ABID: Putting it into practice

WEBINAR 2/3

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Antibody Identification

Session 2/3 – Putting it into practice

Helen Thom

Today’s session overview

- Brief refresher on key tools from session 1
- Case based approach to antibody identification
- Utilise polls to test our understanding

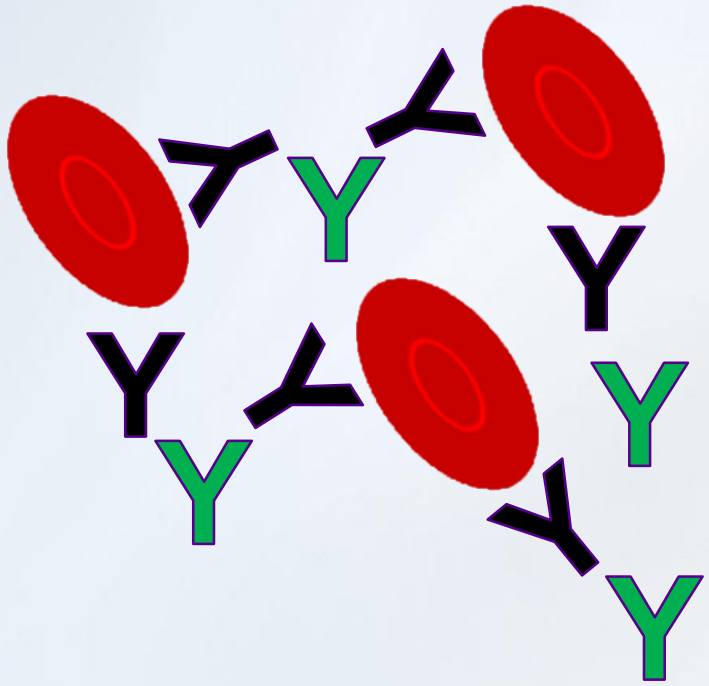
Session 1 recap

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

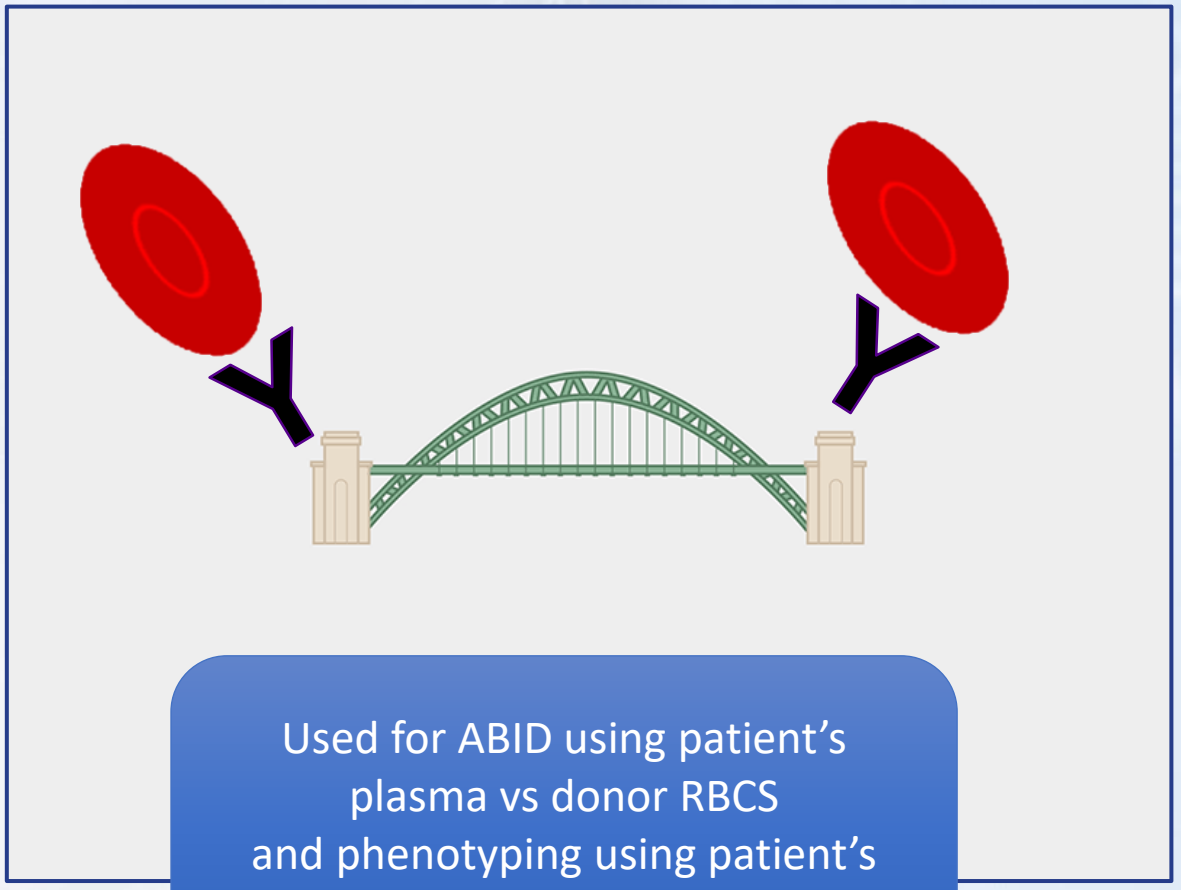
Indirect Antiglobulin Test (IAT)



IgG cannot bridge the gap between RBCs

Anti-IgG

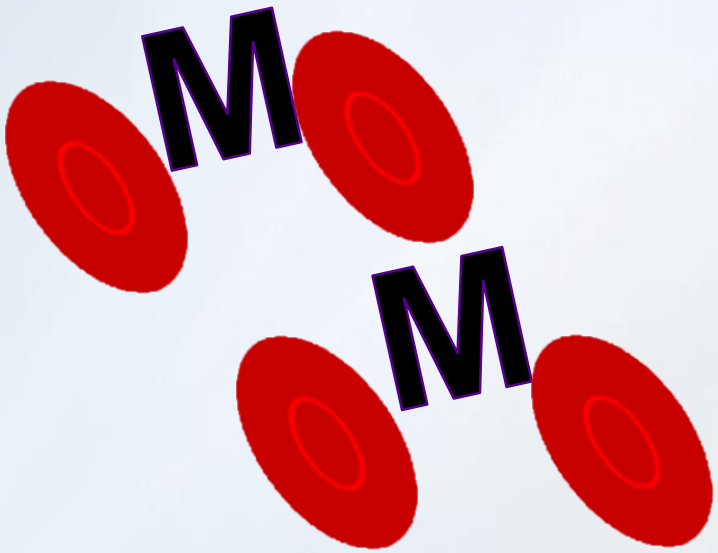
AHG – Anti Human Globulin (IgG and C3d)



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

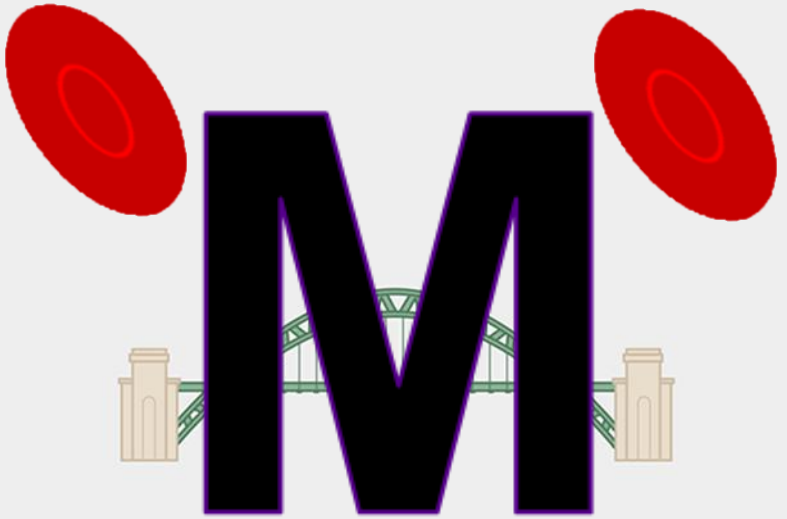
https://dribbble.com/shots/3376611-Tyne-Bridge-Animated

