

Red Cell Immunohaematology

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Caring Expert Quality

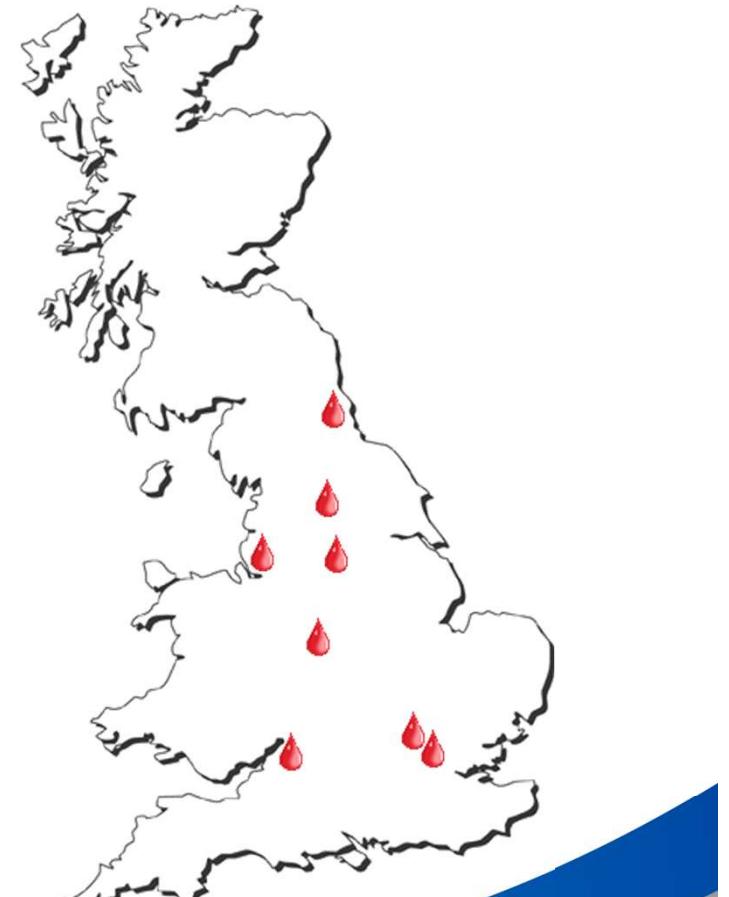


The RCI Laboratories



Red Cell Immunohaematology

- Reference laboratories supporting hospital blood banks in England.
- RCI laboratories at NHSBT centers in
 - Newcastle
 - Leeds
 - Sheffield
 - Liverpool
 - Birmingham
 - Bristol (Filton)
 - London (Colindale and Tooting)



RCI Services

- Blood Grouping Anomalies
- Antibody Investigations
 - Complex antibody investigations
 - Crossmatching
- Antenatal Reference Services
 - Titrations / Quantitations (anti-D, anti-c)
- Fetal-Maternal Haemorrhage
- Transfusion Reaction Investigations

Request for Reference Serology 1A

NHS
Blood and Transplant

Please see reverse for samples required
Place correctly labelled samples in bag, remove protective strip, fold onto bag and seal firmly

At least three points of matching identification must be used on forms and sample tubes (see over)

Please do not use this form for routine antenatal requests

Please write clearly inside text boxes, one character per box and mark option boxes as appropriate

TEAR HERE

Please complete all sections fully. Urgent requests MUST be telephoned.

PATIENT DETAILS		NHS No.		
NHSBT No.				
Surname				
First name				
DOB			Gender (MF) <input type="checkbox"/>	Ethnic origin code (see reverse) <input type="checkbox"/>
First line of address				
Post code			EDD (if pregnant) <input type="checkbox"/>	
Hospital number			Hospital sample ID number	
HOSPITAL & REQUESTER DETAILS				
Full Hospital name				
Hospital (NHS code)			Consultant	<input type="checkbox"/>
Contact name				
Phone			Beep	<input type="checkbox"/>

