Investigation of DAT positive patients – "how much testing is needed?"

Rob Lees RCI Barnsley

NHS
Blood and Transplant

Caring Expert Quality

What is a positive DAT ?

A DAT uses anti-globulins to detect red cell bound Immunoglobulin G (IgG, IgM or IgA)
or human complement component (C3c or C3d) to bring about agglutination

Red cell bound IgG

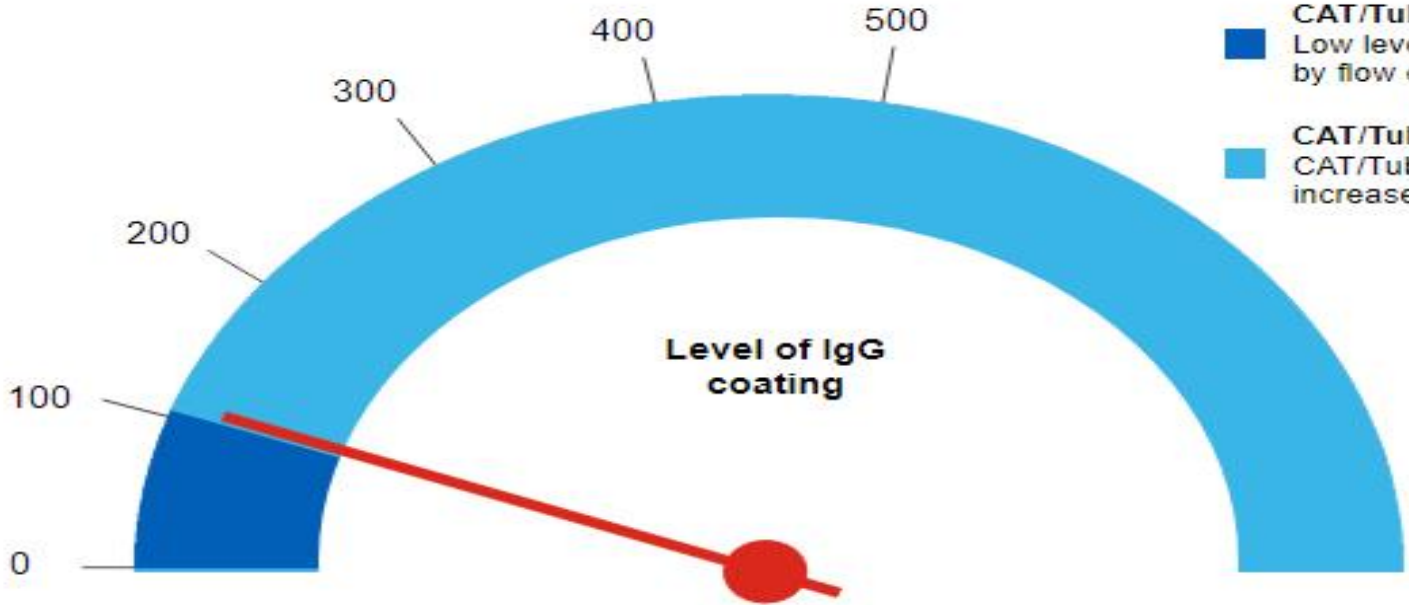Red cell bound C3d

Addition of
polyspecific AHG
(containing anti-IgG
and anti-C3d)

