



Blood and Transplant

Red Cell Antibody Panels

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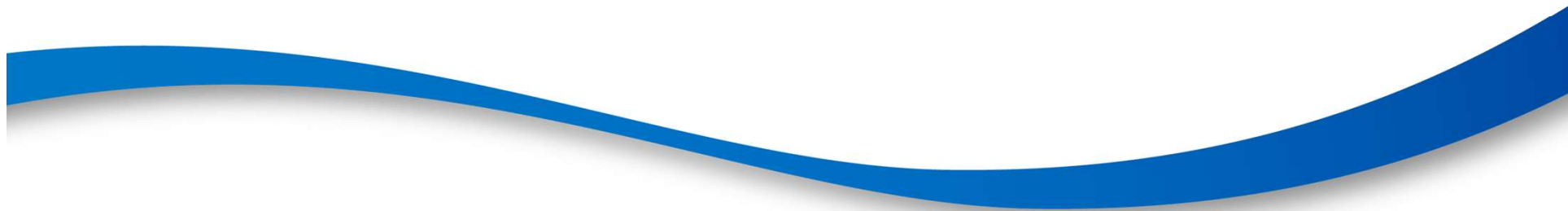
Caring Expert Quality

Interpreting Antigrams



The Rules of the Game I

- BSH Guidelines for pre-transfusion compatibility procedures in blood transfusion laboratories (2012).
- 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

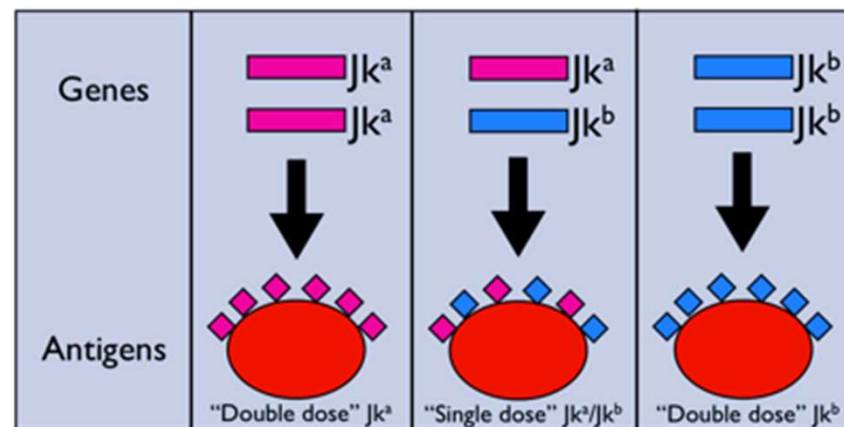
- Papain most commonly used (from Papaya) *
- Papain enhances the reaction strength of
 - Rh system antibodies (anti-D, -C, -c, -E, -e, -Cw)
 - Kidd system antibodies (anti-Jka, -Jkb)
- Papain destroys some red cell antigens; so negative reactions will be observed with
 - MNS system antibodies (anti-M,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