Antibody Investigations



Panels and Cells

- Panel cells from NBSBT reagents
 - Panel 1 and 2
 - Reference Panel 1 (R1R1, for anti-c)
 - Reference Panel 2 (R2R2, for anti-e)
- Rare cells from selected donors
 - From Testing / Rare Cell Exchange Scheme
 - Frozen Cell Bank
 - High frequency negatives, eg. K+k-, U-, Vel-
 - Low frequency positives, eg. Wr(a+), Js(a+), Di(a+)

Serological Techniques

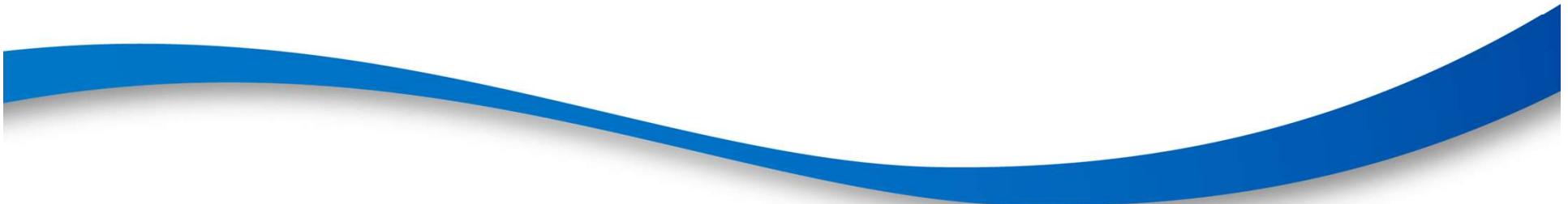
- A variety of techniques can be employed
 - BioRad gel IAT
 - BioRad enzyme IAT
 - LISS Tube IAT
 - Saline (direct agglutination)
 - Capture-R (*Immucor*)
 - BioVue IAT (*Ortho*)
- Vary temperature, incubation times, etc

Adsorptions & Elutions

- DAT performed if auto control positive
 - Monospecific anti-IgG, -IgM, -IgA, -C3d, -C3c
- Autoantibodies can be removed by
 - Autoadsorptions: ZZAP-treated own cells
 - Alloadsorptions: paired reagent cells
- Elutions may be used to ascertain specificity of antibody coating red cells
 - Transfusion Reactions
 - Haemolytic Disease of the Fetus & Newborn

Antibody Neutralisation

- “Nuisance” antibodies can be inhibited or neutralised to reveal any underlying alloantibodies
- Chido-Rogers antibodies
 - Target C4 complement proteins
 - Neutralised by the addition of excess complement (AB serum)
- Knops-McCoy antibodies
 - Target CR1 molecule on red cells
 - Neutralised by the addition of recombinant KNIR reagent



Phenotyping & Genotyping

- Allo or autoantibody?
- Phenotyping
 - Serological testing using antisera
 - Phenotyping cannot be performed if the patient has had a recent transfusion.
- Genotyping
 - DNA Sequencing
 - Consider in: transfusion-dependent patients (eg. sickle cell), patients with multiple antibodies, before starting treatments, eg. Daratumumab

Drug-Related Panreactivity

- In some monoclonal antibody therapies the *drug* causes positive reactions vs all cells
- Daratumumab (Darzalex)
 - Anti-CD38 used in Multiple Myeloma
 - Drug action inhibited using DTT
- Camellia
 - Anti-CD47 used in AML
 - Genotype-matched blood issued

Tertiary Referral

- The International Blood Group Reference Laboratory (IBGRL) is located at NHSBT Filton
 - Samples may be referred to IBGRL from RCI if antibody specificity cannot be ascertained
 - IBGRL receive samples from all over the World.
- The National Frozen Blood Bank is located at NHSBT Liverpool
 - Rare donors are identified by Testing and red cell units frozen
 - Rare, frozen blood can be requested when required
 - eg. Bombay phenotype (with anti-H)