Anti-IgG and/or anti-C3d
binds to the reciprocal
structure, forming an
agglutination matrix
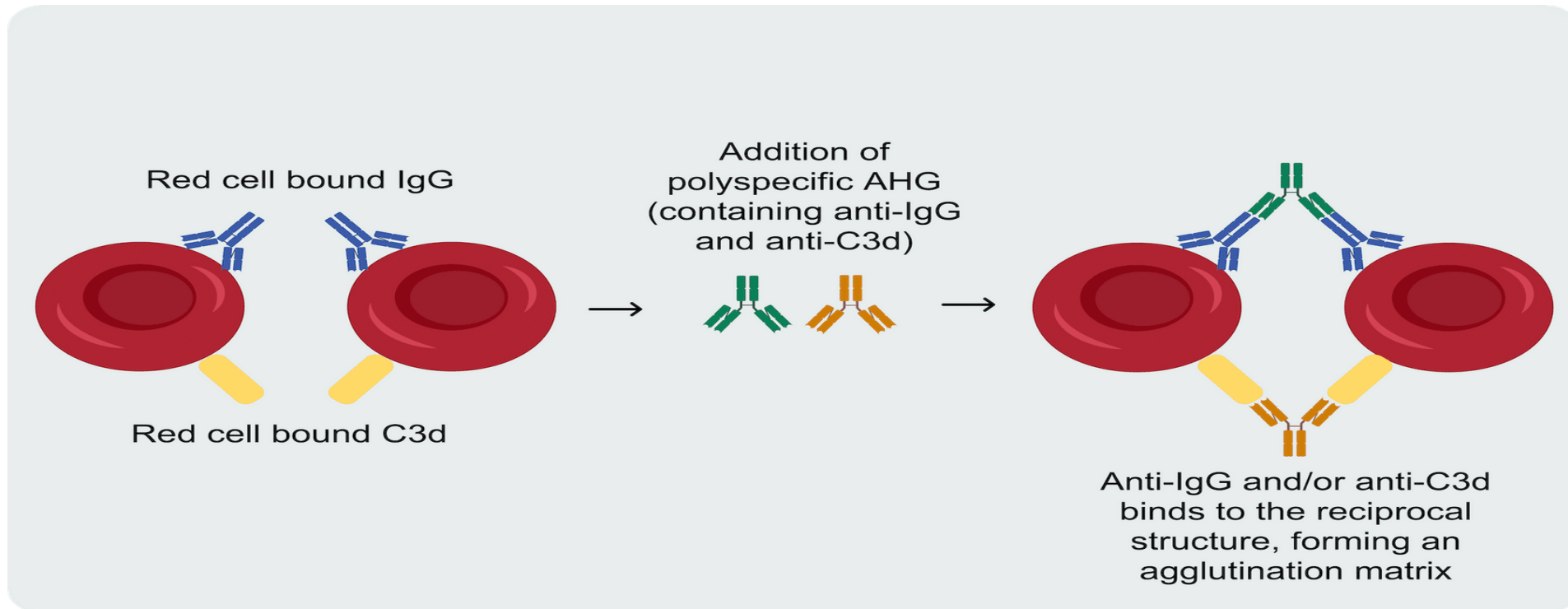
How do you do your DAT?

CAT

TUBE

DAT + / DAT-

NHS
Blood and Transplant

Grade

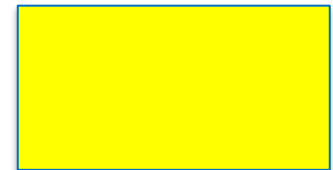0

400

500

300

200

100

0

Level of IgG coating

CAT/Tube DAT negative
Low levels of IgG can be detected by flow cytometry

CAT/Tube DAT positive
CAT/Tube DAT reaction strength increases

What percentage of acutely ill patients do you think are DAT positive?

1-15%

25%-45

50+%

Donor DAT Positive.. Are we bothered?
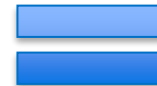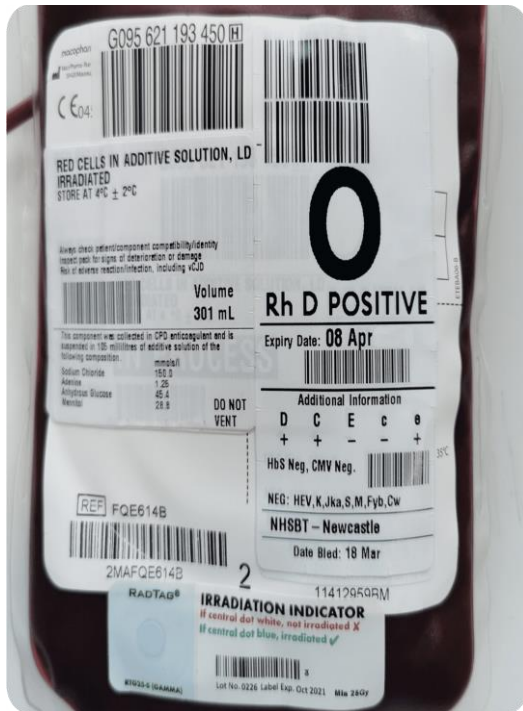
Why alert NHSBT to the Pos DAT unit?

