Not your typical

ABO and Rh (D) type

By: Priscilla Moreira

Australia Red Cross Blood Service - Victoria

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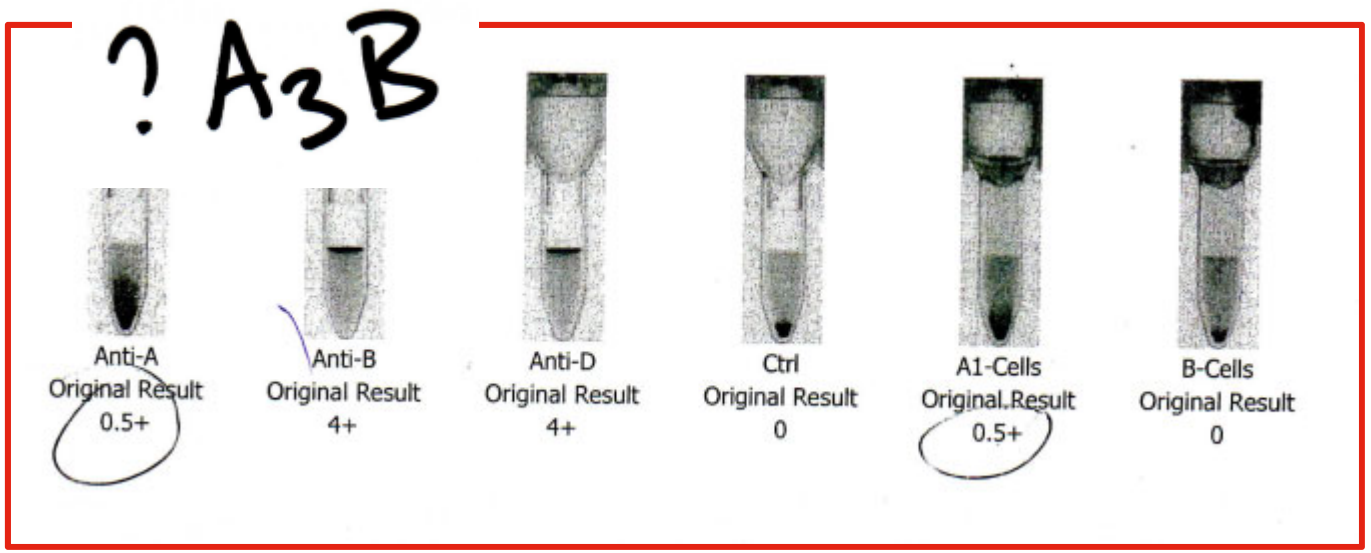
Case 1: Patient Y

- 65 year old male
- No previous transfusion



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Initial group

ABO								Rh (D)					Group	
Cell group				Serum group				IgM	IgM/IgG		Control			
-A	-B	-AB	-A1	A1	A2	B	O		I.S.	IAT	I.S.	IAT	ABO	Rh
0	4+	4+	0	1+	0	0	0	4+	4+	/	0	/	B	Pos

B forward group and weak reactions in the reverse group

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Reverse group and Antibody Screen

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Extended Reagent Red Cell Panel

		Additional Comments	No	IS	15' RT	5' 4°C	RAM IAT		
		A1	1	1+S	1+S	2+	1+		
		A1	2	1+S	1+S	2+	0		
		A2	3	0	0	2+	0		
		A2	4	0	0	2+	0		
		SC1 0	5	0	0	0	0		
		SC2 0	6	0	0	0	0		
		SC3 0	7	0	0	0	0		
		B	8	0	0	0	0		
		B	9	0	0	0	0		
		AUTO	10	0	0	0	0		
			11						
			12						
			13						
			14						
			15						
Pos Control: R1r				0	0	0	1+S		
Neg Control: r r				0	0	0	0		

Anti-A in the plasma, reacting with both A1 and A2 cells at 4°C

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Various ABO typing sera

Reagent Set	FORWARD GROUP				REVERSE GROUP				Testing method
	Anti-A	Anti-B	Anti-AB	Anti-A1	A1	A2	B	O	
Reagents 1	0	4+	4+	0	1+	0	0	0	tube
Reagents 2	★1+	4+			1+s		0		card
Reagents 3	0	4+	4+						tube
Reagents 4	★2+	4+	4+						tube
Reagents 5	0	4+			1+s		0		card

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Adsorption and elution

- Patient's cells failed to adsorb and elute anti-A.
- Unlikely for group to be a weak subgroup of A.

