NHS
Blood and Transplant

OMG!!!

Helen Thom
BMSEDG#9

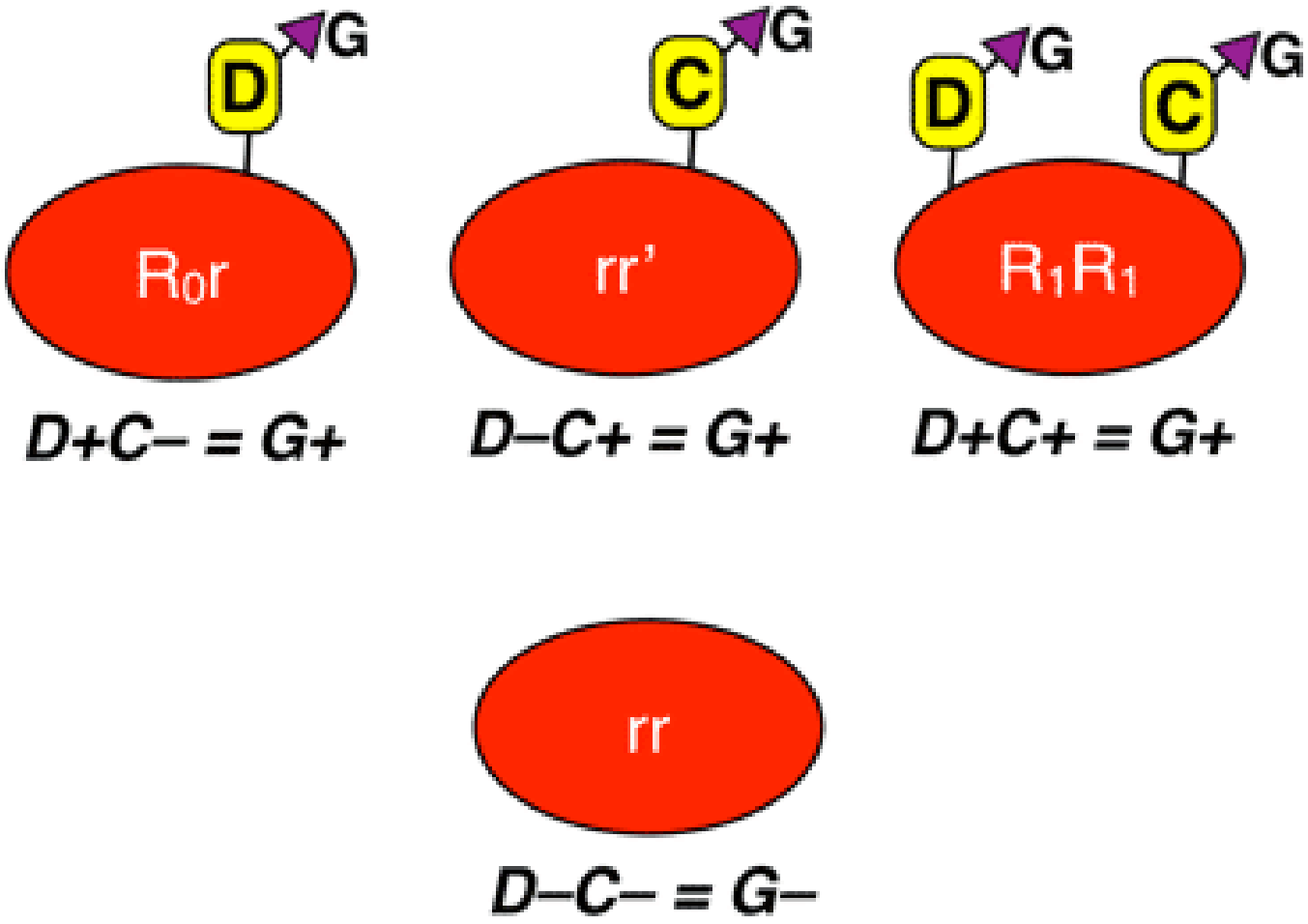
Caring Expert Quality

Objectives

- To try and understand anti-G
- How to distinguish between anti-D, C and G
- Why its important
- Some example scenarios
- Anonymous poll at the end
- Keep an eye out for the clues

So what is the G antigen?

