Serological Case Studies

Interactive !

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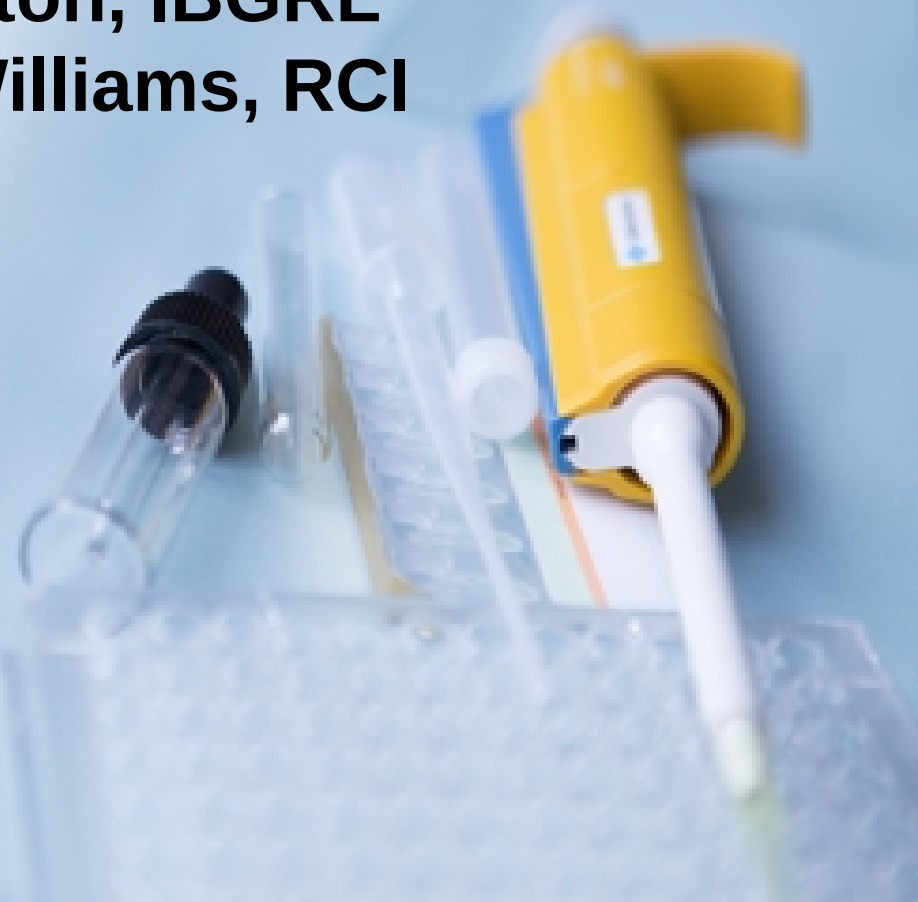


BBTS, Harrogate 2014

NHS Blood and Transplant

Case Study 2

Nicole

Case Study 2

Referral

- 36yr old pregnant female
- Gp A R_1r K–
- DAT–
- Anti-D present
- Additional weaker reactions with all D– cells
- ? Antibody to HFA present

Case Study 2

Referral

- 36yr old pregnant female
- Gp A R_1r K–
- DAT–
- Anti-D present
- Additional weaker reactions with all D– cells
- ? Antibody to HFA present

Any ideas/comments about this information?

Case Study 2: First Panel

	Rh	M	N	S	s	P1	Lu^a	K	k	Kp^a	Le^a	Le^b	Fy^a	Fy^b	Jk^a	Jk^b	Unt IAT	Pap IAT
1	$R_1^wR_1$	0	+	0	+	3	0	0	+	0	+	0	+	0	0	+	4	5
2	R_1R_1	+	0	+	0	1	0	+	+	0	0	+	0	+	+	0	4	5
3	R_2R_2	+	0	0	+	0	0	0	+	0	+	0	0	+	0	+	4	5
4	r'r	0	+	0	+	0	0	0	+	0	0	+	0	+	0	+	2	3
5	r''r	0	+	+	0	2	0	0	+	0	0	+	+	0	+	0	2	3
6	rr	+	0	0	+	2	0	+	0	0	0	+	0	+	0	+	2	3
7	rr	0	+	0	+	0	0	+	+	0	+	0	+	0	0	+	2	3
8	rr	0	+	0	+	0	0	0	+	+	0	+	+	0	+	0	2	3
9	rr	0	+	0	+	4	+	0	+	0	0	0	0	+	+	0	2	3
10	rr	+	0	+	0	2	0	0	+	0	+	0	+	0	0	+	2	3
Auto																	0	0

What could be present?