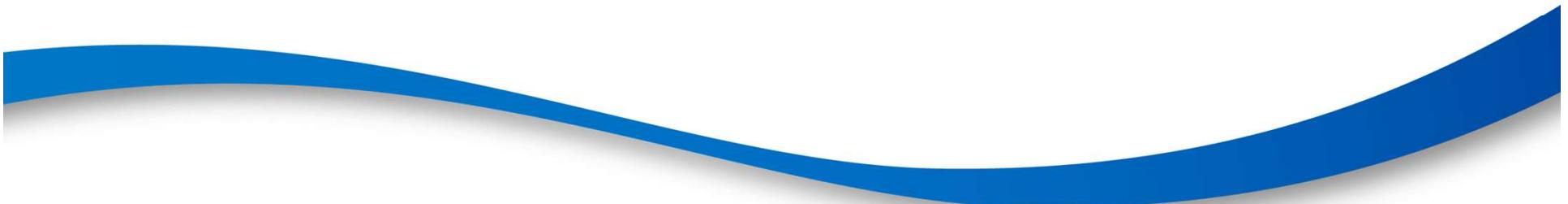


Interpreting Antigrams



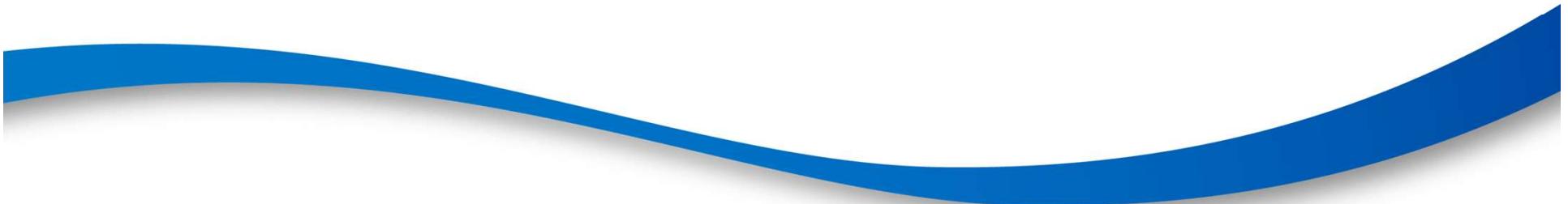
The Rules of the Game I

- BSH Guidelines for pre-transfusion compatibility procedures in blood transfusion laboratories (2002).
- *Always* do your best to exclude all clinically significant antibodies.
 - Excluding anti-Cw, -P1, -Kpa, -Lua, -Lea, Leb not *strictly* necessary if you don't have appropriate cells
- Don't forget your screening results!!!



The Rules of the Game II

- Antibody exclusions:
 - “*...the presence of anti-Jka, anti-Jkb, anti-S, anti-s, anti-Fya and anti-Fyb should be excluded using red cells having homozygous expression of the relevant antigen...*”
 - “**...A single example only of each phenotype is sufficient for exclusion...**”



The Rules of the Game III

- Antibody identification:
 - “*...specificity should only be assigned when **the plasma is reactive with at least two examples of reagent red cells expressing the antigen** and non- reactive with at least two examples of reagent red cells lacking the antigen...*”

