Antibody - Antigen Reactions:

ABO and D typing

Antibody screening and identification

The London and South East Joint Technical Advisory Groups' Transfusion Training Committee

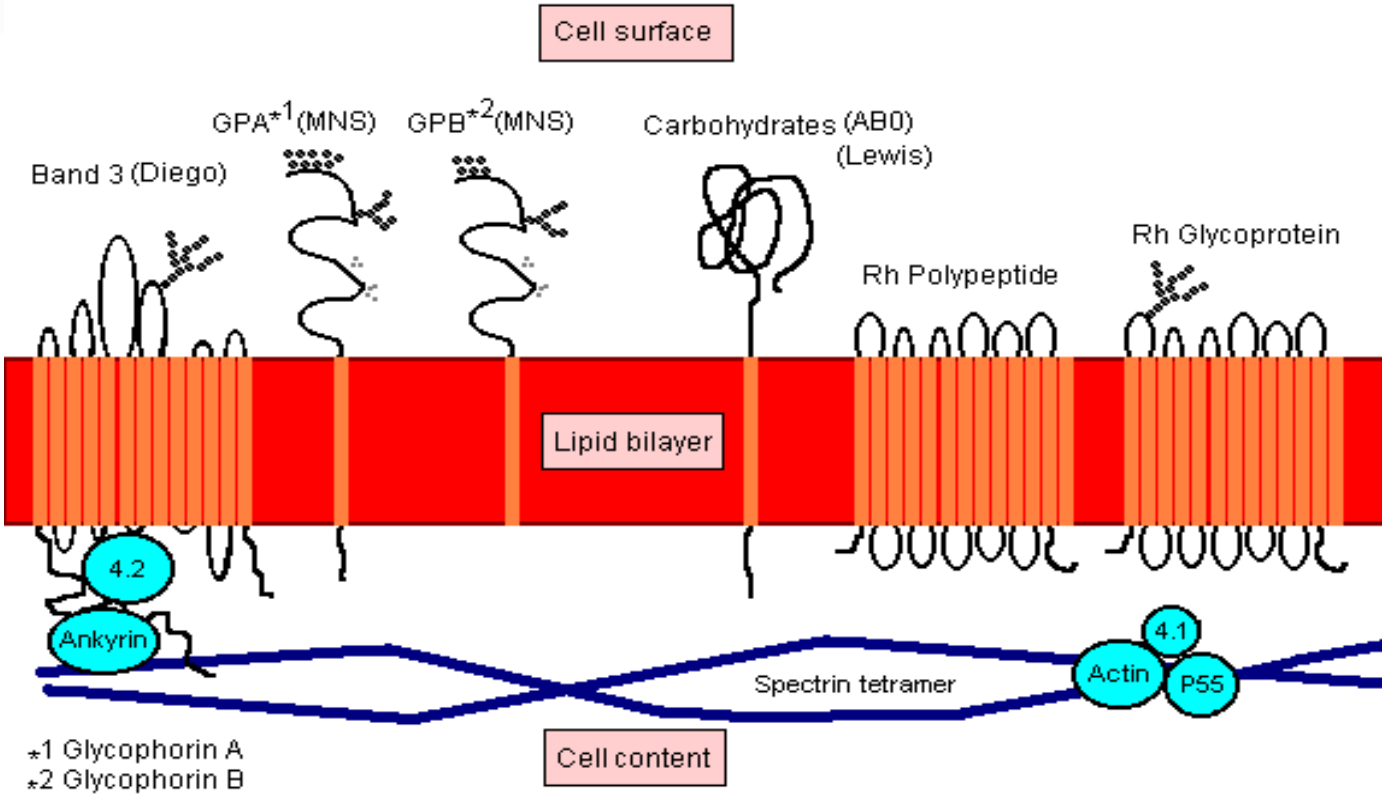
To cover:

- Basics of antigen/antibody reactions
- Why is the ABO group so special?
- D antigen – it's complicated!
- Antibody screen
- Antibody identification
- All you need to know for the workshop!