1) Mixture of antibodies
2) Single specificity
3) Alloanti-D
4) Could be all of the above

Case Study 2: First Panel

	Rh	M	N	S	s	P1	Lu^a	K	k	Kp^a	Le^a	Le^b	Fy^a	Fy^b	Jk^a	Jk^b	Unt IAT	Pap IAT
1	$R_1^wR_1$	0	+	0	+	3	0	0	+	0	+	0	+	0	0	+	4	5
2	R_1R_1	+	0	+	0	1	0	+	+	0	0	+	0	+	+	0	4	5
3	R_2R_2	+	0	0	+	0	0	0	+	0	+	0	0	+	0	+	4	5
4	r'r	0	+	0	+	0	0	0	+	0	0	+	0	+	0	+	2	3
5	r''r	0	+	+	0	2	0	0	+	0	0	+	+	0	+	0	2	3
6	rr	+	0	0	+	2	0	+	0	0	0	+	0	+	0	+	2	3
7	rr	0	+	0	+	0	0	+	+	0	+	0	+	0	0	+	2	3
8	rr	0	+	0	+	0	0	0	+	+	0	+	+	0	+	0	2	3
9	rr	0	+	0	+	4	+	0	+	0	0	0	0	+	+	0	2	3
10	rr	+	0	+	0	2	0	0	+	0	+	0	+	0	0	+	2	3
Auto																	0	0

What would be useful to do next?

Case Study 2

What would be the most useful next step?

1) Extract patient's DNA and sequence *RHD*
2) Investigate patient's D antigen using extended panel of anti-D reagents
3) Test patient's plasma against D+ and D- cord cells
4) Type the patient's cells for High Frequency Antigens
5) Test the patient's plasma with cells treated with a range of enzymes/chemicals
6) Something else

Case Study 2

What we did next

- **Test D+ and D– cord cells**

Cells	IAT	
	Unt	Pap
D+ cord cells	**4**	**5**
D– cord cells	**4**	**5**
D+ adult cells	**4**	**5**
D– adult cells	**2**	**3**
Auto cont.	**0**	**0**

Case Study 2

Cells	IAT	
	Unt	Pap
D+ cord cells	**4**	**5**
D– cord cells	**4**	**5**
D+ adult cells	**4**	**5**
D– adult cells	**2**	**3**
Auto cont.	**0**	**0**

What could these results indicate?

Case Study 2

What would you do next?

1) Match rare null cells against the patient's plasma
2) Type the patient's cells for LW^a
3) Autoabsorption
4) Differential alloabsorptions
5) Test the patient's plasma with cells treated with a range of enzymes/chemicals
6) Something else

Case Study 2

What we did next

1) Match rare null cells against the patient's plasma
2) **Type the patient's cells for LW^a**
3) Autoabsorption
4) Differential alloabsorptions
5) Test the patient's plasma with cells treated with a range of enzymes/chemicals
6) Something else

LW(a-)

If anti-LW^a not available.....

Enzymes & Chemical Modification Study

	Papain	Trypsin	Chymotrypsin	Pronase	AET
Knops	+/-	-	-	+	-
Ch/Rg	-	-	-	-	+
Cromer	+	+	-	+	(+)
Vel	+	+	+	+	+
Rh	+	+	+	+	+
Kell	+	+	+	+	-
JMH	-	-	-	-	-
LW	+	+	+	-	-

Examples of effect of enzyme treatment/chemical modification

Case Study 2

LW(a-)

Given this result what could be present in the patient's plasma?

1) Anti-LW^a
2) Anti-LW^{ab}
3) Anti-D + ?
4) All of the above

Case Study 2

LW(a-)

Given this result what could be present in the patient's plasma?

1) Anti-LW^a
2) Anti-LW^{ab}
3) Anti-D + ?
4) **All of the above**

Why?

Case Study 2

Anti-LW^{a}

- **Patient cells could have the rare LW(a-b+) phenotype and therefore she can make anti-LW^{a}**

Anti-LW^{ab}

- **Patient's LW^{a} antigen could be transiently suppressed due to pregnancy and she could have subsequently made anti-LW^{ab}** (often see a very weak pos DAT but not always)

Anti-D + ?

- **Patient could be D variant with alloanti-D + other antibody/ies**

Case Study 2 - Outcome

Patient's cells: LW(a-b+)

Patient's plasma: anti-LW^a
Anti-D was excluded by compatible D+ LW(a-) cells

Which of the following cells would be compatible with her plasma?

1) **LW(a-b+), LW(a-b-), Rh_{null}, –D–/–D–**
2) **LW(a-b+), LW(a-b-), –D–/–D–**
3) **LW(a-b+), LW(a-b-), Rh_{null}**
4) **Only LW(a-b+) and LW(a-b-)**

Case Study 2

Learning Points

- LW and D antigens have a phenotypic relationship.
  Adults: D– cells have lower expression of LW antigens than D+ cells
  Cord cells: LW strongly expressed in both D– and D+ cells
- LW antigens can be transiently suppressed during pregnancy and patient can make anti-LW^{ab}
  (often see a very weak pos DAT)
- LW(a-b+) individuals are genetically LW(a-) and can make anti-LW^{a}
- Testing pronase treated D+ cells is a useful way of distinguishing between anti-D and anti-LW

Case Study 3

Martin

NHS Blood and Transplant

Case

- 10 year old Philipino boy
- ? Aplastic Anaemia
- Hb <50g/L
- ‘pan-reactive’
- Transfusion history unknown
- O R_1R_1 (c-, E-) K-

