

I'm Not Kidd-ing!

Complexities of Antibody Identification in Patient's with Multiple Antibodies

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NHS Blood and Transplant



Outline

- Antibody Identification
- Why it is important
- Blood availability
- 3 Case Studies





Antibody Screen





Antibody Panels



https://b-s-h.org.uk/guidelines/guidelines/pre-transfusioncompatibility-procedures-in-blood-transfusion-laboratories/

- Consist of red cells from eight or more group O donors
- For commonly encountered clinically significant alloantibodies: 2 antigen pos and 2 antigen neg cells
- 1 x R1R1 (CCDDee) and 1 x R1wR1 (CwCDDee)
- Between them, these two cells should express the antigens K, k, Fya, Fyb, Jka, Jkb, S, s
- One example of each of the phenotypes R2R2 (ccDDEE), r'r (Ccddee) and r"r (ccddEe)
- At least three examples of the phenotype rr (ccddee), including at least one K+, and collectively, homozygous expression of k, Jka, Jkb, S, s, Fya, and Fyb



Papain Treatment

- Can assist with ABID
- Some antigens are sensitive to papain
- Some antigens are enhanced steric hindrance

	Ph.hr		Spender Donor Donneur			Rh	-hr					K	ell			Du	iffy	Ki	dd	Lev	vis	Ρ		M	NS		Lu	th.
	DUEUL		Donatore Donante Dador	D	С	E	С	е	C"	к	k	Kp*	Кр⁵	Jsª	Js"	Fy⁴	Fyn	Jk	JK⁵	Le°	Le ^t	P	М	N	s	s	Luª	Lu°
I	CCD.ee	R ₁ R ₁	251731	+	+	0	0	+	0	+	+	0	+	nt	+	+	+	+	0	0	+	+	+	0	+	0	0	+
	ccD.EE	R_2R_2	032276	+	0	+	+	0	0	0	+	0	+	nt	+	+	0	+	+	+	0	+	0	+	0	+	0	+
	CCD.ee	R ₁ R ₁	114003	+	+	0	0	+	0	0	+	0	+	nt	+	+	+	+	+	0	+	0	+	+	+	+	0	+





Grading



Reading agglutination in tube tests - reaction grades 0 - 5

- Can assist with ABID
- Multiple antibodies
- Enhanced reactions
- Dosage









Antigram - ABID Example



-	Cell	Rh	D	¢	¥	¢	ø	M	×	ø	\$	₽∕	Luª	¥	X	Kpa	Lea	Leb	Fya	Fy	JK ª	Jke	Other	IAT
		R ₁ ^w R ₁	+	+	0	0	+	+	0	+	0	0	0	0	+	0	0	+	+	0	+	0		1
		R ₁ R ₁	+	+	0	0	+	0	+	0	+	0	0	+	+	0	+	0	0	+	0	+		1
t'L	9	R_2R_2	+	0	+	+	0	+	0	+	0	0	0	0	+	0	+	0	0	+	+	0		2
		r'r	0	+	0	+	+	0	+	+	0	0	0	0	+	0	0	+	0	4	Å	0		0
	5	r''r	0	0	+	+	+	0	+	0	+	4	0	0	+	0	0	+	+	0	0	Á		0
	6	rr	0	0	0	+	+	+	0	+	0	4	0	+	0	0	0	+	+	0	0	Á		0
	7	rr	0	0	0	+	+	0	+	0	+	2	+	+	+	0	+	0	0	+	+	0		2
	8	rr	0	0	0	+	+	0	+	0	+	0	0	0	+	+	0	+	0	A	0	+		0
	9	rr	0	0	0	+	+	+	0	0	+	2	0	0	+	0	+	0	+	0	0	+		0
	10	rr	0	0	0	+	+	0	+	0	+	3	0	0	+	0	+	0	+	0	+	0		0
																							Auto	0
																							K control	2

Anti-D and ? Anti-Lua

Not excluded: Anti-C^w

Test additional Lua+ D- cell

Patient's phenotype is B RhD- C- E- K- (B rr K-)



Exclusions















Why is this so important?

