

# **Case studies - How does red cell genotyping benefit patients?**

**Tracey Tomlinson**

Head of Laboratory

RCI Colindale

# Background

- “ Genotyping introduced in RCI late 2014
- “ Fluogene RBC platform
- “ Self-contained system
- “ PCR-SSP method
- “ Results within 90 mins



# vERYfy kit

**Rhesus:**RHD: exons 1, 5, 10, RHDpsi; RHCE: C, C<sup>w</sup>, c, E, e**Kell:**KEL1(K), KEL2(k), KEL3(Kp<sup>a</sup>), KEL4(Kp<sup>b</sup>)**Dombrock:**

DO1(A), DO2(B)

**vERYfy****Kidd**

JK1(A), JK2(B)

**MNS:**

MNS1(M), MNS2(N), MNS3(S), MNS4(s)

**Duffy:**FY1(A), FY2(B), FYnull(A-, B-), FYX(Fy<sup>bweak</sup>)



*Blood and Transplant*

# Case Studies



# Case 1

” Female, DOB 23/02/73

” Referring lab's findings

- . A pos Ror
- . DAT-
- . Antibody screen positive by BioRad IAT, panreactive antibody
- . Diagnosis Pregnant 32/40
- . Transfusions . yes
- . Anti-D Ig given . no
- . Note on bottom of request form . Sorry for small sample

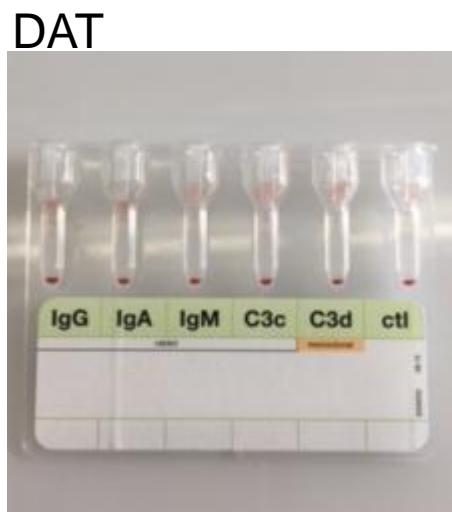
# Case 1

*Blood and Transplant*

Cell	Rh	Rh				MNSs				P1	Lu		Kell		Le		Fy		Jk				
		D	C	E	c	e	C <sup>w</sup>	M	N	S	s	a	b	K	k	Kp <sup>a</sup>	a	b	a	b	a	b	
1	R <sub>1</sub> <sup>w</sup> R <sub>1</sub>	+	+	0	0	+	+	+	0	+	0	0	0	+	0	+	0	+	0	+	0	+	0
2	R <sub>1</sub> R <sub>1</sub>	+	+	0	0	+	0	+	0	0	+	2	0	+	+	+	0	0	+	+	0	0	+
3	R <sub>2</sub> R <sub>2</sub>	+	0	+	+	0	0	0	+	0	+	3	0	+	0	+	0	0	+	0	+	0	+
4	r'r	0	+	0	+	+	0	0	+	+	0	0	+	+	0	+	0	+	0	0	0	0	+
5	r"r	0	0	+	+	+	0	0	+	+	+	3	0	+	0	+	0	0	0	+	+	0	+
6	rr	0	0	0	+	+	0	+	0	+	0	1	0	+	+	0	0	+	0	0	+	+	+
7	rr	0	0	0	+	+	0	0	+	0	+	0	0	+	+	+	0	0	0	+	0	+	0
8	rr	0	0	0	+	+	0	+	+	0	+	0	0	+	0	+	+	0	+	0	0	0	+
9	rr	0	0	0	+	+	0	+	0	+	+	1	0	+	0	+	+	0	+	+	0	+	0
10	rr	0	0	0	+	+	0	+	0	+	0	4	0	+	+	+	0	0	+	0	+	0	+
Auto																							

Results		
IAT	Enz/IAT	
3	3	
3	3	
3	3	
3	3	
3	3	
3	3	
3	3	
3	3	
3	3	
3	3	
3	3	
3	3	
0	N/T	

# Case 1



- “ Phenotype?
- “ Previous transfusions?