Direct Agglutination



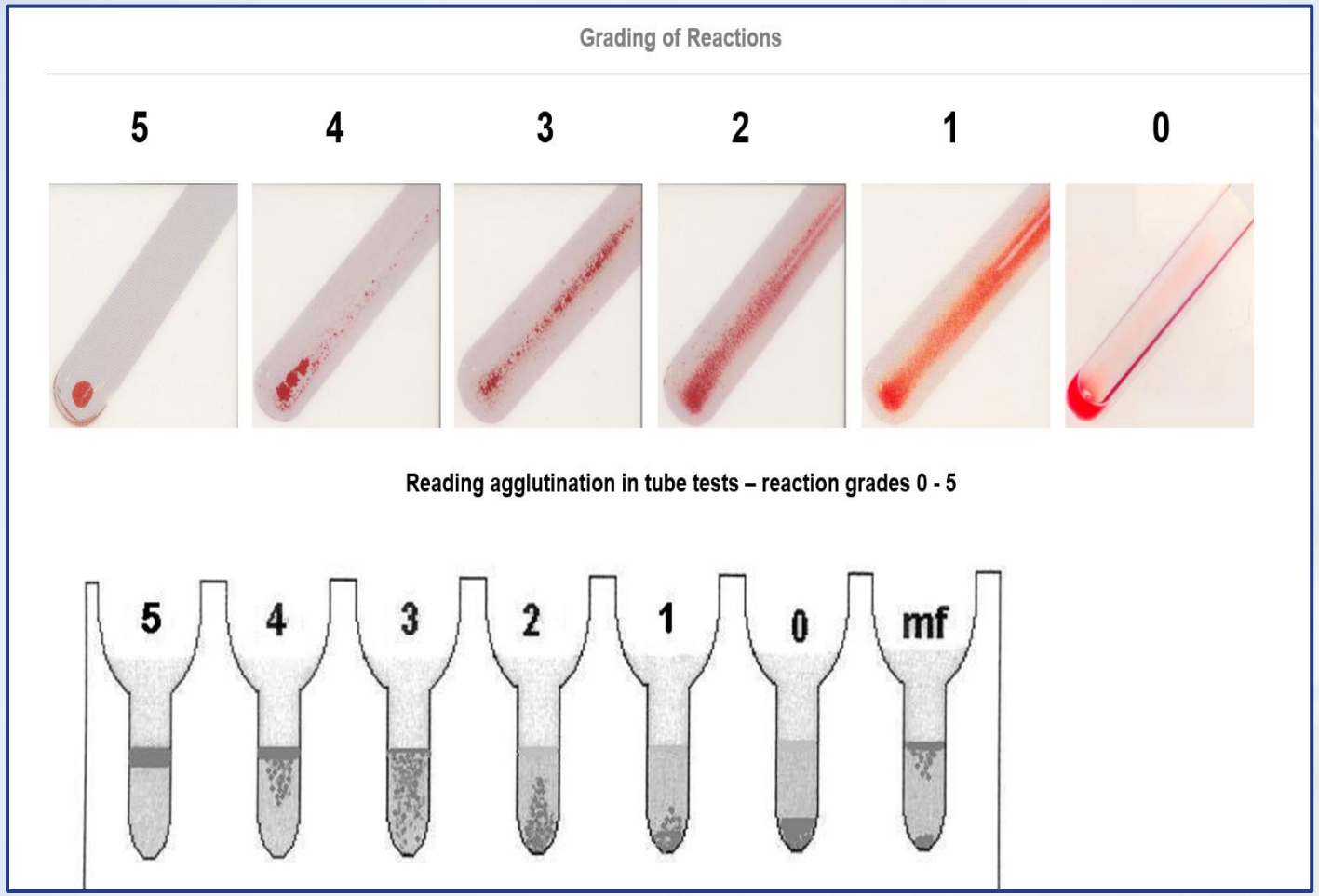
IgM can bridge the gap between RBCs

Does not require AHG



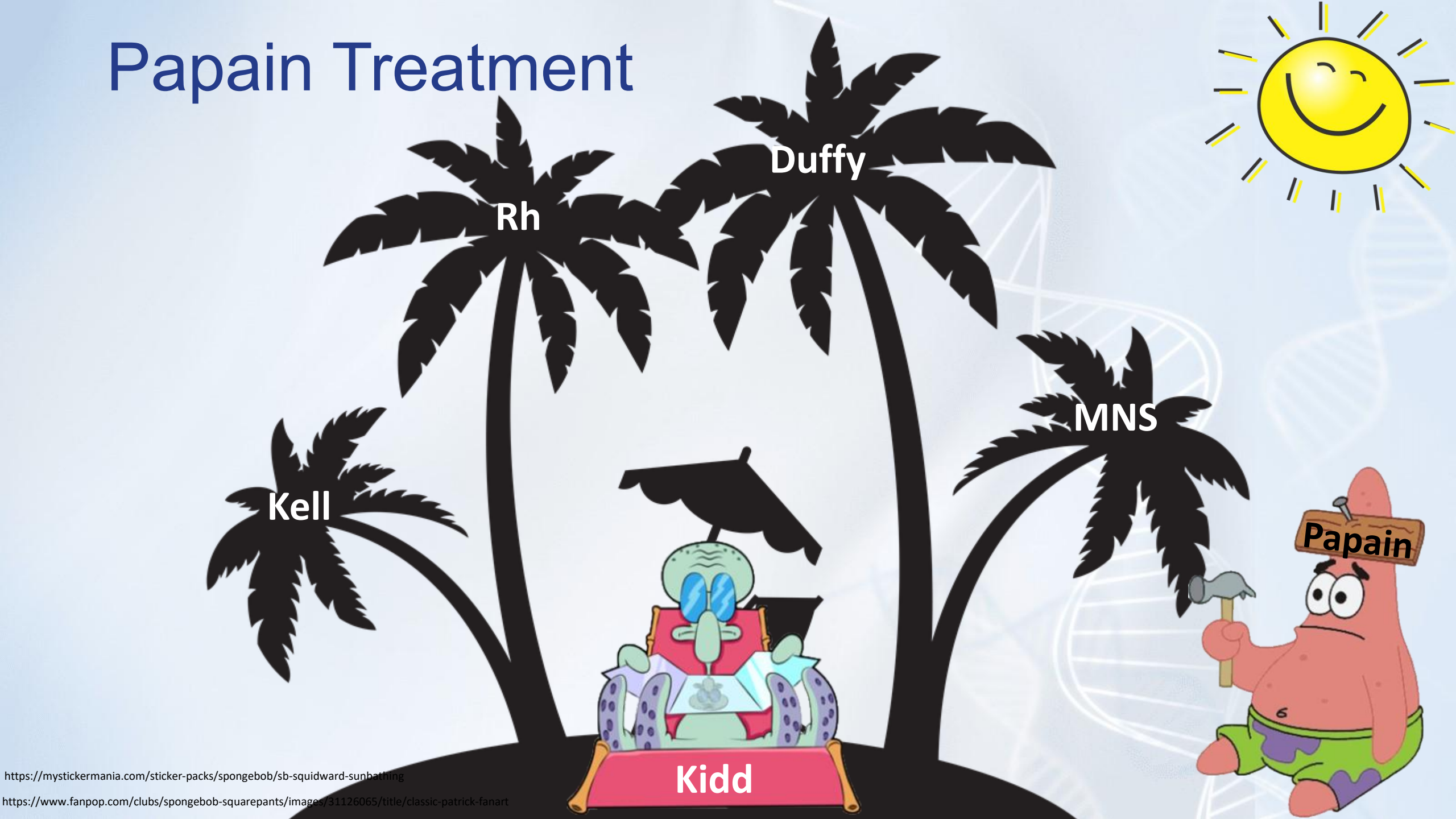
Used for blood grouping, phenotyping using IgM anti-sera and ‘cold’ antibodies

Grading



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

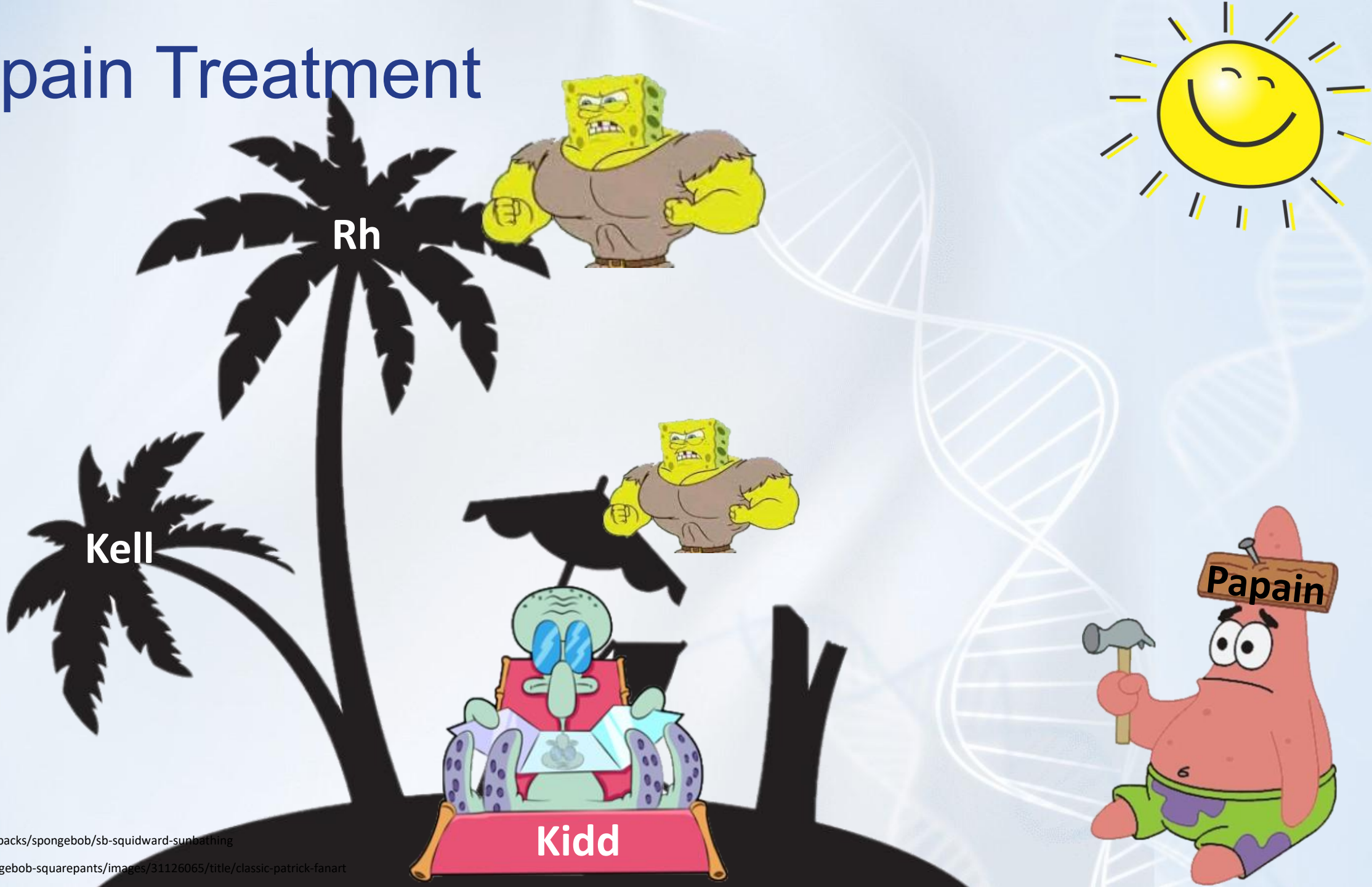
Papain Treatment



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

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

Papain Treatment



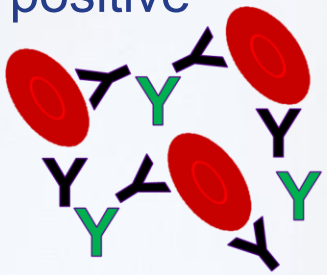
https://mystickermania.com/sticker-packs/spongebob/sb-squidward-sunbathing