- Part of the Rh Blood group System
- Rh12
- Clinically significant
- Enhanced by papain (enzyme treated cells)
- Present on red cells which carry the D antigen or the C antigen or both



https://www.bbguy.org/2016/06/17/want-g-wiz/

NHS Blood and Transplant

Some common G pos and G neg phenotypes

Name	Pheno	G Pos	Name	Pheno	G neg
R1R1	DCe/DCe	D+ C+ G+	rr	dce/dce	D- C- G-
R2R2	DcE/DcE	D+ C- G+	r”r”	dcE/dcE	D- C- G-
r’r	dCe/dCe	D- C+ G+	r”r	dcE/dce	D- C- G-
R1R2	DCe/DcE	D+ C+ G+			
RO	Dce/Dce	D+ C- G+			
R1r	DCe/dce	D+ C+ G+			
R2r	DcE/dce	D+ C- G+			

Not really ‘common’

Anti-D

NHS Blood and Transplant

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**		3	5
2	R_1R_1	+	+	0	0	+	0	+	0	+	**0**	**0**	+	+	**0**	+	**0**	**0**	+	**0**	+		3	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**	/
																						K control	**2**	/

Anti-C

NHS Blood and Transplant

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		3	5
2	R_1R_1	+	+	0	0	+	0	+	0	+	0	0	+	+	0	+	0	0	+	0	+		3	5
3	R_2R_2	+	0	+	+	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	5
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	/
																						K control	2	/

Anti-D + C

NHS Blood and Transplant

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		3	5
2	R_1R_1	+	+	0	0	+	0	+	0	+	0	0	+	+	0	+	0	0	+	0	+		3	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		3	5
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	/
																						K control	2	/

Anti-G (cannot excluded anti-D or C)

NHS Blood and Transplant

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**		3	4
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**	**+**		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**	/
																						K control	**2**	/

What makes us think its anti-G: Spot the difference!

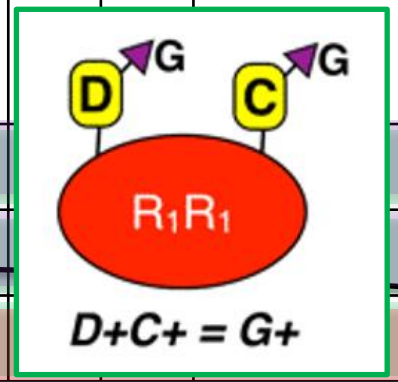
NHS Blood and Transplant

There are other clues which the reference lab would use – but it can get very confusing

Cell	Fy^b	Jk^a	Jk^b	Other	IAT	EIAT
1	0	+	0		3	5
2	+	0	+		3	5
3	+	+	0		4	5
4	+	+	0		3	5

R2R2 cells have more D antigen sites than R1R1 (steric hinderance) so with anti-D + C, you expect to see stronger reaction vs R2R2

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	IAT	EIAT
1	$R_1^wR_1$	+	+	0	0	+	+	0	+	+	0	0	0	+	0	0	+	+	0	4	5
2	R_1R_1	+	+	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	+	3	4
4	r'r	0	+	0	+	+	0	+	+	0	0	0	0	+	0	0	+	0	+	3	4



Weaker reaction vs R2R2 cell *indicates* anti-G. It *does not* exclude anti-D and/or C