## Case 1

- ” Repeat samples request
- ” Patient history
  - . Patient just moved to UK
  - . 7 previous pregnancies

# Case 1

" 4 weeks later



# Case 1



# *Blood and Transplant*

# Case 1

“ Genotype result

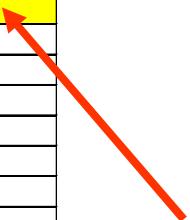
C	c	E	e	K	k	M	N	S	s	Fya	Fyb	Jka	Jkb	Doa	Dob
-	+	-	+	-	+	+	+	-	-	-	-	+	-	+	+

# Case 1

Cell		Results	
		IAT	
1	Fy(a-b-)	3	
2	Fy(a-b-)	3	
3	U-	0	
4	U-	0	
5	Vel-	3	
6	Vel-	3	
7	Lub-	3	
8	Lub-	3	
9	Kpb-	3	
10	Kpb-	3	

# Case 1

Cell		Results	
		IAT	
1	Fy(a-b-)	3	
2	Fy(a-b-)	3	
3	U-	0	
4	U-	0	
5	Vel-	3	
6	Vel-	3	
7	Lub-	3	
8	Lub-	3	
9	Kpb-	3	
10	Kpb-	3	



**Anti-U with titre  
1/32**

# Case 1

” Anti-U

- . First described in 1953
- . Antigen frequency
  - . Caucasians 99.9%
  - . Blacks 99%
- . Clinically significant, can cause HDN and HTR

## Case 2

” Female, DOB 25/03/86

” Referring lab's findings

- . A pos Ror
- . DAT-
- . Antibody screen positive by Capture and IAT. Anti-e
- . Diagnosis Pregnant
- . No prev transfusions & no Anti-D Ig given

## Case 2

### RCI results

- . Group A
- . Discrepant D type
  - . Alba kit confirmed partial D
- . Panel weak anti-e
- . BioRad automated RHK confirmed C-c+E-e+K-
- . DAT negative

# Case 2

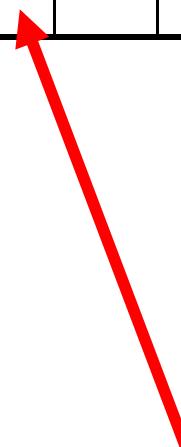
“ Genotype result

C	c	E	e	K	k	M	N	S	s	Fya	Fyb	Jka	Jkb	Doa	Dob
-	+	-		-	+	+	+	-	+	-	-	+	-	-	+

## Case 2

“ Genotype result

C	c	E	e	K	k	M	N	S	s	Fya	Fyb	Jka	Jkb	Doa	Dob
-	+	-		-	+	+	+	-	+	-	-	+	-	-	+



No result



## Case 2

- “ Referred to IBGRL
- “ Genotyping
  - . Confirmed patient as partial D category DAR
  - . Confirmed patient as e var

- “ Serology
  - . Confirmed presence of anti-hrs with titre 1/8

## Case 2

### ” Anti-hrs

- . First reported in 1960
- . Only found in patients of African origin
- . Not generally considered to be clinically significant, but data to support this is limited
- . Anti-hrs resembles anti-e and e-like antibodies are often incorrectly classified as anti-hrs
- . There are no hrs- donors in the UK

## Case 2

- “ DAR
  - . Required prophylactic anti-D
  - . The DAR phenotype was characterised by complete loss of at least 9 of 37 Rh D epitopes
  - . Frequency 1.5% Africans will have the DAR phenotype
  - . 1999 study
    - . 1 out of 4 DAR individuals transfused D + blood developed anti-D

## Case 3

- ” Male, DOB 23/02/33
- ” Referring lab\$ findings
  - . A pos
  - . DAT +
  - . Antibody screen positive by BioRad IAT, panreactive antibody
  - . Transfusions . yes
  - . For surgery . cancelled 2 times already!

# Case 3

*Blood and Transplant*

Cell	Rh	Rh						MNSs				P1	Lu		Kell			Le		Fy		Jk	
		D	C	E	c	e	C <sup>w</sup>	M	N	S	s		a	b	K	k	Kp <sup>a</sup>	a	b	a	b	a	b
1	R <sub>1</sub> <sup>w</sup> R <sub>1</sub>	+	+	0	0	+	+	+	0	+	0	0	0	+	0	+	0	+	0	+	0	+	0
2	R <sub>1</sub> R <sub>1</sub>	+	+	0	0	+	0	+	0	0	+	2	0	+	+	+	0	0	+	+	0	0	+
3	R <sub>2</sub> R <sub>2</sub>	+	0	+	+	0	0	0	+	0	+	3	0	+	0	+	0	0	0	+	0	+	0
4	r'r	0	+	0	+	+	0	0	+	+	0	0	+	+	0	+	0	0	+	0	0	0	+
5	r"r	0	0	+	+	+	0	0	+	+	+	3	0	+	0	+	0	0	0	+	+	0	+
6	rr	0	0	0	+	+	0	+	0	+	0	1	0	+	+	0	0	0	+	0	0	+	+
7	rr	0	0	0	+	+	0	0	+	0	+	0	0	+	+	+	0	0	0	+	0	+	0
8	rr	0	0	0	+	+	0	+	+	0	+	0	0	+	0	+	+	0	0	+	0	0	+
9	rr	0	0	0	+	+	0	+	0	+	+	1	0	+	0	+	+	0	0	+	+	0	+
10	rr	0	0	0	+	+	0	+	0	+	0	4	0	+	+	+	0	0	0	+	0	+	0
Auto																							

