



Blood and Transplant

Red Cell Immunohaematology

Mark Dwight

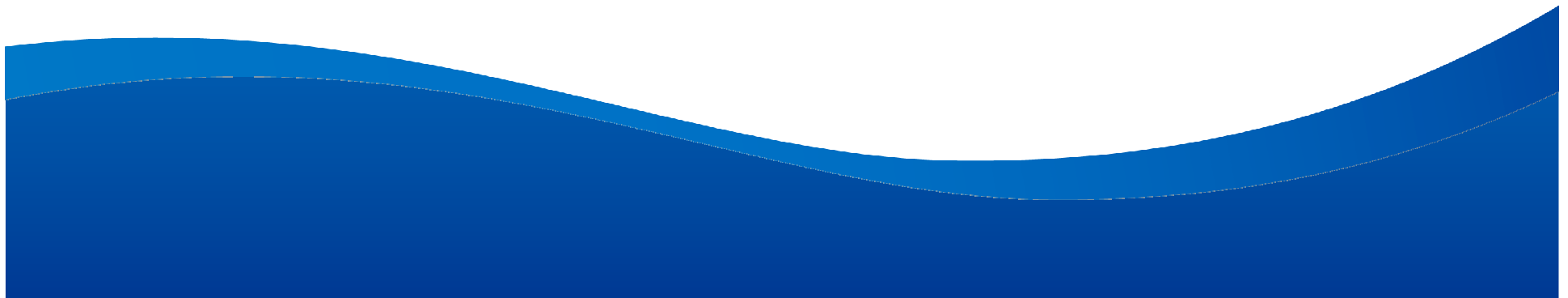
Specialist Biomedical Scientist
RCI Filton