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

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

‘Homozygous’ and ‘heterozygous’ expression

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

We also discussed…

- How antibody screening works
- The make up of antibody panels to allow for inclusions and exclusions
- Clinical significance of antibodies
- Auto control
- Direct Antiglobulin test (DAT)
- How to perform an Elution

Session 2

Putting it into practice

Case 1

- 75-year-old male
- #NOF
- Grouped as A RhD+
- No record of historical alloantibodies
- Never been transfused
- 2 units of red blood cells have been requested

Case 1 Panel

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	3	0	+	0	0	0	+	0	+	0	+		0	0
2	R_1R_1	+	+	0	0	+	+	+	0	+	1	0	0	+	0	+	0	+	0	+	0	HLA+	1	0
3	R_2R_2	+	0	+	+	0	0	+	0	+	3	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	1	0	0	+	0	0	+	+	0	0	+		0	0
6	rr	0	0	0	+	+	0	+	0	+	4	0	+	0	0	0	+	0	+	+	0	HLA+	2	0
7	rr	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	+		0	0
9	rr	0	0	0	+	+	0	+	+	0	1	0	0	+	0	0	+	+	0	+	0		1	0
10	rr	0	0	0	+	+	+	0	0	+	3	+	0	+	0	0	+	0	+	+	0		0	0
																						Auto	0	/
																						K control	2	/

Case 1 - What antibody have you identified?

Case 1

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	3	0	+	0	0	0	+	0	+	0	+		0	0
2	R_1R_1	+	+			+	+	+			1	0	0	+	0	+	0	+	0	+	0		1	0
3	R_2R_2	+	0			0	0	+	0	+	3	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	1	0	0	+	0	0	+	+	0	0	+		0	0
6	rr	0	0			+	0	+	0	+	4	0	+	0	0	0	+	0	+	+	0		2	0
7	rr	0	0			+	+	+			0	0	+	+	0	0	+	W	0	+	0		1	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		1	0
10	rr	0	0	0	+	+	+	0	0	+	3	+	0	+	0	0	+	0	+	+	0		0	0
																						Auto	0	/
																						K control	2	/

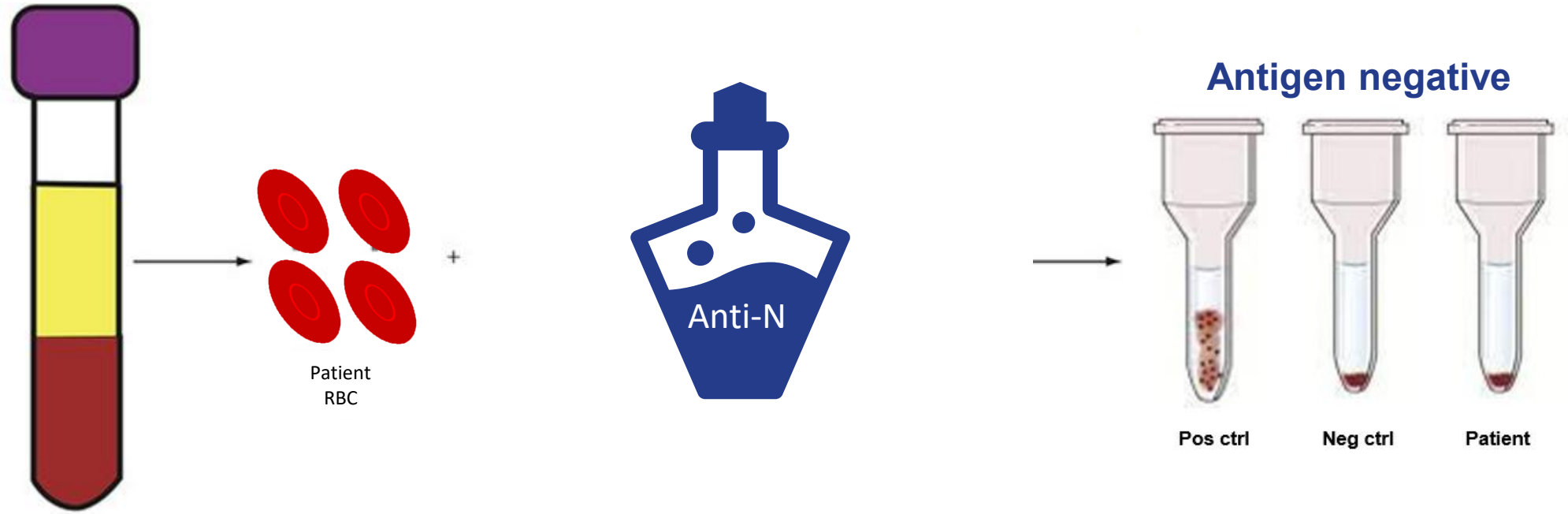
https://uk.pinterest.com/pin/spongebob-squarepants--433541901609503367/

Case 1 - 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	3	0	+	0	0	0	+	0	+	0	+		0	0
2	R_1R_1	+	+	0	0	+	+	+	0	+	1	0	0	+	0	+	0	+	0	+	0	HLA+	1	0
3	R_2R_2	+	0			0	0	+	0	+	3	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	1	0	0	+	0	0	+	+	0	0	+		0	0
6	rr	0	0			+	0	+	0	+	4	0	+	0	0	0	+	0	+	+	0		2	0
7	rr	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	+		0	0
9	rr	0	0	0	+	+	0	+	+	0	1	0	0	+	0	0	+	+	0	+	0		1	0
10	rr	0	0	0	+	+	+	0	0	+	3	+	0	+	0	0	+	0	+	+	0		0	0
																						Auto	0	/
																						K control	2	/

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

Phenotyping – IgM antisera



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

Is alloanti-N clinically significant?

- All MNS alloantibodies are clinically significant
- Alloanti-N is only clinically significant if detected by IAT
- Alloanti-N is only clinically significant when detected by Direct agglutination
- Alloanti-N is not likely to be clinically significant

MNS

MNS
- Destroyed by papain – except anti-U
- Dosage

Anti-M
- Clinically significant if reactive by IAT (37◦C)
- Antigen negative RBC required if detected by IAT

Anti-N
- Unlikely to be clinically significant
- Crossmatch compatible by IAT

Anti-S
- Clinically significant
- Antigen negative RBC required

Anti-s
- Clinically significant
- Antigen negative RBC required

Anti-U
- High Incidence antibody
- Clinically significant
- Antigen negative RBC required

What blood would you select?

- ABO, D and K compatible
- ABO, D and K compatible, N-
- ABO, D and K compatible, extended Rh matched
- ABO, D and K compatible, extended Rh matched, N-

Case 2

- 32-year-old female
- Pregnant
- Booking bloods (11 weeks)
- Grouped as B RhD+ E- K- Fy(b-)
- Previous alloanti-Fy^b which was too weak to titrate in previous pregnancy
- Never been transfused
- ABID

Case 2 panel

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	+		4	0
3	R_2R_2	+	0	+	+	0	+	0	+	0	0	0	0	+	0	+	0	0	+	+	0		4	0
4	r'r	0	+	0	+	+	0	+	+	0	0	0	0	+	0	0	+	0	+	+	0		4	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		4	0
8	rr	0	0	0	+	+	0	+	0	+	0	0	0	+	+	0	+	0	+	0	+		4	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	/
																						K control	2	/

Case 2 - What antibody have you identified?

Case 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	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	+		4	0
3	R_2R_2	+	0	+	+	0	+	0	+	0	0	0	0	+	0			0	+	+	0		4	0
4	r'r	0	+	0	+	+	0	+	+	0	0	0	0	+	0			0	+	+	0		4	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		4	0
8	rr	0	0	0	+	+	0	+	0	+	0	0	0	+	+			0	+	0	+		4	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	/
																						K control	2	/

Case 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	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	+		4	0
3	R_2R_2	+	0	+	+	0	+	0	+	0	0	0	0	+	0	→	→	0	+	+	0	→	4	0
4	r'r	0	+	0	+	+	0	+	+	0	0	0	0	+	0	0	+	0	+	+	0		4	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
7	rr	0	0	0	+	+	0	+	0	+	2	+	+	+	0	+	0	0	+	+	0		4	0
8	rr	0	0	0	+	+	0	+	0	+	0	0	0	+	+	0	+	0	+	0	+		4	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	/
																						K control	2	/

