ABID: Tools of the trade

WEBINAR 1/3

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Antibody Identification

Session 1/3 - Tools of the trade

Helen Thom

Today’s session overview

- Understand the basic principles of antibody identification (ABID)
- Identify key testing tools: panels, IAT/enzyme, DAT, elution
- Use inclusion/exclusion methods to interpret panel results
- Role of red cell phenotyping and genotyping in ABID

Why is ABID so important?

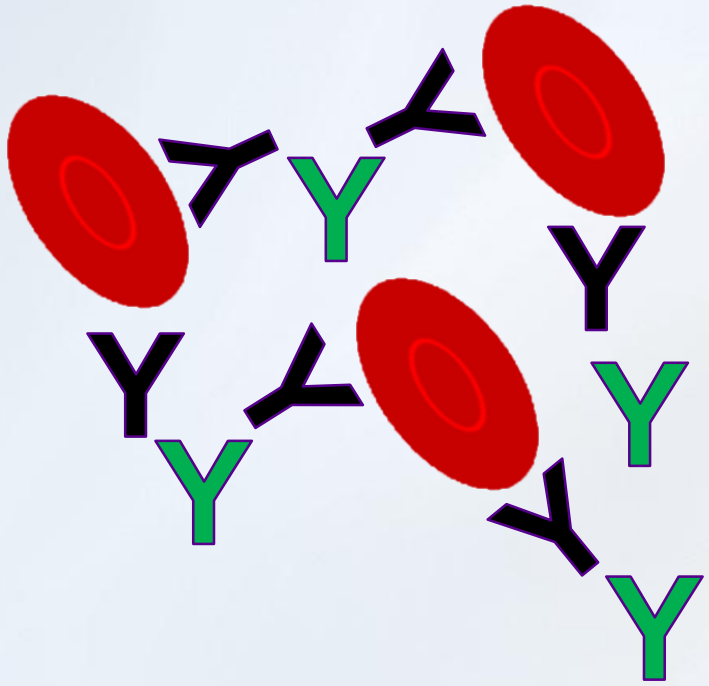
- Multiple blood group antigens
- Not just ABO and RhD
- Red cell antibodies are produced through either pregnancy, transfusion or transplantation
- Antibodies can be clinically significant
- Can cause delayed and immediate Haemolytic Transfusion Reactions (HTR)

The basic principles of ABID

- Unknown antibody with known antigen – ABID
- Or known antibody with unknown antigen – grouping/phenotyping
- Antibody will bind with its corresponding antigen

https://www.google.com/url?sa=i&url=https%3A%2F%2Fspongebob.fandom.com%2Fwiki%2FChum_Bucket&psig

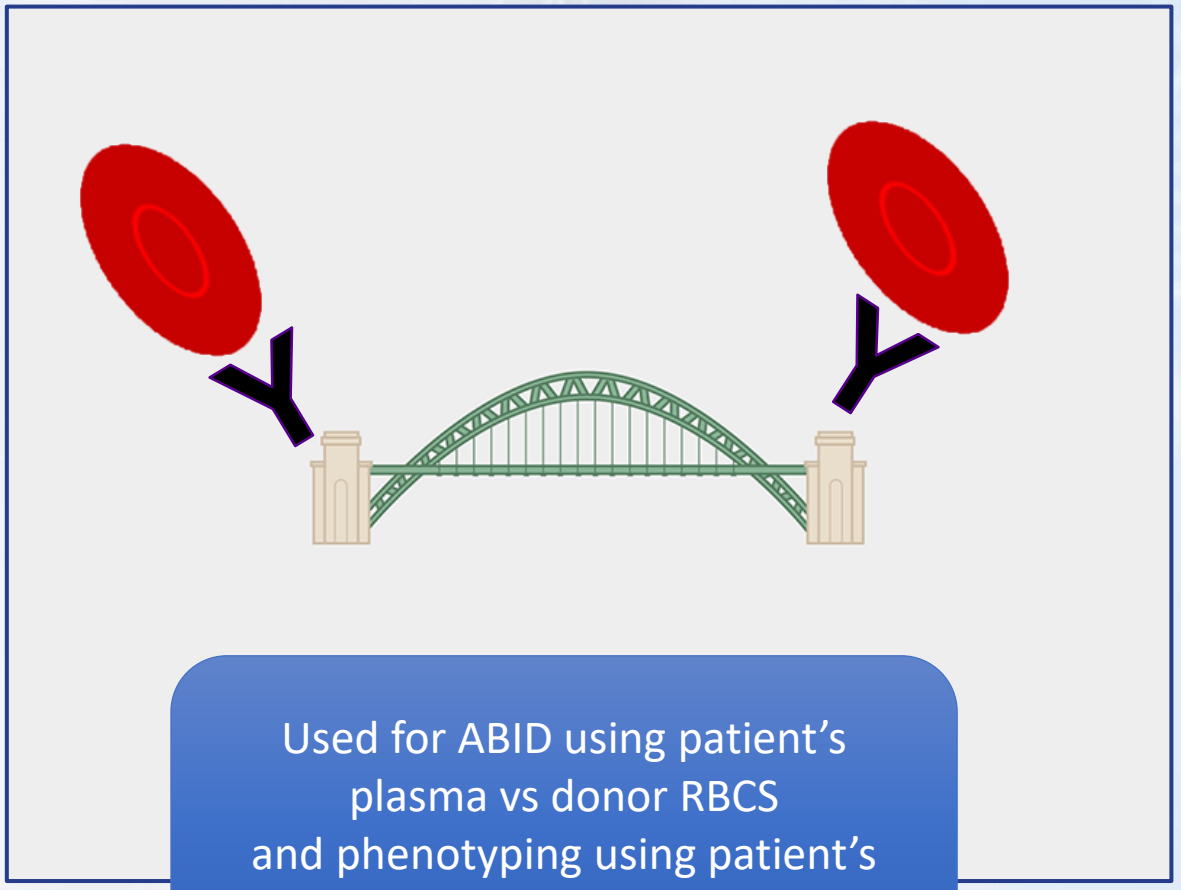
Indirect Antiglobulin Test (IAT)



IgG cannot bridge the gap between RBCs

Anti-IgG

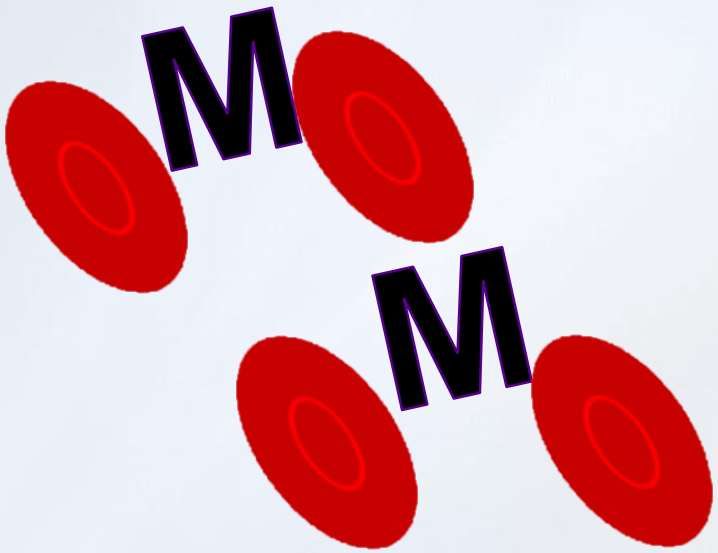
AHG – Anti Human Globulin (IgG and C3d)



Used for ABID using patient's plasma vs donor RBCS and phenotyping using patient's RBCs and IgG anti-sera

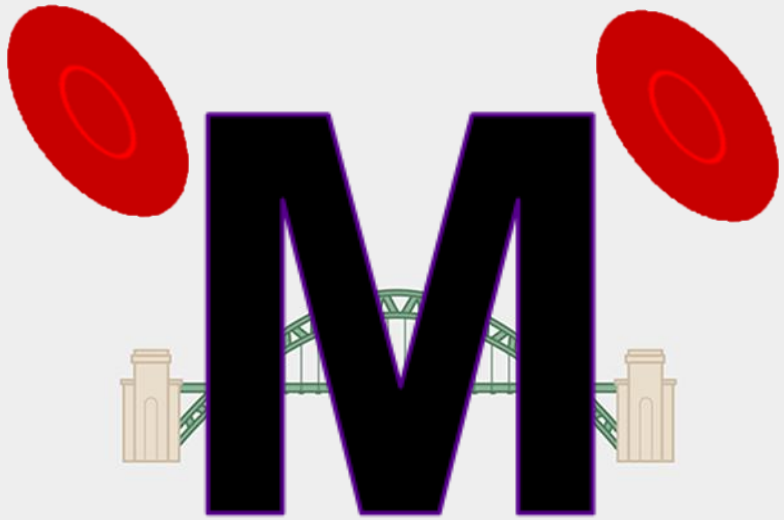
https://dribbble.com/shots/3376611-Tyne-Bridge-Animated

Direct Agglutination



IgM can bridge the gap between RBCs

Does not require AHG



Used for blood grouping, phenotyping using IgM anti-sera and ‘cold’ antibodies

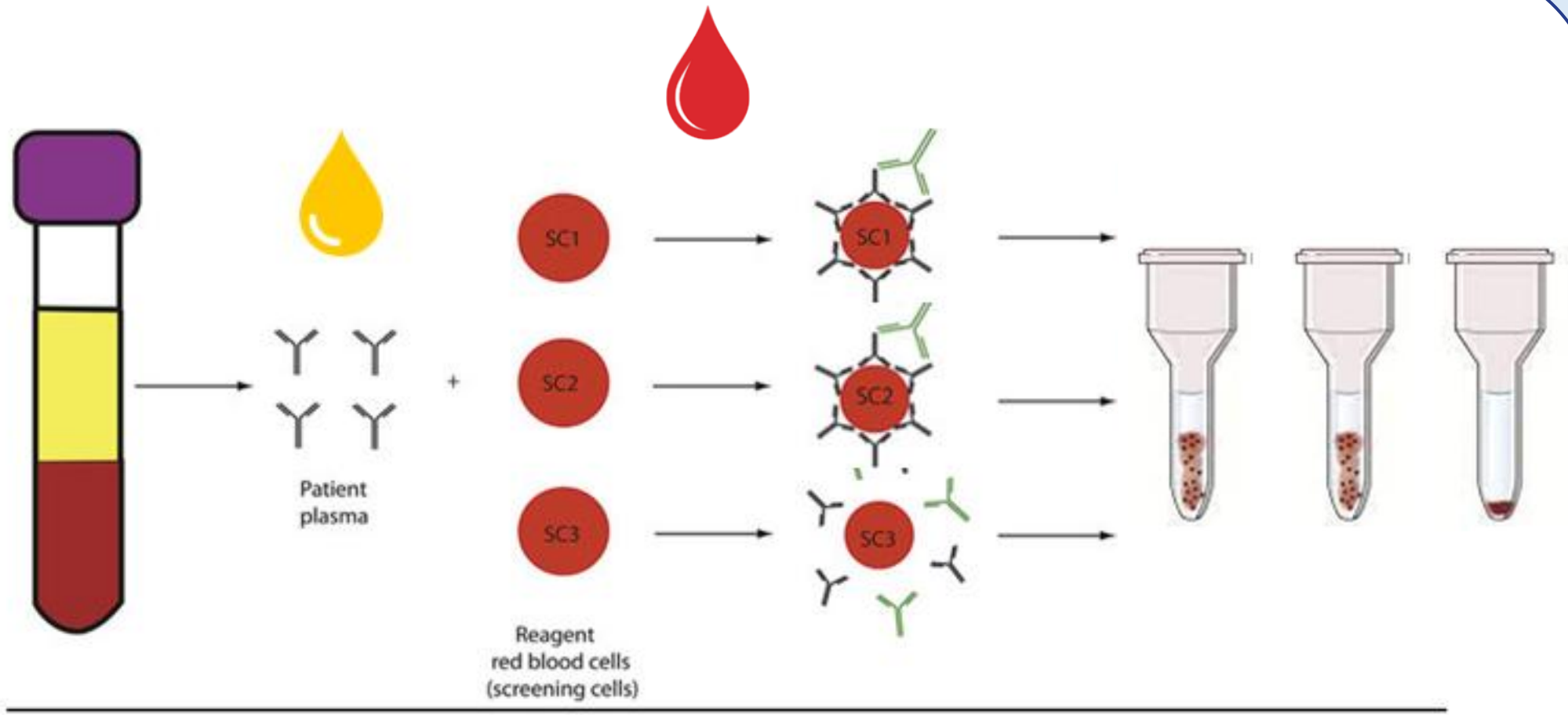
Can you think of some of the tools at our disposal when performing ABID?

Key Testing Tools

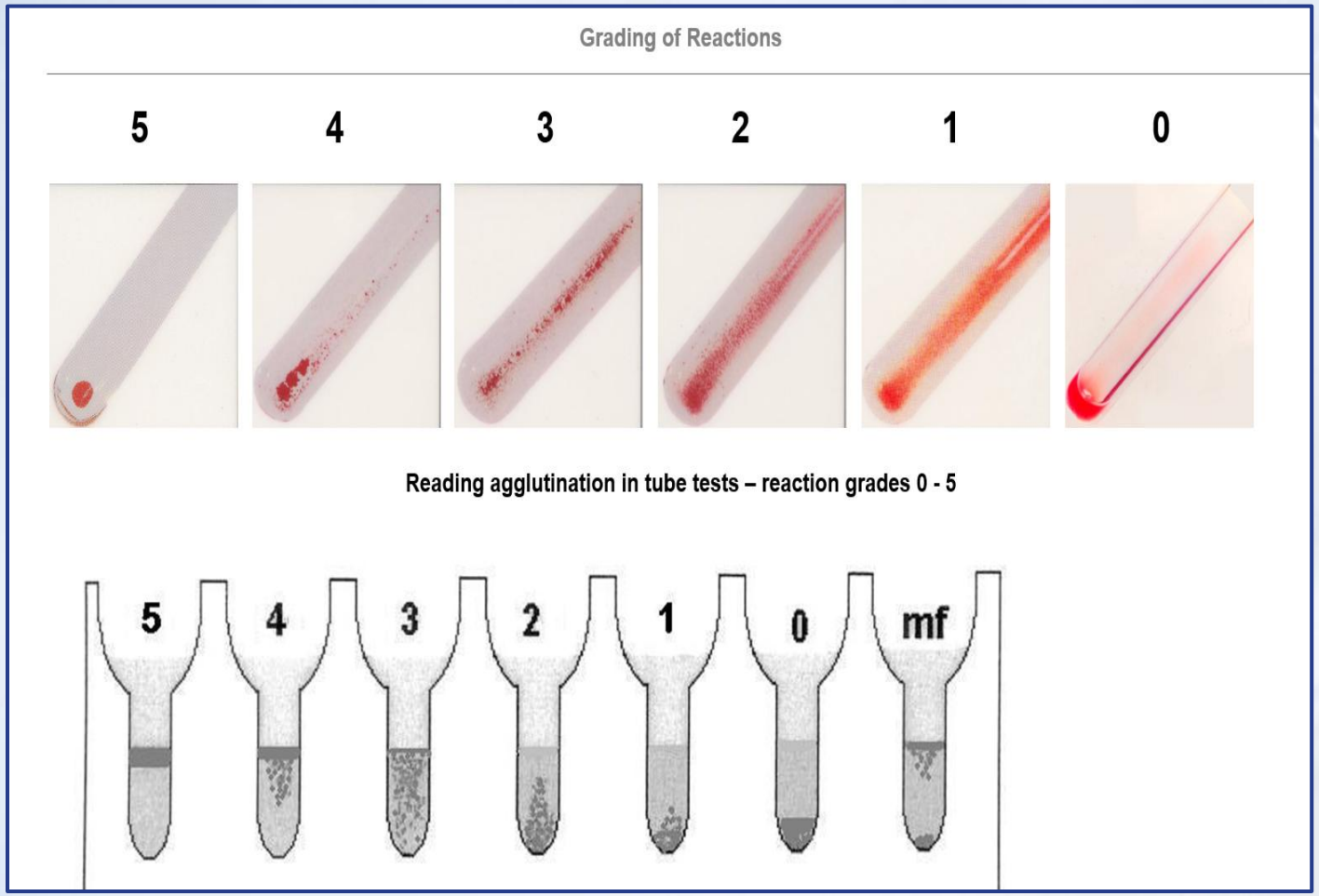
- Patient history
- Antibody screen
- Grading
- Antibody identification
- Inclusions and exclusions
- Antibody panel cells (IAT and enzyme)
- Antigram (panel sheet)
- Clinical significance of antibodies
- Phenotyping/genotyping
- Direct antiglobulin test
- Elution