Caring Expert Quality



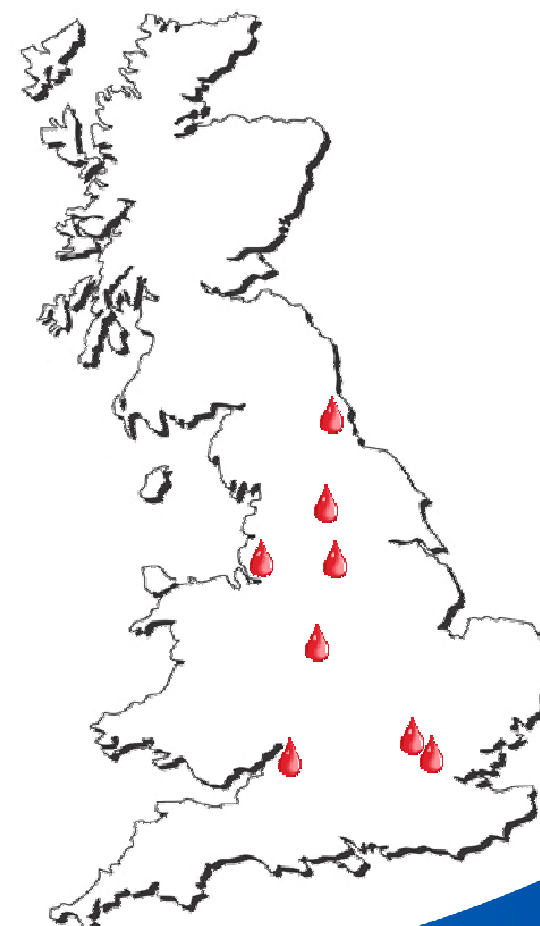
Blood and Transplant

The RCI Laboratories

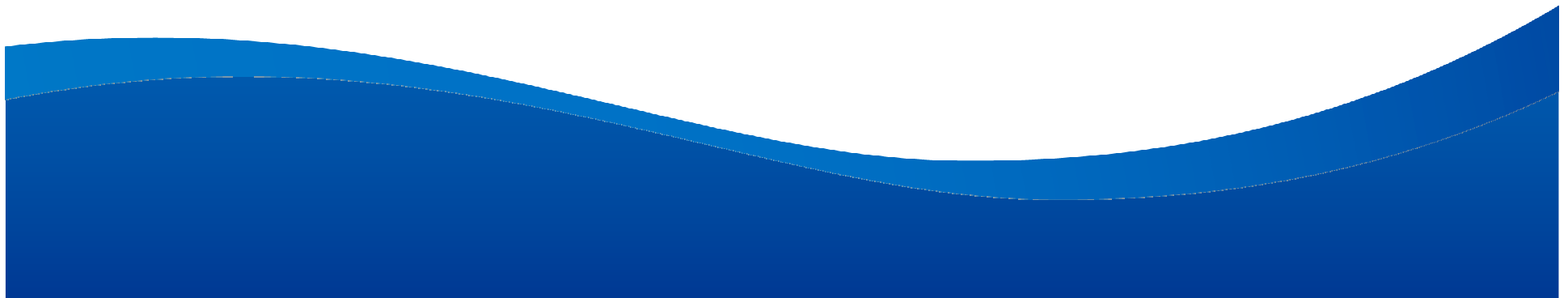


Red Cell Immunohaematology


- 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)