Results			
IAT	Enz/IAT		
5	5		
5	5		
5	5		
5	5		
5	5		
5	5		
5	5		
5	5		
5	5		
5	5		
5	5		
5	N/T		

# Case 3

“ Group

Anti -A	Anti -B	Anti -D	Anti -D	A1	B
+	+	+	+	+	+

“ DAT

IgG	IgA	IgM	C3c	C3d	Ctl
+	+	+	+	+	+

# Case 3

Warm washed cells

“ Group

Anti -A	Anti -B	Anti -D	Anti -D	A1	B
+	0	+	+	+	+

“ DAT

IgG	IgA	IgM	C3c	C3d	Ctl
+	0	+	0	+	0

# Case 3

## Adsorption studies 3 x warm and cold



# Case 3

# *Blood and Transplant*

## Adsorption studies 6 x warm and cold

# Case 3

“ Genotype result

C	c	E	e	K	k	M	N	S	s	Fya	Fyb	Jka	Jkb	Doa	Dob
+	+	-	+	-	+	+	+	-	+	+	+	+	-	-	+

## Case 3

- “ 2 units of A + E- K- S- Jkb- & 1 unit of O + E- K- S-  
Jkb- least incompatible units issued
- “ 2 units transfused without incident

## Case 3

" 6 days later



# Case 3

*Blood and Transplant*

Adsorption studies 6 x warm and cold

Cell	Rh	Rh						MNSs				P1	Lu		Kell				Le		Fy		Jk	
		D	C	E	c	e	C <sup>w</sup>	M	N	S	s		a	b	K	k	Kp <sup>a</sup>	a	b	a	b	a	b	
1	R <sub>1</sub> <sup>w</sup> R <sub>1</sub>	+	+	0	0	+	+	+	0	+	0	0	0	+	0	+	0	+	0	+	0	+	0	
2	R <sub>1</sub> R <sub>1</sub>	+	+	0	0	+	0	+	0	0	+	2	0	+	+	+	0	0	+	+	0	0	+	
3	R <sub>2</sub> R <sub>2</sub>	+	0	+	+	0	0	0	+	0	+	3	0	+	0	+	0	0	+	0	+	0	+	
4	r'r	0	+	0	+	+	0	0	+	+	0	0	+	+	0	+	0	0	+	0	0	0	+	
5	r"r	0	0	+	+	+	0	0	+	+	+	3	0	+	0	+	0	0	+	+	0	+	0	
6	rr	0	0	0	+	+	0	+	0	+	0	1	0	+	+	0	0	+	0	0	+	+	+	
7	rr	0	0	0	+	+	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	+	+	1	0	+	0	+	+	0	+	+	0	+	0	
10	rr	0	0	0	+	+	0	+	0	+	0	4	0	+	+	+	0	0	+	0	+	0	+	
Auto																								

Results			
Abs1	Abs2		
3	3		
1	1		
1	1		
3	3		
3	3		
3	3		
1	1		
1	1		
3	3		
3	3		

# Case 3

*Blood and Transplant*

Adsorption studies 6 x warm and cold

Cell	Rh	Rh					MNSs				P1	Lu		Kell		Le		Fy		Jk		
		D	C	E	c	e	C <sup>w</sup>	M	N	S	s	a	b	K	k	Kp <sup>a</sup>	a	b	a	b	a	b
1	R <sub>1</sub> <sup>w</sup> R <sub>1</sub>	+	+	0	0	+	+	+	0	+	0	0	0	+	0	+	0	+	0	+	0	+
2	R <sub>1</sub> R <sub>1</sub>	+	+	0	0	+	0	+	0	0	+	2	0	+	+	+	0	0	+	+	0	0
3	R <sub>2</sub> R <sub>2</sub>	+	0	+	+	0	0	0	0	+	0	+	3	0	+	0	+	0	0	+	0	+
4	r'r	0	+	0	+	+	0	0	0	+	+	0	0	+	+	0	+	0	0	0	0	+
5	r"r	0	0	+	+	+	0	0	0	+	+	+	3	0	+	0	+	0	0	+	+	0
6	rr	0	0	0	+	+	0	+	0	+	0	1	0	+	+	0	0	+	0	0	+	+
7	rr	0	0	0	+	+	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	+	+	1	0	+	0	+	+	0	+	+	0	+
10	rr	0	0	0	+	+	0	+	0	+	0	4	0	+	+	+	0	0	+	0	+	0
Auto																						