BSH Guidelines: Selection of issue and issue of Red cells

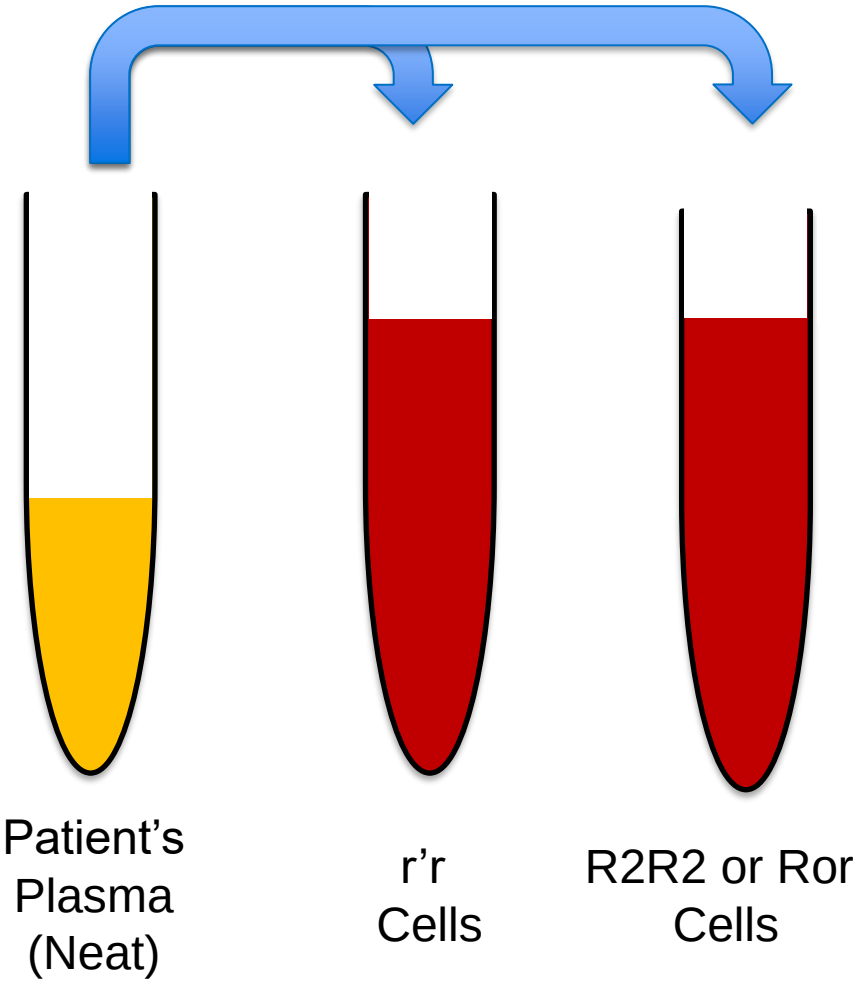
- 7.8.3.D negative red cells should always be selected for:
  - D negative women of childbearing potential (<51 years)
  - D negative patients <18 years old
  - Patients who have formed immune anti-D, even if not currently detectable
  - Transfusion-dependant D negative adults
- 7.10.2. Antigen negative red cells should also be selected when a clinically significant antibody has previously been identified, but cannot be detected or identified in the current sample
- 7.10.3.Patients with anti-D who are rr (ccddee) should receive rr (D- C- E-), K negative blood
- 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://b-s-h.org.uk/guidelines/guidelines/pre-transfusion-compatibility-procedures-in-blood-transfusion-laboratories/

Why does G matter?

- For transfusion purposes in an adult above child bearing potential - it doesn't
  - Select rr (D- C- G-)
- For children, and those of childbearing potential – it does
- The key thing with anti-G is to exclude anti-D (and/or anti-C)
  - Blood selection
  - Prevent sensitisation
  - Eligibility for RAADP
  - Assess risk for HDFN
    - For anti-D/G: need to quant neat and adsorbed plasma
    - Can't test anti-C/G in isolation – titre vs r'r

How to we do it?