Antibody Investigations

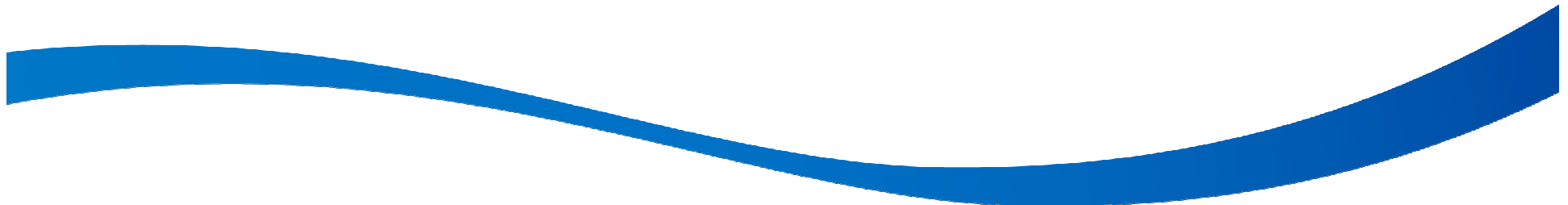


Panels and Cells


- Panel cells from NHSBT 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

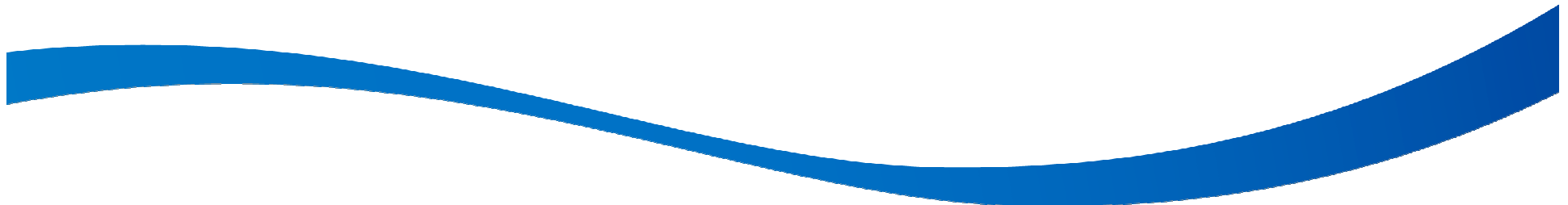


Phenotyping & Genotyping

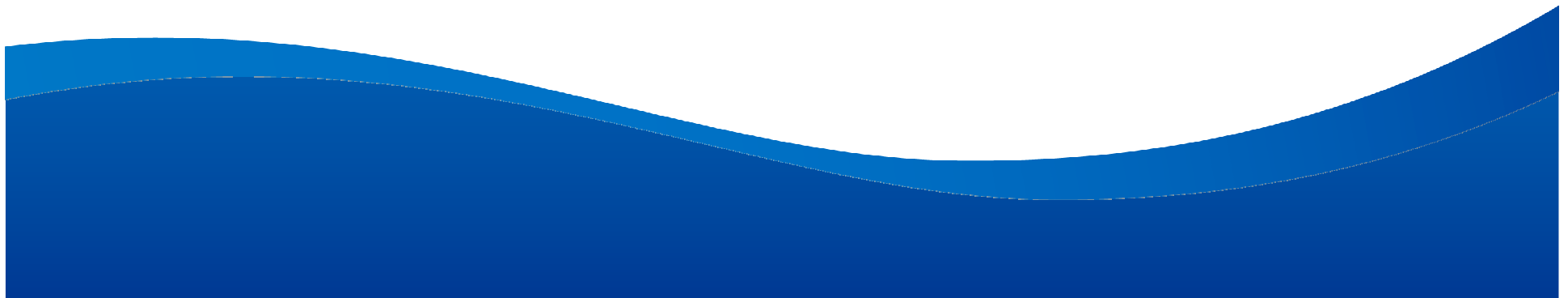
- Allo or autoantibody?
 - Phenotyping
 - Serological testing using antisera
 - Phenotyping cannot be performed if the patient has had a recent transfusion.
 - Genotyping
 - DNA Sequencing
 - Consider in: transfusion-dependent patients (eg. sickle cell), patients with multiple antibodies, before starting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

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!!!



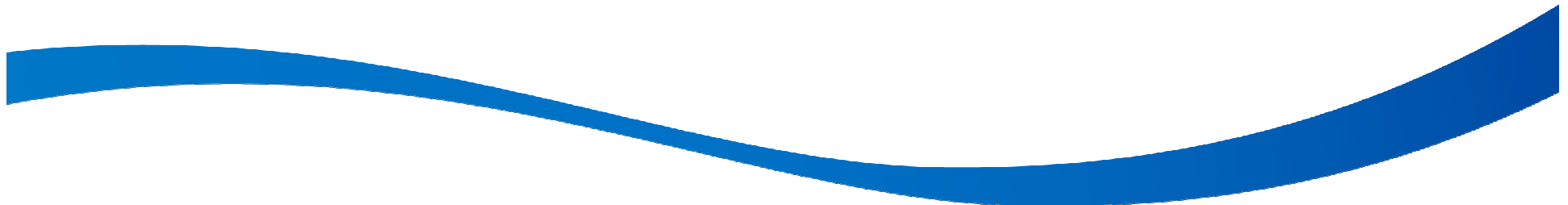
The Rules of the Game II

- 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...”



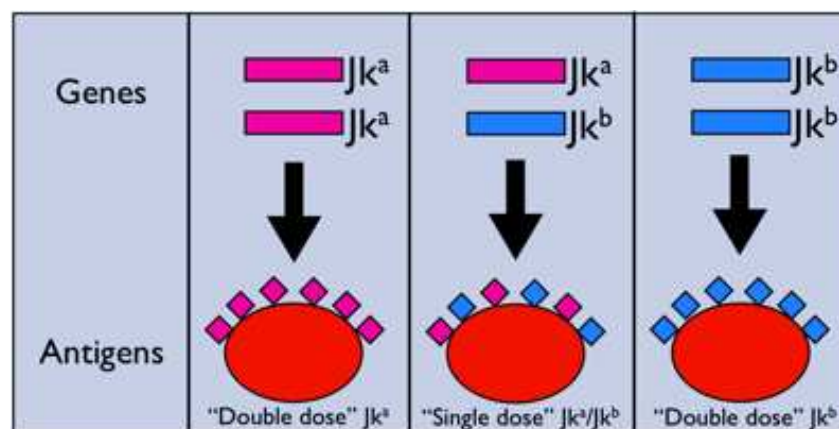
The Enzyme Panel

- 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)