Panel results

NHS Blood and Transplant

NHSBT Reagents **Panel 1** **Antibody Investigation Worksheet**

Sample Name		Requestor		Database Ref No.		Tested by	
Date of Birth		Hosp no		Sample No.		Date Tested	

Product	Lot No.	Product	Lot No.	Product	Lot No.	Product	Lot No.
Panel in Alsevers	R144 3306	Panel in CellStab	R143 3306	Panel in CellMedia	R163 3306	Panel in LISP	R146 3306
Panel Papainised in Alsevers	R154 3306	Panel Papainised in CellStab	R153 3306	Panel Papanised in CellMedia	R173 3306	EXPIRY DATE: 2014.09.18	

	Rh	M	N	S	s	P1	Lu^a	K	k	Kp^a	Le^a	Le^b	Fy^a	Fy^b	Jk^a	Jk^b	Other	IAT		Enz/IAT			
1	$R_1^wR_1$	0	+	0	+	3	0	0	+	0	+	0	+	0	0	+		4		4			
2	R_1R_1	+	0	+	0	1	0	+	+	0	0	+	0	+	+	0		4		4			
3	R_2R_2	+	0	0	+	0	0	0	+	0	+	0	0	+	0	+		4		4			
4	r'r	0	+	0	+	0	0	0	+	0	0	+	0	+	0	+		4		4			
5	r''r	0	+	+	0	2	0	0	+	0	0	+	+	0	+	0		4		4			
6	rr	+	0	0	+	2	0	+	0	0	0	+	0	+	0	+		4		4			
7	rr	0	+	0	+	0	0	+	+	0	+	0	+	0	0	+		4		4			
8	rr	0	+	0	+	0	0	0	+	+	0	+	+	0	+	0		4		4			
9	rr	0	+	0	+	4	+	0	+	0	0	0	0	+	+	0		4		4			
10	rr	+	0	+	0	2	0	0	+	0	+	0	+	0	0	+		4		4			
Auto																		4		4			

Antibody Titre

Dilution:														Cell Id
Anti-														
Archive														
Anti														
Archive														

DAT Batch No

PS	IgG	IgA	IgM	C3c	C3d	Ctl
	4	0	0	0	4	0

Group

Pheno

Conclusion

Entered into Database by:……..Authorised by……

Reagent								
Batch No's								

Pipette batch No's

NHS Blood and Transplant

What to do next?

Adsorption

Elution

More panel cells

Neutralisation

Adsorption

NHS Blood and Transplant

NHSBT Reagents **Panel 1** **Antibody Investigation Worksheet**

Sample Name		**Requestor**		**Database Ref No.**		**Tested by**	
Date of Birth		**Hosp no**		**Sample No.**		**Date Tested**	

Product	**Lot No.**	**Product**	**Lot No.**	**Product**	**Lot No.**	**Product**	**Lot No.**
Panel in Alsevers	**R144 3306**	**Panel in CellStab**	**R143 3306**	**Panel in CellMedia**	**R163 3306**	**Panel in LISP**	**R146 3306**
Panel Papainised in Alsevers	**R154 3306**	**Panel Papainised in CellStab**	**R153 3306**	**Panel Papanised in CellMedia**	**R173 3306**	**EXPIRY DATE: 2014.09.18**	

	Rh	M	N	S	s	P1	Lu^a	K	k	Kp^a	Le^a	Le^b	Fy^a	Fy^b	Jk^a	Jk^b	Other	rr		R1R1			
1	$R_1^wR_1$	0	+	0	+	3	0	0	+	0	+	0	+	0	0	+		4		0			
2	R_1R_1	+	0	+	0	1	0	+	+	0	0	+	0	+	+	0		0		Wk			
3	R_2R_2	+	0	0	+	0	0	0	+	0	+	0	0	+	0	+		4		0			
4	r'r	0	+	0	+	0	0	0	+	0	0	+	0	+	0	+		4		0			
5	r''r	0	+	+	0	2	0	0	+	0	0	+	+	0	+	0		0		0			
6	rr	+	0	0	+	2	0	+	0	0	0	+	0	+	0	+		4		0			
7	rr	0	+	0	+	0	0	+	+	0	+	0	+	0	0	+		4		0			
8	rr	0	+	0	+	0	0	0	+	+	0	+	+	0	+	0		0		Wk			
9	rr	0	+	0	+	4	+	0	+	0	0	0	0	+	+	0		0		0			
10	rr	+	0	+	0	2	0	0	+	0	+	0	+	0	0	+		4		0			
Auto																							

Antibody Titre

Dilution:													Cell Id
Anti-													
Archive													
Anti													
Archive													

Group **Pheno**

Conclusion

DAT Batch No

PS	IgG	IgA	IgM	C3c	C3d	Ctl
	4	0	0	0	4	0

Entered into Database by:……..Authorised by……

Reagent								
Batch No's								

Pipette batch No's

NHS Blood and Transplant

Results

- Adsorbed plasma
- Anti-Jk^b + weak reactions

NHS Blood and Transplant

Next?