Antibody Identification: screen

Antibody Identification: screen



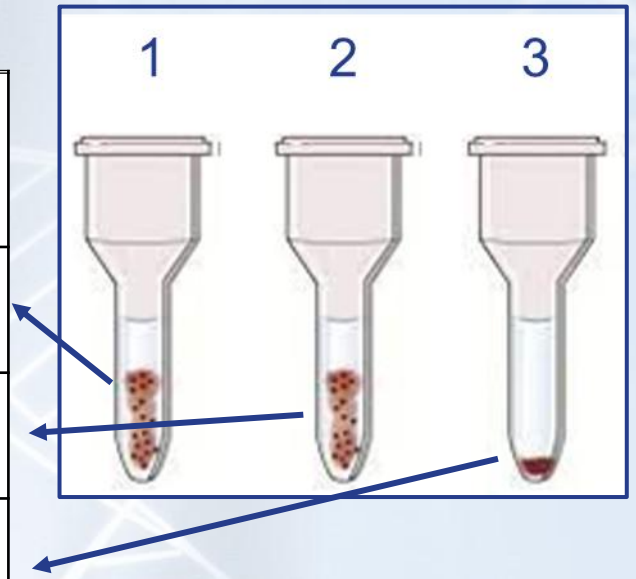
Grading



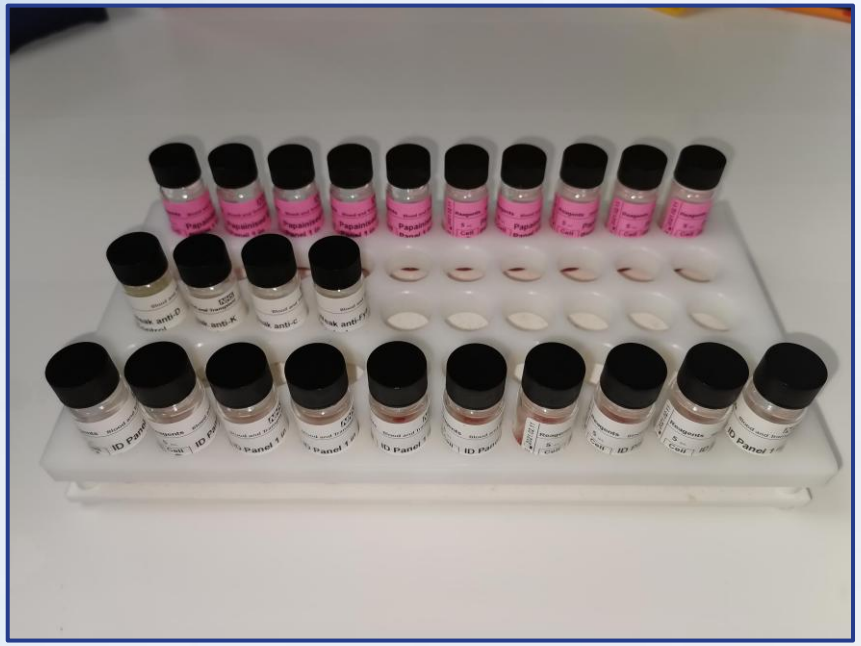
- Can assist with ABID
- Multiple antibodies
- Enhanced reactions
- Dosage

Antibody Identification: screen

Cell	Rh	D	C	E	c	e	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
1	$R_1^wR_1$	+	+	0	0	+	+	0	+	0	0	0	0	+	0	0	+	+	0	+	0		3
2	R_2R_2	+	0	+	+	0	+	0	+	0	0	0	0	+	0	+	0	0	+	+	0		3
3	rr	0	0	0	+	+	+	0	+	0	4	0	+	0	0	0	+	+	0	0	+		0



Panel Cells



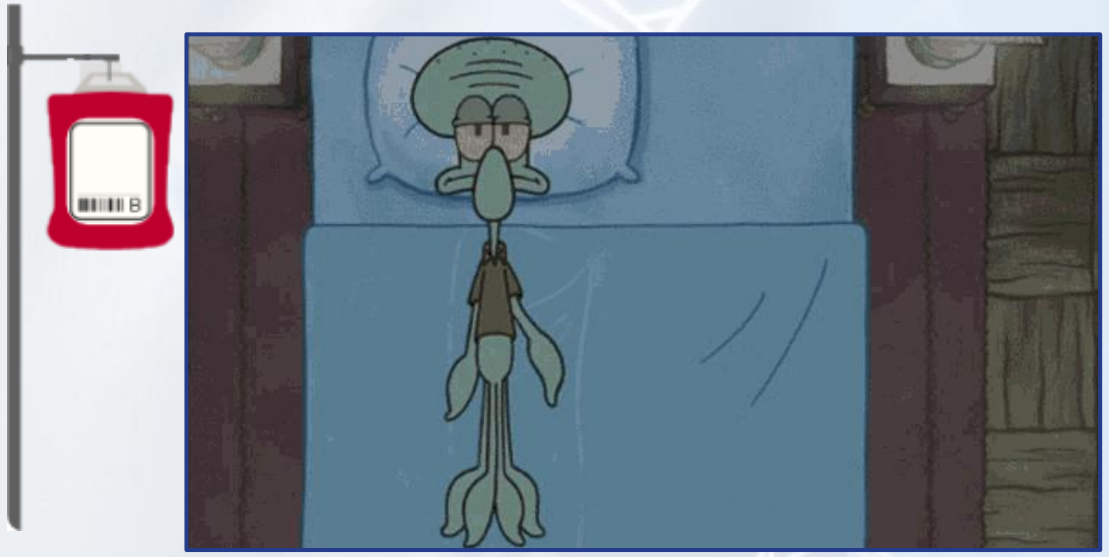
https://b-s-h.org.uk/guidelines/guidelines/pre-transfusion-compatibility-procedures-in-blood-transfusion-laboratories/

- Panels consist of red cells from eight or more group O donors
- For commonly encountered clinically significant alloantibodies: 2 antigen pos and 2 antigen neg cells
- 1 x R1R1 (CCDDee) and 1 x R1wR1 (CwCDDee)
- Between them, these two cells should express the antigens K, k, Fya, Fyb, Jka, Jkb, S, s
- One example of each of the phenotypes R2R2 (ccDDEE), r′r (Ccddee) and r″r (ccddEe)
- At least three examples of the phenotype rr (ccddee), including at least one K+, and collectively, homozygous expression of k, Jka, Jkb, S, s, Fya, and Fyb
- Papainised cells

Antibody Exclusions

- 6.2.5.When one antibody specificity has been identified, it is essential that the presence or absence of additional clinically significant antibodies is established
- 6.2.6.Failure to recognise all of the antibody specificities within a sample may lead to a haemolytic transfusion reaction (SHOT, 1996–2010). In particular, the presence of anti-Jk^a, anti-Jk^b, anti-S, anti-s, anti-Fy^a and anti-Fy^b should be excluded using red cells having homozygous expression of the relevant antigen
- A single example only of each phenotype is sufficient for exclusion

I have a feeling of impending doom!



https://b-s-h.org.uk/guidelines/guidelines/pre-transfusion-compatibility-procedures-in-blood-transfusion-laboratories/

https://tenor.com/view/sleep-roll-alone-bed-squidward-gif-15576803

What do we mean by ‘homozygous’ expression of the relevant antigen?

- The panel cell shows double dose expression
- The panel cell shows single dose expression
- Not sure

What do we mean by ‘homozygous’ expression of the relevant antigen?

- **The panel cell shows double dose expression** – E.g. Jk(a+b-) – stronger reaction

Cell	Rh	D	C	E	c	e	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	$R_1^wR_1$	+	+	0	0	+	+	0	+	0	**0**	**0**	**0**	+	**0**	**0**	+			+	**0**		2	3
2	R_1R_1	+	+	0	0	+	0	+	0	+	**0**	**0**	+	+	**0**	+	**0**	**0**	+	**0**	+		0	0
3	R_2R_2	+	0	+	+	0	+	0	+	0	**0**	**0**	**0**	+	**0**	+	**0**			+	+		0	1

- When the panel cell shows single dose expression, this is heterozygous expression

  E.g. Jk(a+b+) – weaker reaction

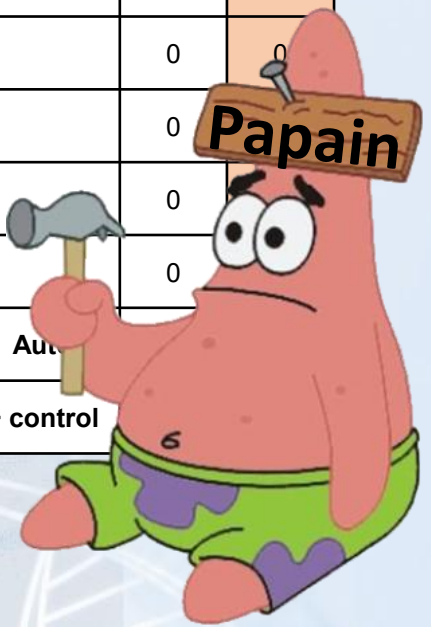
Antibody identification: panels

- IAT
- None enzyme treated cells
- Enzyme treated cells – papainised
- ‘Cold’

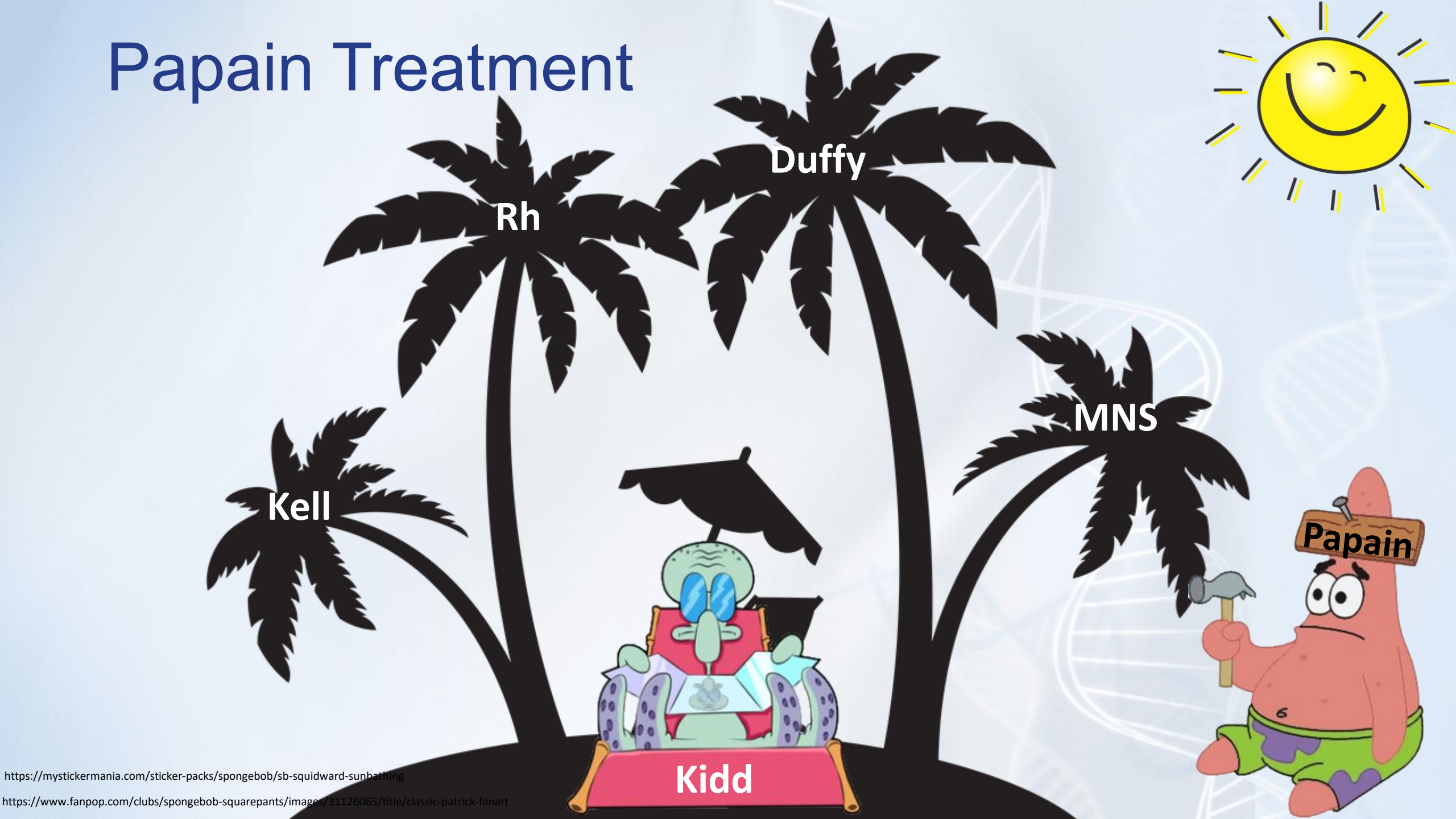
Panel sheet

Red Cells from 8 or more group O donors and full phenotype for each

Cell	Rh	D	C	E	c	e	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	EIAT
1	$R_1^wR_1$	+	+	0	0	+	+	0	+	0	0	0	0	+	0	0	+	+	0	+	0		4	5
2	R_1R_1	+	+	0	0	+	0	+	0	+	0	0	+	+	0	+	0	0	+	0	+		4	5
3	R_2R_2	+	0	+	+	0	+	0	+	0	0	0	0	+	0	+	0	0	+	+	0		4	5
4	r'r	0	+	0	+	+	0	+	+	0	0	0	0	+	0	0	+	0	+	+	0		0	0
5	r''r	0	0	+	+	+	0	+	0	+	4	0	0	+	0	0	+	+	0	0	+		0	0
6	rr	0	0	0	+	+	+	0	+	0	4	0	+	0	0	0	+	+	0	0	+		0	0
7	rr	0	0	0	+	+	0	+	0	+	2	+	+	+	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	+	2	0	0	+	0	+	0	+	0	0	+		0	
10	rr	0	0	0	+	+	0	+	0	+	3	0	0	+	0	+	0	+	0	+	0		0	
																						Aut		
																						+ control		



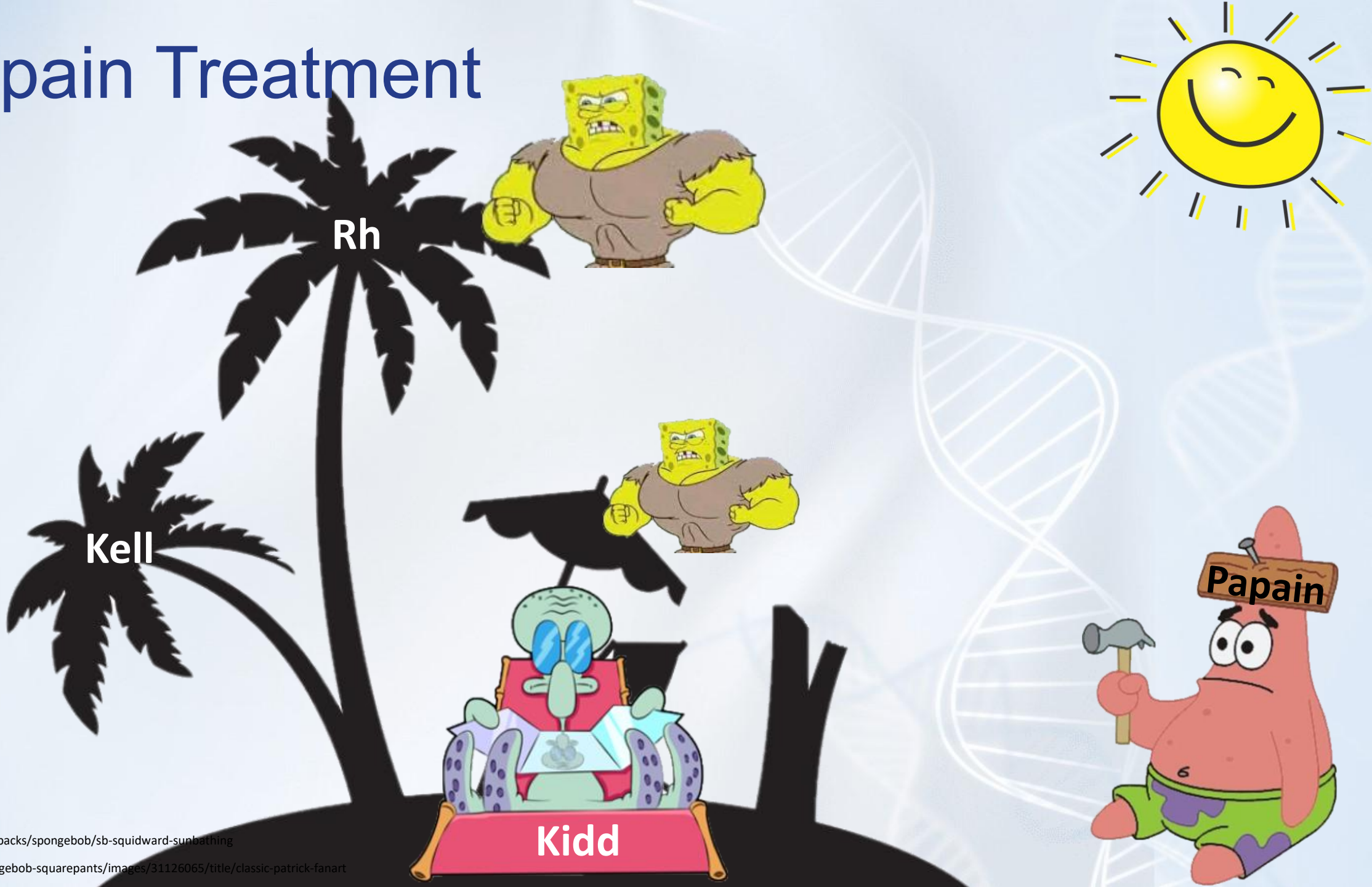
Papain Treatment



https://mystickermania.com/sticker-packs/spongebob/sb-squidward-sunbathing

https://www.fanpop.com/clubs/spongebob-squarepants/images/31126065/title/classic-patrick-fanart