- Multiple blood group antigens
- Not just ABO and RhD
- Red cell antibodies produced through either pregnancy, transfusion or transplantation
- Antibodies can be clinically significant
- Can cause delayed and immediate HTR





ABO Antigens and Antibodies



Naturally Occurring

Additional Red Cell Antigens



- Antibodies against 'non-self'
- Clinically significant
 - Defined by their ability to reduce RBC survival

NHS

Blood and Transplant

- Must select antigen negative RBC
- Stimulated through pregnancy, transfusion or transplantation

Donor RBC

-

Patient's Antibody



Blood on the Shelf?



Don't worry though!

Calculate antigen frequency

- Multiply antigen negative frequencies together
- Then divide the number of units needed by frequency % obtained
- E.g. K- Fy(a-)

• K- ~ 91%

Fy(a-) ~32% 0.91 x 0.32 = 0.29 29%

- 2 units: 2/29 = 7 units
- Also need to consider ABO and RhD
- 100 units

(frequencies based on UK donor population)



Patient 1

- 54 year old Female
- End Stage Renal Failure
- Regular RCI patient
- Known anti-C, anti-K and anti-Jka
- Called out for ABID and 3 unit crossmatch
- Transfused 3 weeks previously
- Set up 3xm O+ C- K- Jk(a-) red cells at the same time
- Crossmatch compatible by IAT



ABID Patient 1

Cell	Rh	D	с	E	с	е	м	N	s	s	P1	Lu ^a	к	k	Kpª	Le ^a	Le ^b	Fy ^a	Fy ^b	Jka	Jk	Other	ΙΑΤ	EIAT
1	R ₁ ^w R ₁	+	+	0	0	+	+	0	+	0	0	0	0	+	0	0	+	+	0	+	0		3	5
2	R ₁ R ₁	+	+	0	0	+	0	+	0	+	0	0	+	+	0	+	0	0	+	0	+		4	5
3	R ₂ R ₂	+	0	+	+	0	+	0	+	0	0	0	0	+	0	+	0	0	+	+	0			3
4	r'r	0 (+	0	+	+	0	+	+	0	0	0	0	+	0	0	+	0	+	+	0		3	5
5	r''r	0	0	+	+	+	0	+	0	+	4	0	0	+	0	0	+	+	0	0	+		0	2
6	rr	0	0	0	+	+	+	0	+	0	4	0	+	0	0	0	+	+	0	0	+		4	5
7	rr	0	0	0	+	+	0	+	0	+	2	+	+	+	0	+	0	0	+	$(\mathbf{+})$	0		3	5
8	rr	0	0	0	+	+	0	+	0	+	0	0	0	+	+	0	+	0	+	0	+		0	2
9	rr	0	0	0	+	+	+	0	0	+	2	0	0	+	0	+	0	+	0	0	+		0	2
10	rr	0	0	0	+	+	0	+	0	+	3	0	0	+	0	+	0	+	0	+	0		1	3
																						Auto	1	
																						K control	2	/

? Known Anti-C plus known anti-K plus known anti-Jka and pan-reactive enzyme antibody

Need to confirm anti-C

Unable to exclude: anti-D, anti-C^w anti-S and anti-Lua

Additional Inclusions and Exclusions

Second panel or other individual cells

										-				-				-	-				
Cell	Rh	D	с	E	с	е	м	N	S	S	P1	Luª	к	k	Kpª	Leª	Le ^b	Fya	Fy ^b	Jk ^a	Jk⊳	Other	ΙΑΤ
1 🤇	R ₁ ^w R ₁	\triangleright	+	0	0	+	+	0	+	0	0	0	0	+	0	0	+	+	0		+		3
2	R_1R_1	+	+	0	0	+	0	+	0	+	0	0	+	+	0	+	0	0	+	+	0		3
3	R ₂ R ₂	1	0)+	+	0	+	0	1	0	0	0	0	+	0	+	0	0	+	0	+		
4	r'r	0	+	0	+	+	0	+	+	0	0	0	0	+	0	0	+	0	+	0	+		3
5	r"r	0	0	+	+	+	0	+	0	+	4	0	0	+	0	0	+	+	0	+	0		1
6	rr	0	0	0	+	+	+	0	+	0	4	0	+	0	0	0	+	+	0	+	0		4
7	rr	0	0	0	+	+	0	+	0	+	2	0	+	+	0	+	0	0	+	0	+		3
8	rr	0	0	0	+	+	0	+	0	+	0	0	0	+	+	0	+	0	+	0	+		0
9	rr	0	0	0	+	+	+	0	0	+	2	0	0	+	0	+	0	+	0	+	0		1
10	rr	0	0	0	+	+	0	+	+	+	3	A	0	+	0	+	0	+	0		+		
																						Auto	1
																						K control	2