Duffy

Duffy
- Destroyed by papain – except anti-Fy^3
- Dosage

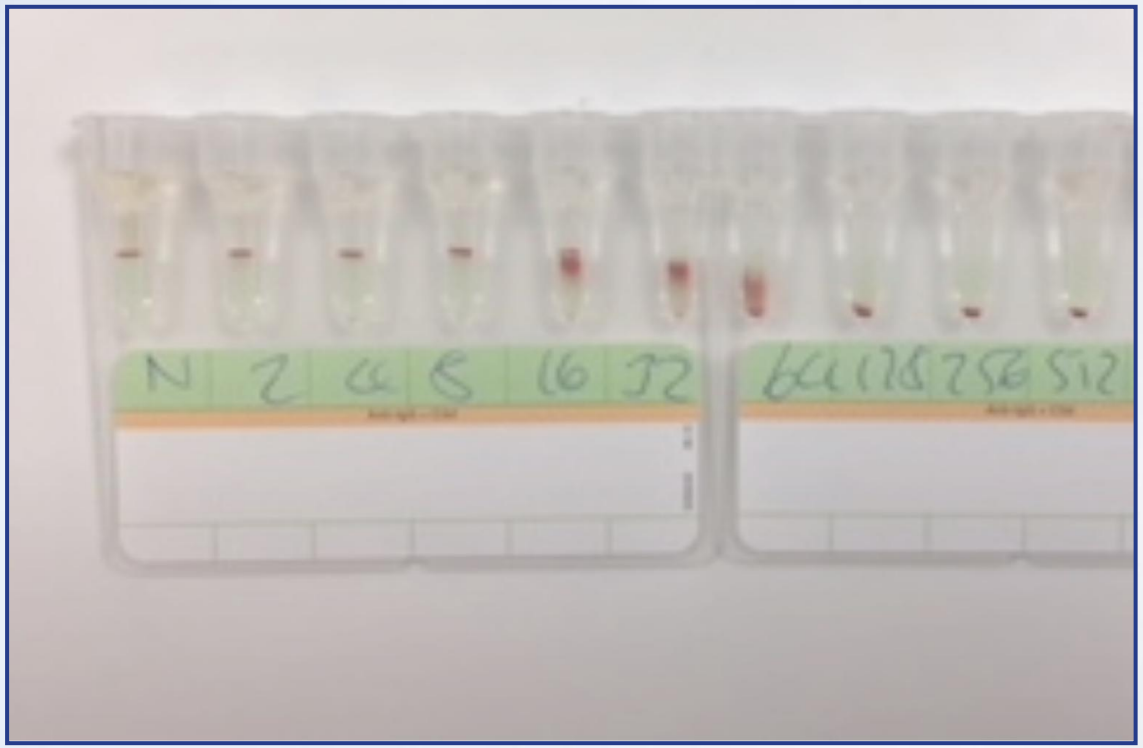
Anti-Fy^a
- Clinically significant
- Antigen negative RBC required

Anti-Fy^b
- Clinically significant
- Antigen negative RBC required

Anti-Fy^3
- High incidence antibody
- Clinically significant
- Antigen negative RBC required: Fy(a-b-)

Doubling dilutions

Titres



‘Heterozygous’ expression

Is this titre clinically significant?

- Yes – this is a clinically significant antibody and titre is ≥32
- Yes - this is a clinically significant antibody and titre is <32
- No - this antibody is not clinically significant and did not require titration

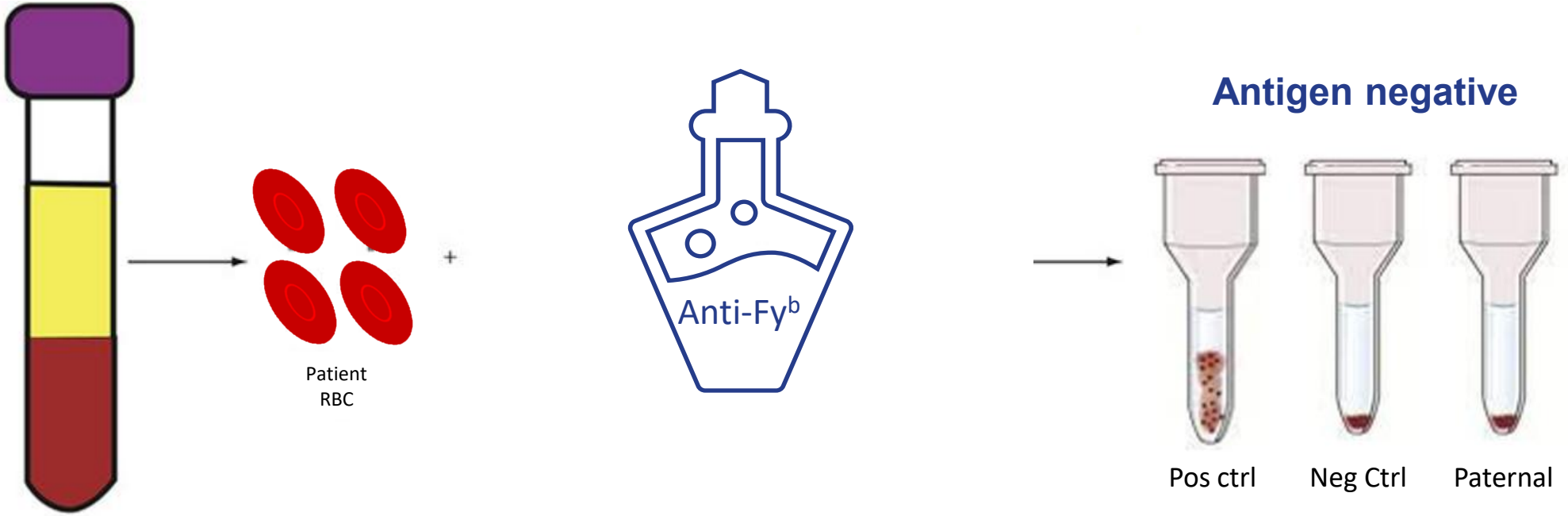
Is there anything else we can consider?

Paternal Phenotype

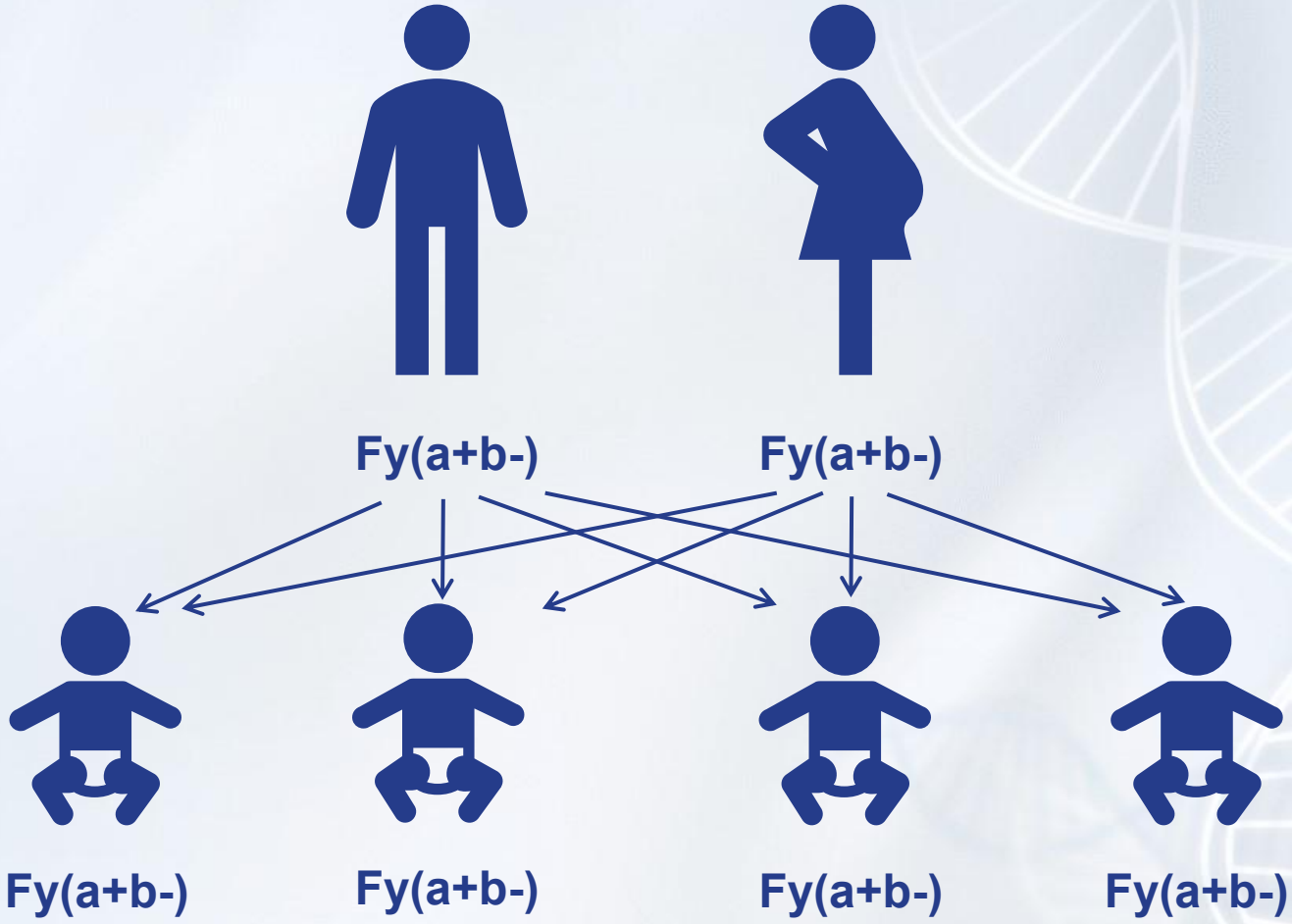
Fetal Genotype
(Cell Free Fetal DNA)

Fetal Genotype
(Chorionic villus sampling or
amniotic fluid)