Go on to develop AIHA

20% to 35%

5% to 10%

60 to 70%
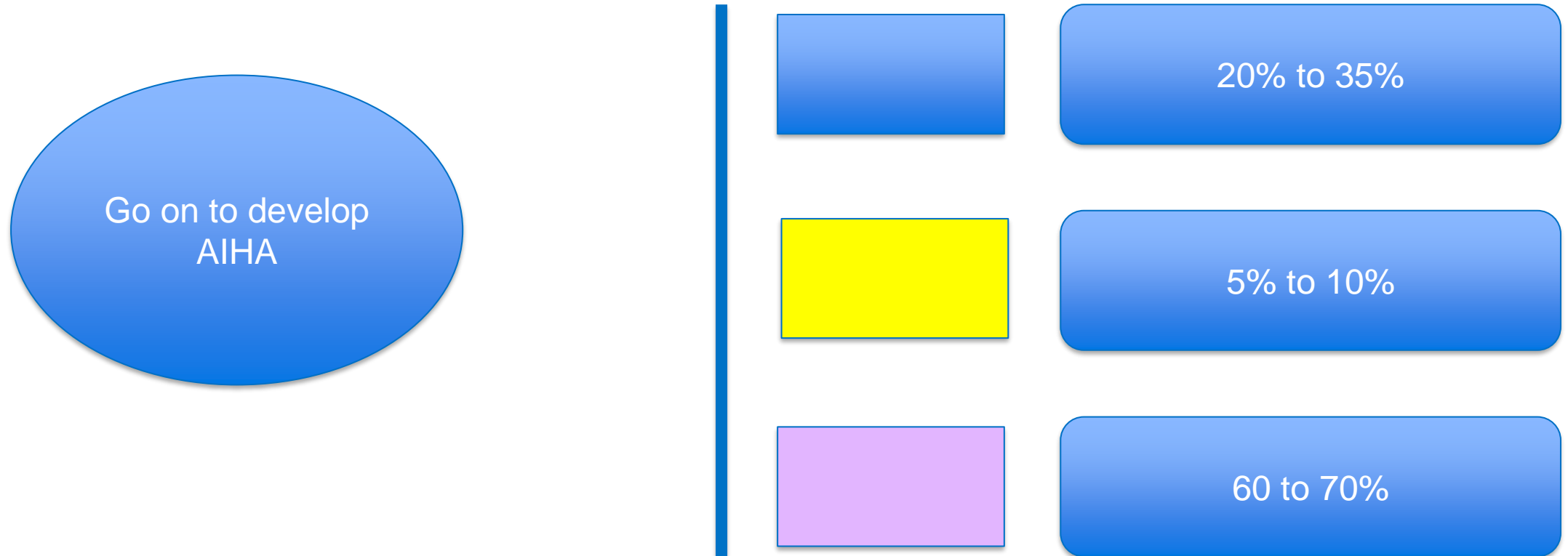
Little **test , BIG impact**

- Diagnostic

- Alerts you to potential issues around grouping (positive control)