Blood and Transplant

NHS

O RhD+ C- K- Jka-



Recently transfused

DAT Results



Crossmatch compatible by IAT

Need to confirm known anti-C and exclude or include anti-D, anti-S, anti-Lua and anti-Cw Low Incidence

Still unable to exclude anti-C^w

Additional Investigations



Direct Antiglobulin Test (DAT) Eluate





Preparing an Eluate

















Patient 1 Eluate Results

Cell	Rh	D	с	E	с	е	М	N	S	s	P1	Lu ^a	к	k	Kpª	Le ^a	Le ^b	Fy ^a	Fy ^b	Jka	Jk♭	Other	Eluate IAT
1	R ₁ ^w R ₁	+	+	0	0	+	+	0	+	0	0	0	0	+	0	0	+	+	0	+	0		0
2	R₁R₁	+	+	0	0	+	0	+	0	+	0	0	+	+	0	+	0	0	+	0	+		1
3	R_2R_2	+	0	+	+	0	+	0	+	0	0	0	0	+	0	+	0	0	+	+	0		1
4	r'r	0	+	0	+	+	0	+	+	0	0	0	0	+	0	0	+	0	+	+	0		1
5	r"r	0	0	+	+	+	0	+	0	+	4	0	0	+	0	0	+	+	0	0	+		0
6	rr	0	0	0	+	+	+	0	+	0	4	0	+	0	0	0	+	+	0	0	+		0
7	rr	0	0	0	+	+	0	+	0	+	2	+	+	+	0	+	0	0	+	+	0		1
8	rr	0	0	0	+	+	0	+	0	+	0	0	0	+	+	0	+	0	+	0	+		1
9	rr	0	0	0	+	+	+	0	0	+	2	0	0	+	0	+	0	+	0	0	+		0
10	rr	0	0	0	+	+	0	+	0	+	3	0	0	+	0	+	0	+	O	+	0		0
																						Auto	1
																						K control	2

New Anti-Fyb in eluate!



The Whole Picture

- Initially set up 3xm O+ C- K- Jk(a-) red cells at the same time as initial ABID
- Crossmatch compatible by IAT
- Patient had anti-Fyb in eluate is this clinically significant? YES
- What would you do next?
- Check the phenotype of the crossmatched units
- Cannot phenotype due to recent transfusion or positive DAT
- Needed to re-crossmatch 3 x O+ C- K- Jk(a-) Fy(b-) Only 2 units in stock
- 500



😂 P	CU11 - Pu	ulse Launcher							- 🗆 ×	
My	Pulse								🚑 🍃 🔀	Ľ
Pul	se user	Helen Louise Thom		Default Printer	\\NDCVK107\Newcastle - NEV	VP02			Date 13/01/2021	
Cur	rent Site	N1 - NHSBT-Newcasti	e	Current Database	NBPULSE - NBS Production	n Database (Live)		Time 11:48	
	стос	к	🗲 Back / 🚑 Report	s 🛛 💐 <u>F</u> ind Program	😼 Manage My Favourites	? Options -	2 Pulse <u>H</u> elp	😨 Help <u>A</u> bout		
	1		Donation Batch I	nformation		1	PCS10			
	2		Stock Validation				PCS28			
	3		Stock Enquiry				PC S50			
	4		Production Batch	1			PCS74			
	5		Request Manage	ement			PC S80			
	6		Stock Movement	s		0	PCS81			
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			63							



NHS Blood and Transplant

What Next?