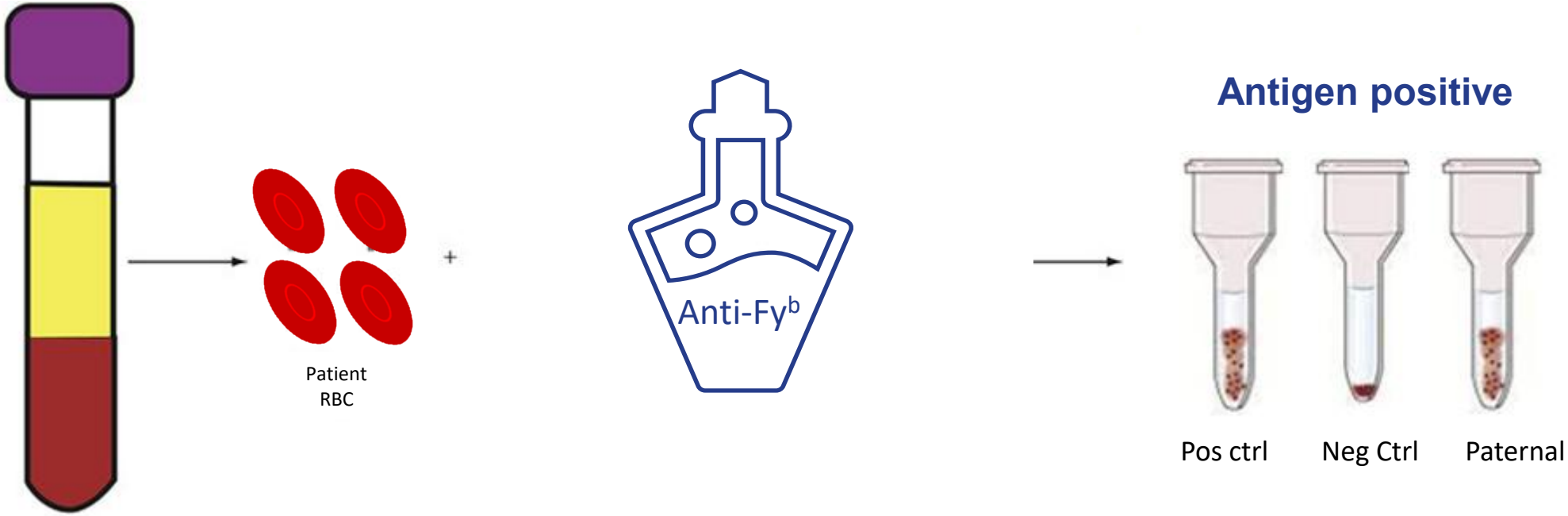
Paternal phenotyping



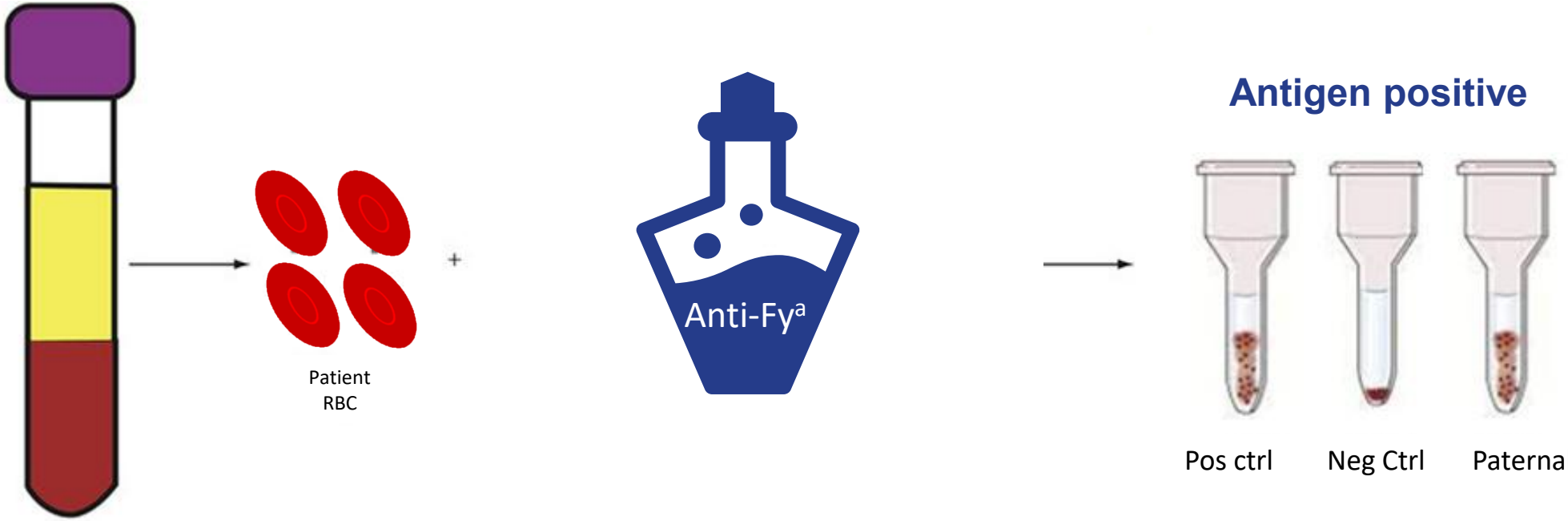
Offspring



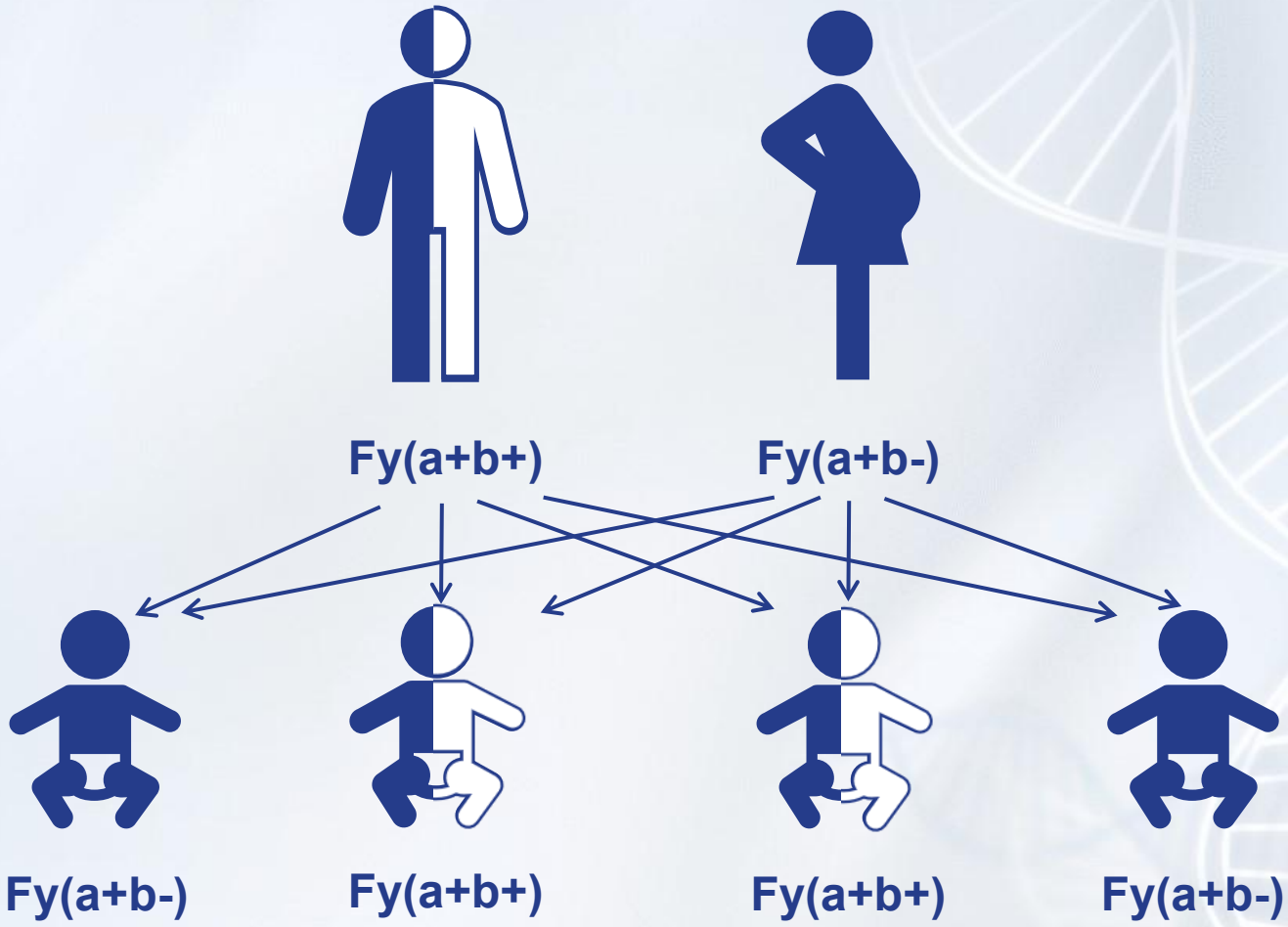
Paternal phenotyping



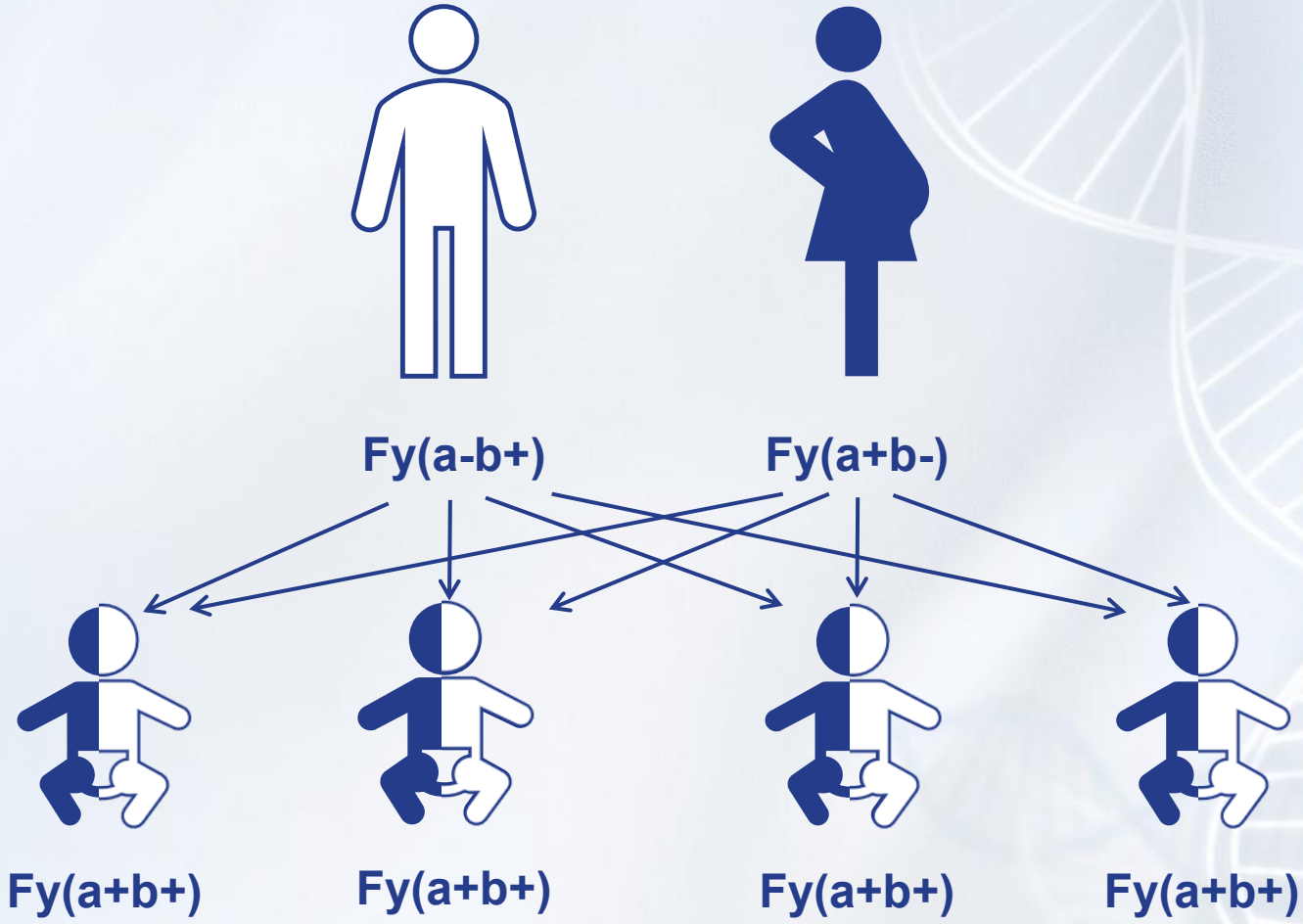
Paternal phenotyping



Offspring



Offspring



Case 3

- 65-year-old female
- Hysterectomy
- Grouped as O RhD+
- No historical antibodies
- Never been transfused
- ABID

Case 3 panel

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	+	+	2	0	0	+	0	0	+	0	+	+	0		0	0
2	R_1R_1	+	+	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		4	5
4	r'r	0	+	0	+	+	0	+	0	+	0	0	0	+	0	0	+	0	+	+	0		2	5
5	r''r	0	0	+	+	+	0	+	+	0	4	0	0	+	0	0	+	+	0	0	+		4	5
6	rr	0	0	0	+	+	+	0	+	0	4	0	+	0	0	0	+	+	0	0	+		3	5
7	rr	0	0	0	+	+	0	+	0	+	2	+	+	+	0	+	0	+	+	+	0		3	5
8	rr	0	0	0	+	+	0	+	0	+	0	0	0	+	+	0	+	0	+	0	+		3	5
9	rr	0	0	0	+	+	+	0	0	+	2	0	0	+	0	+	0	+	0	0	+		3	5
10	rr	0	0	0	+	+	0	+	0	+	3	0	0	+	0	+	0	+	0	+	0		3	5
																						Auto	0	/
																						K control	2	/

Case 3 - What antibody(ies) have you identified?

Case 3 panel

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	+	+	2	0	0	+	0	0	+	0	+	+	0		0	0
2	R_1R_1	+	+	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		4	5
4	r'r	0	+	0	+	+	0	+	0	+	0	0	0	+	0	0	+	0	+	+	0		2	5
5	r''r	0	0	+	+	+	0	+	+	0	4	0	0	+	0	0	+	+	0	0	+		4	5
6	rr	0	0	0	+	+	+	0	+	0	4	0	+	0	0	0	+	+	0	0	+		3	5
7	rr	0	0	0	+	+	0	+	0	+	2	+	+	+	0	+	0	+	+	+	0		3	5
8	rr	0	0	0	+	+	0	+	0	+	0	0	0	+	+	0	+	0	+	0	+		3	5
9	rr	0	0	0	+	+	+	0	0	+	2	0	0	+	0	+	0	+	0	0	+		3	5
10	rr	0	0	0	+	+	0	+	0	+	3	0	0	+	0	+	0	+	0	+	0		3	5
																						Auto	0	/
																						K control	2	/

Is anti Kp^a clinically significant?

Why can’t you exclude anti-S?

- The S+ cell is ‘homozygous’
- The S+ cell is ‘heterozygous’

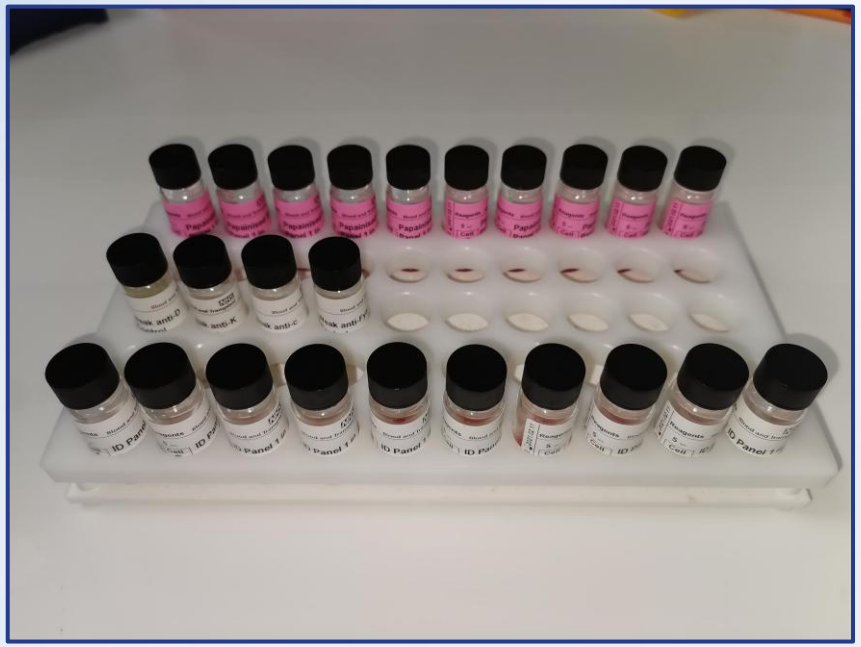
Cell	Rh	D	C	E	c	e	M	N	S	s	P1	Lu^a	K	k	Kp^a	Le^a	Le^b	Fy^a	Fy^b	Jk^a	Jk^b	Other	IAT	EIAT
1	$R_1^wR_1$	+	+	0	0	+	+	0	+	+	2	0	0	+	0	0	+	0	+	+	0		0	0
2	R_1R_1	+	+	0	0	+	0	+	0	+	0	+	+	+	0	+	0	+	0	0	+		0	0

How could we see if anti-E is present?

- Test against c- E+ cells