An antigen

- An antigen can be defined as a substance that, when introduced into the circulation of an individual lacking that antigen, can stimulate the production of a specific antibody
- The antigenic determinant, which is more correctly termed an epitope, is the antibody-binding portion of the antigen

Red cell antigens



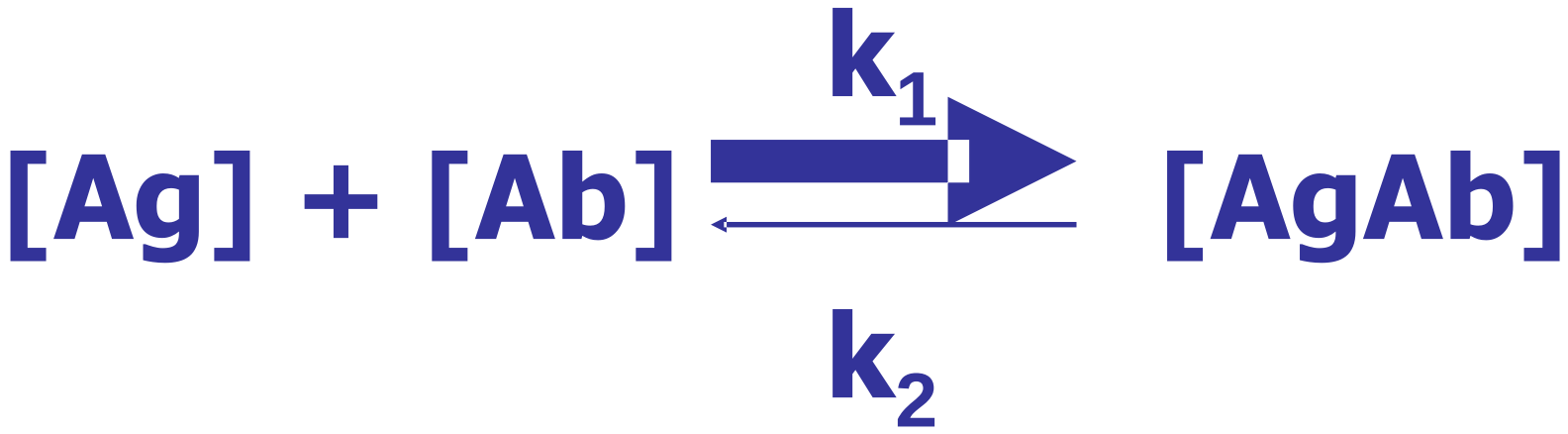
An antibody

- **An antibody can be defined as a serum protein (i.e. an immunoglobulin with specific antigen binding sites) produced as a result of the introduction of a foreign antigen, that has the ability to combine with (and, in many cases, destroy) the cells carrying the antigen that stimulated its production**

The Law of Mass Action governs antigen - antibody reactions

The London and South East Joint Technical Advisory Groups' Transfusion Training Committee

$$[Ag] + [Ab] \underset{k_2}{\overset{k_1}{\rightleftharpoons}} [AgAb]$$


[Ag] + [Ab]
k1
k2
[AgAb]

Nature of antigen-antibody reactions

Non-covalent bonds

- The bonds that hold the antigen to the antibody combining site are all non-covalent in nature. These include hydrogen bonds, electrostatic bonds, Van der Waal's forces and hydrophobic bonds. Multiple bonding between the antigen and the antibody ensures that the antigen will be bound tightly to the antibody.