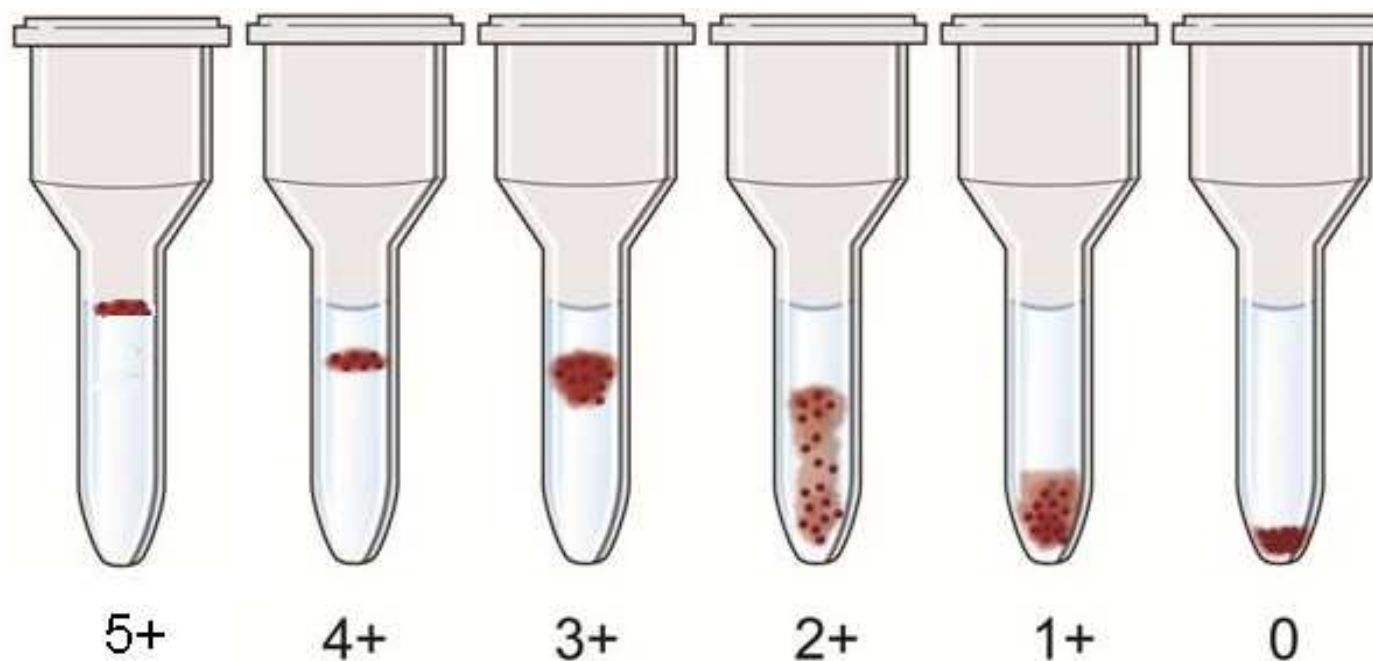


The Dosage Effect

- “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 ^w R ₁	0	+	0	+	5	0	+	+	0	0	+	0	+	0	+	0	0
2	R ₁ R ₁	+	+	+	0	0	0	0	+	0	+	0	+	0	+	0	0	4
3	R ₂ R ₂	0	+	0	+	3	0	0	+	0	+	0	0	+	+	0	0	0
4	r'r	+	0	+	0	3	0	0	+	0	0	+	0	+	+	0	0	0
5	r''r	+	0	+	0	0	0	0	+	0	0	+	+	0	0	+	0	4
6	rr	+	0	0	+	0	0	+	0	0	0	+	0	+	+	0	0	0
7	rr	+	0	+	0	0	0	+	+	0	0	0	+	0	+	0	0	4
8	rr	0	+	0	+	2	0	0	+	+	0	+	+	0	+	0	0	4
9	rr	0	+	+	0	3	0	0	+	0	0	+	+	0	0	+	0	4
10	rr	+	0	+	0	3	+	0	+	0	+	0	0	+	0	+	0	0



Interpreting Antigams

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 ^w R 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