Results		
Abs1	Abs2	
3	3	
1	1	
1	1	
3	3	
3	3	
3	3	
1	1	
1	1	
3	3	
3	3	

# Case 3

*Blood and Transplant*

Adsorption studies 6 x warm and cold

Cell	Rh	Rh					MNSs				P1	Lu		Kell		Le		Fy		Jk		
		D	C	E	c	e	C <sup>w</sup>	M	N	S	s	a	b	K	k	Kp <sup>a</sup>	a	b	a	b	a	b
1	R <sub>1</sub> <sup>w</sup> R <sub>1</sub>	+	+	0	0	+	+	+	0	+	0	0	0	+	0	+	0	+	0	+	0	+
2	R <sub>1</sub> R <sub>1</sub>	+	+	0	0	+	0	+	0	0	+	2	0	+	+	+	0	0	+	+	0	0
3	R <sub>2</sub> R <sub>2</sub>	+	0	+	+	0	0	0	0	+	0	+	3	0	+	0	+	0	0	+	0	+
4	r'r	0	+	0	+	+	0	0	0	+	+	0	0	+	+	0	+	0	0	0	0	+
5	r"r	0	0	+	+	+	0	0	0	+	+	+	3	0	+	0	+	0	0	+	+	0
6	rr	0	0	0	+	+	0	+	0	+	0	1	0	+	+	0	0	+	0	0	+	+
7	rr	0	0	0	+	+	0	0	0	+	0	+	0	+	+	+	0	0	+	0	+	+
8	rr	0	0	0	+	+	0	+	+	0	+	0	0	+	0	+	+	+	0	+	0	+
9	rr	0	0	0	+	+	0	+	0	+	+	1	0	+	0	+	+	0	+	+	0	+
10	rr	0	0	0	+	+	0	+	0	+	0	4	0	+	+	+	0	0	+	0	+	0
Auto																						

Results		
Abs1	Abs2	
3	3	
1	1	
1	1	
3	3	
3	3	
3	3	
1	1	
1	1	
3	3	
3	3	

No anti-S in eluate!!

No change in the DAT

## Case 4

- ” Male, DOB11/08/76
- ” O Pos, known anti-D+Lea+Leb+Fy3+Jkb+Jsa+McCa+Cr1 related antibody
- ” Diagnosis Sickle cell anaemia + surgical
  - . Total hip replacement
  - . Laparoscopic cholecystectomy

## Case 4

### Request

- . For Hb optimisation prior to surgery **12 RBC** units exchange scheduled for the 01/09/16
- . For THR scheduled for the 08/09/16 **2 RBC** units
- . For cholecystectomy scheduled for the 22/09/16 **2 RBC** units

## Case 4

„ NFBB stocks in July 2016

Group O C- E- S- K- Fy(a-b-) Jkb- Jsa-

4!

## Case 4

- “ NHSBT medical team performed a search of suitable donors and arranged for them to donate
- “ RCI staff searched on a daily basis to see if any additional wet units became available

**But still not enough units!**

## Case 4

” No typing anti-Jsa antisera available

**NHS**

*Blood and Transplant*

## Case 4



## Case 4

- “ Discussed with IBGRL to ask whether they would be able to Genotype for Jsa
- “ 6 donations were selected which matched all of the other requirements
- “ Genotyped for Jsa-

## Case 4

- „ 8 units supplied for exchange 01/09/16
- „ 2 units for THR 08/09/16
- „ 2 units for cholecystectomy 22/09/16
- „ Surgery (both) successful



# Summary



In each of the case studies discussed genotyping help to either resolve the case or provide blood quicker then would have been possible using serology only

# Summary

## ” Uses of genotyping in red cell serology

- . Cases where the serology is unclear or especially complex 
- . Cases where the patient has been transfused and cannot be phenotyped 
- . DAT positive 
- . Cases where suitable reagents are not available 
- . To offer clues to help focus the investigation 

# No system is perfect

- “ Expense ✗
- “ Only as good as the kit ✗
- “ What happens if the phenotype and genotype does match ✗
- “ What if the results actually make it more difficult to provide blood? ✗

# References

- „ The Blood Group Antigen Factsbook, M Reid, 2007
- „ Human Blood Groups, G Daniels, 2002
- „ DAR, a new RhD variant involving exons 4 and 5 and 7, Blood, 15:94, 1999