Crossmatch and issue blood

Phenotyping / genotype

Elution

Neutralisation

NHS Blood and Transplant

Serological Typings

	D	C	c	E	e	C^w	M	N	S	s	K	k	Fy^a	Fy^b	Jk^a	Jk^b
Phenotype	+	+	-	-	+	-	+	+	-	n/t	-	+	n/t	n/t	-	-

NHS Blood and Transplant

IBGRL report

- Strongly positive DAT
- Apparent anti-Jk3 reacting at 18ºC by direct agglutination
- Unable to adsorb to see if anti-Jk3 IAT reactive
- Genotype.....

NHS Blood and Transplant

Typings

	D	C	c	E	e	C^w	M	N	S	s	K	k	Fy^a	Fy^b	Jk^a	Jk^b
Phenotype	+	+	-	-	+	-	+	+	-	n/t	-	+	n/t	n/t	-	-
Genotype	+	+	-	-	+	-	+	+	-	+	-	+	+	+	+	-

NHS Blood and Transplant

Urgent – Which blood to Issue?

Jk(b-)

c-, E- Jk(b-)

c-, E-, K-, Jk(b-)

Look for Jk3- units

NHS Blood and Transplant

Up to Date

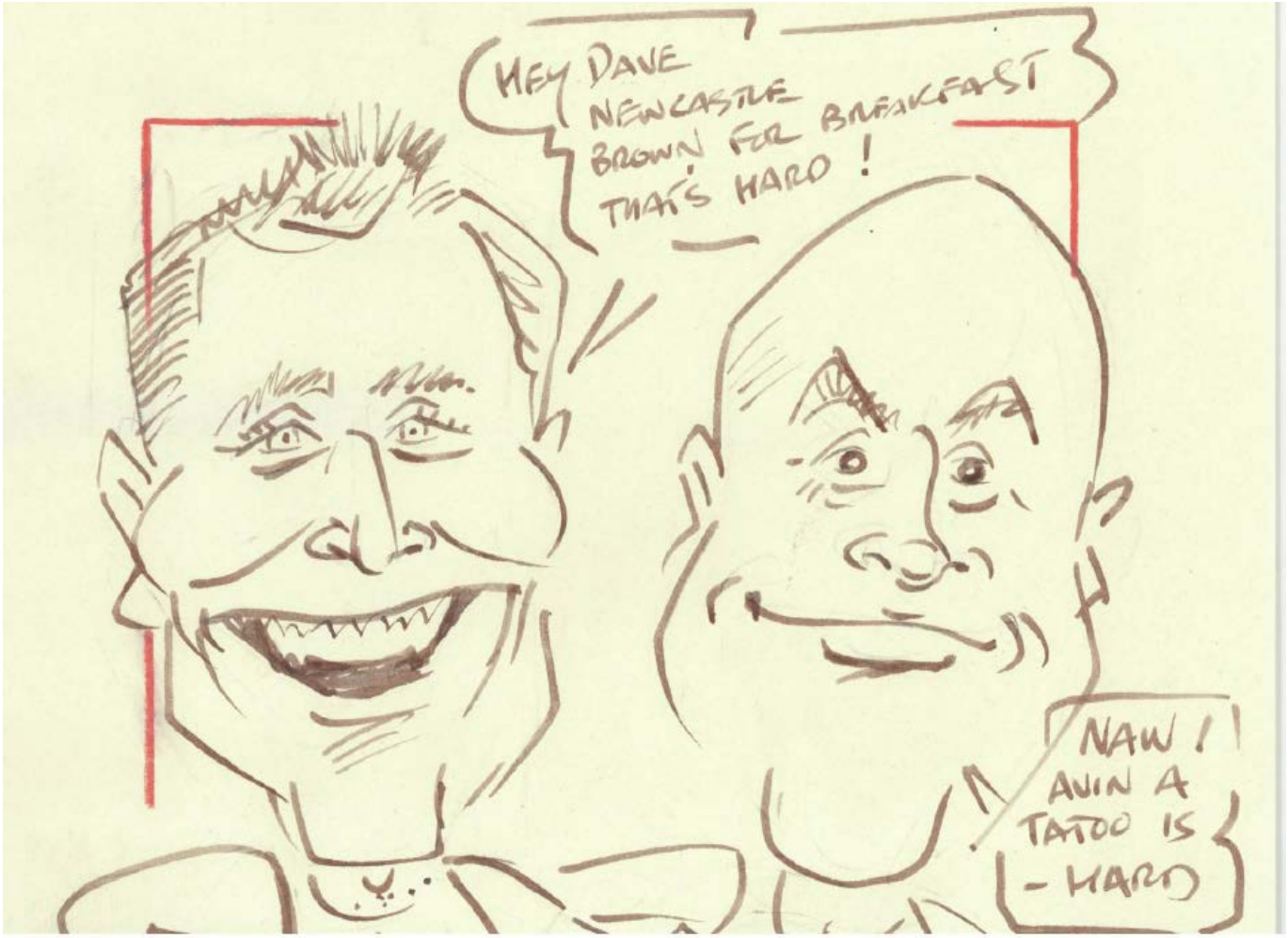
- Presented again this year
- α Thalassaemia
- No alloantibodies in adsorbed plasma
- c-, E-, K-, Jk(b-) blood - no adverse reaction
- Samples for sequencing to IBGRL
- ? Suppression of Jk^a/Jk3 antigens

NHS Blood and Transplant

Learning points

- Geno / Pheno clash
- Value of high quality IgM monoclonals
- Is phenotype always the most important?