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Product inserts, clones used and specific reagent performance characteristics

Reagent Set	FORWARD GROUP				Testing method	
	Anti-A	Anti-B	Anti-AB	Anti-A1		Anti- A Clones
Reagents 1	0	4+	4+	0	tube	4E7, 8F2
Reagents 2	1+	4+			card	MHO4, 3D3
Reagents 3	0	4+	4+		tube	Cell line Birma - 1
Reagents 4	2+	4+	4+		tube	A003
Reagents 5	0	4+			card	Cell line A5

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B(A) phenomenon

- Not frequently encountered - about 1 per year.
- Can be attributed to amino acid polymorphisms in the B glycosyltransferase near (Pro234Ala) or at critical amino acids (Ser235Gly).

Table 2.18 Enzymes with dual A- and B-transferase activity: amino acid substitutions at the four positions (176, 235, 266, 268) characteristic of A and B and at positions 214 and 234.

Phenotype*	Alleles	Amino acids						Shorthand	References
		176	214	234	**235**	**266**	**268**		
A_1	*A101* or *A102*	Arg	Met	Pro	Gly	Leu	Gly	AAAA	
B	*B*	Gly	Met	Pro	Ser	Met	Ala	BBBB	
B(A)	*B(A)01* & *B(A)03*	Gly	Met	Pro	**Gly**	Met	Ala	BABB	[269,293]
B(A)	*B(A)02*	Gly	Met	**Ala**	Ser	Met	Ala	BBBB	[379]
B(A)	*B(A)04*	Gly	**Val**	Pro	Ser	Met	Ala	BBBB	[380]
B(A)	*B(A)05*	Gly	**Thr**	Pro	Ser	Met	Ala	BBBB	[380]

- The B glycosyltransferase in these individuals has an increased capacity to use UDP-N-acetylgalactosamine, as well as UDP-galactose, resulting in small amounts of aberrant A antigen.

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Transfusion

- Patient Y should be treated as group B.

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Case 2: Patient X

- 92 year old female with a pelvic fracture.
- Grouped as O Rh(D) Pos.

ABO								Rh (D)				
Cell group				Serum group				IgM	IgM/IgG		Control	
-A	-B	-AB	-A1	A1	A2	B	O		I.S.	IAT	I.S.	IAT
O	O	O	O	4+	4+	3+	O	4+	3+S	3+S	O	O

- R1r, DAT negative.

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11 Reagent Red Cell panel

Patient X

Cell No	Reference No	RH							KEL			FY		JK		MNS				P1PK	LE		LU	CO	Additional Typings	Cell No	Results			
		Rh Phen.	D	C	E	c	e	C^w	K	k	Kp^a	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s	P1	Le^a	Le^b	Lu^a	Co^b			CAT IAT		CAT IAT	
1	2083263	$R_1^wR_1$	+	+	0	0	+	+	0	+	+	+	0	+	0	+	0	+	+	0	+	0	+	0		1	3+			
2	2146231	R_1R_1	+	+	0	0	+	0	+	+	0	+	0	0	+	+	0	+	0	+	0	+	0	+	*Cs(a-)	2	3+			
3	2195612	R_1R_1	+	+	0	0	+	0	0	+	0	0	+	0	+	0	+	0	+	0	0	+	0	0		3	3+			
4	2002472	R_2R_2	+	0	+	+	0	0	0	+	0	0	+	+	0	0	+	0	+	$+^s$	0	+	0	0		4	3+			
5	2013679	R_2R_2	+	0	+	+	0	0	0	+	+	+	0	+	+	+	0	+	+	0	0	0	0	0		5	3+			
6	2053551	r'r	0	+	0	+	+	0	0	+	0	+	0	+	+	+	0	0	+	0	0	+	0	0	^Ch(a-)	6	0			
7	2260094	r''r	0	0	+	+	+	0	0	+	0	0	+	0	+	+	0	+	0	+	+	0	+	0	#M_1+	7	0			
8	1420103	rr	0	0	0	+	+	0	+	+	0	0	+	+	0	+	+	0	+	$+^s$	+	0	0	0		8	3+			
9	2059833	rr	0	0	0	+	+	0	0	+	0	+	+	+	0	+	0	0	+	0	0	0	0	0		9	0			
10	2058202	rr	0	0	0	+	+	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	0		10	0			
11	2056302	rr	0	0	0	+	+	0	0	+	0	+	0	0	+	0	+	0	+	$+^w$	+	0	0	+		11	0			
Auto																										Auto	0		0	
8	4086345	rr	0	0	0	+	+	0	+	+	0	+	0	+	0	0	+	0	+	0	0	+	0	0	B: 2648 160 01 Exp: 20.2.17	8			3+	
8	3175965	rr	0	0	0	+	+	0	+	+	0	+	+	+	+	+	0	+	+	$+^s$	+	0	0	0	B: 2659 965 01 Exp: 14.3.17	8			3+	
9	1197675 0	rr	0	0	0	+	+	0	0	+	+	+	+	+	+	+	+	+	+	+	0	+	+	0	B: 8660 100 01 Exp: 7.3.17	9			0	
	Reference No																								Reference No					