- Contacted HTL
- Do you really need 3 units for this patient?
- - circumstances slightly different with RCI: time, location etc
- Discussed with HTL. Happy to take 2 units
- RCI will order more and HTL to ring if additional units required
- Red Cell Genotype performed on next sample
- Anti-Fyb confirmed as alloantibody
- Now detected in plasma makes ABID increasingly more difficult. How?



Do they still have symptoms of anaemia?

Are there any signs or symptoms of a transfusion reaction?

Is further transfusion appropriate? Re-check haemoglobin level.



Patient 2

- 36 year old female
- Referred on 30/07/18 from Hospital 1 for routine ABID
- Bled on 29/07/18
- "Confirmation of anti-Fya"
- Previously transfused in 2015
- Patient phenotyped as O+ E- K- Fy(a-) phenotype (if 2 units requested)
- DAT: negative

- RBC units not requested
- Bear in mind it could turn into crossmatch



• Need to test 100 random units for this



ABID Patient 2 (Initial Investigation)

Cell	Rh	D	с	Е	с	е	М	N	s	s	P1	Lu ^a	к	k	Kpª	Le ^a	Le ^b	Fy ^a	Fy ^b	Jka	Jk⊳	Other	IAT	EIAT
1	R ₁ ^w R ₁	+	+	0	0	+	+	0	+	0	0	0	0	+	0	0	+	/+	0	+	0		2	0
2	R₁R₁	+	+	0	0	+	0	+	0	+	0	0	+	+	0	+	0	0	+	0	+		0	0
3	R ₂ R ₂	+	0	+	+	0	+	0	+	0	0	0	0	+	0	+	0	0	+	+	0		0	0
4	r'r	0	+	0	+	+	0	+	+	0	0	0	0	+	0	0	+	0	+	+	0		0	0
5	r"r	0	0	+	+	+	0	+	0	+	4	0	0	+	0	0	+	+	0	0	+		2	0
6	rr	0	0	0	+	+	+	0	+	0	4	0	+	0	0	0	+	+	0	0	+		2	0
7	rr	0	0	0	+	+	0	+	0	+	2	+	+	+	0	+	0	0	+	+	0		0	0
8	rr	0	0	0	+	+	0	+	0	+	0	0	0	+	+	0	+	0	+	0	+		0	0
9	rr	0	0	0	+	+	+	0	0	+	2	0	0	+	0	+	0	+ /	0	0	+		2	0
10	rr	0	0	0	+	+	0	+	0	+	3	0	0	+	0	+	0	\bigvee	0	+	0		2	0
																					(Auto	0) /
																						K control	2	/

Alloanti-Fya. No additional antibodies detected DAT not required. Can phenotype using IgG antisera

2nd Investigation

- Referred on 06/08/18 from Hospital 2 for antibody confirmation
- ABID results from HTL: Anti-Fya
- No diagnosis or transfusion history on form
- HTL to perform crossmatch
- Matched as known patient on LIMS
 - Name
 - Date of birth
 - NHS Number





IH 1000 Results

Bloodgroup: A,B,D Confirmation for P, (DiaClon) (5005)	atients Text time: Processed by: 07/08/2018 08:30 Verified by:	Mick KENNO031	7 transfused
Arti-D VI 50057.5201/18.10/105752	unt insults		
	UR . In	rueg ma	
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Fee.al- D Rh D I AB0	not interpretable		linda m dang
Pierakov ABO Pierakov	not interpretable ASS: DAT:		Under m dang

- Dual population vs anti-D well
- Rang to obtain tx history
- Pt transfused 4 O neg emergency units 29/07/18
- Involved in trauma
- Transferred from Hospital 1
- Transfused prior to ABID results on Sp-Ice
- Obtained donation numbers
- 3/4 units Fy(a+)
- DAT now positive



ABID Patient 2 (2nd Investigation)