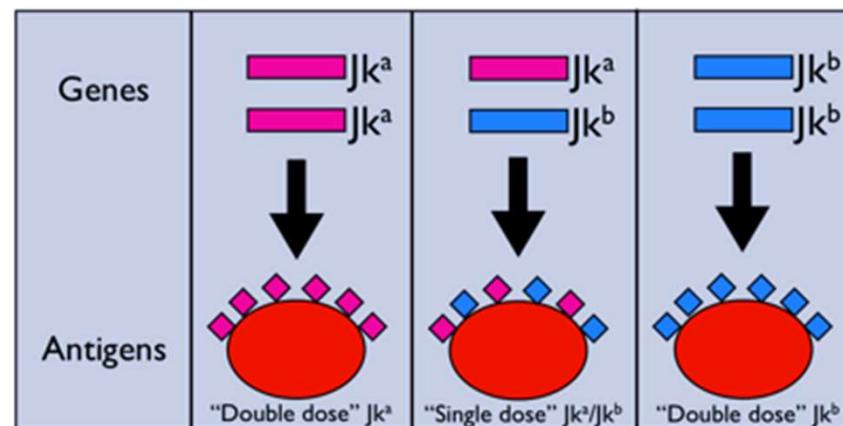


The Enzyme Panel

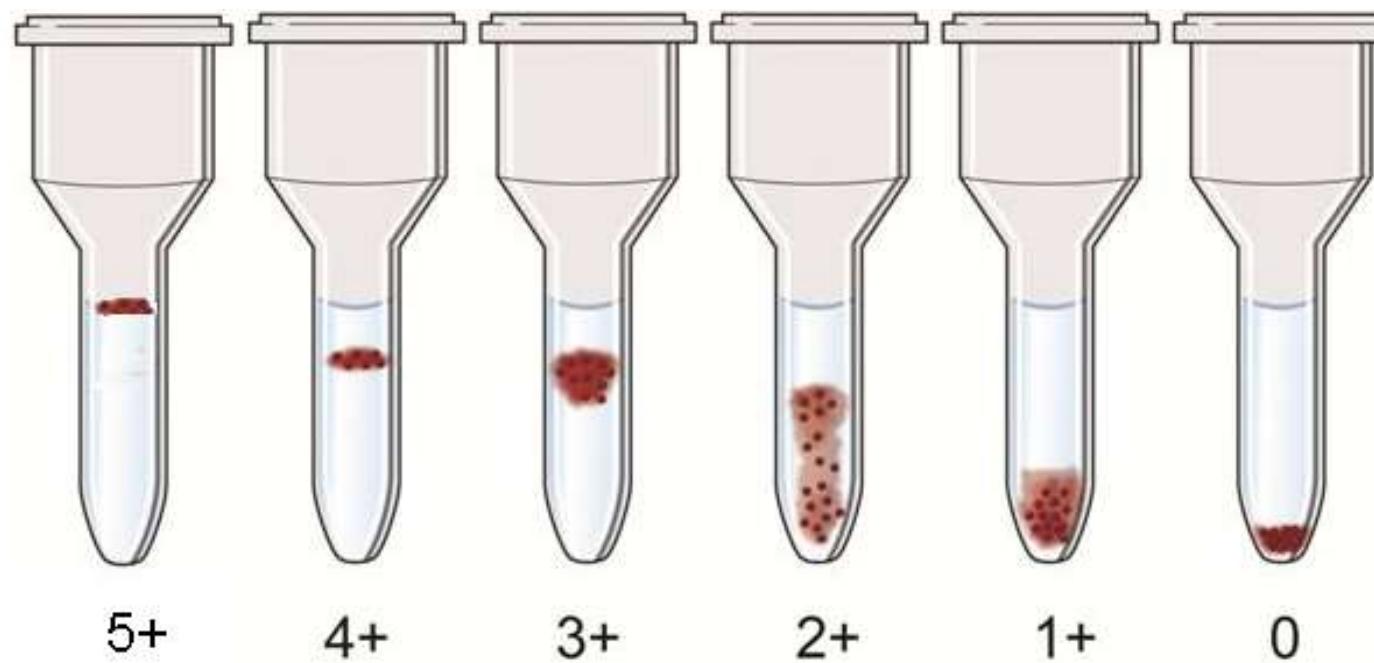
- Papain most commonly used (from Papaya)
- Papain enhances the reaction strength of
 - Rh system antibodies (anti-D, -C, -c, -E, -e, -Cw)
 - Kidd system antibodies (anti-Jka, -Jkb)
- Papain destroys some red cell antigens; so negative reactions will be observed with
 - MNS system antibodies (anti-M, -N, -S, -s)
 - Duffy system antibodies (anti-Fya, -Fyb)

The Dosage Effect

- “Dosage” refers to the effect of seeing stronger reactions with *homozygous* antigen expression compared to *heterozygous* expression.
 - Eg. anti-Jka may react stronger with Jk(a+b-) cells than Jk(a+b+) cells