Anti-D

Anti-K

Auto neg

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But!

- Patient X was Rh(D) pos R1r
- So was this a case of anti-D in a partial D individual or
- An Rh(D) positive patient with anti-LW….

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LW

- LW and D are different antigens but phenotypically related.
- LW is more strongly expressed on D positive than D negative adult cells.
- Rh null cells which lack all Rh antigens, also lack LW.
- LW is strongly expressed on red cells of neonates regardless of the D type.

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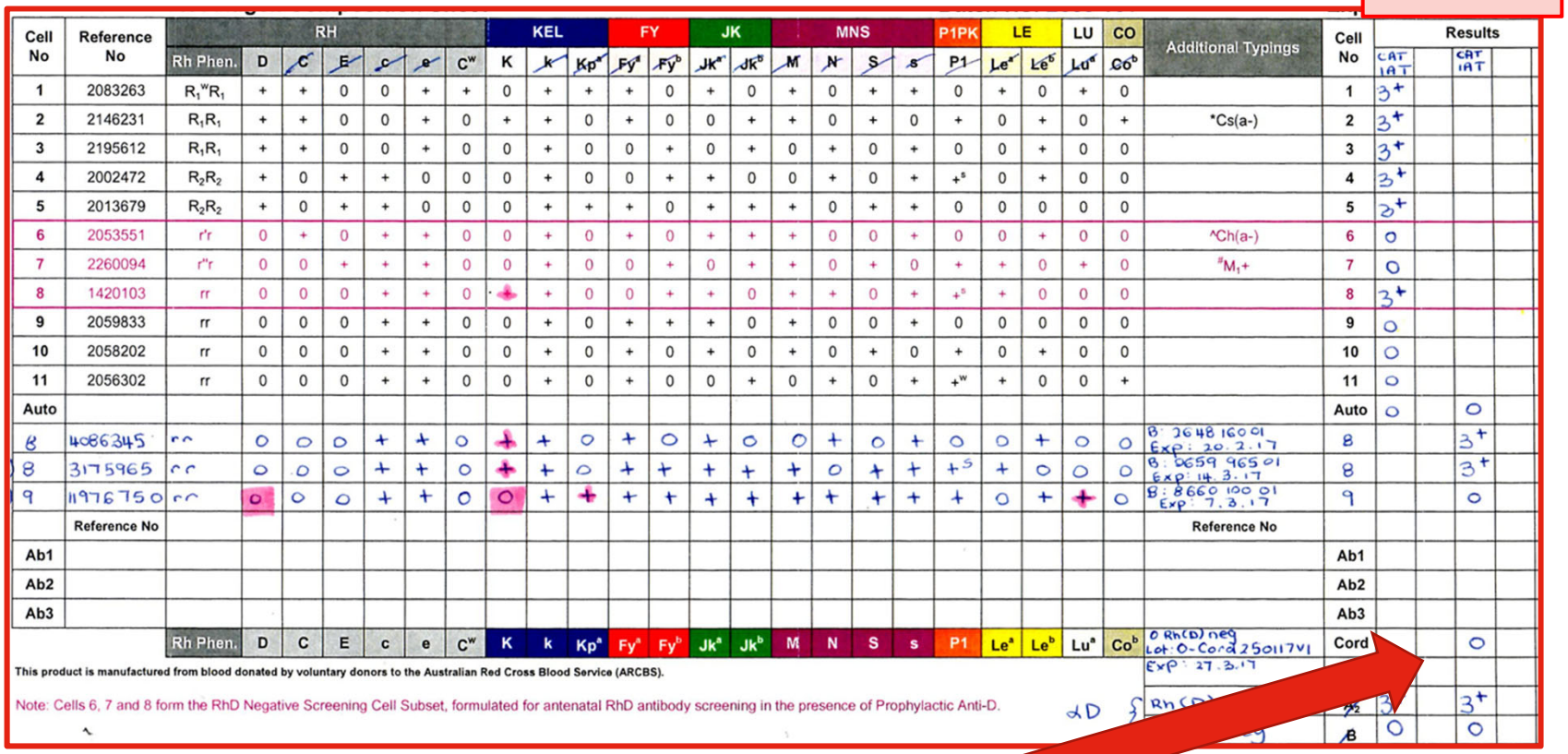
Rh (D) negative cord cell