		-	-	-												-	-	-			-		-		_
Cell	Rh	D	с	E	с	e	м	N	s	s	P1	Lu ^a	к	k	Kpª	Lea	Le ^b	Fy ^a	Fy⁵	Jka	Jk⁵	Other	IAT	EIAT	
1	R ₁ ^w R ₁	+	+	0	0	+	+	0	+	0	0	0	0	+	0	0	+	/+	0	+	/ o \		4	0	1
2	R ₁ R ₁	+	+	0	0	+	0	+	0	+	0	0	+	+	0	+	0	0	+	0	+		0	3	
3	R ₂ R ₂	+	0	+	+	0	+	0	+	0	0	0	0	+	0	+	0	0	+	+	0		0	0	
4	r'r	0	+	0	+	+	0	+	+	0	0	0	0	+	0	0	+	0	+	+	0		0	0	
5	r"r	0	0	+	+	+	0	+	0	+	4	0	0	+	0	0	+	+	0	0	+		4	3	
6	rr	0	0	0	+	+	+	0	+	0	4	0	+	0	0	0	+	+	0	0	+		4	3	
7	rr	0	0	0	+	+	0	+	0	+	2	+	+	+	0	+	0	0	+	+	0		0	0	
8	rr	0	0	0	+	+	0	+	0	+	0	0	0	+	+	0	+	0	+	0	+		0	3	
9	rr	0	0	0	+	+	+	0	0	+	2	0	0	+	0	+	0	+ /	0	0	+/		4	3	
10	rr	0	0	0	+	+	0	+	0	+	3	0	0	+	0	+	0	\bigvee	0	+	∖₀∕	~~	4	0	
																					VV	Auto	3	1	
																						k control	2	/	

Previous anti-Fya plus new anti-Jkb. Both antibodies detected in eluate

Patient 2

- Confirmed anti-Fya
- New anti-Jkb (enzyme only) clinically significant? YES
- Jkb confirmed as alloantibody using pre-tx sample from Hospital 1
- Both antibodies in plasma and eluate
- RCI contacted HTL to suggest we crossmatch
- Issued 4 group O RhD- E- K- Jk(b-) Fy(a-) issued
- Updated antibody card provided





Patient 3

- Referral on 28/08/18
- 23 year old antenatal patient
- "Known anti-Fya we think she's made a Jka"
- Sent in for ABID
- Induction of Labour (IOL)
- HTL have two crossmatch compatible units





But that wasn't all...

- Patient is known alloanti-Fya
- Seen once previously by RCI (from different HTL)
- Previous alloanti-Fya titre: >1000 Does anybody know the risk for HDFN?
- High risk HDFN
- Fetus had received 2 IUTs
- July 2018



Intrauterine transfusion



A fetus may receive a blood transfusion through the umbilical vein in the placenta





Antibody Titres

- Serial dilutions
- Antibodies capable of causing HDFN
- Not anti-D and anti-c
- BSH Guideline for blood grouping and red cell antibody testing in pregnancy
- 'Heterozygous' antigen expression
- <32: low risk HDFN</p>
- ≥32: high risk HDFN
- Kell system antibodies- titre not indicative of HDFN









ABID Patient 3

			-				-																		4
Cell	Rh	D	с	E	с	е	м	N	s	s	P1	Lu ^a	к	k	Kpª	Le ^a	Le ^b	Fy ^a	Fy⁵	Jka	Jk♭	Other	IAT	EIAT	
1	R ₁ ^w R ₁	+	+	0	0	+	+	0	+	0	0	0	0	+	0	0	+	(+)	0	(+)	0		4	(3)	
2	R₁R₁	+	+	0	0	+	0	+	0	+	0	0	+	+	0	+	0	0	+	0	+		0	0	
3	R ₂ R ₂	+	0	+	+	0	+	0	+	0	0	0	0	+	0	+	0	0	+	(+)	0			(3)	
4	r'r	0	+	0	+	+	0	+	+	0	0	0	0	+	0	0	+	0	+	(+)	0			(3)	
5	r"r	0	0	+	+	+	0	+	0	+	4	0	0	+	0	0	+	(+)	0	0	+		4	0	
6	rr	0	0	0	+	+	+	0	+	0	4	0	+	0	0	0	+	(+)	0	0	+		(4)	0	5
7	rr	0	0	0	+	+	0	+	0	+	2	+	+	+	0	+	0	0	+	(+)	0			(3)	
8	rr	0	0	0	+	+	0	+	0	+	0	0	0	+	+	0	+	0	+	0	+		0	0	
9	rr	0	0	0	+	+	+	0	0	+	2	0	0	+	0	+	0	(+)	0	0	+		4	0	
10	rr	0	0	0	+	+	0	+	0	+	3	0	0	+	0	+	0	(+)	0	(\cdot)	0		4	3	
																						Auto	0	/	
																						K control	2	/	