NHS
Blood and Transplant



Case Study 4

Martin

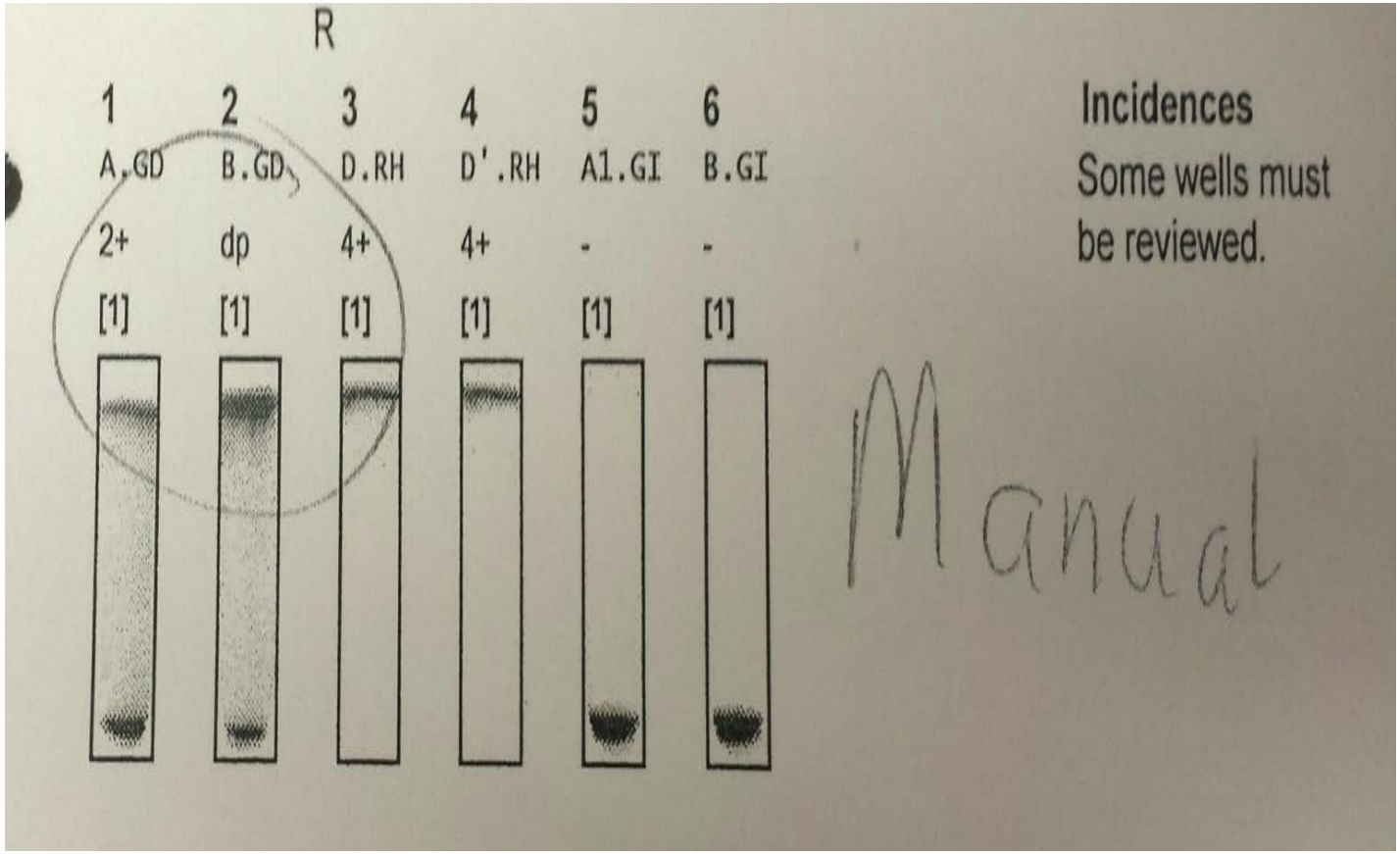
NHS Blood and Transplant

Case

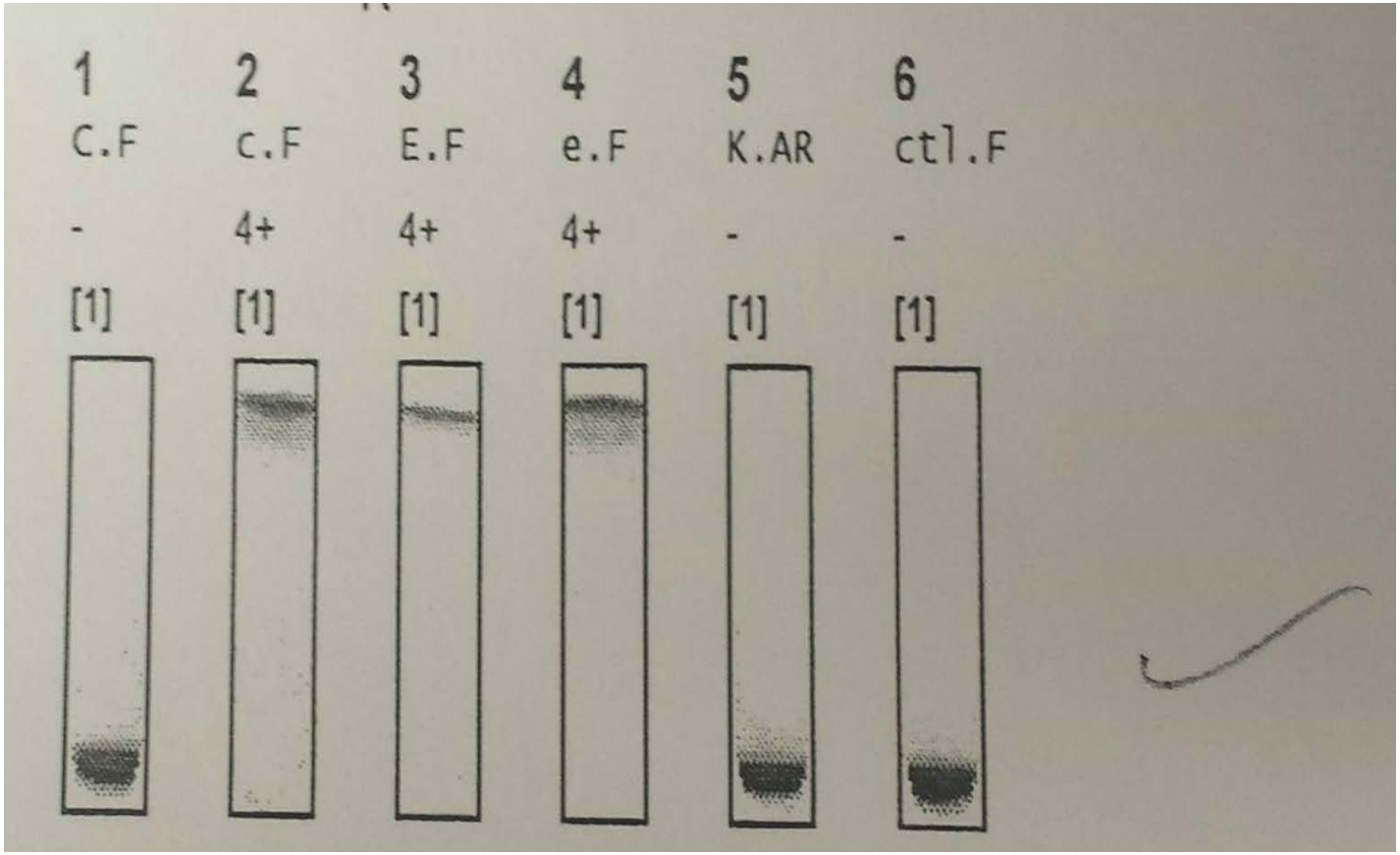
- 62 year old female
- Pericarditis
- ‘? ABO group’
- ‘No known transfusions’
- Negative antibody screen

NHS Blood and Transplant

ABO group (BioRad automated)



NHS
Blood and Transplant



NHS Blood and Transplant

Urgent – Which blood to Issue

O+

A+

B+

AB+

NHS Blood and Transplant

Cause?

- Out of group transfusion / transplant
- Weak subgroup
- Chimerism
- Haematological malignancy

NHS Blood and Transplant

Manual ABO (tubes)

- Red cells;
- v anti-A – 2+MF
- v anti-B – 4+MF
- v anti-A,B – 4+MF
- v anti-A_1 – negative

- Plasma;
- v A_1 cells – negative
- v B cells - negative

NHS Blood and Transplant