Patient X



Cell No	Reference No	RH							KEL			FY		JK		MNS				P1PK	LE		LU	CO	Additional Typings	Cell No	Results	
		Rh Phen.	D	C	E	c	e	C^w	K	k	Kp^a	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s	P1	Le^a	Le^b	Lu^a	Co^b			CAT IAT	CAT IAT
1	2083263	$R_1^wR_1$	+	+	0	0	+	+	0	+	+	+	0	+	0	+	0	+	+	0	+	0	+	0		1	3+	
2	2146231	R_1R_1	+	+	0	0	+	0	+	+	0	+	0	0	+	+	0	+	0	+	0	+	0	+	0	+	*Cs(a-)	2	3+	
3	2195612	R_1R_1	+	+	0	0	+	0	0	+	0	0	+	0	+	0	+	0	+	0	0	+	0	+	0	0		3	3+	
4	2002472	R_2R_2	+	0	+	+	0	0	0	+	0	0	+	+	0	0	+	0	+	+s	0	+	0	0		4	3+	
5	2013679	R_2R_2	+	0	+	+	0	0	0	+	+	+	0	+	+	+	0	+	+	0	0	0	0	0		5	3+	
6	2053551	r'r	0	+	0	+	+	0	0	+	0	+	0	+	+	+	0	0	+	0	0	+	0	0	^Ch(a-)	6	0	
7	2260094	r''r	0	0	+	+	+	0	0	+	0	0	+	0	+	+	0	+	0	+	+	0	+	0	#M_1+	7	0	
8	1420103	rr	0	0	0	+	+	0	+	+	0	0	+	+	0	+	+	0	+	+s	+	0	0	0		8	3+	
9	2059833	rr	0	0	0	+	+	0	0	+	0	+	+	+	0	+	0	0	+	0	0	0	0	0		9	0	
10	2058202	rr	0	0	0	+	+	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	0	0		10	0	
11	2056302	rr	0	0	0	+	+	0	0	+	0	+	0	0	+	0	+	0	+	+w	+	0	0	+		11	0	
Auto																										Auto	0	0
8	4086345	rr	0	0	0	+	+	0	+	+	0	+	0	+	0	0	+	0	+	0	0	+	0	0	0	B: 2648 160 01 Exp: 20.2.17	8		3+
8	3175965	rr	0	0	0	+	+	0	+	+	0	+	+	+	+	+	0	+	+	+s	+	0	0	0		B: 2659 965 01 Exp: 14.3.17	8		3+
9	1197675	rr	0	0	0	+	+	0	0	+	+	+	+	+	+	+	+	+	+	+	0	+	+	0		B: 8660 100 01 Exp: 7.3.17	9		0
	Reference No																									Reference No			
Ab1																											Ab1		
Ab2																											Ab2		
Ab3																											Ab3		
		Rh Phen.	D	C	E	c	e	C^w	K	k	Kp^a	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s	P1	Le^a	Le^b	Lu^a	Co^b	O Rh(D) neg Lot: O-Cord 250117V1 Exp: 27.3.17	Cord		0
																									Rh(D)	½	3	3+
																										B	0	0

This product is manufactured from blood donated by voluntary donors to the Australian Red Cross Blood Service (ARCBS).