- Test against c+ E- cells
- Test against c+ E+ cells
- You can tell it's present from the reaction strength
- Perform phenotyping

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	+	+	2	0	0	+	0	0	+	0	+	+	0		0	0
2	R_1R_1	+	+	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		4	5
4	r'r	0	+	0	+	+	0	+	0	+	0	0	0	+	0	0	+	0	+	+	0		2	5
5	r''r	0	0	+	+	+	0	+	+	0	4	0	0	+	0	0	+	+	0	0	+		4	5

Panel cells



- “Primary panel“
  - Cellstab – column agglutination technology (CAT)
  - Alsevers – tube technique
- Secondary panel
- Screening cells (2 or 3 cells) – CAT and tube
- Rare cells – negative for high incidence antigens
- R1R1 panel – used when a patient has alloanti-c
- R2R2 panel – used when a patient has alloanti-e
- DTT treated panel cells – used for patients on monoclonal antibody therapy (anti-CD38) – session 3

Rh nomenclature

Name	Phenotype	Frequency (based on UK donor population)
R_1	DCe	42%
R_2	DcE	14%
R_O	Dce	3%
R_Z	DCE	<1%
r	dce	39%
r’	dCe	1%
r’’	dcE	1%
r^y	dCE	<1%

Adapted from Introduction to Transfusion Science, The Rh Blood Group System, p161

Rh shorthand from panels

Rh	D	C	E	c	e
R_1R_1	+	+	0	0	+
R_2R_2	+	0	+	+	0
r'r	0	+	0	+	+
r''r	0	0	+	+	+
rr	0	0	0	+	+
R1Rz	+	+	+	0	+
Rzr'	+	+	+	0	+
RoRo	+	0	0	+	+

Case 3 - R1R1 panel

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
2	**R_1R_1**	+	+	0	0	+	0	+	0	+	**0**	**0**	+	+	**0**	+	**0**	**0**	+	**0**	+		0
3	**R1Rz**	+	+	+	0	+	+	0	+	0	**0**	**0**	**0**	+	**0**	+	**0**	**0**	+	+	**0**		4
4	Rzr'	+	+	+	0	+	0	+	+	0	**0**	**0**	**0**	+	**0**	**0**	+	**0**	+	+	**0**		4
5	RoRo	+	0	0	+	+	0	+	0	+	**4**	**0**	**0**	+	**0**	**0**	+	+	**0**	**0**	+		4
6	R1R1	+	+	0	0	+	+	0	+	0	**4**	**0**	+	**0**	**0**	**0**	+	+	**0**	**0**	+		0
7	**R1R1**	+	+	0	0	+	0	+	0	+	**2**	+	+	+	**0**	+	**0**	**0**	+	+	**0**		0
8	R1R1	+	+	0	0	+	0	+	+	0	**0**	**0**	**0**	+	+	**0**	+	**0**	+	**0**	+		0
9	R1R1	+	+	0	0	+	+	0	0	+	**2**	0	**0**	+	**0**	+	**0**	+	**0**	**0**	+		0
10	R1R1	+	+	0	0	+	0	+	0	+	**3**	**0**	**0**	+	**0**	+	**0**	+	**0**	+	**0**		0
																						Auto	**0**
																						K control	**2**