Gel IAT Grading



Interpreting Antigrams

Cell	Rh	M	N	S	s	P1	Lu ^a	K	k	Kp ^a	Le ^a	Le ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	
1	R ₁ wR ₁	0	+	0	+	5	0	+	+	0	0	+	0	+	0	+	0
2	R ₁ R ₁	+	+	+	0	0	0	0	+	0	+	0	+	0	+	0	4
3	R ₂ R ₂	0	+	0	+	3	0	0	+	0	+	0	0	+	+	0	0
4	r'r	+	0	+	0	3	0	0	+	0	0	+	0	+	+	0	0
5	r"r	+	0	+	0	0	0	0	+	0	0	+	+	0	0	+	4
6	rr	+	0	0	+	0	0	+	0	0	0	+	0	+	+	0	0
7	rr	+	0	+	0	0	0	+	+	0	0	0	+	0	+	0	4
8	rr	0	+	0	+	2	0	0	+	+	0	+	+	0	+	0	4
9	rr	0	+	+	0	3	0	0	+	0	0	+	+	0	0	+	4
10	rr	+	0	+	0	3	+	0	+	0	+	0	0	+	0	+	0



Interpreting Antigrams

Cell	Rh	M	N	S	s	P1	Lu ^a	K	k	Kp ^a	Le ^a	Le ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	
1	R ₁ wR ₁	0	+	0	+	5	0	+	+	0	0	+	0	+	0	+	0
2	R ₁ R ₁	+	+	+	0	0	0	0	+	0	+	0	+	0	+	0	4
3	R ₂ R ₂	0	+	0	+	3	0	0	+	0	+	0	0	+	+	0	0
4	r'r	+	0	+	0	3	0	0	+	0	0	+	0	+	+	0	0
5	r"r	+	0	+	0	0	0	0	+	0	0	+	+	0	0	+	4
6	rr	+	0	0	+	0	0	+	0	0	0	+	0	+	+	0	0
7	rr	+	0	+	0	0	0	+	+	0	0	0	+	0	+	0	4
8	rr	0	+	0	+	2	0	0	+	+	0	+	+	0	+	0	4
9	rr	0	+	+	0	3	0	0	+	0	0	+	+	0	0	+	4
10	rr	+	0	+	0	3	+	0	+	0	+	0	0	+	0	+	0

Interpreting Antigrams

Cell	Rh	M	N	S	s	P1	Lu ^a	K	k	Kp ^a	Le ^a	Le ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	
1	R_{1w}R₁	0	+	0	+	5	0	+	+	0	0	+	0	+	0	+	0
2	R ₁ R ₁	+	+	+	0	0	0	0	+	0	+	0	+	0	+	0	4
3	R₂R₂	0	+	0	+	3	0	0	+	0	+	0	0	+	+	0	0
4	r'r	+	0	+	0	3	0	0	+	0	0	+	0	+	+	0	0
5	r''r	+	0	+	0	0	0	0	+	0	0	+	+	0	0	+	4
6	rr	+	0	0	+	0	0	+	0	0	0	+	0	+	+	0	0
7	rr	+	0	+	0	0	0	+	+	0	0	0	+	0	+	0	4
8	rr	0	+	0	+	2	0	0	+	+	0	+	+	0	+	0	4
9	rr	0	+	+	0	3	0	0	+	0	0	+	+	0	0	+	4
10	rr	+	0	+	0	3	+	0	+	0	+	0	0	+	0	+	0



Your Turn!



Panel Sheet Exercise

- “IAT” = BioRad Gel IAT results
- “ENZ” = BioRad Enzyme results (papainised cells)
- Effect of Papain (enzyme panel)
 - Enhances reactions with anti-D, -C, -c, -E, -e, -C^w, -Jka, -Jkb
 - Destroys M, N, S, s, Fya, Fyb antigens