Note: Cells 6, 7 and 8 form the RhD Negative Screening Cell Subset, formulated for antenatal RhD antibody screening in the presence of Prophylactic Anti-D.

Non reactive

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Range of anti-D reagents

	IgM		IgM		IgM		IgM		IgM/IgG			Rh Control			IgM/IgG		
No.		ctl		ctl											15'@ 37		
	IS	~~37C~~	IS	~~37C~~	IS	37C	IS	37C	IS	37C	IAT	IS	37C	IAT	IS	37C	IAT
R_1r	4^+	0	4^+	0											4^+	/	/
$D+^w$	2^{+s}	0	2^+	0											2^+	1^+	3^+
rr	0	0	0	0											0	0	0
Patient X	4^+	0	4^+	0											4^+	/	/

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Partial Rh (D) typing kit

Patient X

Check our website for the most up-to-date version of the reaction profile, supplementary information and recent findings. www.quotientbd.com

Kit ID	Anti-D Cell Line	Weak D Type 1 and 2[a]	DII & DNU	DIII	DIV	DV*	DCS	DVI	DVII	DOL	DFR	DMH	DAR†	DAR-E	DHK‡ & DAU-4	DBT	Ro^{Har} §	Test Result: Pos Cont.	Neg Cont.		
A	LHM76/58	+	+	+	+	+/0	+	0	+	+	+	+	+	0	0	0	(+)/0	4+	0	3+	4+
B	LHM76/59	+	+	+	0	+	+	+	+	+	+	+	+	+	+	0	0	4+	0	4+	4+
C	LHM174/102	(+)/0	+	+	0	0	+	0	+	0	0	+	0	0	0	0	0	4+	0	+/−	4+
D	LHM50/2B	+	+	+	+	+	+	0	+	+	+	+	+	+	+	0	0	4+	0	4+	4+
E	LHM169/81	+	+	+	0	0	+	0	+	+	+	+	0	0	0	0	0	4+	0	3+	4+
F	ESD1	+	+	+	0	+	+	+	+	+	+	+	+	+	+	0	0	4+	0	3+	3+
G	LHM76/55	+	+	+	0	+	+	+	+	+	+	+	+	+	+	0	0	4+	0	3+	4+
H	LHM77/64	+	0	+	0	+	+	+	+	+	+	+	+	+	+/0	0	0	4+	0	2+	4+
I	LHM70/45	(+)/0	+	+	0	0	0	0	+	0	0	0	0	0	0	0	0	4+	0	+/−	4+
J	LHM59/19	+	+	+	+	+	+	0	0	0	0	(+)	0	(+)	+	+	0	4+	0	2+	4+
K	LHM169/80	+	+	+	+	+	+	0	+	+	+	+	+	+	0	0	0	4+	0	4+	4+
L	LHM57/17	+	+	+	+	+	0	0	+	+	0	+	+	0	0	+	0	3+	0	0	3+s