Inclusions – panel 1 for anti-c

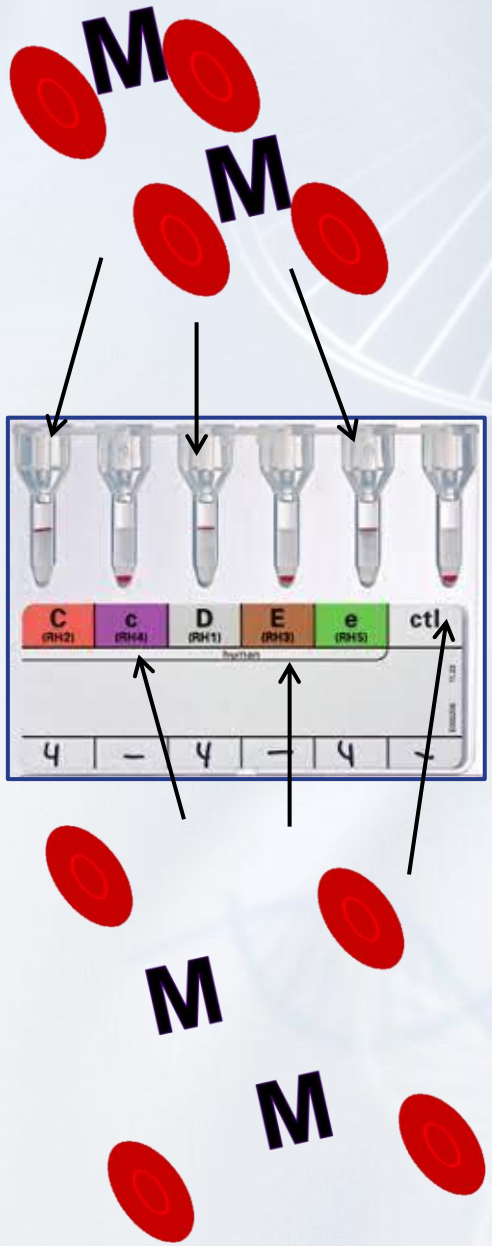
Cell	Rh	D	C	E	c	e	M	N	S	s	P1	Lu^a	K	k	Kp^a	Le^a	Le^b	Fy^a	Fy^b	Jk^a	Jk^b	Other	IAT	EIAT
1	$R_1^wR_1$	+	+	0	0	+	+	0	+	+	2	0	0	+	0	0	+	0	+	+	0		0	0
2	R_1R_1	+	+	0	0	+	0	+	0	+	0	+	+	+	0	+	0	+	0	0	+		0	0
3	R_2R_2	+	0	+	+	0	+	0	+	0	0	0	0	+	0	+	0	0	+	+	0		4	5
4	r'r	0	+	0	+	+	0	+	0	+	0	0	0	+	0	0	+	0	+	+	0		2	5
5	r''r	0	0	+	+	+	0	+	+	0	4	0	0	+	0	0	+	+	0	0	+		4	5
6	rr	0	0	0	+	+	+	0	+	0	4	0	+	0	0	0	+	+	0	0	+		3	5
7	rr	0	0	0	+	+	0	+	0	+	2	+	+	+	0	+	0	+	+	+	0		3	5
8	rr	0	0	0	+	+	0	+	0	+	0	0	0	+	+	0	+	0	+	0	+		3	5
9	rr	0	0	0	+	+	+	0	0	+	2	0	0	+	0	+	0	+	0	0	+		3	5
10	rr	0	0	0	+	+	0	+	0	+	3	0	0	+	0	+	0	+	0	+	0		3	5
																						Auto	0	/
																						K control	2	/

Inclusions – R1R1 panel for anti-E

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
2	**R_1R_1**	+	+	0	0	+	0	+	0	+	**0**	**0**	+	+	**0**	+	**0**	**0**	+	**0**	+		0
3	**R1Rz**	+	+	+	0	+	+	0	+	0	**0**	**0**	**0**	+	**0**	+	**0**	**0**	+	+	**0**	→	4
4	Rzr'	+	+	+	0	+	0	+	+	0	**0**	**0**	**0**	+	**0**	**0**	+	**0**	+	+	**0**	→	4
5	RoRo	+	0	0	+	+	0	+	0	+	**4**	**0**	**0**	+	**0**	**0**	+	+	**0**	**0**	+		4
6	R1R1	+	+	0	0	+	+	0	+	0	**4**	**0**	+	**0**	**0**	**0**	+	+	**0**	**0**	+		0
7	**R1R1**	+	+	0	0	+	0	+	0	+	**2**	+	+	+	**0**	+	**0**	**0**	+	+	**0**		0
8	R1R1	+	+	0	0	+	0	+	+	0	**0**	**0**	**0**	+	+	**0**	+	**0**	+	**0**	+	→	0
9	R1R1	+	+	0	0	+	+	0	0	+	**2**	0	**0**	+	**0**	+	**0**	+	**0**	**0**	+	→	0
10	R1R1	+	+	0	0	+	0	+	0	+	**3**	**0**	**0**	+	**0**	+	**0**	+	**0**	+	**0**		0
																						Auto	**0**
																						K control	**2**

Phenotyping

- Requires Anti-c, E and K phenotypes
- Extended Rh/K phenotype
- Autocontrol is negative
- Using IgM antisera in this case, but always be mindful of autocontrol



Rh

Rh

- Enhanced by papain
- Match for extended Rh once Rh antibody detected

Anti-D

- Clinically significant – most immunogenic after ABO
- Most significant in terms of HDFN
- Antigen negative RBC required

Anti-C/c

- Both clinically significant
- Both implicated in HDFN, particularly anti-c
- Antigen negative RBC required

Anti-E/e

- Clinically significant
- Both implicated in HDFN
- Antigen negative RBC required

Others

- Some are clinically significant, some may be clinically significant
- e.g anti-Cw
- Check BSH guidelines

Anti-e panel example

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		3	4
2	R_1R_1	+	+	0	0	+	0	+	0	+	0	0	+	+	0	+	0	0	+	0	+		3	4
3	R_2R_2	+	0	+	+	0	+	0	+	0	0	0	0	+	0	+	0	0	+	+	+		0	0
4	r'r	0	+	0	+	+	0	+	+	0	0	0	0	+	0	0	+	0	+	+	0		3	4
5	r''r	0	0	+	+	+	0	+	0	+	4	0	0	+	0	0	+	+	0	0	+		3	4
6	rr	0	0	0	+	+	+	0	+	0	4	0	+	0	0	0	+	+	0	0	+		3	4
7	rr	0	0	0	+	+	0	+	0	+	2	+	+	+	0	+	0	0	+	+	0		3	4
8	rr	0	0	0	+	+	0	+	0	+	0	0	0	+	+	0	+	0	+	0	+		3	4
9	rr	0	0	0	+	+	+	0	0	+	2	0	0	+	0	+	0	+	0	0	+		3	4
10	rr	0	0	0	+	+	0	+	0	+	3	0	0	+	0	+	0	+	0	+	0		3	4
																						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	EIAT
1	$R_1^wR_1$	+	+	0	0	+	+	0	+	0	0	0	0	+	0	0	+	+	0	+	0		3	4
2	R_1R_1	+	+	0	0	+	0	+	0	+	0	0	+	+	0	+	0	0	+	0	+		3	4
3	R_2R_2	+	0	+	+	0	+	0	+	0	0	0	0	+	0	+	0	0	+	+	+		0	0
4	r'r	0	+	0	+	+	0	+	+	0	0	0	0	+	0	0	+	0	+	+	0		3	4
5	r''r	0	0	+	+	+	0	+	0	+	4	0	0	+	0	0	+	+	0	0	+		3	4
6	rr	0	0	0	+	+	+	0	+	0	4	0	+	0	0	0	+	+	0	0	+		3	4
7	rr	0	0	0	+	+	0	+	0	+	2	+	+	+	0	+	0	0	+	+	0		3	4
8	rr	0	0	0	+	+	0	+	0	+	0	0	0	+	+	0	+	0	+	0	+		3	4
9	rr	0	0	0	+	+	+	0	0	+	2	0	0	+	0	+	0	+	0	0	+		3	4
10	rr	0	0	0	+	+	0	+	0	+	3	0	0	+	0	+	0	+	0	+	0		3	4
																						Auto	0	/
																						+ control	2	/

R2R2 Panel example

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	R2RZ	+	+	+	+	0	+	0	+	+	0	0	0	+	0	0	+	+	0	+	0		0
2	R2RZ	+	+	+	+	0	0	+	0	+	0	0	+	+	0	+	0	0	+	0	+		0
3	R2r”	+	0	+	+	0	+	0	+	0	0	0	0	+	0	+	0	0	+	+	0		0
4	R2R2	+	0	+	+	0	0	+	+	0	0	0	0	+	0	0	+	0	+	+	0		0
5	R2R2	+	0	+	+	0	0	+	0	+	4	0	0	+	0	0	+	+	0	0	+		0
6	R2R2	+	0	+	+	0	+	0	+	0	4	0	+	0	0	0	+	+	0	0	+		0
7	R2R2	+	0	+	+	0	0	+	0	+	2	+	+	+	0	+	0	0	+	+	0		0
8	R2R2	+	0	+	+	0	0	+	0	+	0	0	0	+	+	0	+	0	+	0	+		0
9	R2R2	+	0	+	+	0	+	0	0	+	2	0	0	+	0	+	0	+	0	0	+		0
10	R2R2	+	0	+	+	0	0	+	0	+	3	0	0	+	0	+	0	+	0	+	0		0
																						Auto	0
																						K control	2

Case 4

- 25-year-old female
- RTC
- Grouped as O RhD+
- No historical antibodies
- 1 previous pregnancy
- 4 units RBC

Case 4 panel

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	+	0	+		0	0
3	R_2R_2	+	0	+	+	0	+	0	+	0	0	0	0	+	0	+	0	0	+	+	+		0	0
4	r'r	0	+	0	+	+	0	+	+	0	0	0	0	+	0	0	+	0	+	+	0		2	4
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	+		2	4
7	rr	0	0	0	+	+	0	+	0	+	2	+	+	+	0	+	0	0	+	+	0		2	4
8	rr	0	0	0	+	+	0	+	0	+	0	0	0	+	+	0	+	0	+	0	+		2	4
9	rr	0	0	0	+	+	+	0	0	+	2	0	0	+	0	+	0	+	0	0	+		2	4
10	rr	0	0	0	+	+	0	+	0	+	3	0	0	+	0	+	0	+	0	+	0		2	4
																						Auto	0	/
																						+ control	2	/

Case 4 - What antibody have you identified?