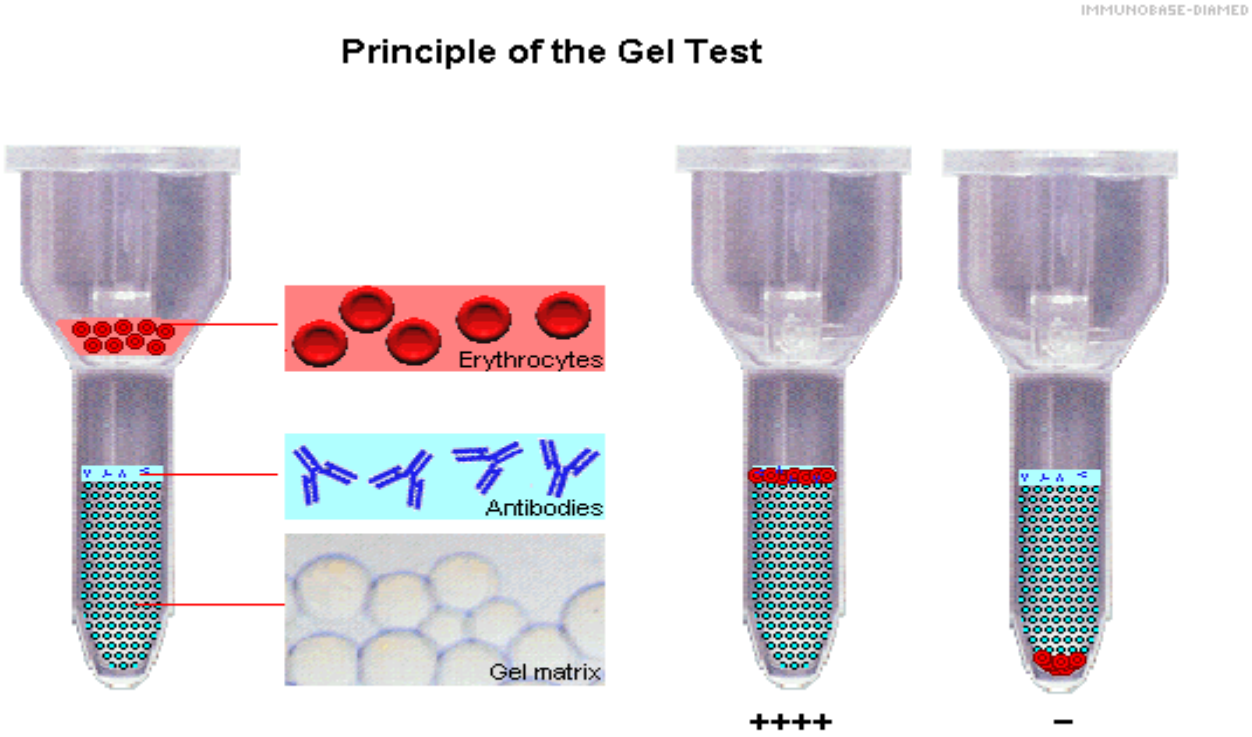
Reversibility

- Since antigen-antibody reactions occur via non-covalent bonds, they are by their nature reversible.

Antibody/antigen reactions in vitro take place in two distinct phases.

- Sensitisation **(antigen and antibody combine)**
- Agglutination **(the way in which antigen/ antibody reactions are observed)**

One example of agglutination ie column technology



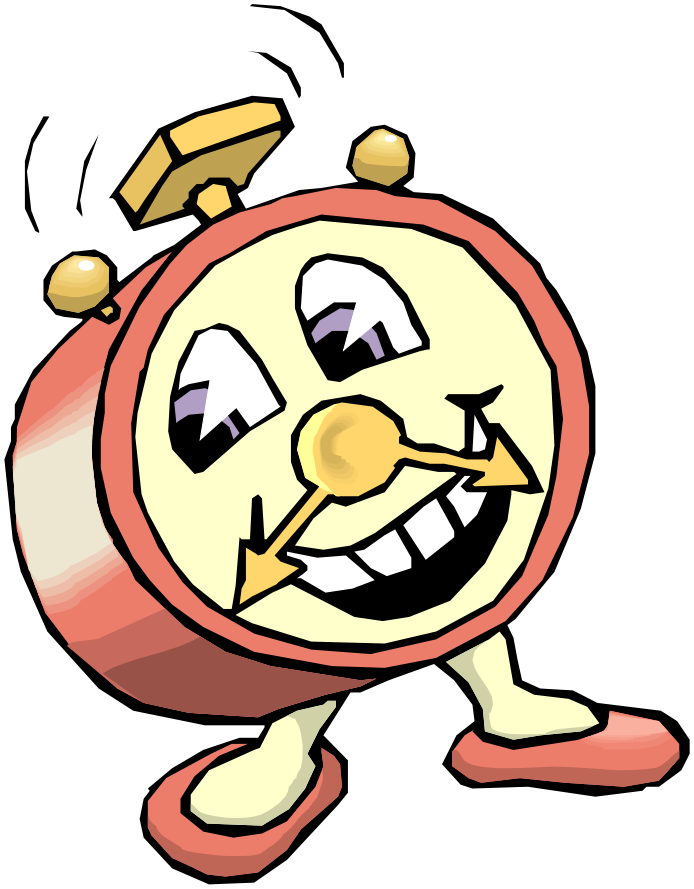
Factors affecting antibody/antigen reactions