Sequential adsorptions with papainised cells

Mix patients plasma with r'r – D- C+ G+

Mix patients plasma with R2R2/Ror – D+ C- G+

Incubate for 10 mins at 37°C
Centrifuge
Remove plasma – save each aliquot
Repeat adsorptions using each of the absorbed plasma – r'r, R2R2/Ror

Antigen positive adsorptions cells remove the corresponding antibody from the patient's plasma

Think antibody/antigen interactions

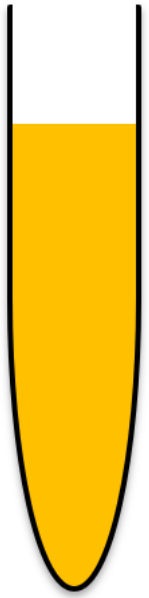
What you end up with….

Neat Plasma

Haven't adsorbed

Could contain anti-D, C and G

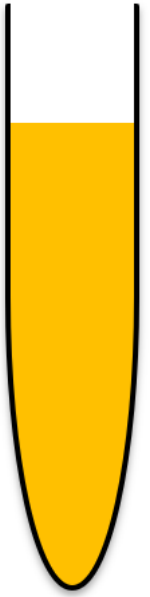
r'r (D-C+G+) Adsorbed Plasma



Have adsorbed out anti-C and G

Could contain anti-D

Ror (D+C-G+) Adsorbed Plasma



Have adsorbed out anti-D and G

Could contain anti-C

If you have anti-D, C and G

NHS Blood and Transplant

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	Neat IAT	r'r IAT	Ror IAT
1	$R_1^wR_1$	+	+	0	0	+	+	0	+	+	0	0	0	+							0	4	2	3
2	R_1R_1	+	+	0	0	+	0	+	0	+	0	0	+	+							+	4	2	3
3	R_2R_2	+	0	+	+	0	+	0	+	0	0	0	0	+							0	3	2	0
4	r'r	0	+	0	+	+	0	+	+	0	0	0	0	+	0	0	+	0	+	+	0	3	0	3
5	r''r	0	0	+	+	+	0	+	0	+	4	0	0	+							+	0	0	0
6	rr	0	0	0	+	+	+	0	+	0	4	0	+	0							+	0	0	0
7	rr	0	0	0	+	+	0	+	0	+	2	+	+	+							0	0	0	0
8	rr	0	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
10	rr	0	0	0	+	+	0	+	0	+	3	0	0	+							0	0	0	0
																					Auto	0	/	/
																					K control	2	/	/

r'r plasma:
removed
anti-C and G
leaves anti-D behind

Ror plasma:
removed
anti-D and G
leaves anti-C behind

Neat plasma
Therefore contains
anti-D+C+G

If you have anti-D and G

NHS Blood and Transplant

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	Neat IAT	r'r IAT	Ror IAT
1	$R_1^wR_1$	+	+	0	0	+	+	0	+	+	0	0	0	+							0	4	4	0
2	R_1R_1	+	+	0	0	+	0	+	0	+	0	0	+	+							+	4	4	0
3	R_2R_2	+	0	+	+	0	+	0	+	0	0	0	0	+							0	3	3	0
4	r'r	0	+	0	+	+	0	+	+	0	0	0	0	+	0	0	+	0	+	+	0	3	0	0
5	r''r	0	0	+	+	+	0	+	0	+	4	0	0	+							+	0	0	0
6	rr	0	0	0	+	+	+	0	+	0	4	0	+	0							+	0	0	0
7	rr	0	0	0	+	+	0	+	0	+	2	+	+	+							0	0	0	0
8	rr	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	0
10	rr	0	0	0	+	+	0	+	0	+	3	0	0	+							0	0	0	0
																					Auto	0	/	/
																					K control	2	/	/

r'r plasma:
removed
anti-C and G
leaves anti-D behind

Ror plasma:
removed
anti-D and G
Nothing left behind
Therefore does not contain anti-C