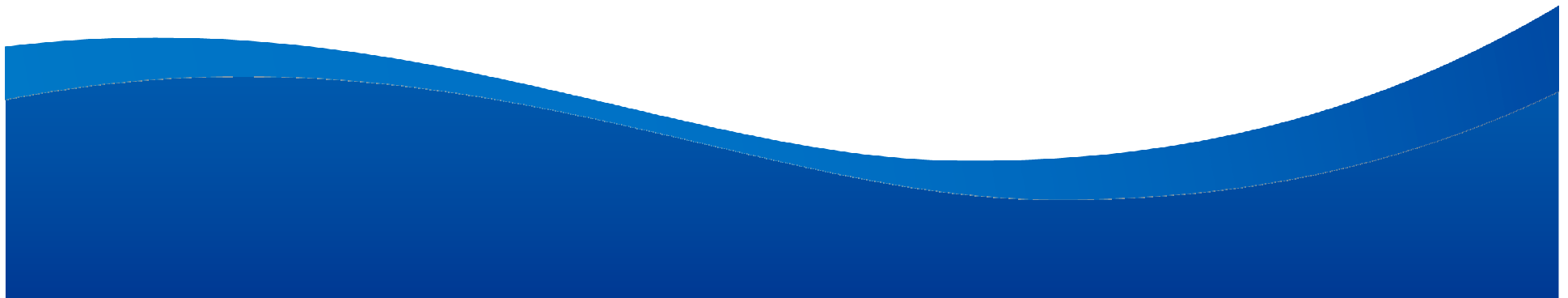
Cell	Rh	M	N	S	s	P1	Lu ^a	K	k	Kp ^a	Le ^a	Le ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b		
1	R₁wR₁	0	+	0	+	5	0	+	+	0	0	+	0	+	0	+	0	0
2	R ₁ R ₁	+	+	+	0	0	0	0	+	0	+	0	+	0	+	0	+	4
3	R₂R₂	0	+	0	+	3	0	0	+	0	+	0	0	+	+	0	+	0
4	r'r	+	0	+	0	3	0	0	+	0	0	+	0	+	+	0	+	0
5	r''r	+	0	+	0	0	0	0	+	0	0	+	+	0	0	+	+	4
6	rr	+	0	0	+	0	0	+	0	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





Blood and Transplant

Your Turn!



Panel Sheet Exercise

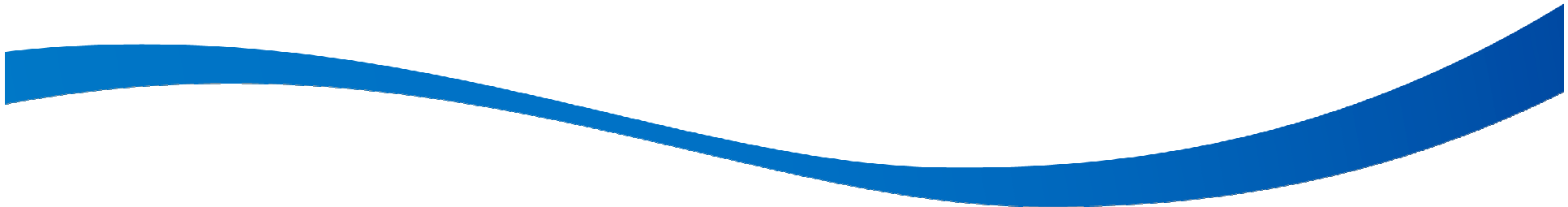
- “IAT” = BioRad Gel IAT results
- “ENZ” = BioRad Enzyme results (papainised cells)
- Effect of Papain (enzyme panel)
 - Enhances reactions with anti-D, -C, -c, -E, -e, -C^w, -Jka, -Jkb
 - Destroys M, N, S, s, Fya, Fyb antigens



Panel 1

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

	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	IRF	EN2						
1	R ₁ ^w R ₁	+	+	0	0	+	+	+	+	0	+	4	0	+	+	0	+	0	0	+	+	0		0	0						
2	R ₁ R ₁	+	+	0	0	+	0	+	0	+	0	3	0	0	+	0	0	+	+	0	0	+		0	0						
3	R ₂ R ₂	0	+	+	+	0	0	0	+	0	+	2	0	0	+	+	0	+	+	0	0	+		3	5						
4	r'r	+	0	0	+	+	0	0	+	0	+	2	0	0	+	0	+	0	0	+	0	+	Kna-	0	0						
5	r''r	0	0	+	+	+	0	+	0	+	0	1	0	0	+	0	+	0	0	+	+	0		4	5						
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	+		0	0						
8	rr	0	0	0	+	+	0	+	+	0	+	0	0	0	+	+	+	0	+	0	+	0		0	0						
9	rr	0	0	0	+	+	0	+	0	0	+	0	+	0	+	0	0	+	0	+	0	+		0	0						
10	rr	0	0	0	+	+	0	0	+	+	0	3	0	0	+	0	0	+	0	+	+	0		0	0						
																							AUTO	0	/						
Reagent																															
Lot No.																															