Papain Treatment



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https://www.fanpop.com/clubs/spongebob-squarepants/images/31126065/title/classic-patrick-fanart

Antigram example - exclusions

Cell	Rh	D	~~C~~	~~E~~	~~c~~	~~e~~	~~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	EIAT
1	$R_1^wR_1$	+	+	0	0	+	+	0	+	0	0	0	0	+	0	0	+	+	0	+	+		4	5
2	R_1R_1	+	+	0	0	+	0	+	0	+	0	0	+	+	0	+	0	0	+	0	+		4	5
3	R_2R_2	+	0	+	+	0	+	0	+	0	0	0	0	+	0	+	0	0	+	+	0		4	5
4	r'r	0	+	0	+	+	0	+	+	0	0	0	0	+	0	0	+	0	+	+	0		0	0
5	r''r	0	0	+	+	+	0	+	0	+	4	0	0	+	0	0	+	+	0	0	+		0	0
6	rr	0	0	0	+	+	+	0	+	0	4	0	+	0	0	0	+	+	0	0	+		0	0
7	rr	0	0	0	+	+	0	+	0	+	2	+	+	+	0	+	0	0	+	+	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	+	2	0	0	+	0	+	0	+	0	0	+		0	0
10	rr	0	0	0	+	+	0	+	0	+	3	0	0	+	0	+	0	+	0	+	0		0	0
																						Auto	0	/
																						+ control	2	/

75yo male. Transfused 6 years ago

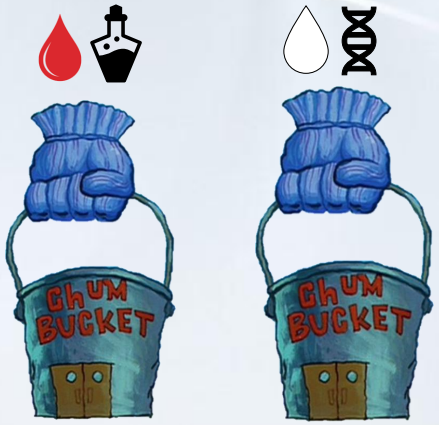
Antibody inclusion

Cell	Rh	D	~~C~~	~~E~~	~~c~~	~~e~~	~~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	EIAT
1	$R_1^wR_1$	+	+	0	0	+	+	0	+	0	0	0	0	+	0	0	+	+	0	+	0		4	5
2	R_1R_1	+	+	0	0	+	0	+	0	+	0	0	+	+	0	+	0	0	+	0	+		4	5
3	R_2R_2	+	0	+	+	0	+	0	+	0	0	0	0	+	0	+	0	0	+	+	0		4	5
4	r'r	0	+	0	+	+	0	+	+	0	0	0	0	+	0	0	+	0	+	+	0		0	0
5	r''r	0	0	+	+	+	0	+	0	+	4	0	0	+	0	0	+	+	0	0	+		0	0
6	rr	0	0	0	+	+	+	0	+	0	4	0	+	0	0	0	+	+	0	0	+		0	0
7	rr	0	0	0	+	+	0	+	0	+	2	+	+	+	0	+	0	0	+	+	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	+	2	0	0	+	0	+	0	+	0	0	+		0	0
10	rr	0	0	0	+	+	0	+	0	+	3	0	0	+	0	+	0	+	0	+	0		0	0
																						Auto	0	/
																						+ control	2	/

75yo male. Transfused 6 years ago

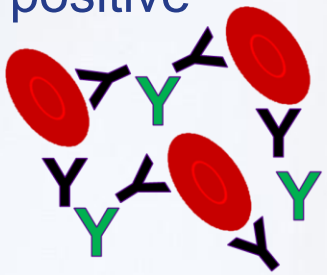
What is the patient's phenotype?

Pheno vs geno



Phenotype

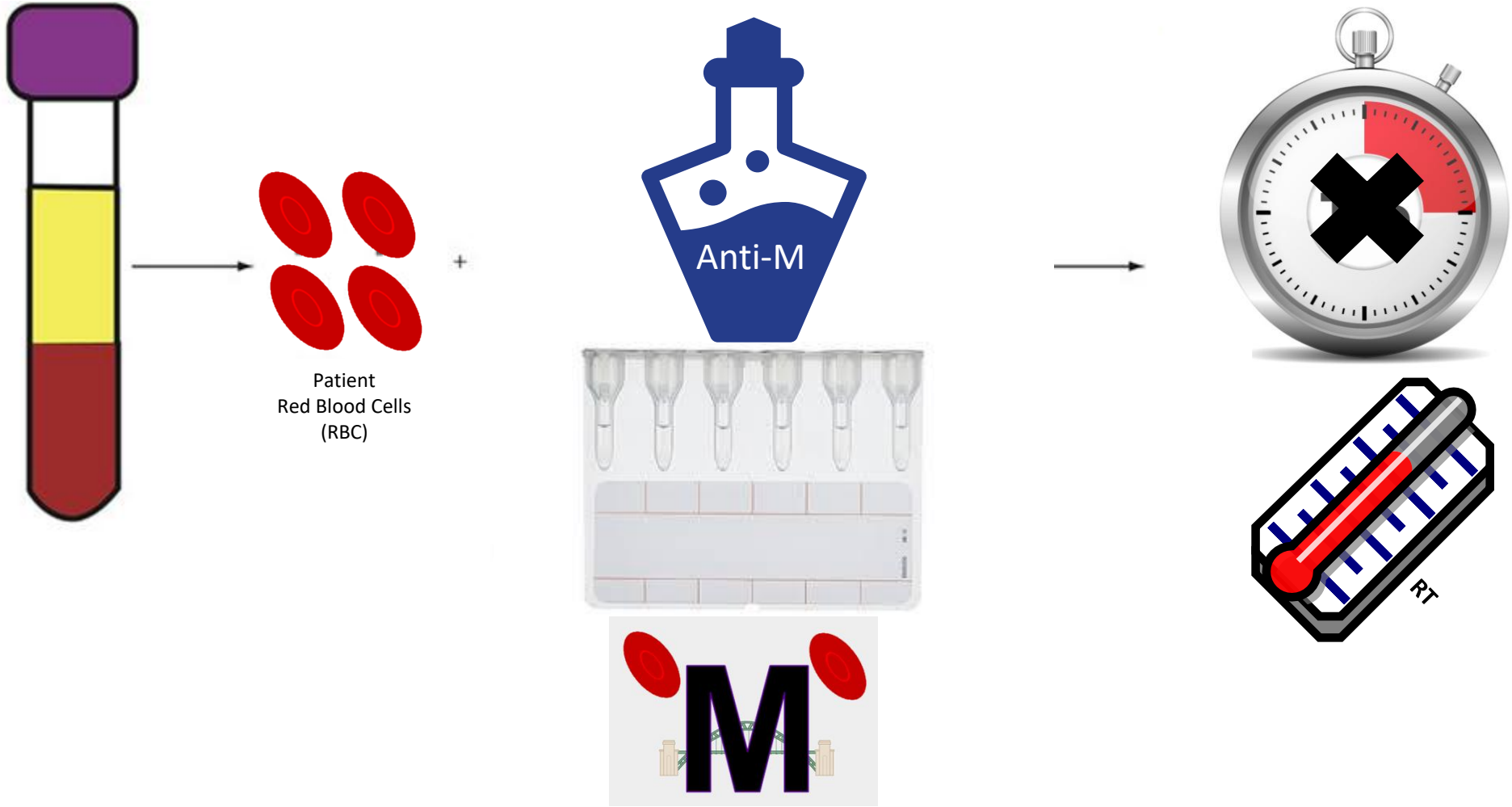
- Performed using unknown patient's red cells with known antisera
- Shows which antigens are expressed on the red cells – useful in paternal phenotyping too!
- Cannot be performed following recent transfusion (3 months or less) due to dual population of RBCs
- IgG positive DAT can cause false positive result with anti-IgG antisera



Genotype

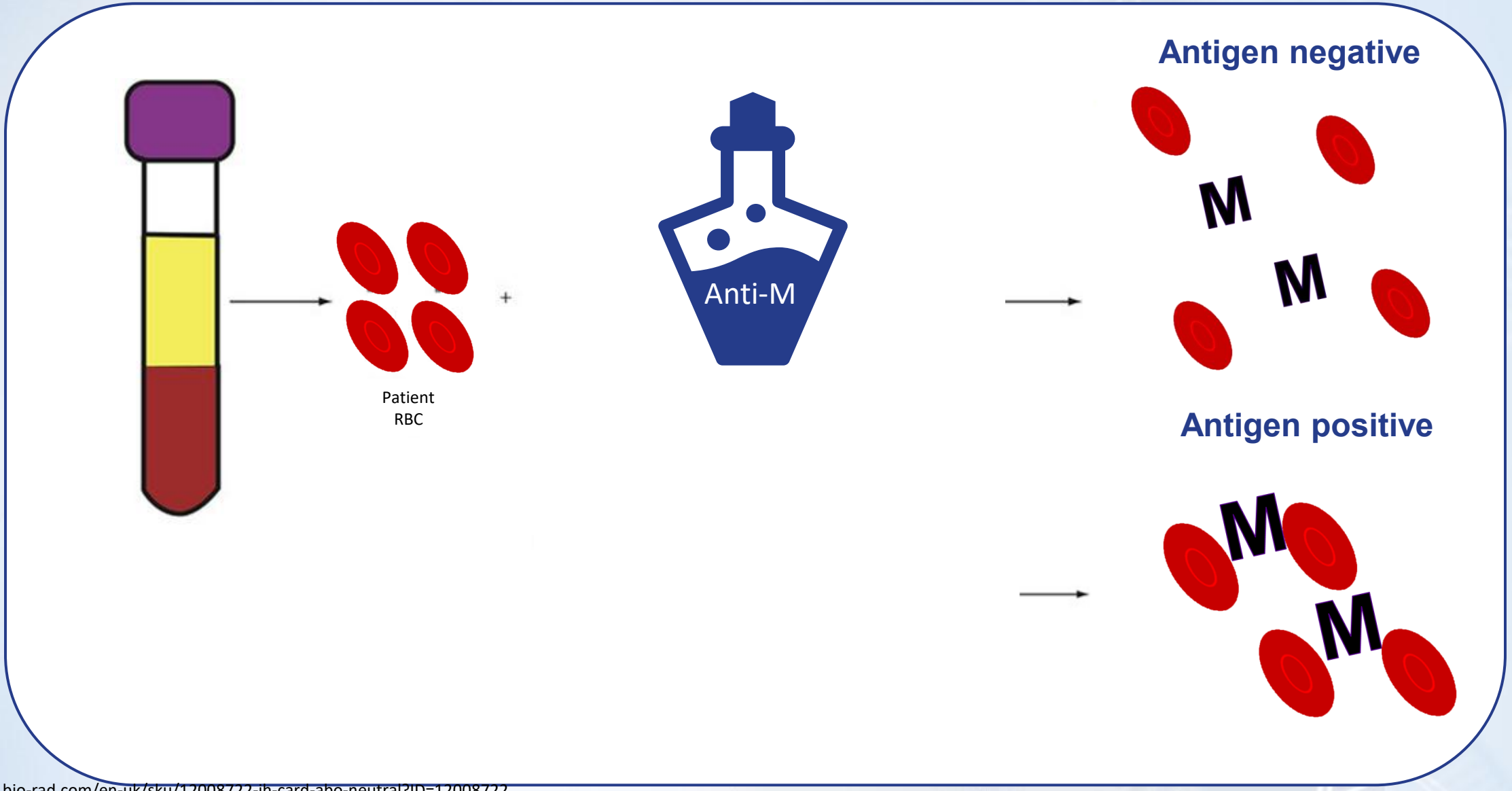
- Performed using patient's DNA – extracted from white cells
- Shows which genes are present – may not be expressed on red cells. This gives you a predicted red cell phenotype
- Can be performed following recent transfusion
- Can be performed with IgG DAT Pos patient
- Can also be used to determine the genotype for haemoglobinopathy patients – performed at IBGRL
- Also used for fetal DNA