- Ionic strength
- Incubation time
- Temperature
- Number of antigen sites (on red cells)
- Proximity of red cells
- pH
- Antigen and antibody concentration
- Antibody affinity

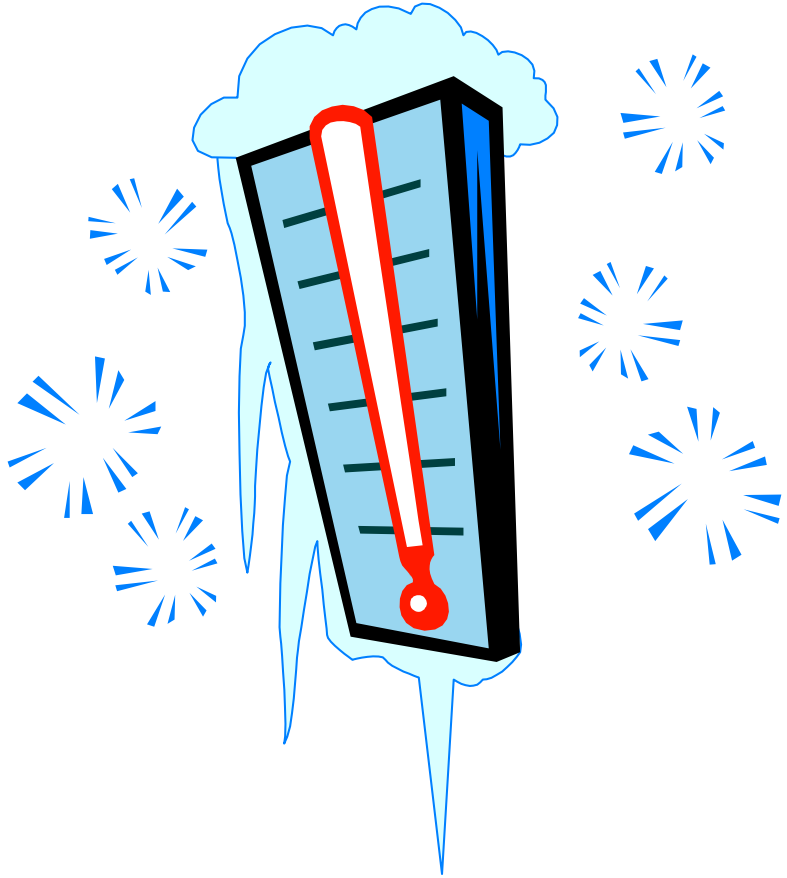
Ionic strength of the medium

- Lower ionic strengths increase the likelihood of a successful approach of a positively charged immunoglobulin to the antigen on the red cell surface, as the density of the positively charged ionic cloud is reduced. Thus, the rate of association is increased
- The rate of association of antibody with antigen may be enormously increased by lowering ionic strength
- The initial rate of association between Anti-D and the D antigen is increased 1000 fold by reducing the ionic strength from 0.17 to 0.03