- Informs / directs your investigation

- Alerts you to possible transfusion reactions or bone marrow failure

- It highlights the need to investigate further and potentially perform an eluate
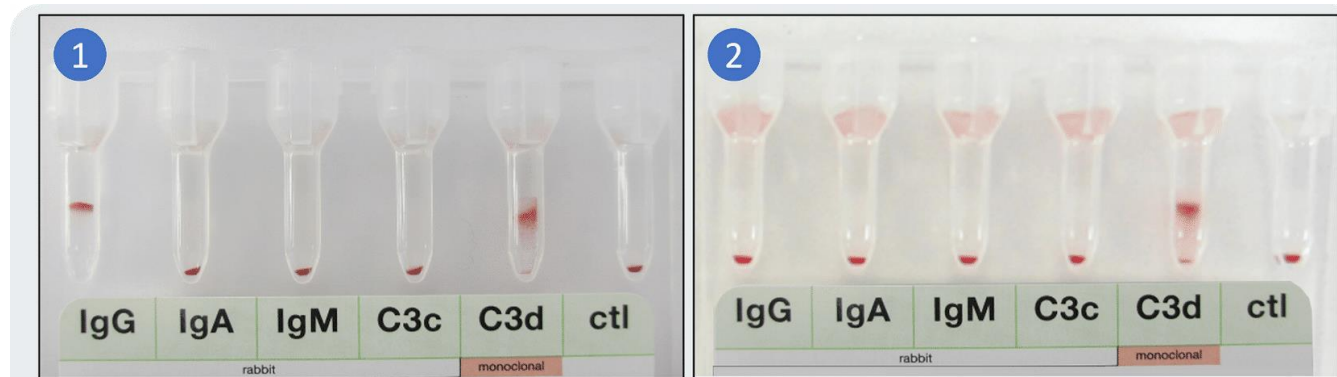
Eluates
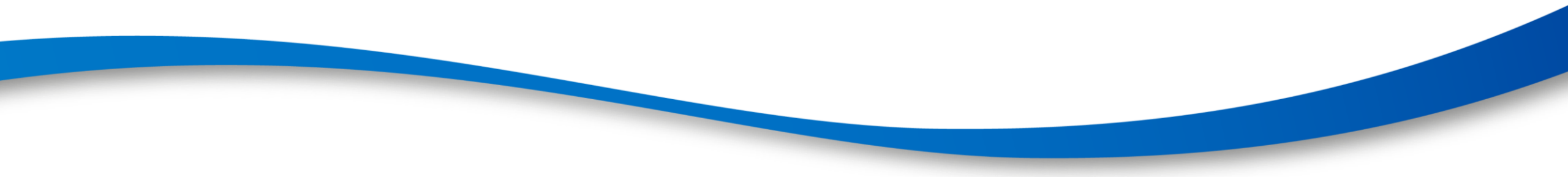
Elucidate the eluate
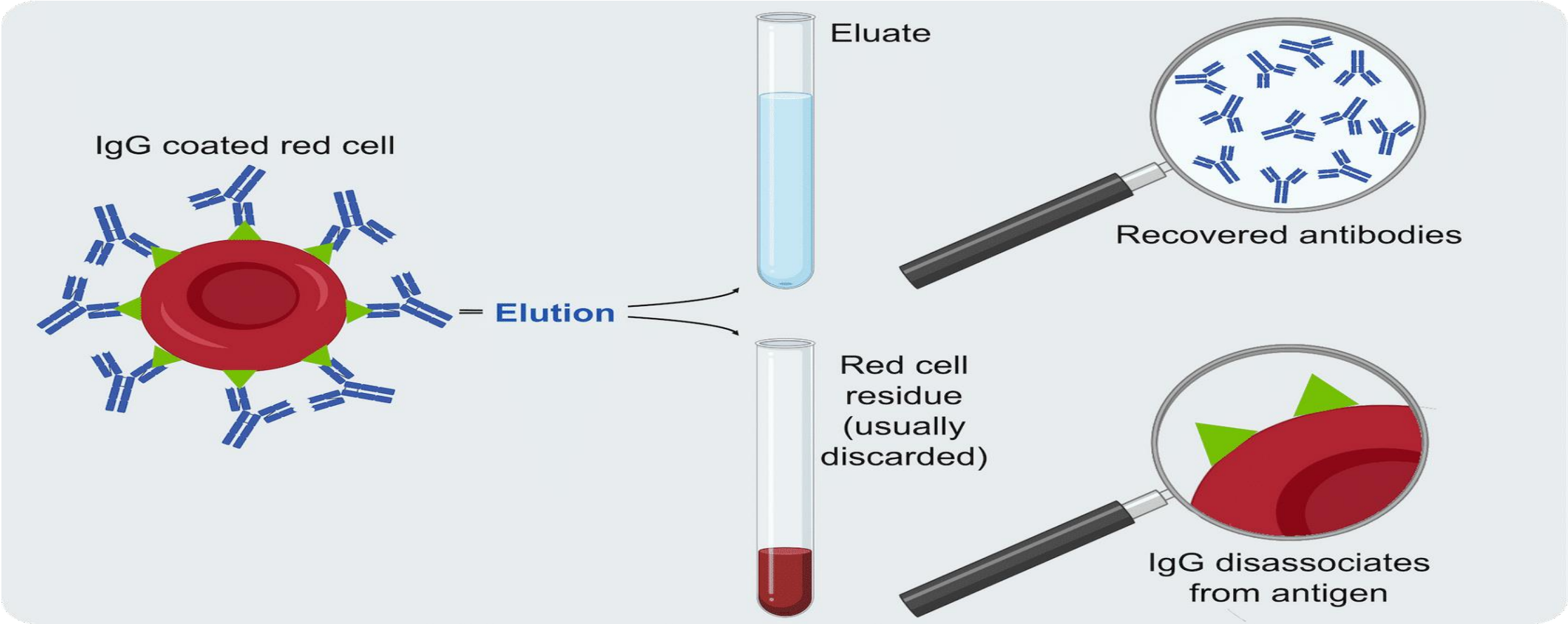
Question ?

Solvent
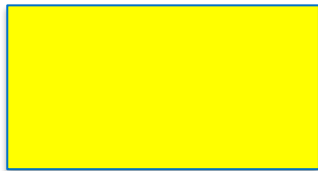
Freeze and thaw

Acid
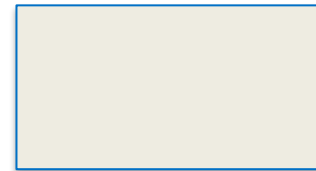
SHeat

Acid elution is the current technique used across RCI.

This lowers the pH of antibody and antigen proteins such that they become negatively charged and repel each other (the acid eluting reagent is likely to be pH 1.5-3.0, depending on the specific method). This disrupts the tertiary structure of proteins, causing unfolding and loss of complementarity between antigen and antibody.