Phenotyping – IgM antisera



https://www.bio-rad.com/en-uk/sku/12008722-ih-card-abo-neutral?ID=12008722

Phenotyping – IgM antisera



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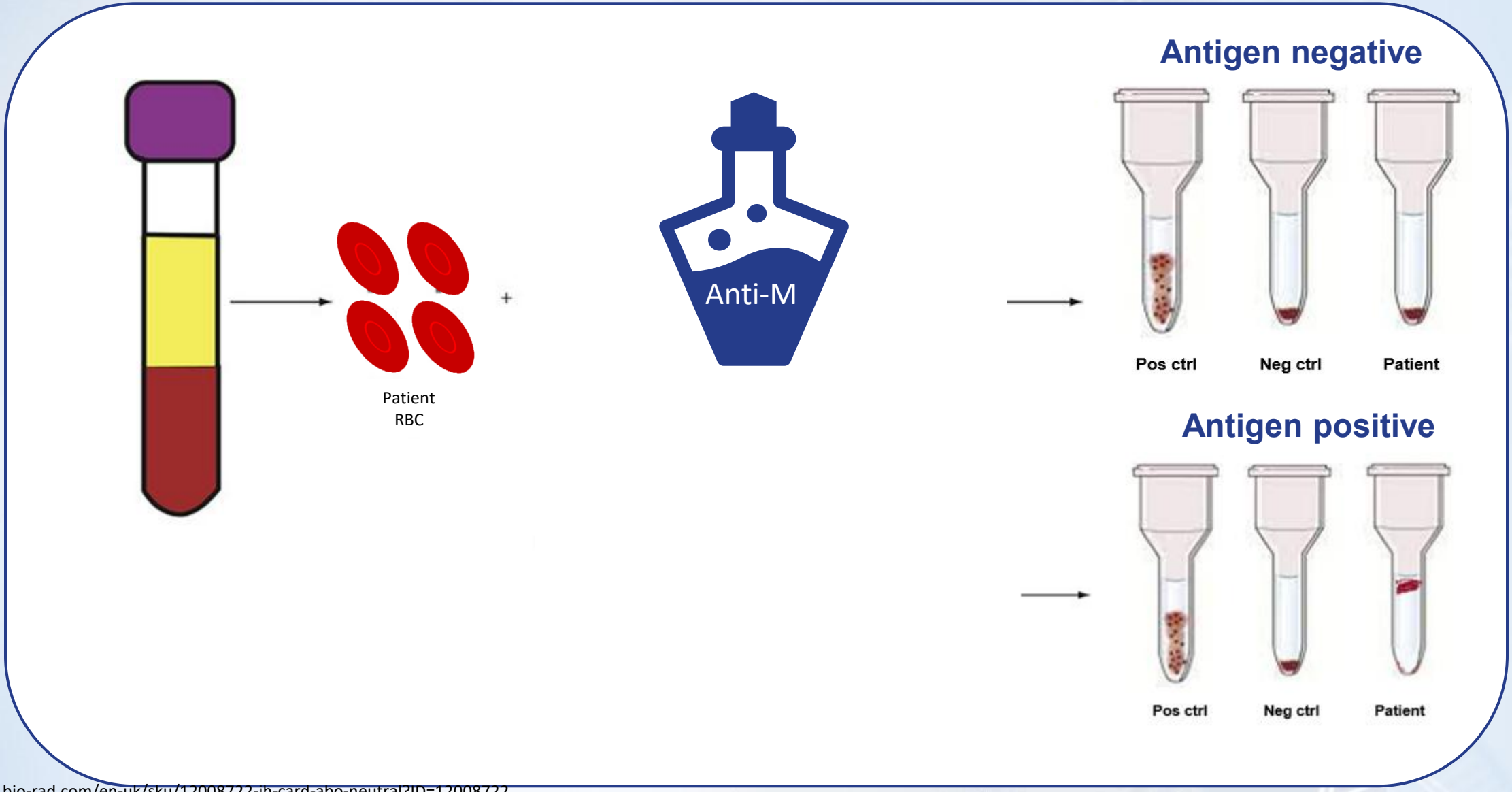
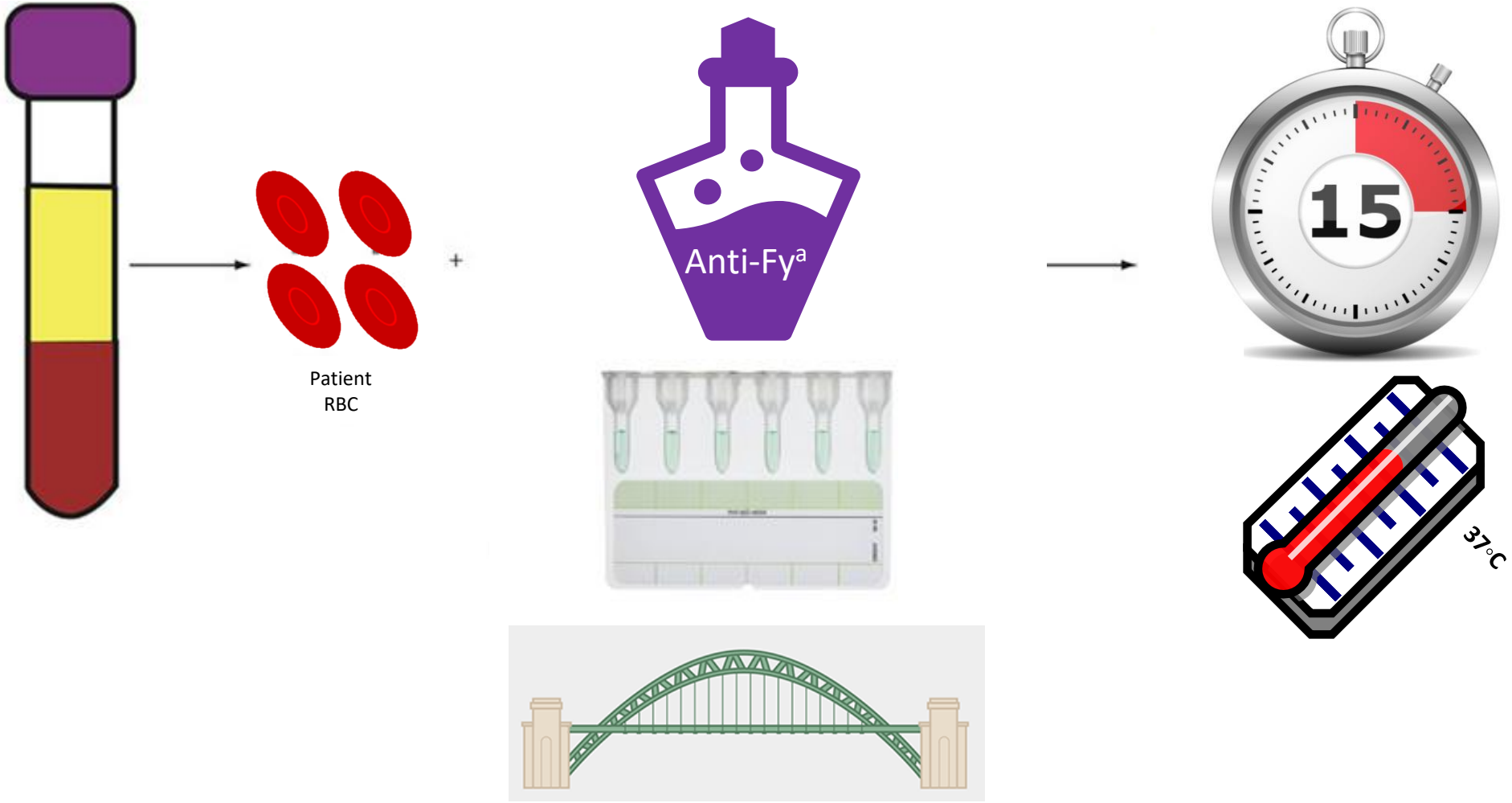
Phenotyping – IgM antisera

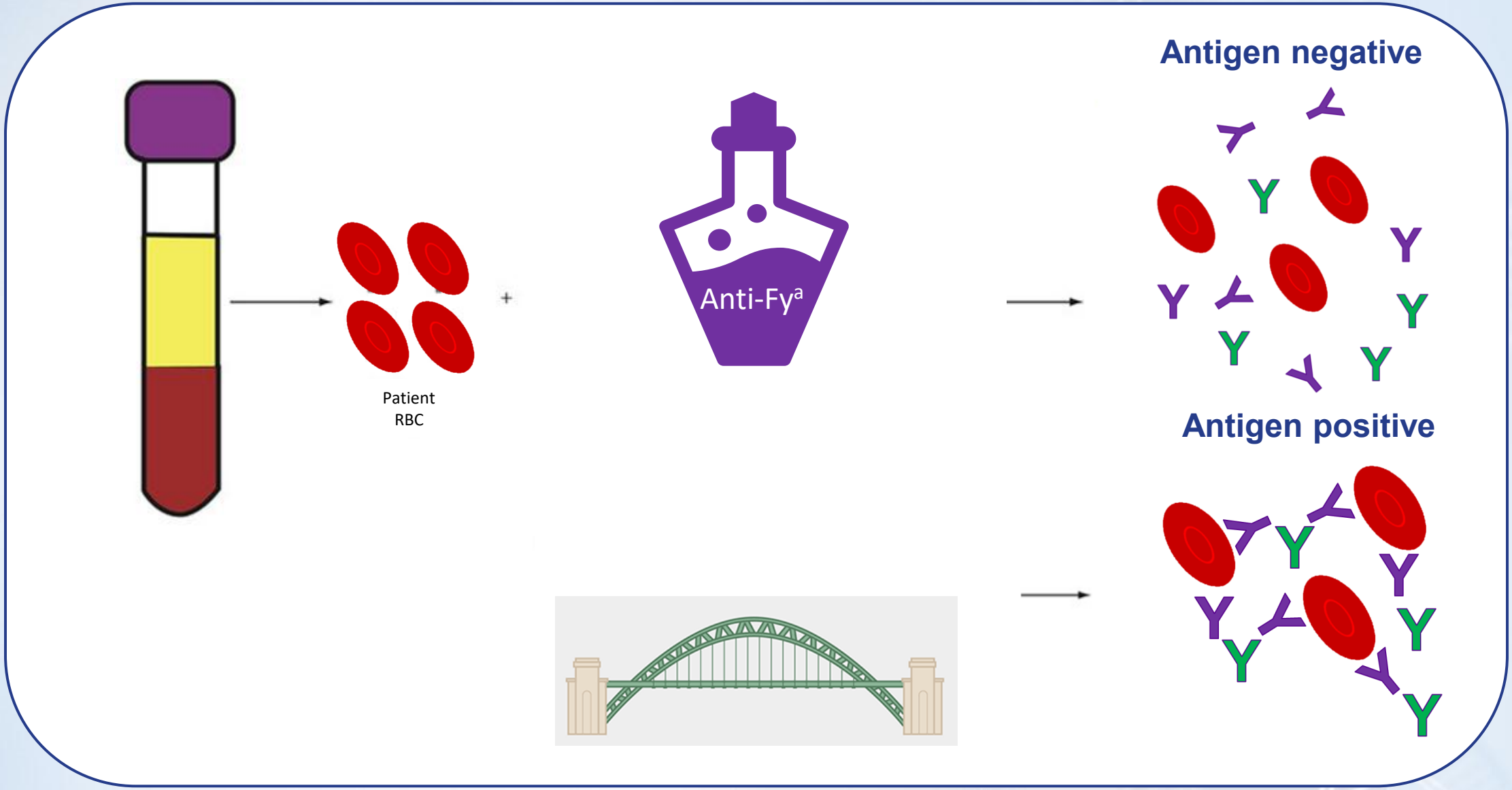


https://www.bio-rad.com/en-uk/sku/12008722-ih-card-abo-neutral?ID=12008722

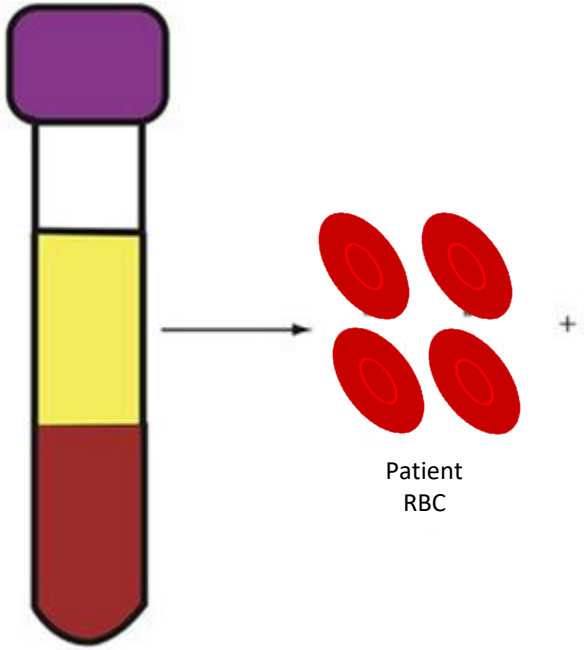
Phenotyping – IgG antisera



Phenotyping – IgG antisera



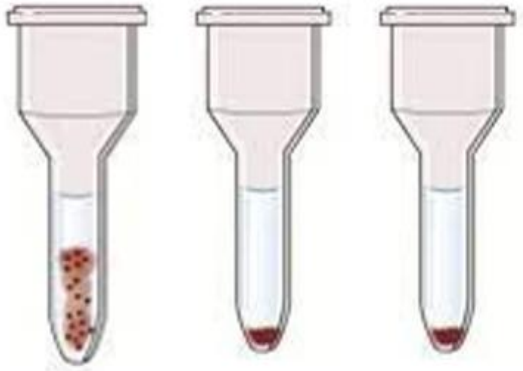
Phenotyping – IgG antisera



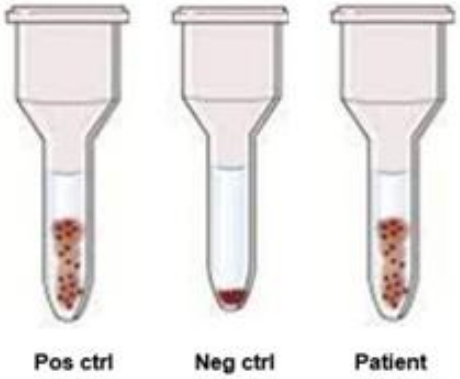
Patient
RBC

+

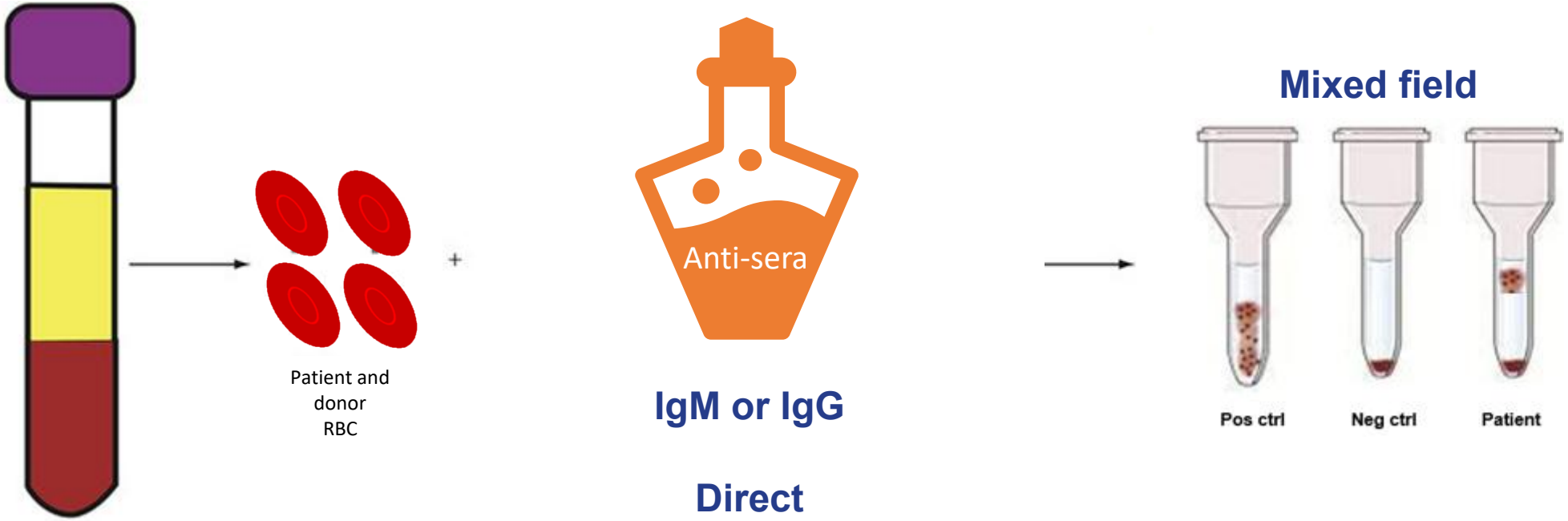
Antigen negative



Antigen positive



Phenotyping – recently transfused

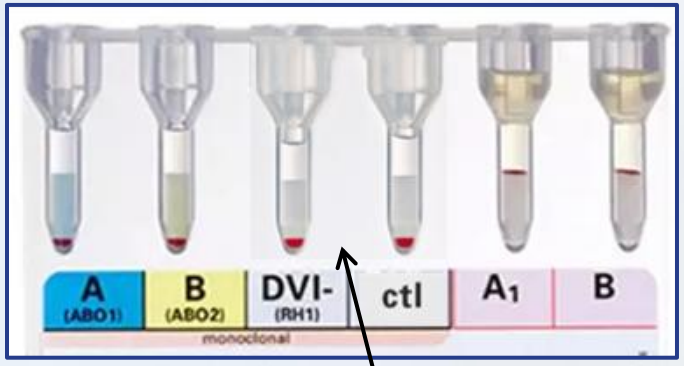


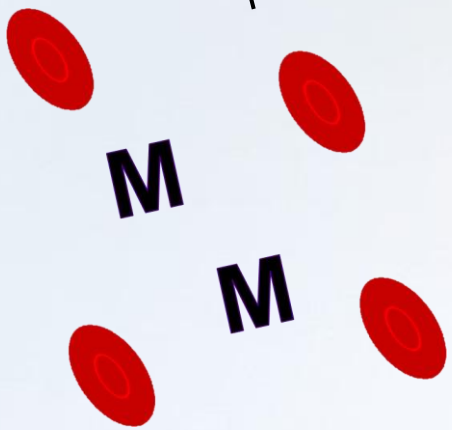
Our patient

- Anti-D detected: what is the D phenotype?
- Phenotype performed using patient's red cells (unknown) with anti-D antisera (known)
- Shows which antigens are expressed on the red cells – alloantibody or autoantibody
- Helps to determine the clinical significance
- Cannot be performed following recent transfusion due to dual population of RBCs – **patient transfused 6 years ago**
- **Auto control is negative**
- In this case, the D phenotyping is done alongside ABO

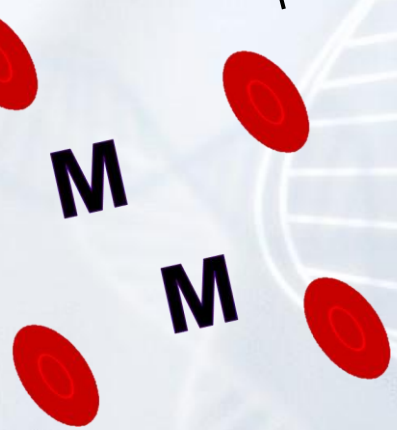
Our patient

Long Group





Short Group



Alloanti-D

Knowing the clinically significance of antibodies

ISBT:

“A clinically significant antibody can be defined as one capable of causing accelerated destruction of a significant proportion of transfused cells, or one capable of crossing the placenta and causing haemolytic disease of the fetus and newborn”

https://onlinelibrary.wiley.com/doi/abs/10.1111/voxs.12325#:~:text=A%20clinically%20significant%20antibody%20can,of%20the%20fetus%20and%20newborn.