Incubation time

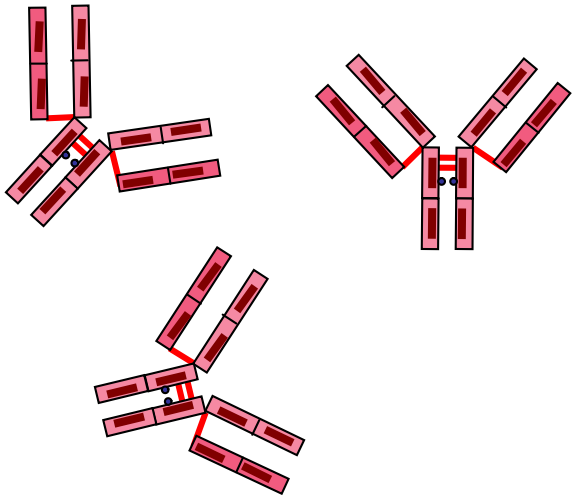


Temperature



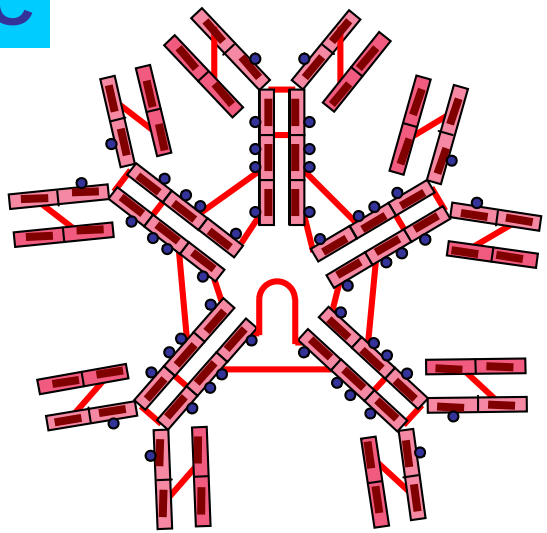
"Warm" and "cold" antibodies

37°C



"Warm" antibodies
(mostly IgG)

0-4°C



"Cold" antibodies
(mostly IgM)

The number of antigen sites on a red cell differs from one antigen specificity to another, by the age of the individual and, even from individual to individual.

Examples

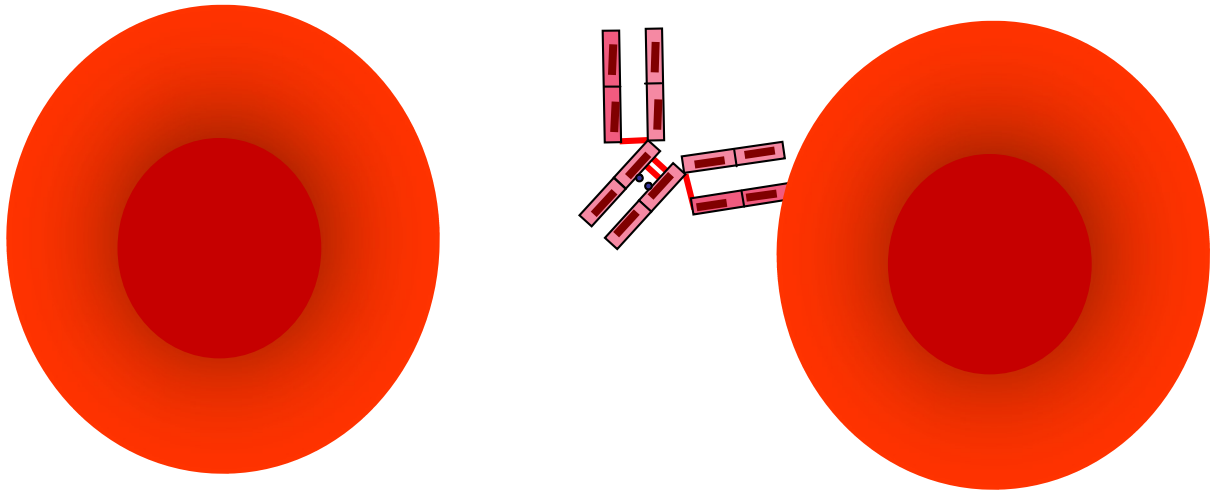
- The adult A_1 red cell has between 810 000 and 1 170 000 A_1 antigen sites per red cell.
- There are only 14 000 copies of Kidd antigens on each red cell.

- The adult A_1 red cell has between 810 000 and 1 170 000 antigen sites per red cell.
- The red cells of a newborn will only carry between 250 000 and 370 000 copies of the A_1 antigen.