Neat plasma
Therefore contains anti-D+G

If you have anti-C and G

NHS Blood and Transplant

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

r'r plasma:
removed
anti-C and G
Nothing left behind
Therefore does not contain anti-D

Ror plasma:
removed
anti-D and G
Leaves behind anti-C

Neat plasma
Therefore contains
anti-C+G

If you have anti-G

NHS Blood and Transplant

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

r'r plasma:
removed
anti-C and G
Nothing left behind
Therefore does not
contain anti-D

Ror plasma:
removed
anti-D and G
Nothing left behind
Therefore does not
contain anti-C

Neat plasma
Therefore contains
anti-G

NHS Blood and Transplant

TYPICAL RESULTS

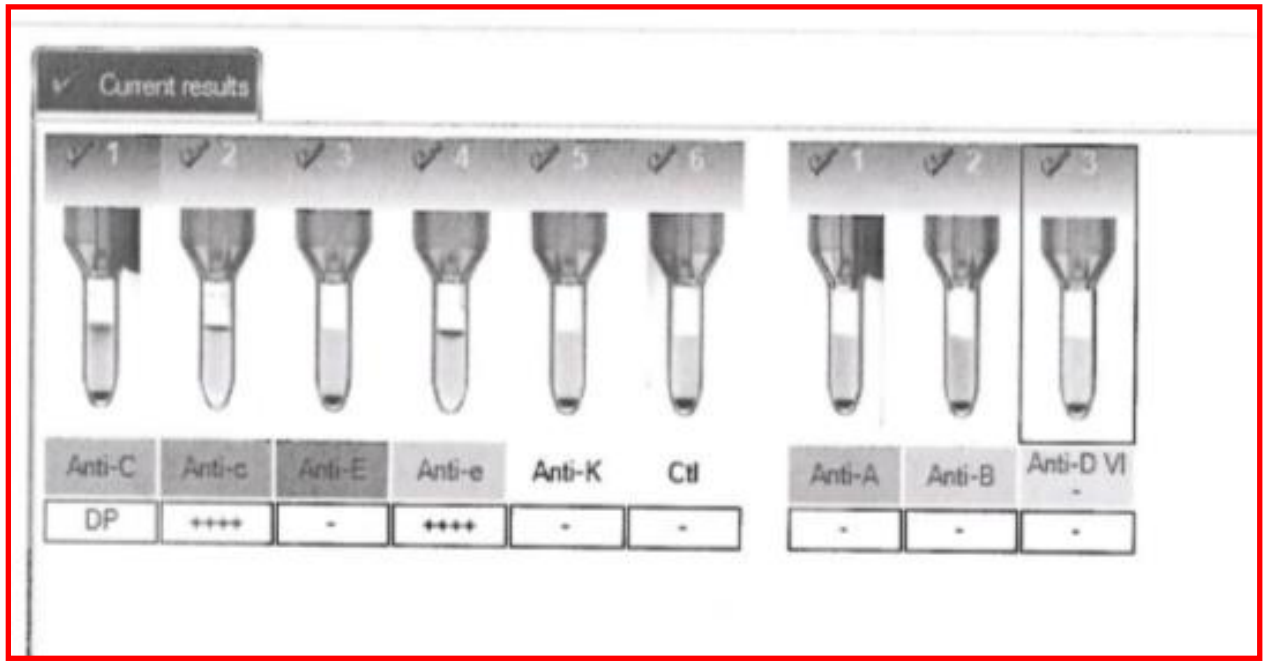
Absorbed with	Test Cells		Conclusion
	r’	R2R2 / R_o	
r’	0	0	**Anti-G only**
R2R2 / R_o	0	0	
r’	0	+	**Anti-D + G**
R2R2 / R_o	0	0	
r’	0	0	**Anti-C + G**
R2R2 / R_o	+	0	
r’	0	+	**Anti-D + C +/- G**
R2R2 / R_o	+	0	

Case Scenario 1

- 65 year old male
- Hb 75
- 2 units RBC requested
- ABID: presents as apparent anti-D+C
- What would you do next?
  - Confirm anti-C using second cell
  - Select ABO and K compatible, D- C- E-

Case Scenario 2

- 7 year old female
- Multi transfused since birth
- No previous alloantibodies
- ABID: apparent anti-D+C
- Extended Rh phenotype mixed field vs anti-C well
- What would you do next?