Panel 1

Instructions for use can be found at <http://www.blood.co.uk/reagents>

Panel 1

Instructions for use can be found at <http://www.blood.co.uk/reagents>

Anti-E

Panel 2

Instructions for use can be found at <http://www.blood.co.uk/reagents>

Panel 2

Instructions for use can be found at <http://www.blood.co.uk/reagents>

Anti-Fya Can't exclude anti-Kpa

Panel 3

Instructions for use can be found at <http://www.blood.co.uk/reagents>

Panel 3

Instructions for use can be found at <http://www.blood.co.uk/reagents>

Anti-Jka

Can't exclude anti-Cw

Panel 4

Instructions for use can be found at <http://www.blood.co.uk/reagents>

	Rh	C	D	E	c	e	C ^w	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															
1	R ₁ ^w R ₁	+	+	0	0	+	+	+	+	0	+	4	0	+	+	0	+	0	0	+	+	0		3	5															
2	R ₁ R ₁	+	+	0	0	+	0	+	0	+	0	3	0	0	+	0	0	+	+	0	0	+		3	5															
3	R ₂ R ₂	0	+	+	+	0	0	0	+	0	+	2	0	0	+	+	0	+	+	0	0	+		3	0															
4	r'r	+	0	0	+	+	0	0	+	0	+	2	0	0	+	0	+	0	0	+	0	+	Kna-	3	5															
5	r''r	0	0	+	+	+	0	+	0	+	0	1	0	0	+	0	+	0	0	+	+	0		0	0															
6	rr	0	0	0	+	+	0	+	0	+	0	4	0	+	0	0	0	+	+	0	0	+		0	0															
7	rr	0	0	0	+	+	0	0	+	0	+	1	0	+	+	0	0	+	+	w	0	+		3	0															
8	rr	0	0	0	+	+	0	+	+	0	+	0	0	0	+	+	+	0	+	0	+	0		3	0															
9	rr	0	0	0	+	+	0	+	0	0	+	0	+	0	0	0	+	0	+	0	+			3	0															
10	rr	0	0	0	+	+	0	0	+	+	0	3	0	0	+	0	0	+	0	+	+	0		0	0															
Reagent													DAT Profile		Anti-IgG		IgA		IgM		C3c		C3d		Ctrl															
Lot No.													Result																											

AUTO

0 /

Panel 4

Instructions for use can be found at <http://www.blood.co.uk/reagents>

Anti-C, Anti-s

Can't exclude anti-Cw, Lua, Kpa

Panel 5

	Rh	C	D	E	c	e	C ^w	M	N	S	s	P1	Lu ^a	K	k	Kp ^a	Le ^a	Le ^b	Fy ^a	Fy ^b	JK ^a	JK ^b	Other	RF			
1	R1wR1	+	+	0	0	+	+	0	+	0	+	0	0	0	+	0	+	0	0	+	0	+	Bgb+	0			
2	R1wR1	+	+	0	0	+	+	+	+	0	+	0	0	0	+	0	0	+	+	0	+	0	Cob+	3			
3	R1Rz	+	+	+	0	+	0	+	+	0	+	0	0	0	+	0	0	+	0	+	0	+	0	0	0		
4	R1Rz	+	+	+	0	+	0	0	+	0	+	4	0	0	+	0	0	+	+	0	+	0	0	0	0		
5	Ror	0	+	0	+	+	0	+	0	0	+	4	0	0	+	0	0	+	0	0	+	0	Dob- Goa+	5			
6	R1R1	+	+	0	0	+	0	0	+	+	0	1	0	+	0	0	0	0	w	0	0	+	0	0	0		
7	R1R1	+	+	0	0	+	0	+	0	0	+	0	0	+	+	0	0	+	0	+	+	0	0	3			
8	R1R1	+	+	0	0	+	0	0	+	0	+	2	0	0	+	+	0	+	+	0	+	0	+	0	0		
9	R1R1	+	+	0	0	+	0	+	0	+	0	3	0	0	+	0	+	0	0	+	0	+	0	+	3		
10	R1R1	+	+	0	0	+	0	+	+	0	+	3	+	0	+	0	0	+	+	0	0	0	+	Cob+	0		

Panel 5

Anti-M, Anti-c

**Need two M- c+ cells to confirm anti-c.
Anti-M showing dosage**

Panel 6

Instructions for use can be found at <http://www.blood.co.uk/reagents>