Example Eluate case

	Rh	C^W	C	c	D	E	e	M	N	S	s	P$_1$	Lu^a	K	k	Kp^a	Le^a	Le^b	Fy^a	Fy^b	Jk^a	Jk^b	Other	IAT	Enz IAT
1	R$_1$^{W}R$_1$	+	+	0	+	0	+	+	+	0	+	0	0	0	+	0	+	0	+	0	+	0		0	0
2	R$_1$ R$_1$	0	+	0	+	0	+	+	0	+	0	2+	+	+	+	0	0	0	0	+	0	+		3	2
3	R$_2$ R$_2$	0	0	+	+	+	0	+	+	0	+	0	0	0	+	0	0	+	0	+	0	+		0	0
4	r`r	0	+	+	0	0	+	+	+	0	+	4+	0	0	+	0	0	0	0	+	0	+	Yk (a-)	0	0
5	r``r	0	0	+	0	+	+	+	+	+	0	5+	0	0	+	0	0	+	0	+	+	0		0	0
6	r r	0	0	+	0	0	+	0	+	+	0	0	0	0	+	0	0	+	+	+	0	+		0	0
7	r r	0	0	+	0	0	+	+	+	+	+	4+	0	+	+	0	+	0	+	0	+	0		2	2
8	r r	0	0	+	0	0	+	0	+	0	+	2+	+	0	+	0	0	+	+	0	0	+		0	0
9	r r	0	0	+	0	0	+	0	+	0	+	5+	+	0	+	+	0	+	0	+	+	0		0	0
10	r r	0	0	+	0	0	+	+	0	0	+	4+	0	+	0	0	0	+	0	+	0	+		3	2
Auto																								**2**	

Lets imagine…

The desired result

	Rh	C^W	C	c	D	E	e	M	N	S	s	P$_1$	Lu^a	K	k	Kp^a	Le^a	Le^b	Fy^a	Fy^b	Jk^a	Jk^b	Other	IAT	Enz IAT
1	R$_1$^{W}R$_1$	+	+	0	+	0	+	+	+	0	+	0	0	0	+	0	+	0	+	0	+	0		0	0
2	R$_1$ R$_1$	0	+	0	+	0	+	+	0	+	0	2+	+	+	+	0	0	0	0	+	0	+		3	2
3	R$_2$ R$_2$	0	0	+	+	+	0	+	+	0	+	0	0	0	+	0	0	+	0	+	0	+		0	0
4	r`r	0	+	+	0	0	+	+	+	0	+	4+	0	0	+	0	0	0	0	+	0	+	Yk (a-)	0	0
5	r``r	0	0	+	0	+	+	+	+	+	0	5+	0	0	+	0	0	+	0	+	+	+	0		0	0
6	r r	0	0	+	0	0	+	0	+	+	0	0	0	0	+	0	0	+	+	+	0	+		0	0
7	r r	0	0	+	0	0	+	+	+	+	+	4+	0	+	+	0	+	0	+	0	+	0		2	2
8	r r	0	0	+	0	0	+	0	+	0	+	2+	+	0	+	0	0	+	+	0	0	+		0	0
9	r r	0	0	+	0	0	+	0	+	0	+	5+	+	0	+	+	0	+	0	+	+	0		0	0
10	r r	0	0	+	0	0	+	+	0	0	+	4+	0	+	0	0	0	+	0	+	0	+		3	2
Auto																								2	