Blood selection

BSH Guidelines – clinical significance of red cell antibodies			
System	Specificity	Likely clinical significance in transfusion	Recommendation for selection of red cells for transfusion
Rh	Anti-D	Yes	Antigen negative

- 7.3.3. An IAT crossmatch must be used: If the patient's plasma contains or has been known to contain, red cell alloantibodies of likely clinical significance. This recommendation is based on the need to:
  - provide assurance that the phenotype of the donor red cells correct;
  - detect additional specificities which may have been masked or undetected in antibody identification;
  - provide assurance that the assigned antibody specificity is correct
- 7.10.4. Patients with other Rh antibodies should be additionally matched for C, c, E and e in order to prevent further Rh alloimmunisation, provided this does not impede delivery of effective transfusion support

https://onlinelibrary.wiley.com/doi/full/10.1111/j.1365-3148.2012.01199.x

Our patient

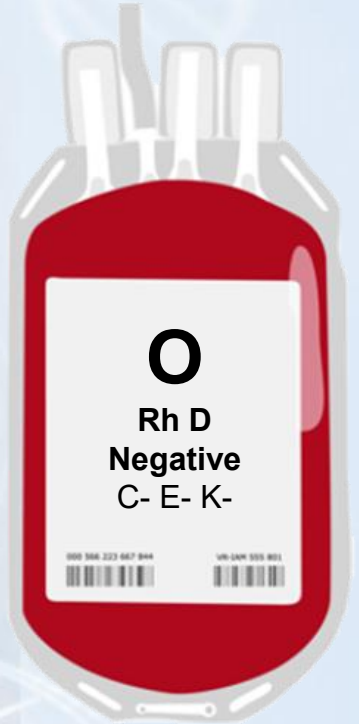
- Alloanti-D
- Clinically significant
- Requires extended Rh/K phenotype
- Autocontrol is negative
- Using IgM antisera in this case, but always be mindful of autocontrol and transfusion history
- Include reagent control (ctrl) when phenotyping – shows results are valid

What blood would you select?

- Group O D –
- Group O D- C- E-
- Group O D- C- E- K-
- Group O D+
- Not sure

What blood would you select?

- 7.10.1.Red cells should be selected which have been phenotyped and found negative for the relevant antigen. It is good practice to give K negative red cells to these patients because it is sometimes difficult to exclude anti-K in the presence of other antibodies and easy to select K negative units
- 7.10.3.Patients with anti-D who are rr (ccddee) should receive rr (D- C- E-), K negative blood



Key Testing Tools so far

- Patient history
- Antibody screen
- Grading
- Antibody identification
- Inclusions and exclusions
- Antibody panel cells (IAT and enzyme)
- Antigram (panel sheet)
- Clinical significance of antibodies
- Phenotyping/genotyping
- **More grading examples**
- **Direct antiglobulin test**
- **Elution**

Grading example - dosage

Cell	Rh	D	C	E	c	e	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	$R_1^wR_1$	+	+				+	0	+	0	3	0	+	0	0	0	+	0	+	0	+		3	0
2	R_1R_1	+	+	0	0	+	+	+			1	0	0	+	0	+	0	+	0	+	0		1	0
3	R_2R_2	+	0	+	+	0	0	+	0	+	3	0	0	+	0	+	0	0	+	+	0		0	0
4	r'r	0	+	0	+	+	0	+	0	+	0	0	0	+	0	+	0	+	0	0	+		0	0
5	r''r	0	0				+	0	+	0	1	0	0	+	0	0	+	+	0	0	+		3	0
6	rr	0	0	0	+	+	0	+	0	+	4	0	+	0	0	0	+	0	+	+	0	HLA+	0	0
7	rr	0	0	0	+	+	+	+			0	0	+	+	0	0	+	W	0	+	0		1	0
8	rr	0	0				+	0	0	+	0	0	0	+	+	0	+	+	0	0	+		3	0
9	rr	0	0	0	+	+	0	+	+	0	1	0	0	+	0	0	+	+	0	+	0		0	0
10	rr	0	0				+	0	0	+	3	+	0	+	0	0	+	0	+	+	0		3	0
																						Auto	0	/
																						K control	2	/

https://uk.pinterest.com/pin/spongebob-squarepants--433541901609503367/

Grading example – dosage/temperature

Cell	Rh	D	C	E	c	e	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	$R_1^wR_1$	+	+	0	0	+	+	0	←		3	0	+	0	0	0	+	0	+	0	+	→	1	0
2	R_1R_1	+	+	0	0	+	+	+	←		1	0	0	+	0	+	0	+	0	+	0	→	0	0
3	R_2R_2	+	0	+	+	0	0	+	0	+	3	0	0	+	0	+	0	0	+	+	0		0	0
4	r'r	0	+	0	+	+	0	+	0	+	0	0	0	+	0	+	0	+	0	0	+		0	0
5	r''r	0	0	+	+	+	+	0	←		1	0	0	+	0	0	+	+	0	0	+	→	1	0
6	rr	0	0	0	+	+	0	+	0	+	4	0	+	0	0	0	+	0	+	+	0	HLA+	0	0
7	rr	0	0	0	+	+	0	+	0	+	0	0	+	+	0	0	+	W	0	+	0		0	0
8	rr	0	0	0	+	+	+	0	←		0	0	0	+	+	0	+	+	0	0	+	→	1	0
9	rr	0	0	0	+	+	0	+	+	0	1	0	0	+	0	0	+	+	0	+	0		0	0
10	rr	0	0	0	+	+	+	+	←		3	+	0	+	0	0	+	0	+	+	0	→	0	0
																						Auto	0	/
																						K control	2	/

https://uk.pinterest.com/pin/spongebob-squarepants--433541901609503367/

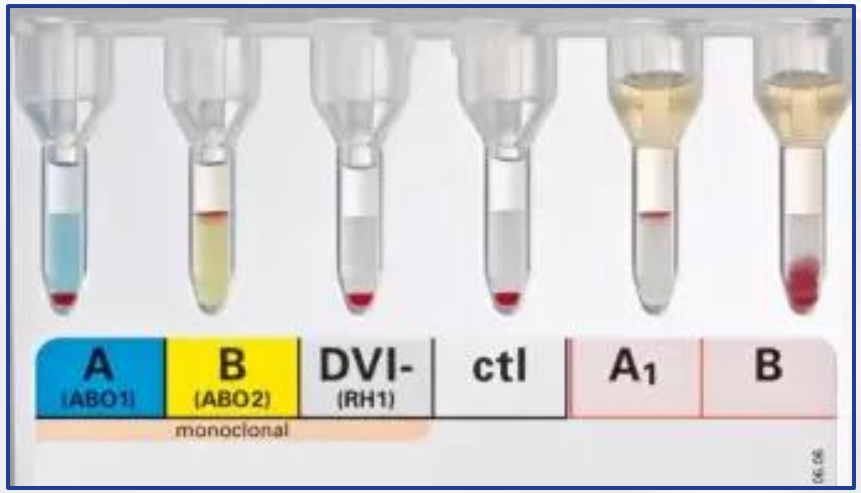
Grading example - dosage/temperature

Cell	Rh	D	C	E	c	e	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	Cold IAT
1	$R_1^wR_1$	+	+	0	0	+	+	0			3	0	+	0	0	0	+	0	+	0	+		1	0	2
2	R_1R_1	+	+	0	0	+	+	+			1	0	0	+	0	+	0	+	0	+	0		0	0	1
3	R_2R_2	+	0	+	+	0	0	+	0	+	3	0	0	+	0	+	0	0	+	+	0		0	0	0
4	r'r	0	+	0	+	+	0	+	0	+	0	0	0	+	0	+	0	+	0	0	+		0	0	0
5	r''r	0	0	+	+	+	+	0			1	0	0	+	0	0	+	+	0	0	+		1	0	2
6	rr	0	0	0	+	+	0	+	0	+	4	0	+	0	0	0	+	0	+	+	0	HLA+	0	0	0
7	rr	0	0	0	+	+	0	+	0	+	0	0	+	+	0	0	+	W	0	+	0		0	0	0
8	rr	0	0	0	+	+	+	0			0	0	0	+	+	0	+	+	0	0	+		1	0	2
9	rr	0	0	0	+	+	0	+	+	0	1	0	0	+	0	0	+	+	0	+	0		0	0	0
10	rr	0	0	0	+	+	+	+			3	+	0	+	0	0	+	0	+	+	0		0	0	2
																						Auto	0	/	
																						K control	2	/	

https://uk.pinterest.com/pin/spongebob-squarepants--433541901609503367/

Grading example - dosage/temperature

- Cold antibody may interfere with ABO grouping



Grading example – papain enhanced

Cell	Rh	D	C	E	c	e	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 IAT
1	[illegible]	+	+	0	0	+	+	0	+	0	3	0	+	0	0	0	+	0	+	0	+		3	4
2	[illegible]	+	+	0	0	+	+	+	0	+	1	0	0	+	0	+	0	+	0	+	0	HLA+	3	4
3	R_2R_2	+	0	+	+	0	0	+	0	+	3	0	0	+	0	+	0	0	+	+	0		0	0
4	[illegible]	+	+	0	+	[illegible]	[illegible]	[illegible]	0	+	0	0	0	+	0	+	0	+	0	0	+		2	4
5	r''r	0	0	+	+	+	+	0	+	0	1	0	0	+	0	0	+	+	0	0	+		0	0
6	rr	0	0	0	+	+	0	+	0	+	4	0	+	0	0	0	+	0	+	+	0	HLA+	0	0
7	rr	0	0	0	+	+	0	+	0	+	0	0	+	+	0	0	+	W	0	+	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	1	0	0	+	0	0	+	+	0	+	0		0	0
10	rr	0	0	0	+	+	+	0	0	+	3	+	0	+	0	0	+	0	+	+	0		0	0
																						Auto	0	/
																						K control	2	/

Grading example – multiple antibodies

Cell	Rh	D	C	E	c	e	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	$R_1^wR_1$	+	+				+	0	+	0	0	0	0	+	0	0	+	+	0	+	0		2	0
2	R_1R_1	+	+	0	0	+	0	+	0	+			+	+	0	+	0	0	+	+	0		3	3
3	R			+	+	0	+			0	0	0	0	+	0	+	0	0	+	+	+		3	4
4	r'r	0	+	0	+	+	0	+	+	0	0	0	0	+	0	0	+	0	+	+	+		0	0
5	r'			+	+	+			0	+	4	0	0	+	0	0	+	+	0	0	+		2	4
6	rr	0	0				+	0	+	0			+	0	0	0	+	+	0	0	+		3	3
7	rr	0	0	0	+	+	0	+	0	+			+	+	0	+	0	0	+	+	0		3	3
8	rr	0	0	0	+	+	0	+	0	+	0	0	0	+	+	0	+	0	+	+	+		0	0
9	rr	0	0	0	+	+	0	+	0	+	2	+	0	+	0	+	0	+	0	+	0		0	0
10	rr	0	0				+	+			3	0	0	+	0	+	0	+	0	+	0		0	0
																						Auto	0	/
																						+ control	2	/

Exclusions

Cell	Rh	D	C	E	c	e	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	$R_1^wR_1$	+	+	0	0	+	+	0	+	0	0	0	0	+	0	0	+	+	0	+	0		2	0
2	R_1R_1	+	+	0	0	+	0	+	0	+	0	0	+	+	0	+	0	0	+	+	0		3	3
3	R_2R_2	+	0	+	+	0	+	0	+	0	0	0	0	+	0	+	0	0	+	+	+		3	4
4	r'r	0	+	0	+	+	0	+	+	0	0	0	0	+	0	0	+	0	+	+	+		0	0
5	r''r	0	0	+	+	+	0	+	0	+	4	0	0	+	0	0	+	+	0	0	+		2	4
6	rr	0	0	0	+	+	+	0	+	0	4	0	+	0	0	0	+	+	0	0	+		3	3
7	rr	0	0	0	+	+	0	+	0	+	2	0	+	+	0	+	0	0	+	+	0		3	3
8	rr	0	0	0	+	+	0	+	0	+	0	0	0	+	+	0	+	0	+	+	+		0	0
9	rr	0	0	0	+	+	0	+	0	+	2	+	0	+	0	+	0	+	0	+	0		0	0
10	rr	0	0	0	+	+	+	+	0	+	3	0	0	+	0	+	0	+	0	+	0		0	0
																						Auto	0	/
																						+ control	2	/

Can you exclude anti-D?

Cell	Rh	D	C	E	c	e	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	$R_1^wR_1$	+	+	0	0	+	+	0	+	0	**0**	**0**	**0**	+	**0**	**0**	+	+	**0**	+	**0**		2	0
2	R_1R_1	+	+	0	0	+	0	+	0	+	**0**	**0**	+	+	**0**	+	**0**	**0**	+	+	**0**		3	3
3	R_2R_2	+	0	+	+	0	+	0	+	0	**0**	**0**	**0**	+	**0**	+	**0**	**0**	+	+	+		3	4
4	r'r	0	+	0	+	+	0	+	+	0	**0**	**0**	**0**	+	**0**	**0**	+	**0**	+	+	+		0	0

- Yes - Anti-D is not being masked by any other antibodies
- Yes - Rh antibodies can be excluded by enzyme technique
- No – you are unable to exclude anti-D on this panel

Can you exclude anti-D?

Cell	Rh	D	C	E	c	e	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	$R_1^wR_1$	+	+	0	0	+	+	0	+	0	0	0	0	+	0	0	+	+	0	+	0		2	0
2	R_1R_1	+	+	0	0	+	0	+	0	+	0	0	+	+	0	+	0	0	+	+	0		3	3
3	R_2R_2	+	0	+	+	0	+	0	+	0	0	0	0	+	0	+	0	0	+	+	+		3	4
4	r'r	0	+	0	+	+	0	+	+	0	0	0	0	+	0	0	+	0	+	+	+		0	0

- **Yes - Rh antibodies can be excluded by enzyme technique**
- 6.2.7.It is acceptable to exclude Rh antibodies using validated techniques incorporating enzyme treated cells

https://onlinelibrary.wiley.com/doi/full/10.1111/j.1365-3148.2012.01199.x

Can you exclude anti-Jk^b?

~~Jk^a~~	Jk^b	Other	IAT	ENZ
+	0		2	0
+	0		3	3
+	+		3	4
+	+		0	0
0	+		2	4
0	+		3	3
+	0		3	3
+	+		0	0
+	0		0	0
+	0		2	0
		Auto	0	/
		+ control	2	/

- Yes – the E- M- K- Jk(b+) cells are homozygous
- No – the E- M- K- Jk(b+) cells are heterozygous

Can you exclude anti-Jk^b?