[a]Refer to website for supplementary information

All strongly positive

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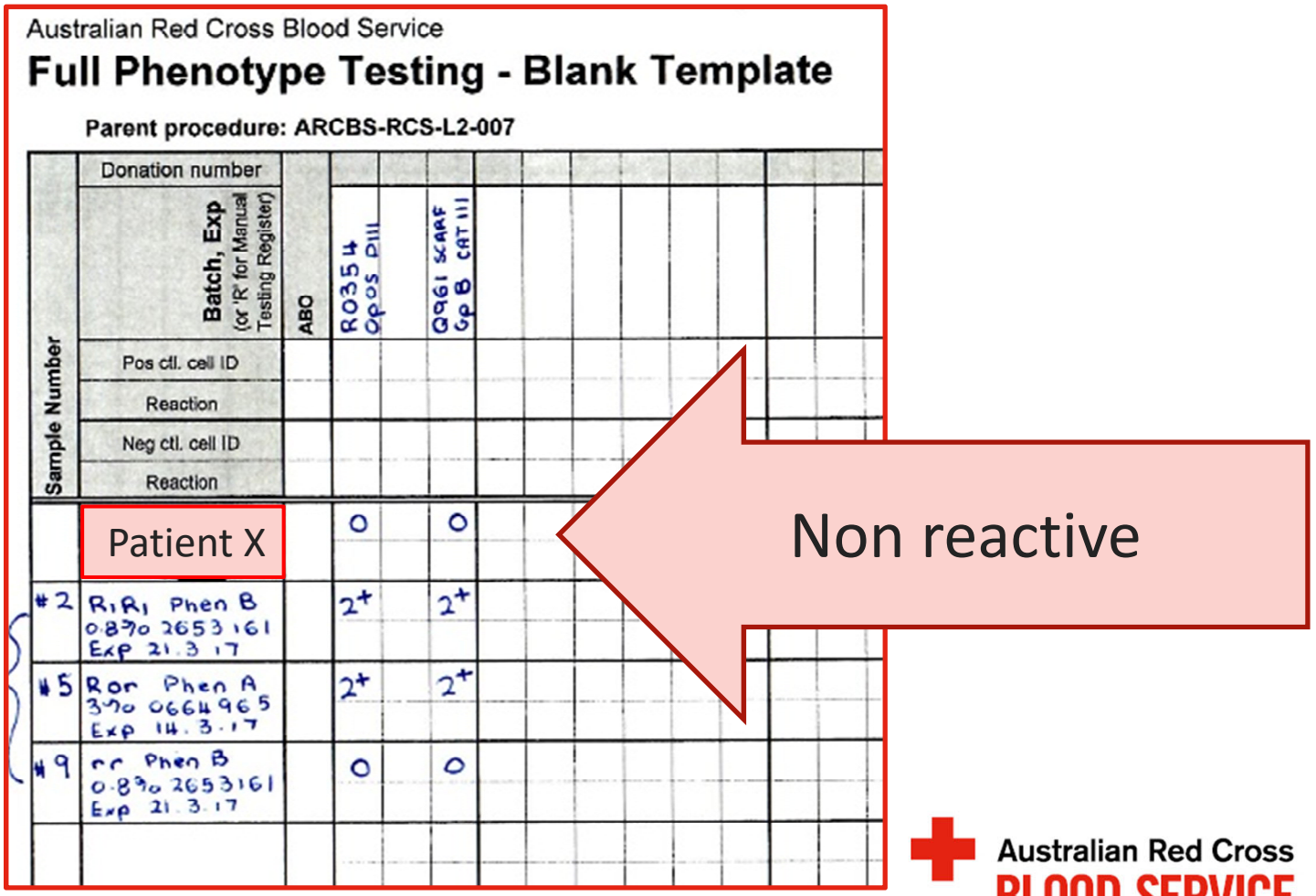
Human Source anti-D



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Full Phenotype Testing - Blank Template

Parent procedure: ARCBS-RCS-L2-007

Sample Number	Donation number	ABO	R0354 Qpos PIII	Q961 SCARF Gp B CAT III
	Batch, Exp (or 'R' for Manual Testing Register)			
	Pos ctl. cell ID			
	Reaction			
	Neg ctl. cell ID			
	Reaction			
	Patient X		0	0
#2	R1R1 Phen B 0870 2653161 Exp 21.3.17		2+	2+
#5	Ror Phen A 370 0664965 Exp 14.3.17		2+	2+
#9	rr Phen B 0870 2653161 Exp 21.3.17		0	0

Non reactive

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RHD genotyping – serological findings confirmed!

DNA extracted from the EDTA sample was genotyped using the BioArray Solutions RHD BeadChipTM Kit.

The Immucor BioArray System TM analyses the signal intensity for each specific marker to determine the predicted RhD phenotype.

RhD Phenotypical Variant: DIIIc (hemizygous or homozygous)

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Partial D Category DIII

- This is a result of the exon 3 of RHD being replaced with that of the RHCE.