DAT + , Eluate required

True → **or** ← **false**

Post BMT / organ transplant

Cases of suspected HDFN

To resolve ABO anomalies

Because the Hospital have written "eluate" on the request form

True

True

False / True

False / True
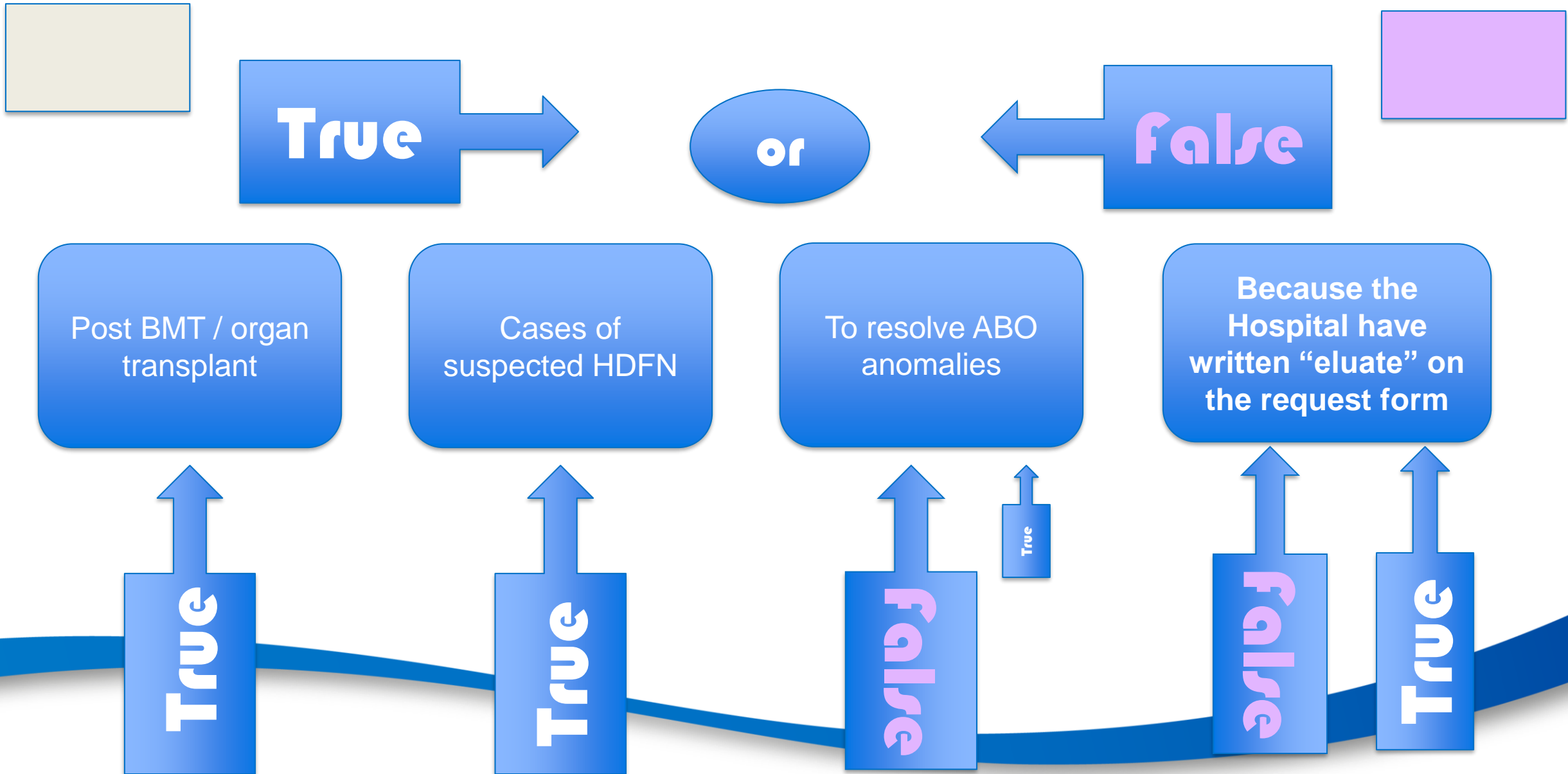
RCI Rules

Known patients , but increasing transfusion requirement	Change in serology / DAT from last sample	No change in serology or transfusion for 3 months, but increased signs of haemolysis	When absorbtions fail and transfused within 3 months

DAT- ve , Eluate required?

| Suspected transfusion reaction, **pre** trx sample , DAT negative | Suspected transfusion reaction, **post** trx sample , DAT negative | Part of Drug induced AIHA investigation |
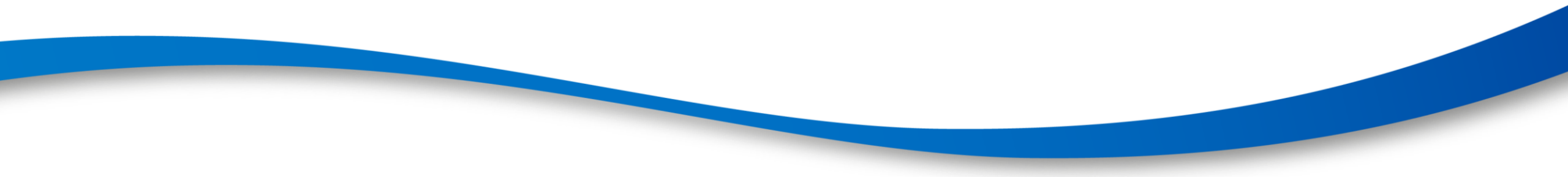
Low affinity antibody below the level of detection , but enough antibody to detect by IAT