~~Jk^a~~	Jk^b	Other	IAT	ENZ
+	0		2	0
+	0		3	3
+	+		3	4
+	+		0	0
0	+		2	4
0	+		3	3
+	0		3	3
+	+		0	0
+	0		0	0
+	0		0	0
		Auto	0	/
		+ control	2	/

- **No – the E- M- K- Jk(b+) cells are heterozygous – Jk(a+b+)**

Exclusions

Cell	Rh	D	C	E	c	e	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	$R_1^wR_1$	+	+	0	0	+	+	0	+	0	0	0	0	+	0	0	+	+	0	+	0		2	0
2	R_1R_1	+	+	0	0	+	0	+	0	+	0	0	+	+	0	+	0	0	+	+	0		3	3
3	R_2R_2	+	0	+	+	0	+	0	+	0	0	0	0	+	0	+	0	0	+	+	+		3	4
4	r'r	0	+	0	+	+	0	+	+	0	0	0	0	+	0	0	+	0	+	+	+	X	0	0
5	r''r	0	0	+	+	+	0	+	0	+	4	0	0	+	0	0	+	+	0	0	+		2	4
6	rr	0	0	0	+	+	+	0	+	0	4	0	+	0	0	0	+	+	0	0	+		3	3
7	rr	0	0	0	+	+	0	+	0	+	2	0	+	+	0	+	0	0	+	+	0		3	3
8	rr	0	0	0	+	+	0	+	0	+	0	0	0	+	+	0	+	0	+	+	+	X	0	0
9	rr	0	0	0	+	+	0	+	0	+	2	+	0	+	0	+	0	+	0	+	0		0	0
10	rr	0	0	0	+	+	+	+	0	+	3	0	0	+	0	+	0	+	0	+	0		0	0
																						Auto	0	/
																						+ control	2	/

Exclusions

- You can exclude anti-D
- You cannot exclude anti-Jk^b
- You would need to test E- M- K- Jk(a-b+) cells to exclude anti-Jk^b
- For the anti-E, M and K, you need 2 positive and 2 negative examples of each antibody to confirm their presence
- Or, if these were known historical antibodies, just one example is sufficient for inclusion
- Depending on the transfusion history, you would also phenotype the patient (if phenotype unknown)
- We need to know the patient history

Patient history

- 60-year-old female
- Hysterectomy
- Multiparous
- Grouped as O RhD+
- No record of historical alloantibodies
- Never been transfused
- 2 units of red blood cells have been requested

Inclusions

Cell	Rh	D	C	E	c	e	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	$R_1^wR_1$	+	+	0	0	+	+	0	+	0	0	0	0	+	0	0	+	+	0	+	0		2	0
2	R_1R_1	+	+	0	0	+	0	+	0	+	0	0	+	+	0	+	0	0	+	+	0		3	3
3	R_2R_2	+	0	+	+	0	+	0	+	0	0	0	0	+	0	+	0	0	+	+	+		3	4
4	r'r	0	+	0	+	+	0	+	+	0	0	0	0	+	0	0	+	0	+	+	+		0	0
5	r''r	0	0	+	+	+	0	+	0	+	4	0	0	+	0	0	+	+	0	0	+		2	4
6	rr	0	0	0	+	+	+	0	+	0	4	0	+	0	0	0	+	+	0	0	+		3	3
7	rr	0	0	0	+	+	0	+	0	+	2	0	+	+	0	+	0	0	+	+	0		3	3
8	rr	0	0	0	+	+	0	+	0	+	0	0	0	+	+	0	+	0	+	+	+		0	0
9	rr	0	0	0	+	+	0	+	0	+	2	+	0	+	0	+	0	+	0	+	0		0	0
10	rr	0	0	0	+	+	+	+	0	+	3	0	0	+	0	+	0	+	0	+	0		0	0
																						Auto	0	/
																						+ control	2	/

Inclusions

Cell	Rh	D	C	E	c	e	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	$R_1^wR_1$	+	+	0	0	+	+	0	+	0	0	0	0	+	0	0	+	+	0	+	0		2	0
2	R_1R_1	+	+	0	0	+	0	+	0	+	0	0	+	+	0	+	0	0	+	+	0		3	3
3	R_2R_2	+	0	+	+	0	+	0	+	0	0	0	0	+	0	+	0	0	+	+	+		3	4
4	r'r	0	+	0	+	+	0	+	+	0	0	0	0	+	0	0	+	0	+	+	+		0	0
5	r''r	0	0	+	+	+	0	+	0	+	4	0	0	+	0	0	+	+	0	0	+		2	4
6	rr	0	0	0	+	+	+	0	+	0	4	0	+	0	0	0	+	+	0	0	+		3	3
7	rr	0	0	0	+	+	0	+	0	+	2	0	+	+	0	+	0	0	+	+	0		3	3
8	rr	0	0	0	+	+	0	+	0	+	0	0	0	+	+	0	+	0	+	+	+		0	0
9	rr	0	0	0	+	+	0	+	0	+	2	+	0	+	0	+	0	+	0	+	0		0	0
10	rr	0	0	0	+	+	+	+	0	+	3	0	0	+	0	+	0	+	0	+	0		0	0
																						Auto	0	/
																						+ control	2	/

Inclusions

Cell	Rh	D	C	E	c	e	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	$R_1^wR_1$	+	+	0	0	+	+	0	+	0	0	0	0	+	0	0	+	+	0	+	0		2	0
2	R_1R_1	+	+	0	0	+	0	+	0	+	0	0	+	+	0	+	0	0	+	+	0		3	3
3	R_2R_2	+	0	+	+	0	+	0	+	0	0	0	0	+	0	+	0	0	+	+	+		3	4
4	r'r	0	+	0	+	+	0	+	+	0	0	0	0	+	0	0	+	0	+	+	+		0	0
5	r''r	0	0	+	+	+	0	+	0	+	4	0	0	+	0	0	+	+	0	0	+		2	4
6	rr	0	0	0	+	+	+	0	+	0	4	0	+	0	0	0	+	+	0	0	+		3	3
7	rr	0	0	0	+	+	0	+	0	+	2	0	+	+	0	+	0	0	+	+	0		3	3
8	rr	0	0	0	+	+	0	+	0	+	0	0	0	+	+	0	+	0	+	+	+		0	0
9	rr	0	0	0	+	+	0	+	0	+	2	+	0	+	0	+	0	+	0	+	0		0	0
10	rr	0	0	0	+	+	+	+	0	+	3	0	0	+	0	+	0	+	0	+	0		0	0
																						Auto	0	/
																						+ control	2	/

Inclusion/exclusion

Cell	Rh	D	C	E	c	e	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	EIAT
1	**$R_1^wR_1$**	+	+	0	0	+	0	+	+	0	**0**	**0**	**0**	+	**0**	**0**	+	+	**0**	**0**	+		0	0
2	**R_1R_1**	+	+	0	0	+	+	+	0	+	**0**	**0**	+	+	**0**	+	**0**	**0**	+	+	**0**		3	3
3	**R_2R_2**	+	0	+	+	0	0	0	+	0	**0**	**0**	**0**	+	**0**	+	**0**	**0**	+	+	**0**		3	4
4	**r'r**	0	+	0	+	+	0	+	+	0	**0**	**0**	**0**	+	**0**	**0**	+	**0**	+	**0**	+		0	0
5	**r''r**	0	0	+	+	+	0	+	0	+	**4**	**0**	**0**	+	**0**	**0**	+	+	**0**	+	+		2	4
																						+ control	2	/

Blood selection

BSH Guidelines – clinical significance of red cell antibodies			
System	Specificity	Likely clinical significance in transfusion	Recommendation for selection of red cells for transfusion
Rh	Anti-E	Yes	Antigen negative
Kell	Anti-K	Yes	Antigen negative
MNS	Anti-M (active at 37°C)	Yes	Antigen negative

- 7.10.4.Patients with other Rh antibodies should be additionally matched for C, c, E and e in order to prevent further Rh alloimmunisation, provided this does not impede delivery of effective transfusion support

https://onlinelibrary.wiley.com/doi/full/10.1111/j.1365-3148.2012.01199.x

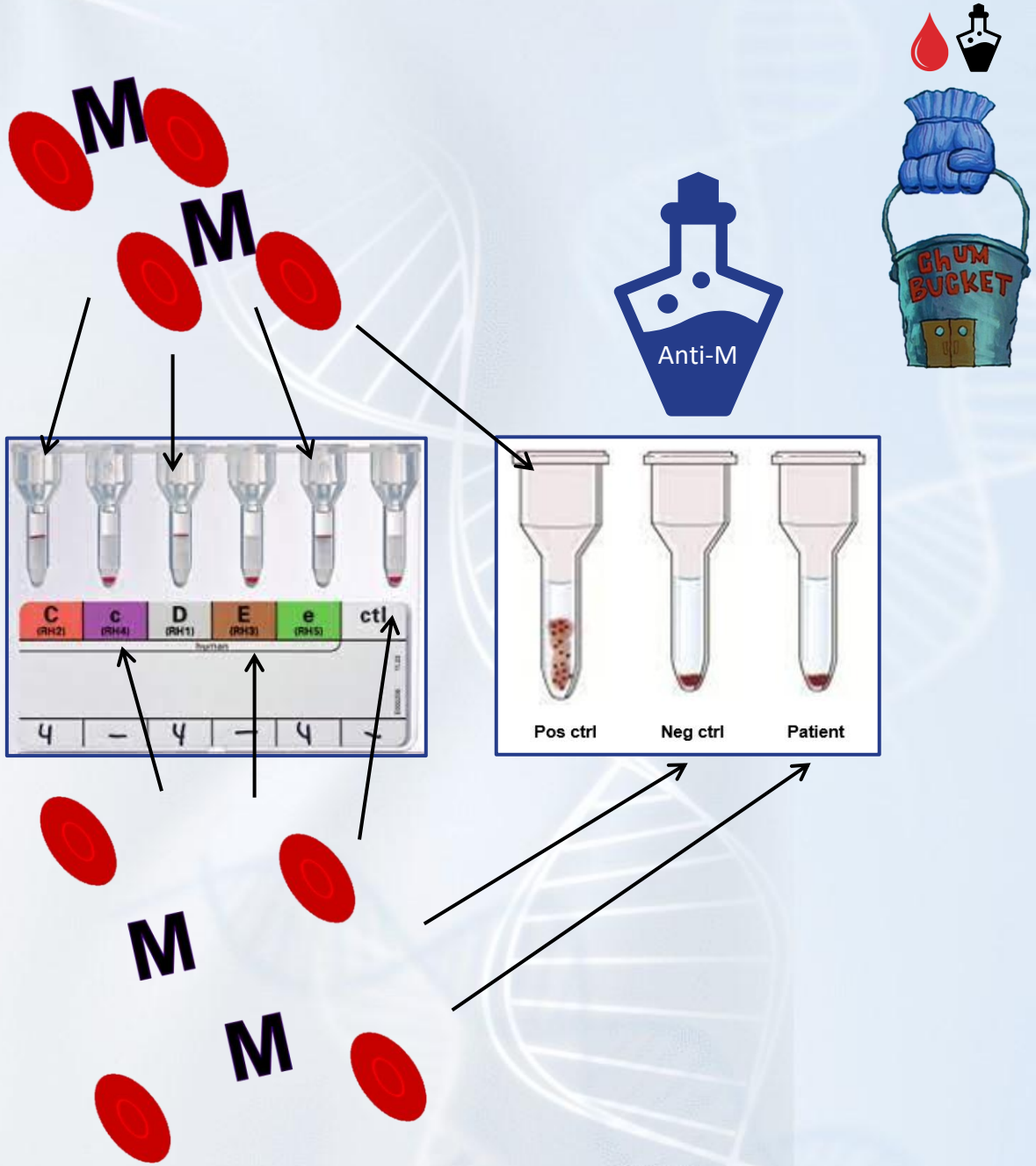
Phenotyping

- Anti-E, M and K detected: what are the phenotypes?
- Phenotype performed using patient's red cells (unknown) with anti-E, M and K antisera (known)
- Shows which antigens are expressed on the red cells – alloantibodies, autoantibodies or both
- Helps to determine the clinical significance
- Cannot be performed following recent transfusion due to dual population of RBCs – **patient never transfused**
- **Auto control is negative**

Our patient

- Requires Anti-E, M and K phenotypes
- Also requires extended Rh/K phenotype
- Autocontrol is negative
- Using IgM antisera in this case, but always be mindful of autocontrol and transfusion history

- Alloanti-E, M and K
- Clinically significant

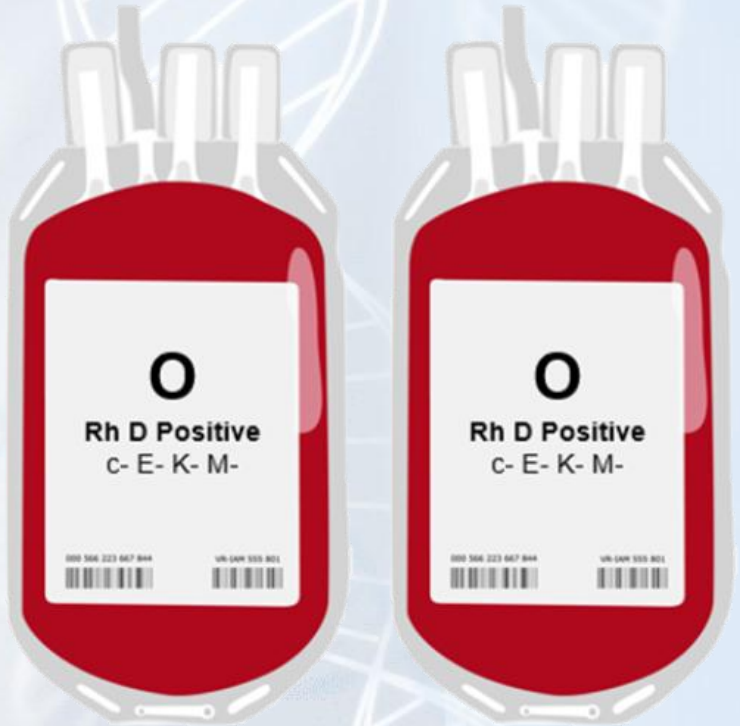


What blood would you select?

- Group O D+ E- K- M-
- Group O D+ c- E- K- M-
- Group O D- C- E- K- M-
- Not sure

What blood would you select?

- 7.10.1.Red cells should be selected which have been phenotyped and found negative for the relevant antigen.
- 7.10.4. Patients with other Rh antibodies should be additionally matched for C, c, E and e in order to prevent further Rh alloimmunisation, provided this does not impede delivery of effective transfusion support



Grading example – papain sensitive

Cell	Rh	D	C	E	c	e	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 IAT
1	$R_1^wR_1$	+	+	0	0	+	+	0	+	0	3	0	+	0	0	0	+	0	+	0	+		0	0
2	R_1R_1	+	+	0	0	+	+	+	0	+	1	0	0	+	0	+	0	+	0	+	0	→	2	0
3	R_2R_2	+	0	+	+	0	0	+	0	+	3	0	0	+	0	+	0	0	+	+	0		0	0
4	r'r	0	+	0	+	+	0	+	0	+	0	0	0	+	0	+	0	+	0	0	+	→	2	0
5	r''r	0	0	+	+	+	+	0	+	0	1	0	0	+	0	0	+	+	0	0	+	→	2	0
6	rr	0	0	0	+	+	0	+	0	+	4	0	+	0	0	0	+	0	+	+	0	HLA+	0	0
7	rr	0	0	0	+	+	0	+	0	+	0	0	+	+	0	0	+	W	0	+	0	→	1	0
8	rr	0	0	0	+	+	+	0	0	+	0	0	0	+	+	0	+	+	0	0	+	→	2	0
9	rr	0	0	0	+	+	0	+	+	0	1	0	0	+	0	0	+	+	0	+	0	→	2	0
10	rr	0	0	0	+	+	+	0	0	+	3	+	0	+	0	0	+	0	+	+	0		0	0
																				→		Auto	3	/
																						K control	2	/