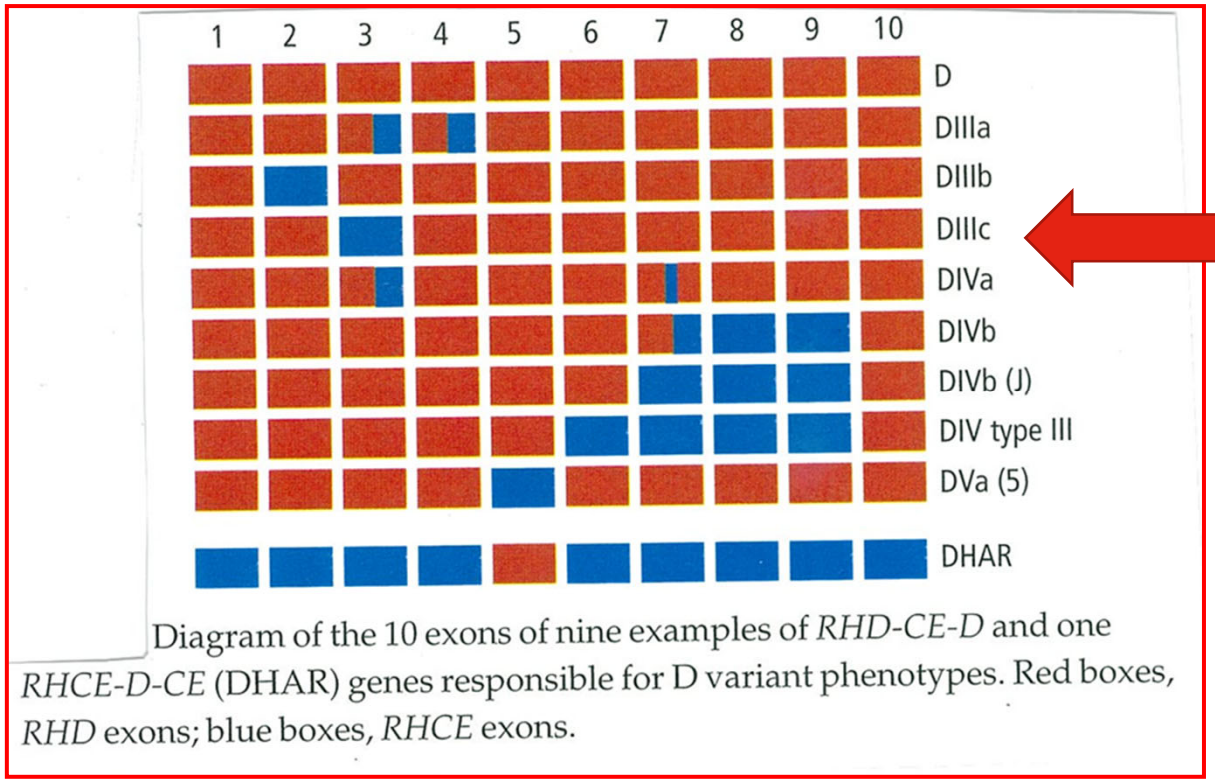

Diagram of the 10 exons of nine examples of *RHD-CE-D* and one *RHCE-D-CE* (DHAR) genes responsible for D variant phenotypes. Red boxes, *RHD* exons; blue boxes, *RHCE* exons.

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Partial D Category DIII

- The DIII phenotype is not frequently encountered in our lab.
- DIII red cells although reactive with all monoclonal anti-D must lack at least one D epitope resulting in the ability to make allo anti-D.
- Patients with this phenotype should be treated as Rh (D) negative for transfusion purposes and in the antenatal setting.

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References

- Carton JP, Rouger P 1995. Blood Cell Biochemistry. Molecular Basis of Human Blood Group Antigens. New York and London: Plenum Press.

- Daniels G 2013. Human Blood Groups. New Jersey: Wiley-Blackwell.

- Daniels G and Bromilow I 2010. Essential Guide to Blood Groups. New Jersey: Wiley-Blackwell.

- Roback J, Combs MR, Grossman BJ, Hillyer CD 2008. Technical Manual. USA: AABB.

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