Anti-Fya and anti-Jka Unable to exclude: Anti-S, anti-M, anti-C^w What w

What would you do next?



What next?

- Additional exclusions or inclusions:
 - Test C^w+ Fy(a-) Jk(a-) M- cell by IAT
 - Test C^w+ Jk(a-) Papainised cell
 - Test Fy(a-) Jk(a-) S+ M- cells
 - Test Fy(a-) Jk(a-) S- M+ cells
 - Or test Fy(a-) Jk(a-) S+ M+ cells(all 'homozygous' expression)
- Phenotype might be antigen positive

Think about HDFN risk





ABID Results - Mother

- Alloanti-Fya
- Confirmed anti-Jka
- 4 units crossmatched
- A RhD+ E- K- Jk(a-) Fy(a-)
- Crossmatch compatible by IAT
- To cover IOL



- Must also assess risk for HDFN
- Alloanti-Fya titre >1000
 - High risk HDFN
- Alloanti-Jka titre Neat
 - Unlikely to contribute to HDFN

What about the baby?

- HTL had ordered 2 x O rr K- Fy(a-) exchange units for 28/08/18
- Must meet specific criteria which are?
- One donor specifically bled on 25/08/18
- HTL rang to ask if anti-Jka has been produced, so I asked have they considered blood for baby?
- "We were just gonna wait and see what you found"
- "IOL today"
- Told them NOT to induce as no blood available
- Pt only 32/40
- Pulse search by HSD: no units available





Plasma reduced with haematocrit of 0.5–0.6 (NHSBT 0.5–0.55) to reduce the risk of post-exchange polycythaemia

In CPD anticoagulant Less than 5 days old Irradiated (essential if previous IUT) CMV negative Sickle screen negative Usually produced as group O (with low-titre haemolysins)

RhD negative (or RhD identical with neonate) and Kell negative

Red cell antigen negative for maternal alloantibodies

IAT crossmatch compatible with maternal plasma

https://www.transfusionguidelines.org/transfusion-handbook/10effective-transfusion-in-paediatric-practice/10-2-neonataltransfusion



NHS Blood and Transplant



10:00-16:00 28/08/18

- Several telephone calls
- Manchester to source units or suitable donor
- HTL consultant
- NHSBT consultant



- HTL
- telling them not to induce
- that there was no blood available for baby
- being told I best find some as they had induced mother!!!
- explaining how its manufactured
- being told it was a matter of life and death – why do you think I said not to induce mother?

We got there in the end!

- Pulse search by RCI sourced 3 Orr K- Fy(a-) Jk(a-) exchange units
- 1 Sheffield, 2 Filton but all expired 30/08/18 at 23:59
- Sent up via Blue Light
- Needed phenotyping confirmed (on call)
- HTL took all 3 for exchange
- C-section 30/08/18
- Baby exchanged and doing well
- Anti-Fya and Jka in baby's eluate







Conclusion

- 3 investigations, 3 different patient types
 - -ESRF
 - -Trauma
 - -Antenatal







- All required further work but not all on the bench
- Always look at the bigger picture: investigating the patient, not just the sample!
- All 3 involved BMS Empowerment
 - Do you need that many units?
 - Can we take over?
 - What about the baby?
- All 3 made me say "Are Fy-ing Kidd-ing me?"