PCR-SSP Genotype

Reaction No.	1	2	3	4	5	6#	7	8		
PCR Product (Size in bp)	134	133	194	193	195	194	170	170		
Specificity	O^1	non O^1	O^2	non O^2	B	non B	A^2	non A^2		
Examples for results:									*Genotype	*Phenotype
Position 1–positive (O^1)	+	-	-	+	-	+	-	+	O^1O^1	O
	+	+	+	+	-	+	-	+	O^1O^2	O
	+	+	-	+	+	+	-	+	O^1B	B
	+	+	-	+	-	+	-	+	O^1A	A
	+	+	-	+	-	+	+	+	O^1A^2	A_2
Position 3-positive (O^2)	-	+	+	-	-	+	-	+	O^2O^2	O
	-	+	+	-	+	+	-	+	$**O^2B$	B
	-	+	+	+	-	+	-	+	O^2A	A
	-	+	+	+	-	+	+	+	O^2A^2	A_2
Position 5-positive (B)	-	+	-	-	+	-	-	+	**BB	B
	-	+	-	+	+	+	-	+	AB	AB
	-	+	-	+	+	+	+	+	A^2B	A_2B
Position 2/4/6-positive (non $O^1/O^2/B$)	-	+	-	+	-	+	-	+	AA	A
	-	+	-	+	-	+	+	+	AA^2	A
	-	+	-	+	-	+	+	-	A^2A^2	A_2
Results:										
[redacted]	—	+	—	+	+	+	—	+	AB.	