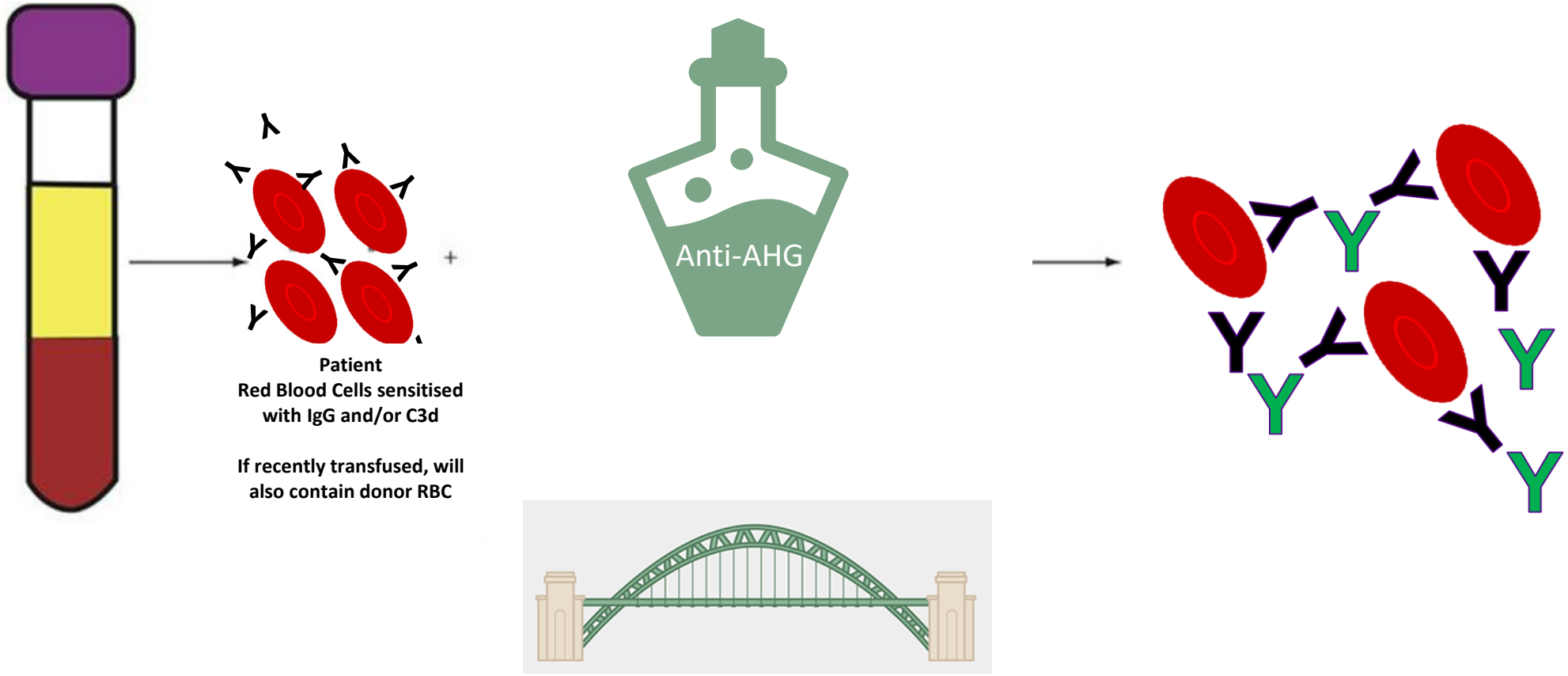
Auto control

- Testing patient's red cells versus patient's plasma
- A positive auto control in a patient who has been *not* been transfused recently could be due to autoimmune conditions, medication, or 'just one of those things'
- A positive auto control in a patient who *has* been transfused in the last 6 weeks can indicate sensitisation of donor RBC - formation of new alloantibody(ies)
- These can potentially cause transfusion reactions, so it is important to identify them
- A DAT is required, and if IgG positive, an elution may need to be performed
- If patient has haemolysis or clinical signs indicate a transfusion reaction, elution should be performed even if DAT/auto is negative

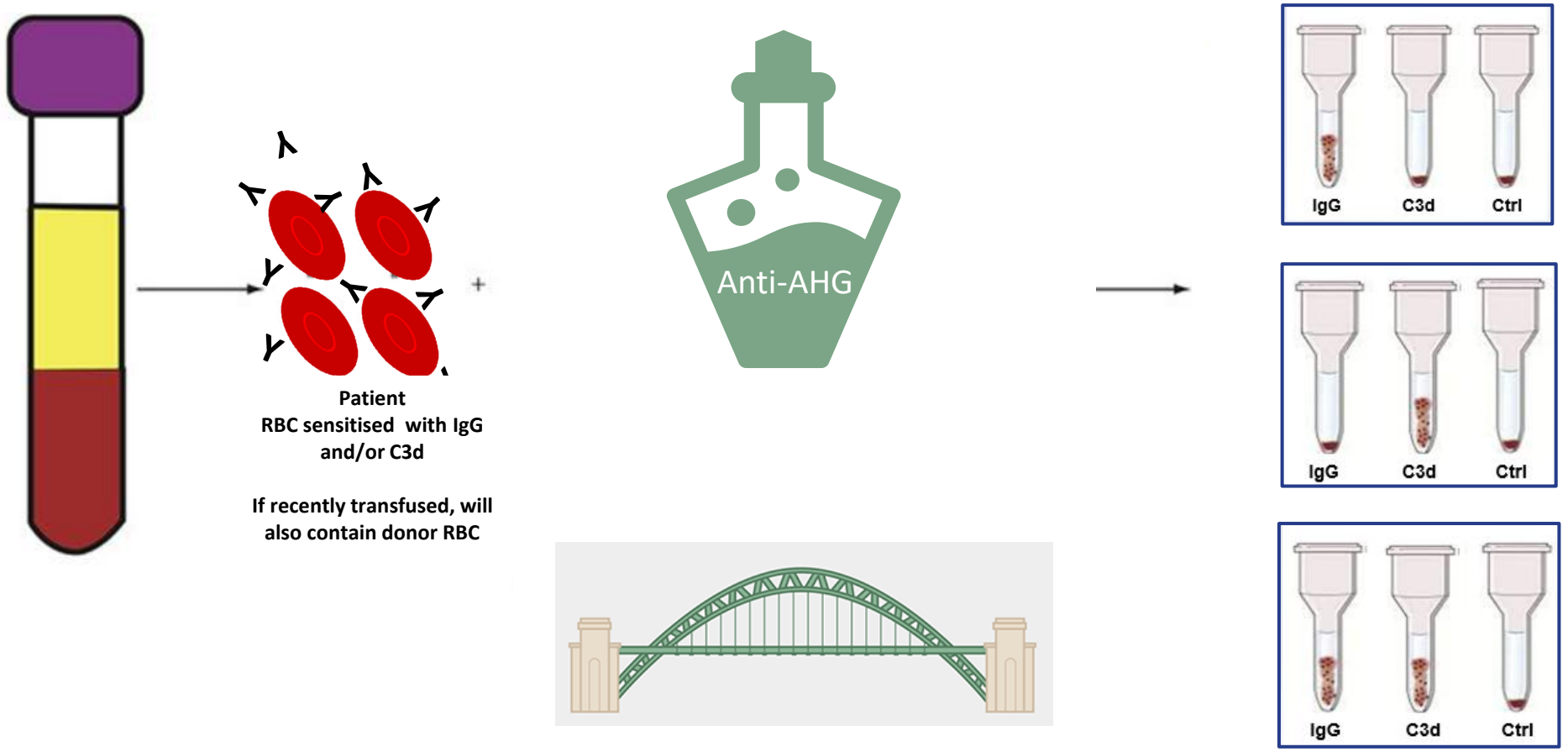
Direct Antiglobulin Test (DAT)

- DAT shows you what is bound to red cells *in vivo* – sensitised
- IgG positive shows there is IgG antibody bound to red cells
- If the patient has been recently transfused, this will be a mix of donor and patient cells
- If transfused in the last 6 weeks and IgG positive, an elution is most likely required
- C3d positive shows there is complement bound to red cells
- Interpretation of positive DATs must include the patient's history, clinical data, and the results of other laboratory tests

Positive DAT



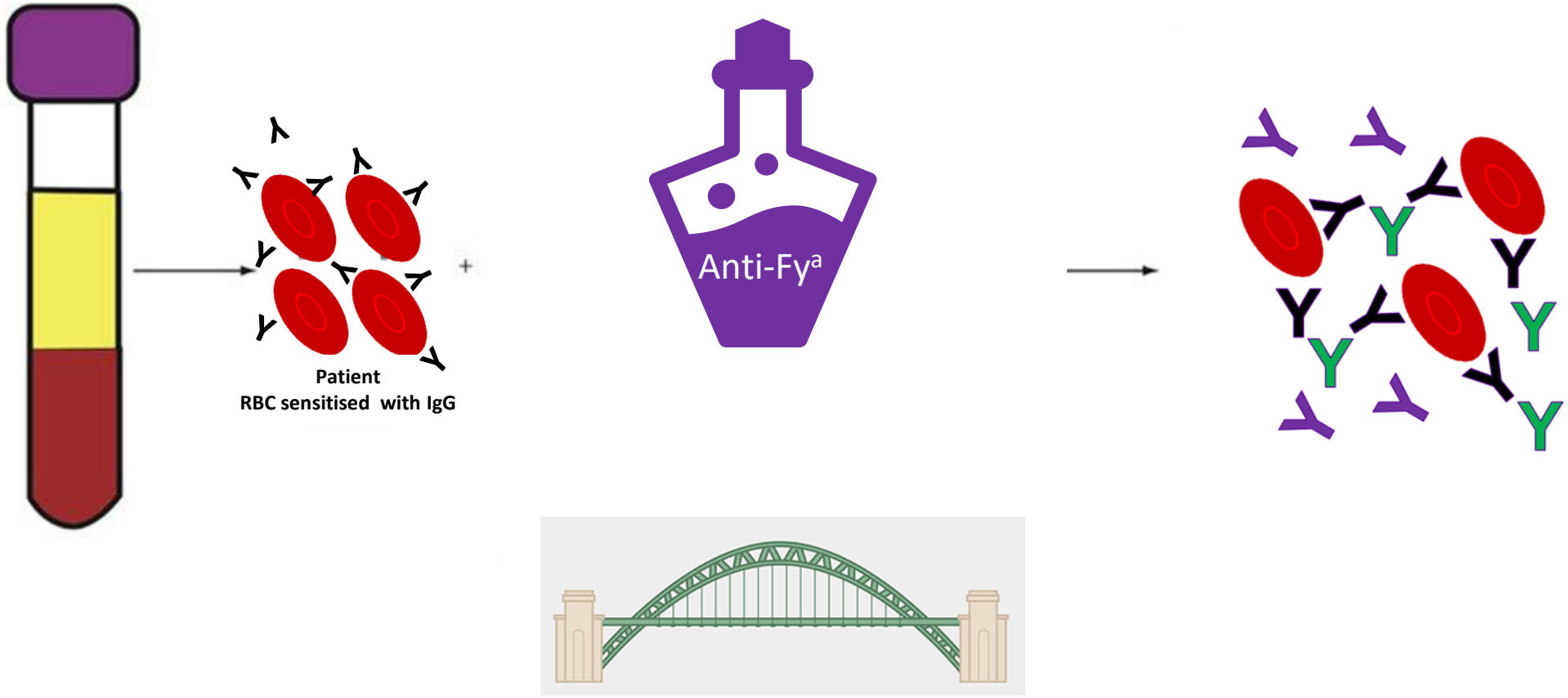
Positive DAT



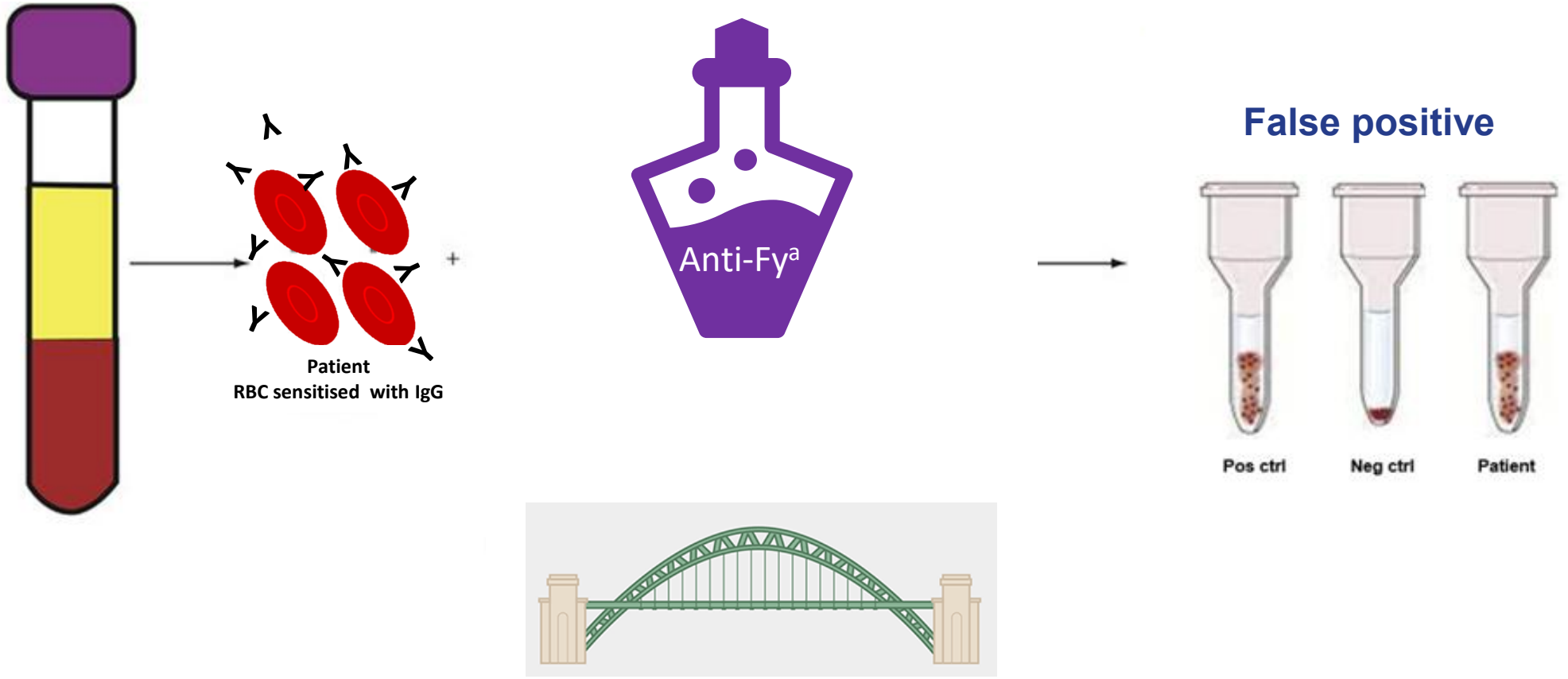
Patient
RBC sensitised with IgG and/or C3d

If recently transfused, will also contain donor RBC

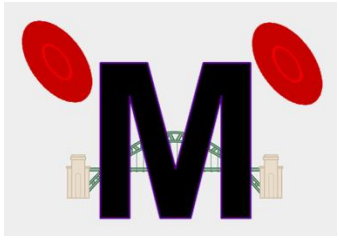
Phenotyping – IgG antisera, IgG pos DAT, not recently transfused



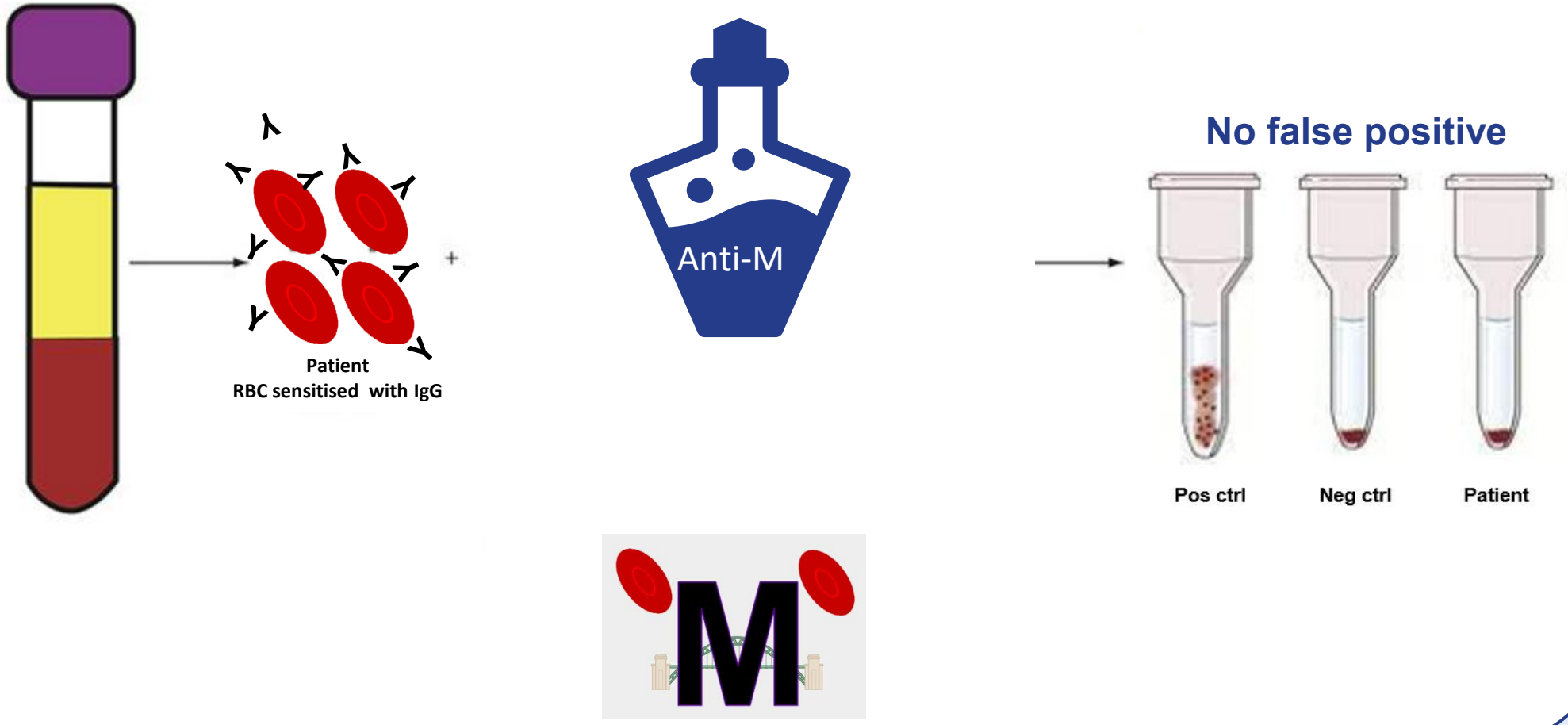
Phenotyping – IgG antisera, IgG pos DAT, not recently transfused