Case 4 panel - exclusions

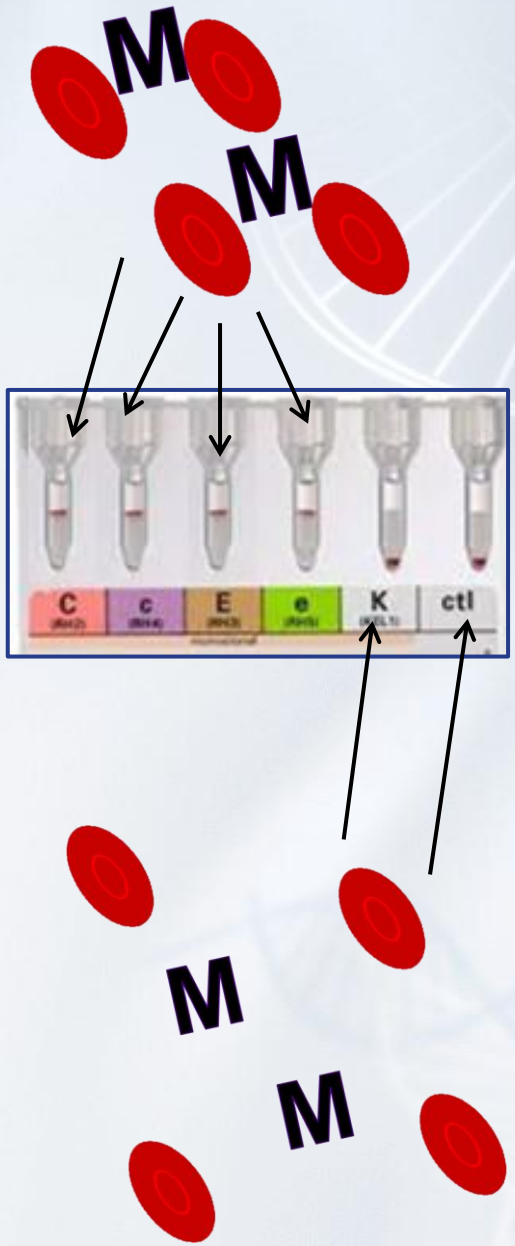
Cell	Rh	D	C	E	c	e	M	N	S	s	P1	Lu^a	K	k	Kp^a	Le^a	Le^b	Fy^a	Fy^b	Jk^a	Jk^b	Other	IAT	EIAT
1	$R_1^wR_1$	+	+	0	0	+	+	0	+	0	4	+	0	+	+	0	+	+	0	+	0		0	0
2	R_1R_1	+	+	0	0	+	0	+	0	+	0	0	+	+	0	+	0	0	+	0	+		0	0
3	R_2R_2	+	0	+	+	0	+	0	+	0	0	0	0	+	0	+	0	0	+	+	+		0	0
4	r'r	0	+	0	+	+	0	+	+	0	0	0	0	+	0	0	+	0	+	+	0		2	4
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	+		2	4
7	rr	0	0	0	+	+	0	+	0	+	2	+	+	+	0	+	0	0	+	+	0		2	4
8	rr	0	0	0	+	+	0	+	0	+	0	0	0	+	0	0	+	0	+	0	+		2	4
9	rr	0	0	0	+	+	+	0	0	+	2	0	0	+	0	+	0	+	0	0	+		2	4
10	rr	0	0	0	+	+	0	+	0	+	3	0	0	+	0	+	0	+	0	+	0		2	4
																						Auto	0	/
																						+ control	2	/

What would you do next?

Phenotyping

- Requires extended Rh/K phenotype
- Autocontrol is negative
- Using IgM antisera in this case, but always be mindful of autocontrol

- R1R2 – most likely phenotype



What the f?

f negative		f positive	
Name	Pheno	Name	Pheno
R1R1	DCe/DCe	rr	dce/dce
R2R2	DcE/DcE	r”r	dcE/dce
R1R2	DCe/DcE	r’r	dCe/dce
r’r’	dCe/dCe	RO	Dce/Dce
r”r”	dcE/dcE	R1r	DCe/dce
r’r”	dCe/dcE	R2r	DcE/dce

Case 4 - R1R1 panel

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
2	R_1R_1	+	+	0	0	+	0	+	0	+	0	0	+	+	0	+	0	0	+	0	+		0
3	R1Rz	+	+	+	0	+	+	0	+	0	0	0	0	+	0	+	0	0	+	+	0		0
4	Rzr'	+	+	+	0	+	0	+	+	0	0	0	0	+	0	0	+	0	+	+	0		0
5	RoRo	+	0	0	+	+	0	+	0	+	4	0	0	+	0	0	+	+	0	0	+		2
6	R1R1	+	+	0	0	+	+	0	+	0	4	0	+	0	0	0	+	+	0	0	+		0
7	R1R1	+	+	0	0	+	0	+	0	+	2	+	+	+	0	+	0	0	+	+	0		0
8	R1R1	+	+	0	0	+	0	+	+	0	0	0	0	+	+	0	+	0	+	0	+		0
9	R1R1	+	+	0	0	+	+	0	0	+	2	0	0	+	0	+	0	+	0	0	+		0
10	R1R1	+	+	0	0	+	0	+	0	+	3	0	0	+	0	+	0	+	0	+	0		0
																						Auto	0
																						K control	2

Back to original panel

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	+	0	+		0	0
3	R_2R_2	+	0	+	+	0	+	0	+	0	0	0	0	+	0	+	0	0	+	+	+		0	0
4	r'r	0	+	0	+	+	0	+	+	0	0	0	0	+	0	0	+	0	+	+	0		2	4
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	+		2	4
7	rr	0	0	0	+	+	0	+	0	+	2	+	+	+	0	+	0	0	+	+	0		2	4
8	rr	0	0	0	+	+	0	+	0	+	0	0	0	+	+	0	+	0	+	0	+		2	4
9	rr	0	0	0	+	+	+	0	0	+	2	0	0	+	0	+	0	+	0	0	+		2	4
10	rr	0	0	0	+	+	0	+	0	+	3	0	0	+	0	+	0	+	0	+	0		2	4
																						Auto	0	/
																						+ control	2	/

Case 4 - 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	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	+	0	+		0	0
3	R_2R_2	+	0	+	+	0	+	0	+	0	0	0	0	+	0	+	0	0	+	+	+		0	0
4	r'r	0	+	0	+	+	0	+	+	0	0	0	0	+	0	0	+	0	+	+	0		2	4
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	+		2	4
7	rr	0	0	0	+	+	0	+	0	+	2	+	+	+	0	+	0	0	+	+	0		2	4
8	rr	0	0	0	+	+	0	+	0	+	0	0	0	+	+	0	+	0	+	0	+		2	4
9	rr	0	0	0	+	+	+	0	0	+	2	0	0	+	0	+	0	+	0	0	+		2	4
10	rr	0	0	0	+	+	0	+	0	+	3	0	0	+	0	+	0	+	0	+	0		2	4
																						Auto	0	/
																						+ control	2	/

Is alloanti-f clinically significant?

- All Rh alloantibodies are clinically significant
- Alloanti-f is only clinically significant if detected by IAT
- Alloanti-f is clinically significant
- Not sure

Rh

Anti-f

- Clinically significant
- Implicated in HDFN
- Antigen negative RBC required

Compound Antibody

- Compound antibody
- Also known anti-ce

Phenotype

- Individuals are f negative when the c and e are on different genes

What blood would you select?

- Group O DCe/DCe (R1R1) K-
- Group O DcE/DcE (R2R2) K-
- Group O DCe/DcE (R1R2) K-
- Group O Dce/Dce (Ro) K-
- Group O dce/dce (rr) K-
- Not sure

What blood would you select?

- **Group O DCe/DCe (R1R1) K-**
- **Group O DcE/DcE (R2R2) K-**
- **Group O DCe/DcE (R1R2) K-**
- **All f (ce) negative – c and e are on different genes**
- Group O Dce/Dce (Ro) K-
- Group O dce/dce (rr) K-
- **These are f positive as the c and e are on the same gene**

Question for the group

“Why do pregnant women get weaker or completely lose their reverse group, I see this a lot”

Next time…

- High incidence antibodies and finding blood
- Plasma modification with case examples
  - Alloadsorptions
  - Neutralisation
  - DTT (RBC and plasma)

https://slidesdocs.com/background/science-technology-spiral-simple-powerpoint-background_dbc76465dc

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)