* The specificity in table does not consider rare blood groups. You will find closer explanations about this in the product insert.

** An exception to the general reaction pattern is represented by the non o^2 reaction, in case of a o^2o^2, BB or BO^2

NHS Blood and Transplant

What else can you do?

Report as ?? and recommend Gp O

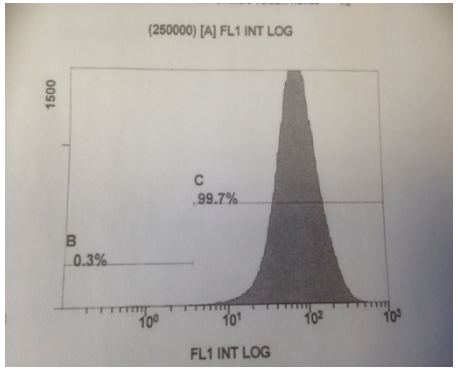
Flow cytometry

Do a ‘better’ ABO genotype

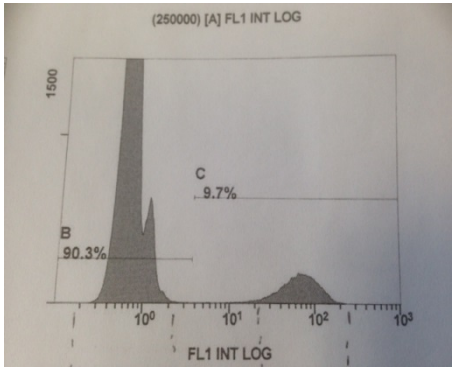
Lectin panel

Flow Cytometry

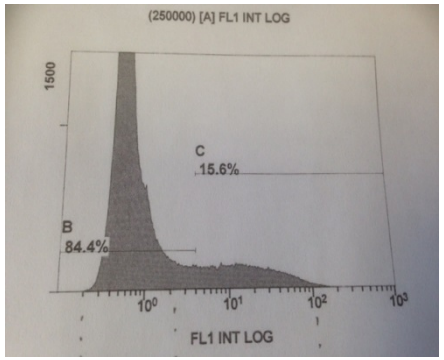
NHS Blood and Transplant



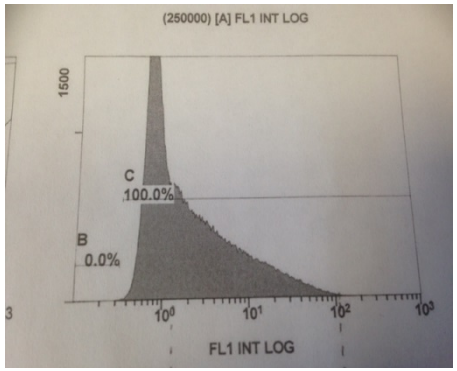
Group A control



Typical dual population



Patient v anti-A



Patient v anti-B

NHS Blood and Transplant

Cause

1) Out of group transfusion / transplant
2) Weak subgroup
3) Chimerism
4) Haematological malignancy

NHS Blood and Transplant

Urgent

Blood to select

O+

A+

B+

AB+

NHS
Blood and Transplant

NHS
Blood and Transplant

Case Study 5

Nicole

Case Study 5

Referral

- **45yr old male, knee surgery scheduled**
- **ABO group cannot be determined**

Forward group: looks like group AB with slight weakening of A antigen

Reverse group: anti-A present (bit weaker than normal)

Case Study 5

Results

Cells	Anti-A	Anti-A	Anti-A	Anti-A	Anti-A1	Anti-B	Anti-A,B	Anti-H	Patient's plasma
Patient	3+	2+	3+	2+	0	4+	4+	4+	0
A_1	4+	4+	4+	4+	4+	0	4+	3+	3+
A_2	4+	4+	4+	4+	0	0	4+	5+	2+
B	0	0	0	0	0	4+	4+	4+	0
O	0	0	0	0	0	0	0	5+	0

Reminder....

H expression

O, A_w, B_w
A_2
B
A_2B
A_1
A_1B
O_h (H-)

Case Study 5

What <u>needs</u> to be done next?

1) *ABO* genotype
2) Nothing, can report now
3) Test cells with a larger anti-A panel
4) Determine the possible A subgroup
5) Something else

Why?

Case Study 5

How would you report?

1) Group AB
2) Group A_xB with anti-A
3) Group $A_{weak}B$ with anti-A
4) Group B
5) ABO group could not be determined

Case Study 5

Learning Points

- Allelic enhancement is an effect of gene interaction, resulting in enhanced expesssion of weak *A* or *B* alleles in *AB* heterozygotes

  eg. $A^x B$ individuals may appear to have A_2B phenotype
- It is not neccessary to determine A and B subgroups definitively, to provide information to aid clinical decisions
- *ABO* genotyping by allelic discrimination assay cannot predict A and B subgroups (*ABO* sequencing required).