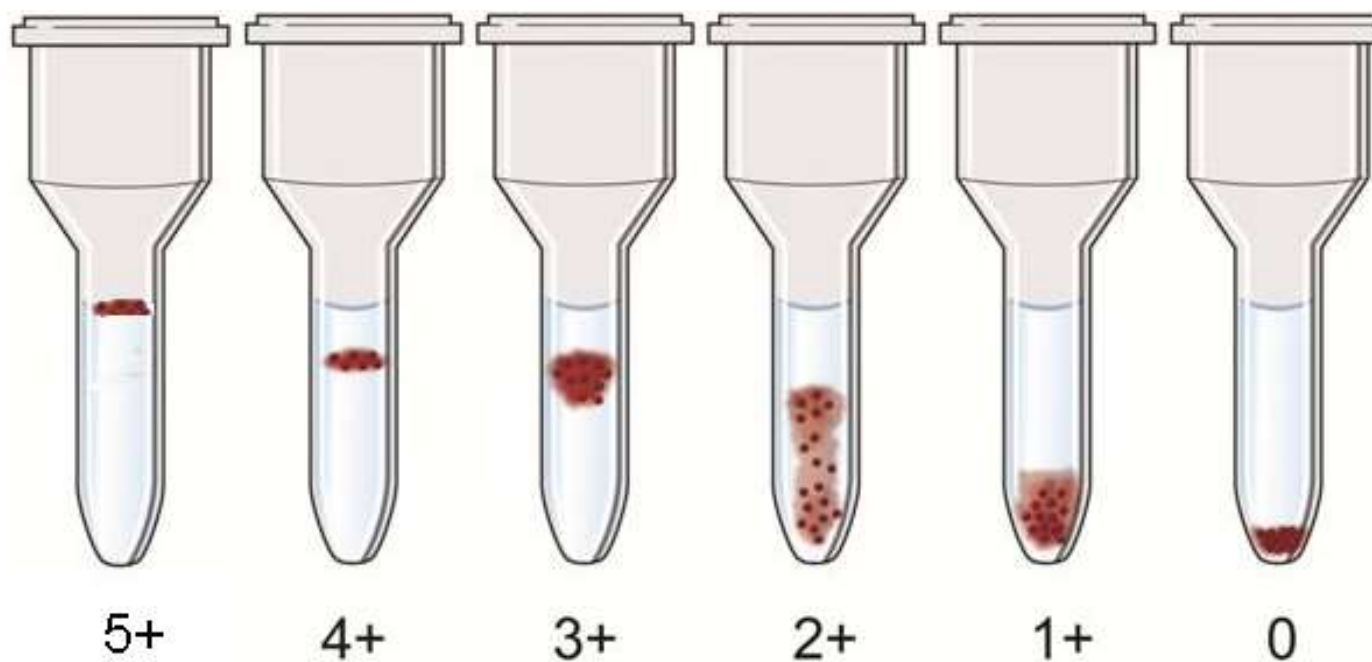
* Ortho BioVue panels may use ficin

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 in RCI



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 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 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	0	4
3	R₂R₂	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





Blood and Transplant

Your Turn!



Panel Sheet Exercise

- Key
 - “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
- NOTE: Case E has two panel sheets!





Blood and Transplant

Answers



Case A

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	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.																																			

Case B

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	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	+		3	0							
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-	0	0							
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	+		3	0							
7	rr	0	0	0	+	+	0	0	+	0	+	1	0	+	+	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	+		0	0							
10	rr	0	0	0	+	+	0	0	+	+	0	3	0	0	+	0	0	+	0	+	+	0		0	0							
																							ALTO	0	/							
Reagent																																
Lot No.																																

Anti-Fya
Can't exclude anti-Kpa

Case E: 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	19F	Enz										
1	R ₁ ^w R ₁	+	+	0	0	+	+	0	+	0	+	2	0	0	+	0	+	0	+	0	+	0	Cob+	0	0										
2	R ₁ R ₁	+	+	0	0	+	0	+	0	+	0	0	0	+	+	0	0	+	0	+	0	+		3	0										
3	R ₂ R ₂	0	+	+	+	0	0	0	+	0	+	2	0	0	+	0	0	0	+	0	+	0	HLA +	5	5										
4	r'r	+	0	0	+	+	0	+	0	0	+	0	0	0	+	0	0	+	0	+	+	0		5	5										
5	r'r	0	0	+	+	+	0	+	0	+	0	3	0	0	+	0	0	+	0	+	0	+		5	5										
6	rr	0	0	0	+	+	0	+	+	0	+	4	0	+	0	0	0	+	0	+	+	0		5	5										
7	rr	0	0	0	+	+	0	0	+	0	+	4	0	+	+	0	+	0	+	0	0	+		5	5										
8	rr	0	0	0	+	+	0	0	+	0	+	4	+	0	+	+	0	0	+	0	0	+		5	5										
9	rr	0	0	0	+	+	0	0	+	+	0	0	0	0	+	0	0	+	w	0	+	0		5	5										
10	rr	0	0	0	+	+	0	+	0	+	0	4	+	0	+	0	+	0	+	0	+	0	Cob+	5	5										
																							Auto	0	/										

Case F: 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	APT	CMZ										
1	R ₁ ^w R ₁	+	+	0	0	+	+	+	0	+	0	0	0	+	+	0	+	0	0	+	0	+		3	5										
2	R ₁ R ₁	+	+	0	0	+	0	0	+	0	+	0	0	0	+	0	0	+	+	0	+	0		3	5										
3	R ₂ R ₂	0	+	+	+	0	0	+	0	+	0	2	0	0	+	0	+	0	+	0	0	+	HLA+	3	5										
4	r'r	+	0	0	+	+	0	0	+	0	+	0	0	0	+	0	+	0	+	+	+	0		3	5										
5	r''r	0	0	+	+	+	0	0	+	0	+	4	+	0	+	0	0	+	0	+	+	0		3	5										
6	rr	0	0	0	+	+	0	+	0	+	0	1	0	+	0	0	0	+	+	0	+	0		3	5										
7	rr	0	0	0	+	+	0	0	+	0	+	3	0	+	+	0	+	0	0	+	+	0	Cob+	3	5										
8	rr	0	0	0	+	+	0	0	+	0	+	0	0	0	+	+	0	+	+	0	+	0		3	5										
9	rr	0	0	0	+	+	0	+	0	0	+	2	+	0	+	0	0	+	0	+	0	+		3	5										
10	rr	0	0	0	+	+	0	0	+	+	0	3	0	0	+	0	+	0	+	0	0	+	Cob+	3	5										
																							Auto	3											