- Unit was C+ (therefore G+)
- G invest – showed anti-C and G (n/s)
- Select ABO compatible, D- C- E- K-

Case Scenario 3

- Pregnant woman at 28 weeks' gestation
- RhD negative
- Second pregnancy
- Negative screen at booking
- ABID: apparent anti-D+C
- No RAADP
- Never transfused
- What would you do next? Refer to RCI

Case Scenario 3 continued

- Potential outcomes:
  - Anti-D + C – quant anti-D and titre anti-C
  - Anti-D + C + G – quant anti-D vs neat and adsorbed, titre anti-C/G
  - Anti-D + G – quant anti-D vs neat and adsorbed, titre anti-G
  - Anti-C + G – titre anti-C/G – quant not required
  - Anti-G – titre anti-G – quant not required
- Quantitate neat and adsorbed plasma: the presence of anti-G can elevate quant result (R1R1 cells used) – need to assess risk of HDFN (using BSH guidelines) and eligibility for RAADP

- Can't titre C/G in isolation – titre vs r'r and report combined result

Case Scenario 3 continued

Antibody Quantification

Table 1. The significance of levels of anti-D

Anti-D concentration	Predicted clinical outcome
Less than 4 IU mL^{-1}	HDFN unlikely, continue to monitor
4–15 IU mL^{-1}	Moderate risk of HDFN, requiring referral to a fetal medicine specialist
More than 15 IU mL^{-1}	High risk of HDFN requiring referral, as above

Antibody Titration

Anti-C

Anti-G

Anti-C/G – can't titre in isolation

r'r cell: D- C+ G+

- <32: low risk HDFN
- ≥32: high risk HDFN

https://b-s-h.org.uk/guidelines/guidelines/blood-grouping-and-antibody-testing-in-pregnancy/

Example of Quant Results for anti-D with G (+/-C)

- Neat plasma: 5.0 IU/mL
  - elevated due to the presence of anti-G
- r'r adsorbed plasma: <0.2 IU/mL
  - Removed anti-C and G – leaves anti-D
  - N/S anti-D (likely prophylaxis)
  - Check history
- This patient is eligible for RAADP

- Neat plasma: 5.0 IU/mL
  - elevated due to the presence of anti-G
- r'r adsorbed plasma: 4.0IU/mL
  - Removed anti-C and G – leaves anti-D
  - In this case, there is an alloanti-D
  - Moderate risk HDFN
- This patient is NOT eligible for RAADP

Propholicious?

NHS Blood and Transplant

Antibodies	Anti-D specificity and level using adsorbed plasma		Eligible for RAADP
Anti-D + C	N/S	<0.4 IU/mL	Yes
Anti-D + C	ALLO	Any level	No
Anti-D + C + G	N/S	0.4 IU/mL	Yes
Anti-D + C + G	ALLO	Any level	No
Anti-D + G	N/S	<0.4 IU/mL	Yes
Anti-D + G	ALLO	Any level	No
Anti-C + G	N/A	N/A	Yes
Anti-G	N/A	N/A	Yes

It isn't as clear cut as this, but it gives you an idea of the eligibility for RAADP
The values obtained using the adsorbed plasma help to inform these decisions
RCI medics and Clinical Scientists are available to make the clinical decisions

Recap of the Clues

Weaker reaction vs R2R2 cell *indicates* anti-G

Patient's phenotype: must be G negative (rr, r"r" or r"r)

Elevated quant result

You will probably have a headache!

Thank you for listening

- I hope this has helped you to understand anti-G
- Please take our poll
- All answers anonymised