Grade

0

400
500
300
200
100
0

Level of IgG coating

CAT/Tube DAT negative
Low levels of IgG can be detected by flow cytometry

CAT/Tube DAT positive
CAT/Tube DAT reaction strength increases
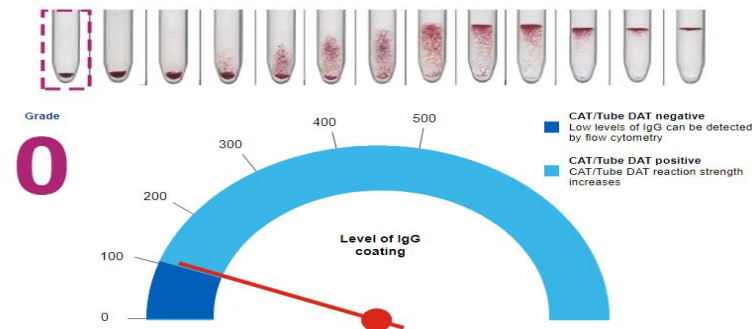
Great when it works..but

- 9/10 just pulls off non specific IgG

- Negative eluate—great!

- Won't remove compliment

- Difficult to produce an eluate in presence of strong cold auto

- Takes approximately an hour to prepare and test

Pan-reactive eluates, now what ?

IgG coated red cell = Elution → Eluate → Recovered antibodies

Red cell residue (usually discarded) → IgG disassociates from antigen
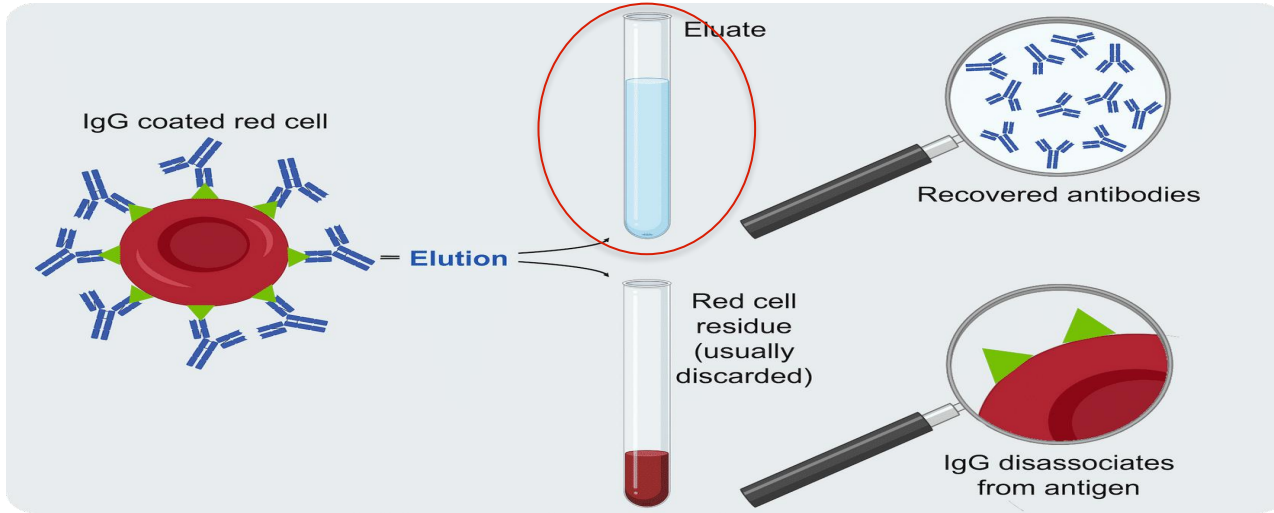
	Rh	C^W	C	c	D	E	e	M	N	S	s	P_1	Lu^a	K	k	Kp^a	Le^a	Le^b	Fy^a	Fy^b	Jk^a	Jk^b	Other	IAT	Enz IAT	Eluate
1	$R_1^W R_1$	+	+	0	+	0	+	+	+	0	+	0	0	0	+	0	+	0	+	0	+	0		0	0	3
2	$R_1 R_1$	0	+	0	+	0	+	+	0	+	0	2+	+	+	+	0	0	0	0	+	0	+		3	2	3
3	$R_2 R_2$	0	0	+	+	+	0	+	+	0	+	0	0	0	+	0	0	+	0	+	0	+		0	0	3
4	r`r	0	+	+	0	0	+	+	+	0	+	4+	0	0	+	0	0	0	0	+	0	+	Yk (a-)	0	0	3
5	r``r	0	0	+	0	+	+	+	+	+	0	5+	0	0	+	0	0	+	0	+	+	0		0	0	3
6	r r	0	0	+	0	0	+	0	+	+	0	0	0	0	+	0	0	+	+	+	0	+		0	0	3
7	r r	0	0	+	0	0	+	+	+	+	+	4+	0	+	+	0	+	0	+	0	+	0		2	2	3
8	r r	0	0	+	0	0	+	0	+	0	+	2+	+	0	+	0	0	+	+	0	0	+		0	0	3
9	r r	0	0	+	0	0	+	0	+	0	+	5+	+	0	+	+	0	+	0	+	+	0		0	0	3
10	r r	0	0	+	0	0	+	+	0	0	+	4+	0	+	0	0	0	+	0	+	0	+		3	2	3
Auto																								2		