Dosage

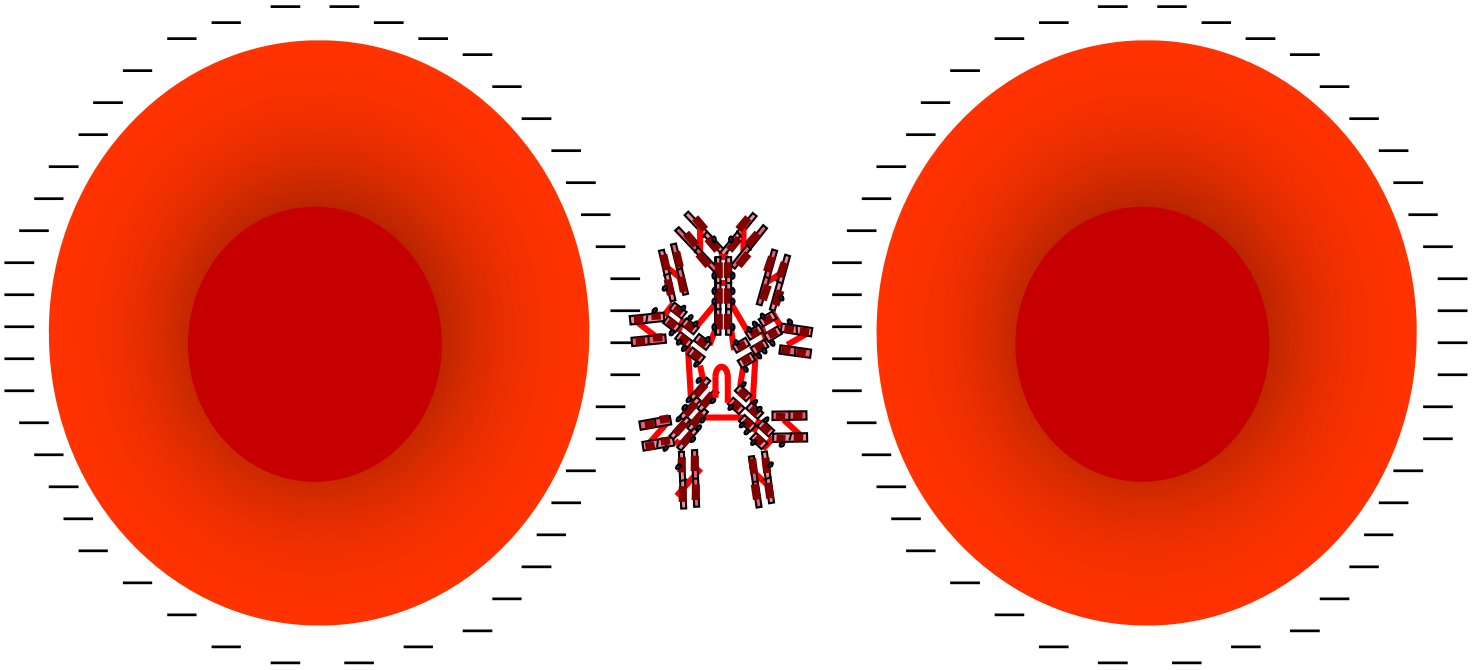
- Many antigens are expressed on the red cell more frequently if the genes are inherited in a double dose (homozygous)
- Examples of some antibodies may react more strongly or readily or only with, the "homozygous" red cells expressing the antigen