Panel 1

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	IRF	EN2					
1	R ₁ ^w R ₁	+	+	0	0	+	+	+	+	0	+	4	0	+	+	0	+	0	0	+	+	0		0	0					
2	R ₁ R ₁	+	+	0	0	+	0	+	0	+	0	3	0	0	+	0	0	+	+	0	0	+		0	0					
3	R ₂ R ₂	0	+	+	+	0	0	0	+	0	+	2	0	0	+	+	0	+	+	0	0	+		3	5					
4	r'r	+	0	0	+	+	0	0	+	0	+	2	0	0	+	0	+	0	0	+	0	+	Kna-	0	0					
5	r''r	0	0	+	+	+	0	+	0	+	0	1	0	0	+	0	+	0	0	+	+	0		4	5					
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	+		0	0					
8	rr	0	0	0	+	+	0	+	+	0	+	0	0	0	+	+	+	0	+	0	+	0		0	0					
9	rr	0	0	0	+	+	0	+	0	0	+	0	+	0	+	0	0	+	0	+	0	+		0	0					
10	rr	0	0	0	+	+	0	0	+	+	0	3	0	0	+	0	0	+	0	+	+	0		0	0					
																							AUTO	0	/					
Reagent																														
Lot No.																														

Anti-E

Panel 3

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	END	IAT IS ELUOPE
1	R ₁ ^w R ₁	+	+	0	0	+	+	+	+	0	+	4	0	+	+	0	+	0	0	+	+	0		0	0	1
2	R ₁ R ₁	+	+	0	0	+	0	+	0	+	0	3	0	0	+	0	0	+	+	0	0	+		0	0	0
3	R ₂ R ₂	0	+	+	+	0	0	0	+	0	+	2	0	0	+	+	0	+	+	0	0	+		0	0	0
4	r'r	+	0	0	+	+	0	0	+	0	+	2	0	0	+	0	+	0	0	+	0	+	Kna-	0	0	0
5	r''r	0	0	+	+	+	0	+	0	+	0	1	0	0	+	0	+	0	0	+	+	0		0	0	1
6	rr	0	0	0	+	+	0	+	0	+	0	4	0	+	0	0	0	+	+	0	0	+		0	0	0
7	rr	0	0	0	+	+	0	0	+	0	+	1	0	+	+	0	0	+	+	w	0	+		0	0	0
8	rr	0	0	0	+	+	0	+	+	0	+	0	0	0	+	+	+	0	+	0	+	0		0	0	1
9	rr	0	0	0	+	+	0	+	0	0	+	0	+	0	+	0	0	+	0	+	0	+		0	0	0
10	rr	0	0	0	+	+	0	0	+	+	0	3	0	0	+	0	0	+	0	+	+	0		0	0	1
																							Auto	3	/	/
Reagent												DAT Profile	Anti-IgG	IgA	IgM	C3c	C3d	Ctrl								
Lot No.												Result	3mf	0	0	0	0	0								

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				
																							Au.D	0	/				
Reagent														DAT Profile	Anti-IgG	IgA	IgM	C3c	C3d	Ctrl									
Lot No.														Result															

Anti-C, Anti-s
Can't exclude anti-Cw, Lua, Kpa

References & Photo Credits

- Papaya: <http://mightymacadirect.com/media/sites/173/images/maca8.png>
- Dosage: <http://www.bbguy.org/wp-content/uploads/2016/03/Dosageexample.png>
- Pipetting: http://www.ztm.si/uploads/IMG_0939.jpg
- Giant Microbes Erythrocyte: <http://dkleeklie156t.cloudfront.net/cdn/142231/media/catalog/product/cache/2/image/1012.5x879/9df78eab33525d08d6e5fb8d27136e95/r/e/redbloodcell.jpg>