Gel Panel - IAT

1 2 3 4 5 6 7 8 9 10

Pan reactive Eluates- useful ?
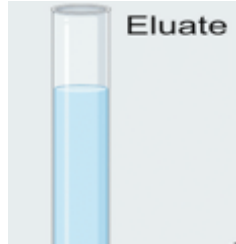
Eluate

	Rh	C^W	C	c	D	E	e	M	N	S	s	P_1	Lu^a	K	k	Kp^a	Le^a	Le^b	Fy^a	Fy^b	Jk^a	Jk^b	Other	Eluate IAT
1	$R_1^W R_1$	+	+	0	+	0	+	+	+	0	+	0	0	0	+	0	+	0	+	0	+	0		5
2	$R_1 R_1$	0	+	0	+	0	+	+	0	+	0	2+	+	+	+	0	0	0	0	+	0	+		5
3	$R_2 R_2$	0	0	+	+	+	0	+	+	0	+	0	0	0	+	0	0	+	0	+	0	+		5
4	r`r	0	+	+	0	0	+	+	+	0	+	4+	0	0	+	0	0	0	0	+	0	+	Yk (a-)	5
5	r``r	0	0	+	0	+	+	+	+	+	0	5+	0	0	+	0	0	+	0	+	+	0		5
6	r r	0	0	+	0	0	+	0	+	+	0	0	0	0	+	0	0	+	+	+	0	+		5
7	r r	0	0	+	0	0	+	+	+	+	+	4+	0	+	+	0	+	0	+	0	+	0		5
8	r r	0	0	+	0	0	+	0	+	0	+	2+	+	0	+	0	0	+	+	0	0	+		5
9	r r	0	0	+	0	0	+	0	+	0	+	5+	+	0	+	+	0	+	0	+	+	0		5
10	r r	0	0	+	0	0	+	+	0	0	+	4+	0	+	0	0	0	+	0	+	0	+		5
Auto																								

Absorb the Eluate !

Commercial cells (OR₁R₁, OR₂R₂ and Orr) - designed for adsorptions

Eluate **+** 3 x O R₁R₁ cells ① ② ③ **&** 3 x O rr cells ⑦ ⑧ ⑨ **=**

(c) Andy Miller, 2007

	Rh	C^W	C	c	D	E	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	Eluate IAT	R1R1 Abs	rr Abs
1	R₁^{W}R₁	+	+	0	+	0	+	+	+	0	+	0	0	0	+	0	+	0	+	0	+	0		5	**0**	**3**
2	R₁ R₁	0	+	0	+	0	+	+	0	+	0	2+	+	+	+	0	0	0	0	+	0	+		5	**0**	**3**
3	R₂ R₂	0	0	+	+	+	0	+	+	0	+	0	0	0	+	0	0	+	0	+	0	+		5	**0**	**3**
4	r`r	0	+	+	0	0	+	+	+	0	+	4+	0	0	+	0	0	0	0	+	0	+	Yk (a-)	5	**0**	**0**
5	r``r	0	0	+	0	+	+	+	+	+	0	5+	0	0	+	0	0	+	0	+	+	0		5	**0**	**0**
6	r r	0	0	+	0	0	+	0	+	+	0	0	0	0	+	0	0	+	+	+	0	+		5	**0**	**0**
7	r r	0	0	+	0	0	+	+	+	+	+	4+	0	+	+	0	+	0	+	0	+	0		5	**0**	**0**
8	r r	0	0	+	0	0	+	0	+	0	+	2+	+	0	+	0	0	+	+	0	0	+		5	**0**	**0**
9	r r	0	0	+	0	0	+	0	+	0	+	5+	+	0	+	+	0	+	0	+	+	0		5	**4**	**4**
10	r r	0	0	+	0	0	+	+	0	0	+	4+	0	+	0	0	0	+	0	+	0	+		5	**0**	**0**
Auto																										

Market Research

NHS Blood and Transplant

Should RCI Absorb every Eluate ?

Yes

No

Thank You