Blood and Transplant

Red Cell Immunohaematology

Mark Dwight MSc CSci MIBMS

Advanced Specialist Biomedical Scientist
RCI Filton

Caring Expert Quality



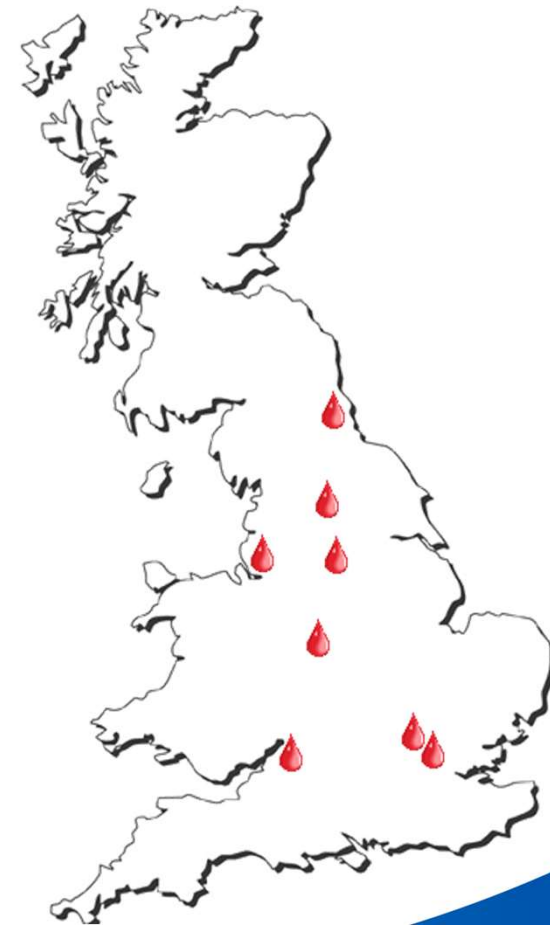
Blood and Transplant

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)



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 
Red Cell Immunohaematology 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.	<input type="text"/>	<input type="text"/>	<input type="text"/>
Surname	<input type="text"/>		
First name	<input type="text"/>		
DOB	<input type="text"/>	Gender (MF) <input type="checkbox"/>	Ethnic origin code (see reverse) <input type="checkbox"/>
First line of address	<input type="text"/>		
Post code	<input type="text"/>	EDD (if pregnant) <input type="checkbox"/>	<input type="text"/>
Hospital number	<input type="text"/>	Hospital sample ID number <input type="text"/>	

HOSPITAL & REQUESTER DETAILS	
Full Hospital name	<input type="text"/>
Hospital (H&H code)	<input type="text"/> Consultant <input type="text"/>
Contact name	<input type="text"/>
Phone	<input type="text"/> Bleep <input type="text"/>



Blood and Transplant

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 freq negatives, eg. K+k-, U-, Vel-
 - Low freq positives, eg. Wr(a+), Js(a+), Di(a+)



Serological Techniques

- A variety of techniques are 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



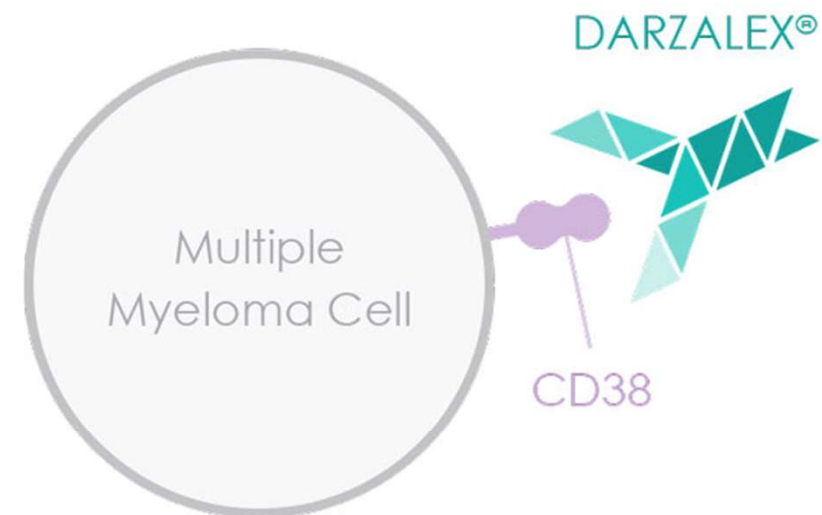
Phenotyping & Genotyping

- 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)



Thank you!