Phenotyping – IgM antisera, IgG pos DAT, not recently transfused

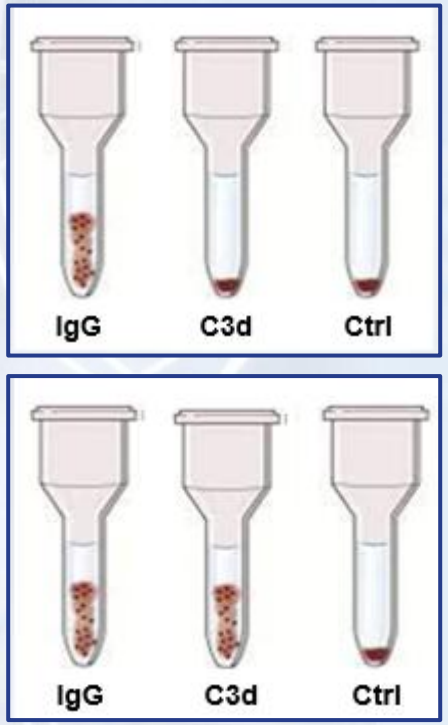


Phenotyping – IgM antisera, IgG pos DAT, not recently transfused

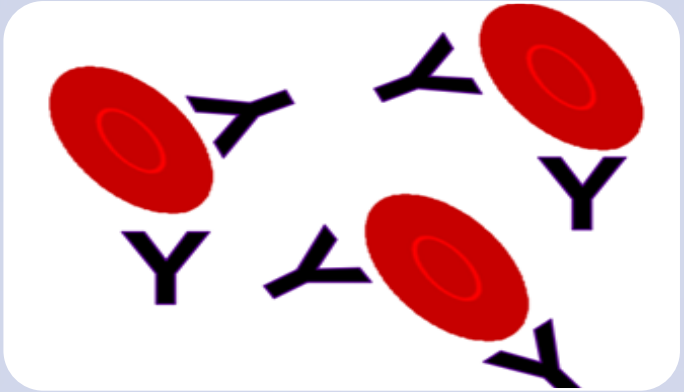


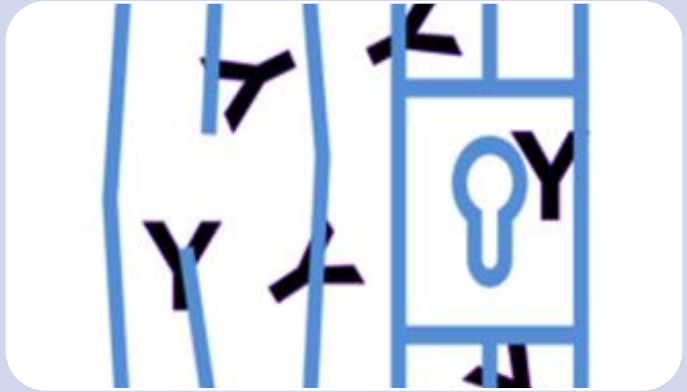
What about those patient's who have been recently transfused with IgG positive DAT?

- Elution will likely be required
- Remember, interpretation of positive DATs must include the patient's history, clinical data, and the results of other laboratory tests



Acid elution





Rh	D	C	E	c	e	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
R_1R_1	+	+	0	0	+	+	0	+	0	0	+	+	0	0	+	+	0	+	0			Wk
R_1R_2	+	+	0	0	+	+	0	+	0	4	0	0	+	0	+	0	0	+	0	+		Wk
R_2R_2	+	0	+	+	0	0	+	+	0	3	0	0	+	0	+	0	0	+	+	0		0
r'r'	0	+	0	+	+	0	+	0	+	2	0	0	+	0	0	+	0	+	+	0		Wk
r"r"	0	0	+	+	+	+	0	+	0	1	0	0	+	0	0	+	+	0	0	+		0
rr	0	0	0	+	+	0	+	0	+	3	0	+	0	0	0	+	+	+	0	+		0
rr	0	0	0	+	+	+	0	+	0	0	+	+	+	0	0	+	0	+	+	0		0
rr	0	0	0	+	+	0	+	0	+	3	0	0	+	+	+	0	+	0	0	+		0
rr	0	0	0	+	+	+	0	0	+	4	+	0	+	0	0	+	+	0	+	0		0
rr	0	0	0	+	+	0	+	+	0	1	0	0	+	0	+	0	0	+	0	+		0
																					Auto	1
																					K control	2

IgG DAT Pos &
Recently Transfused

Antibodies bound to RBC - ‘sensitised’

Mix of donor and patient RBC

It’s the donor red cells you are eluting alloantibody from

Acid elution technique

Releases bound antibodies from sensitised red cells

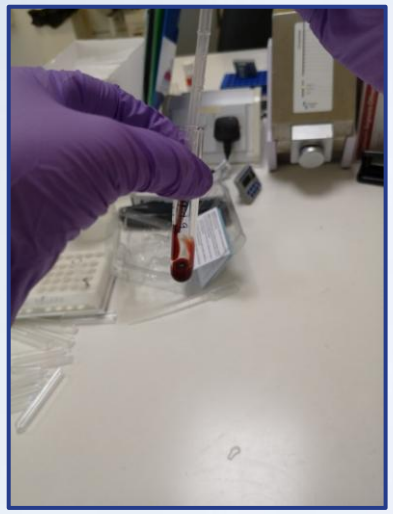
Performed when tx ≤6 weeks
Reduction in tx interval
Increase in DAT strength

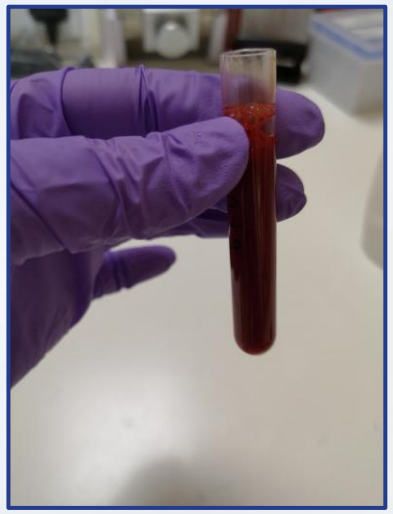
Perform ABID on elution

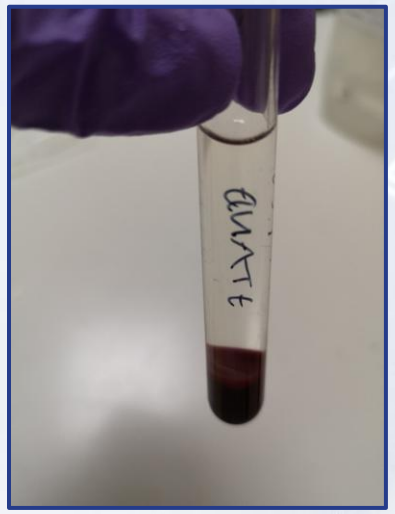
Allows you to ‘see’ antibodies eluted from RBC

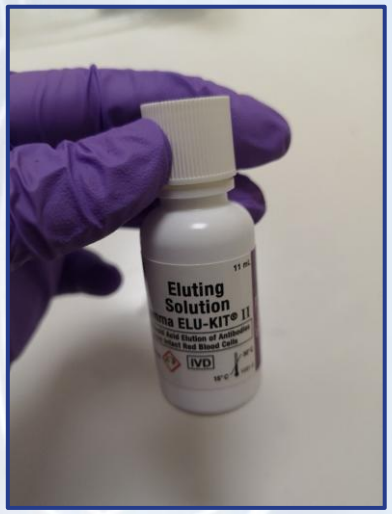
If any clinically significant alloantibodies are identified, even if not present in the patient plasma, antigen negative blood must be selected

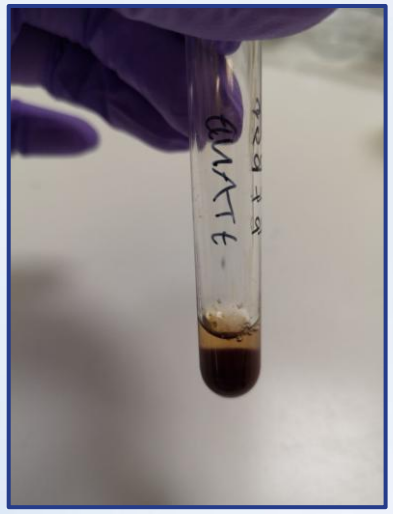
Preparing an eluate

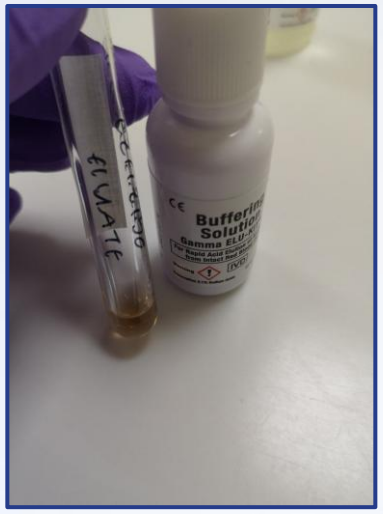












Let’s revisit the first example

- 75yo male
- Transfused 6 years ago
- Patient’s phenotype is O RhD negative: alloanti-D. No other known alloantibodies
- They have now been transfused and a new sample is sent to the lab

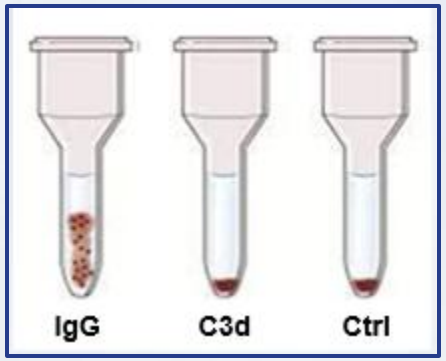
ABID

Cell	Rh	D	~~C~~	~~E~~	~~c~~	~~e~~	~~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	EIAT
1	$R_1^wR_1$	+	+	0	0	+	+	0	+	0	0	0	0	+	0	0	+	+	0	+	0		4	5
2	R_1R_1	+	+	0	0	+	0	+	0	+	0	0	+	+	0	+	0	0	+	0	+		4	5
3	R_2R_2	+	0	+	+	0	+	0	+	0	0	0	0	+	0	+	0	0	+	+	0		4	5
4	r'r	0	+	0	+	+	0	+	+	0	0	0	0	+	0	0	+	0	+	+	0		0	0
5	r''r	0	0	+	+	+	0	+	0	+	4	0	0	+	0	0	+	+	0	0	+		0	0
6	rr	0	0	0	+	+	+	0	+	0	4	0	+	0	0	0	+	+	0	0	+		0	0
7	rr	0	0	0	+	+	0	+	0	+	2	+	+	+	0	+	0	0	+	+	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	+	2	0	0	+	0	+	0	+	0	0	+		0	0
10	rr	0	0	0	+	+	0	+	0	+	3	0	0	+	0	+	0	+	0	+	0		0	0
																						Auto	3	/
																						+ control	2	/

75yo male. Transfused 1 week ago

Patient's phenotype is O RhD negative: alloanti-D. No other known alloantibodies

DAT



Elution required

Elution ABID

Cell	Rh	D	C	E	c	e	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	LWS
1	$R_1^wR_1$	+	+	0	0	+	+	0	+	0	0	0	0	+	0	0	+	+	0	+	0		2	0
2	R_1R_1	+	+	0	0	+	0	+	0	+	0	0	+	+	0	+	0	0	+	0	+		0	0
3	R_2R_2	+	0	+	+	0	+	0	+	0	0	0	0	+	0	+	0	0	+	+	0		2	0
4	r'r	0	+	0	+	+	0	+	+	0	0	0	0	+	0	0	+	0	+	+	0		2	0
5	r''r	0	0	+	+	+	0	+	0	+	4	0	0	+	0	0	+	+	0	0	+		0	0
6	rr	0	0	0	+	+	+	0	+	0	4	0	+	0	0	0	+	+	0	0	+		0	0
7	rr	0	0	0	+	+	0	+	0	+	2	+	+	+	0	+	0	0	+	+	0		2	0
8	rr	0	0	0	+	+	0	+	0	+	0	0	0	+	+	0	+	0	+	0	+		0	0
9	rr	0	0	0	+	+	+	0	0	+	2	0	0	+	0	+	0	+	0	0	+		0	0
10	rr	0	0	0	+	+	0	+	0	+	3	0	0	+	0	+	0	+	0	+	0		2	0
																						Auto	3	/
																						+ control	2	/

Blood selection

BSH Guidelines – clinical significance of red cell antibodies			
System	Specificity	Likely clinical significance in transfusion	Recommendation for selection of red cells for transfusion
Rh	Anti-D	Yes	Antigen negative
Kidd	Anti-Jk^{a}	Yes	Antigen negative

https://onlinelibrary.wiley.com/doi/full/10.1111/j.1365-3148.2012.01199.x

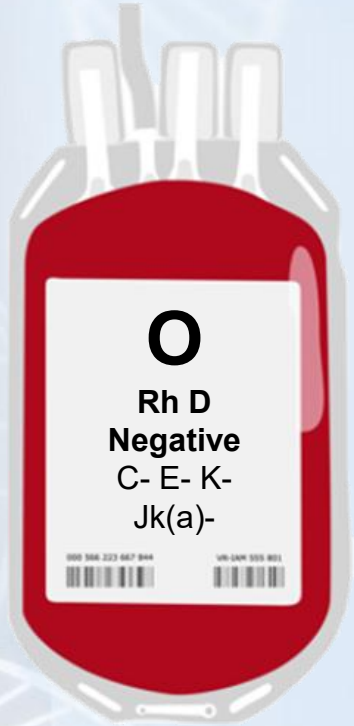
Blood selection

- 7.10.1.Red cells should be selected which have been phenotyped and found negative for the relevant antigen. It is good practice to give K negative red cells to these patients because it is sometimes difficult to exclude anti-K in the presence of other antibodies and easy to select K negative units
- 7.10.3.Patients with anti-D who are rr (ccddee) should receive rr (D- C- E-), K negative blood

- Patient has a known alloanti-D. Previous phenotype: O RhD- C- E- K-

For the anti-Jka:

- The patient has been recently transfused
- Mix of both donor and patient cells, so cannot Jk(a) phenotype – false positive
- Requires antigen negative blood – clinically significant antibody
- Antibody would be reported as not-specified
- Perform phenotyping 3 months post transfusion



Key Testing Tools

- Patient history
- Antibody screen
- Grading
- Antibody identification
- Inclusions and exclusions
- Antibody panel cells (IAT and enzyme)
- Antigram (panel sheet)
- Clinical significance of antibodies
- Phenotyping/genotyping
- Direct antiglobulin test
- Elution

Next time…

- ABID – Putting it into practice
- Interactive session covering case studies
- Panel sheets will be supplied prior to the session
- Use polls to provide answers
- Opportunity for questions

What is your favourite antibody?

Thank you

Certificates of attendance and details of subsequent webinars will be shared in the next few days

Register to the Only Cells Webinar mailing list (scan QR code)