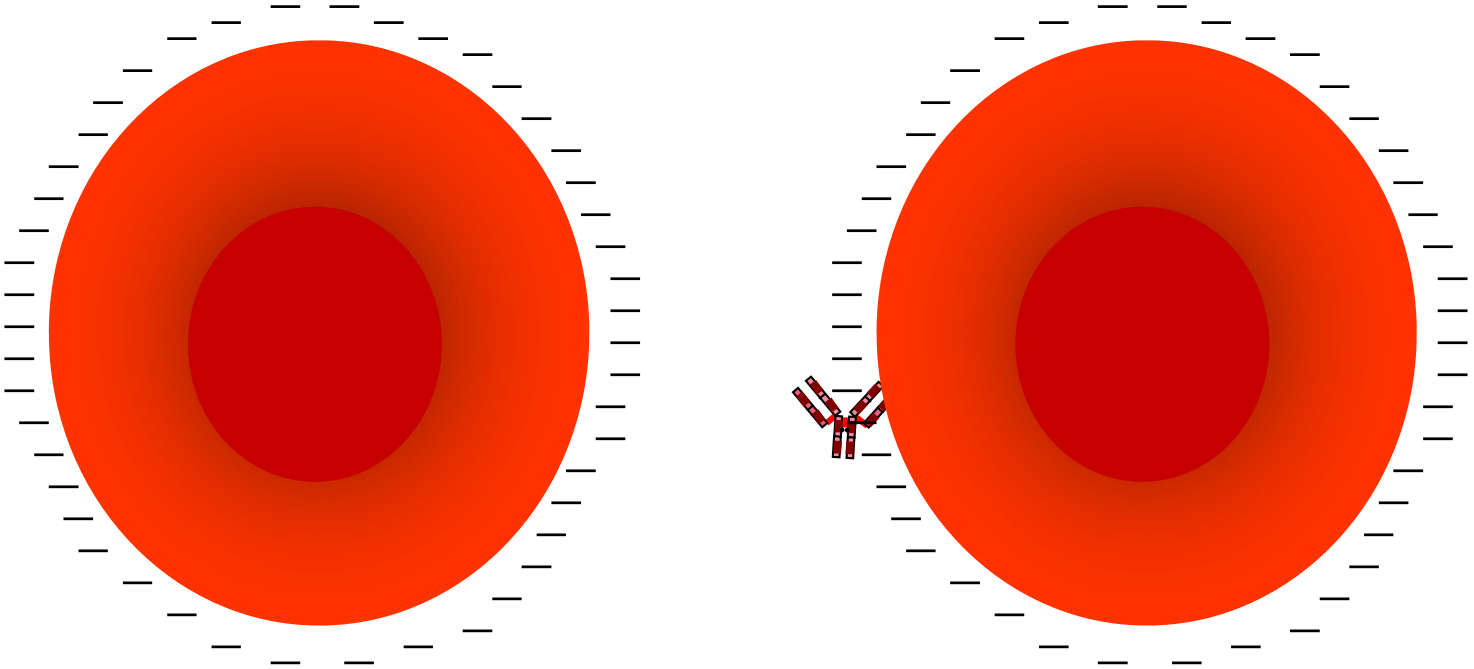
Proximity of red cells



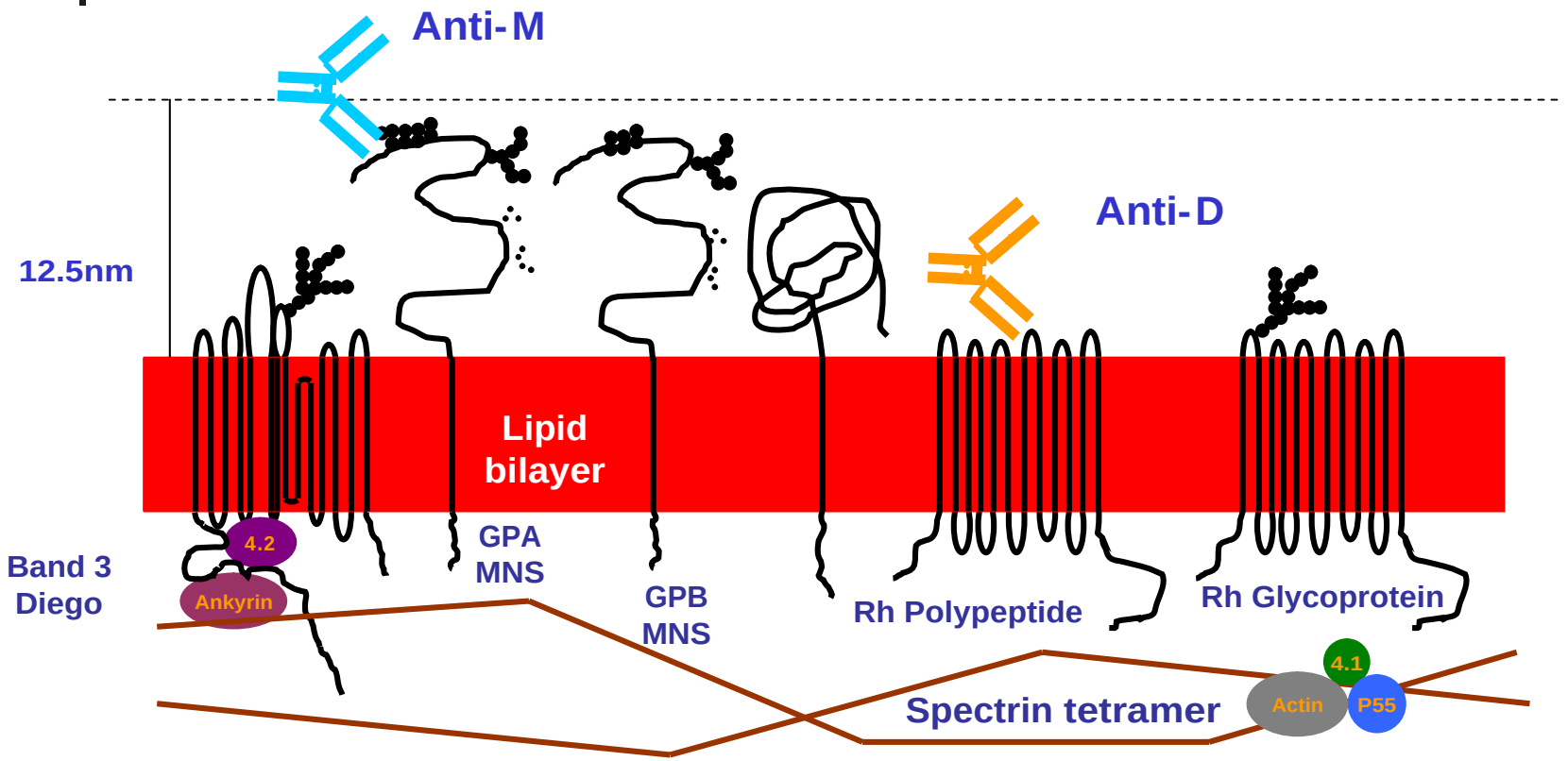
This can be brought about in several different ways