Case Study 6

Nicole

Case Study 6

Referral

- 82yr old female, hip replacement scheduled
- Gp O R_1r K–, Fy(a-b+), M+N+S-s+, Jk(a+b+)
- All cells incompatible by IAT, no reactivity with papain treated cells
- Weakly positive DAT [IgG (+)]
- ? Antibody to HFA

Case Study 6: First Panel

	Rh	M	N	S	s	P1	Lu^a	K	k	Kp^a	Le^a	Le^b	Fy^a	Fy^b	Jk^a	Jk^b	Unt IAT	Pap IAT
1	$R_1^wR_1$	0	+	0	+	3	0	0	+	0	+	0	+	0	0	+	3	0
2	R_1R_1	+	0	+	0	1	0	+	+	0	0	+	0	+	+	0	3	0
3	R_2R_2	+	0	0	+	0	0	0	+	0	+	0	0	+	0	+	3	0
4	r'r	0	+	0	+	0	0	0	+	0	0	+	0	+	0	+	3	0
5	r''r	0	+	+	0	2	0	0	+	0	0	+	+	0	+	0	3	0
6	rr	+	0	0	+	2	0	+	0	0	0	+	0	+	0	+	3	0
7	rr	0	+	0	+	0	0	+	+	0	+	0	+	0	0	+	3	0
8	rr	0	+	0	+	0	0	0	+	+	0	+	+	0	+	0	3	0
9	rr	0	+	0	+	4	+	0	+	0	0	0	0	+	+	0	3	0
10	rr	+	0	+	0	2	0	0	+	0	+	0	+	0	0	+	3	0
Auto																	(+)	0

Any ideas based on case history and initial panel?

Case Study 6

What would you do next?

1) Type the patient's cells for papain sensitive HFAs
2) Match cells lacking papain sensitive HFAs, against the patient's plasma
3) Test the patient's plasma with cells treated with enzymes/chemicals
4) Make an eluate from the patient's cells
5) Something else

Case Study 6

What we did next

- **Type the patient's cells for papain sensitive HFAs**

	Anti-Yt^{a}	Anti-JMH	Anti-Ge2	Anti-In^{b}	AB serum control
Patient	**4**	**(+)**	**4**	**4**	**(+)**
Pos Control	**4**	**4**	**4**	**4**	**0**

Case Study 6

	Rh	M	N	S	s	P1	Lu^a	K	k	Kp^a	Le^a	Le^b	Fy^a	Fy^b	Jk^a	Jk^b	Unt IAT	Pap IAT
1	$R_1^wR_1$	0	+	0	+	3	0	0	+	0	+	0	+	0	0	+	3	0
2	R_1R_1	+	0	+	0	1	0	+	+	0	0	+	0	+	+	0	3	0
3	R_2R_2	+	0	0	+	0	0	0	+	0	+	0	0	+	0	+	3	0
4	r'r	0	+	0	+	0	0	0	+	0	0	+	0	+	0	+	3	0
5	r''r	0	+	+	0	2	0	0	+	0	0	+	+	0	+	0	3	0
6	rr	+	0	0	+	2	0	+	0	0	0	+	0	+	0	+	3	0
7	rr	0	+	0	+	0	0	+	+	0	+	0	+	0	0	+	3	0
8	rr	0	+	0	+	0	0	0	+	+	0	+	+	0	+	0	3	0
9	rr	0	+	0	+	4	+	0	+	0	0	0	0	+	+	0	3	0
10	rr	+	0	+	0	2	0	0	+	0	+	0	+	0	0	+	3	0
Auto																	(+)	0

What is most likely present?

1) Anti-Yt^a
2) Anti-Ge2
3) Anti-JMH
4) Anti-In^b

Case Study 6

What would you do next?

1) **Nothing, case is solved**
2) **Match JMH– cells against the patient's plasma**
3) **Test the patient's plasma with cells treated with a range of enzymes/chemicals**
4) **Carry out *SEMA7A* sequencing**
5) **Something else**

Case Study 6

What we did next

- **Matched JMH– cells against the patient's plasma**

Cells	IAT	
	Patient Plasma	AB Serum
JMH-	**0**	**0**
JMH-	**w**	**w**
JMH-	**0**	**0**
Pos cont.	**4**	**0**
Auto cont.	**(+)**	**(+)**

Case Study 6 - Outcome

Patient's plasma: anti-JMH
Anti-Fy^a, -S were excluded by compatible Fy(a+b–) S+s– JMH– cells

In this case anti-JMH is most likely an

1) **Autoantibody**
2) **Alloantibody**

Case Study 6

Learning Points

- Autoanti-JMH is often found in the plasma of elderly individuals with an acquired JMH-/wk phenotype and the DAT is often weakly positive (but not always!)
- Individuals with an acquired JMH-/wk phenotype will have a normal *SEMA7A* gene sequence
- JMH-variant individuals have mutated *SEMA7A* genes and can make alloanti-JMH (DAT will be negative)
- JMH variant cells are positive with anti-JMH from individuals with the acquired JMH-/wk phenotype but negative with anti-JMH from individuals with the same JMH variant

NHS Blood and Transplant

Thank You For Contributing!