0	0
7	rr	0	0	0	+	+	+	0	+	+	+	+	0	+	0	+	0	0	0
8	rr	0	0	0	+	+	0	+	+	0	0	+	0	+	+	0	0	0	0
9	rr	0	0	0	+	+	0	+	0	+	0	+	+	0	+	+	0	0	0
10	rr	0	0	0	+	+	+	+	+	0	0	+	0	+	+	0	0	0	0
11	R_1R_1	+	+	0	0	+	+	+	+	+	+	+	0	+	+	+	0	0	0

There's another issue…

- IRL likes to do no more than 4-6 adsorptions to prevent any dilution of the patient's plasma

- Due to the limited sample, the plasma adsorbed 4 times using Ficin treated cells was used again to do an additional 4 adsorptions with untreated cells…
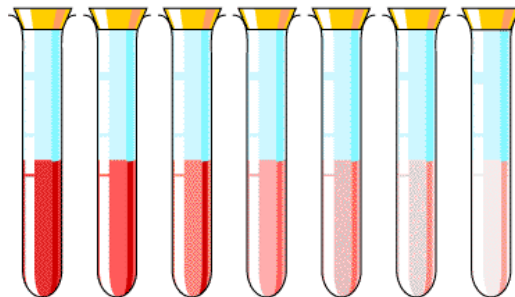
Sample sent to NYBC

- Per the requesting facility, a sample was sent to New York Blood Center

- NYBC was able to provide us with a genotype:

  C-, E-, c+, e+, K-, Fy (a+b-), Jk (a+b-), S+, s+

- With what little sample was available, NYBC was able to duplicate our results and confirm that there were no underlying alloantibodies.

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In the mean time…

- Due to the patient's hemolysis, IRL recommended that transfusion be withheld unless utterly necessary

- Patient was stable at a 6.8 g Hgb 2 days later
  - units matching the patient's genotype were found and held

- Patient "pretty stable" 4 days later

- Additional units were not needed from CBC
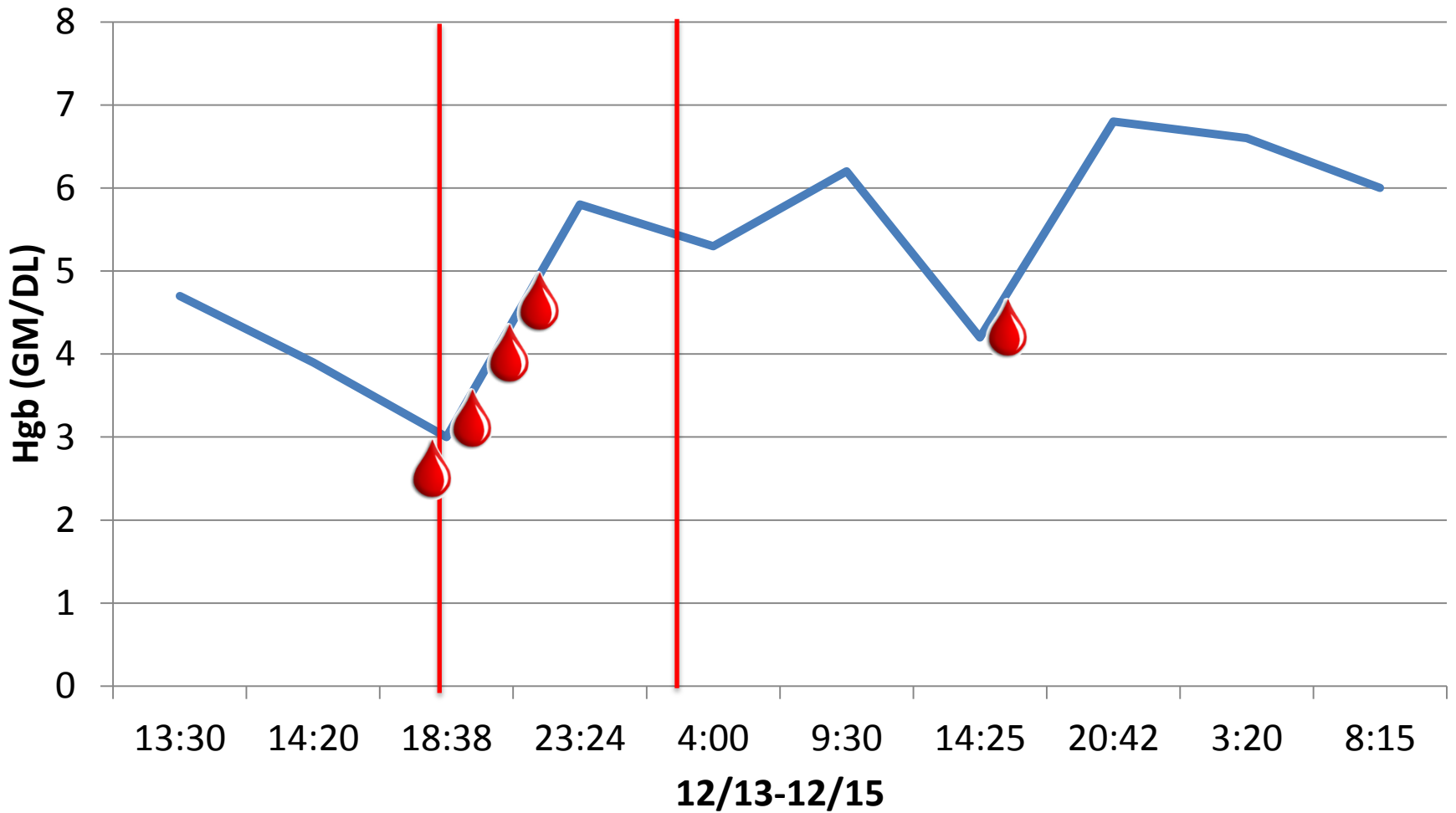
Work-up breakdown

- ABO/Rh
- DAT
- 1 Elution
- 1 Phenotype
- A total of 6 panels ran with 24 adsorptions
- A total of 10 hours of tech time

Further patient investigation

- 2007
  - Diagnosed with Lymphoproliferative disease
  - Wegner's granulomatosis – an autoimmune disorder
  - Subsequent renal failure – kidney transplant in 2008
- Patient was transferred from an outside facility due to jaundice and anemia
- Unfortunately the patient was unable to overcome the hemolytic event and passed away

Tips on what to transfuse when dealing with warm autoantibodies

- Random, Crossmatch incompatible unit(s)

- Random unit(s) tested with alloadsorbed plasma – will still be XM incompatible

- Phenotypically matched – becoming a preferred practice

Questions?

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