- **Centrifugation will increase gravitational force and bring the red cells into closer proximity with each other by mechanical means.**
- **Proteolytic enzymes will bring the red cells into closer proximity with each other by chemical means.**
- **Anti-human globulin (AHG) will bring the red cells into closer proximity with each other by immunological means.**

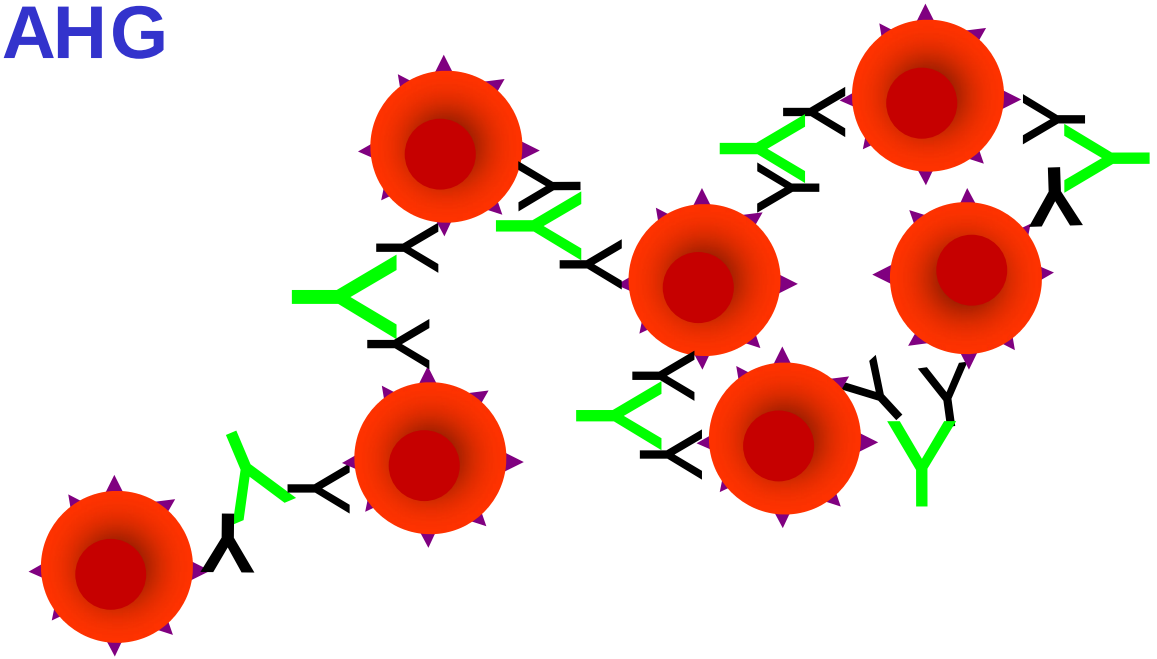




Presentation of the antigen

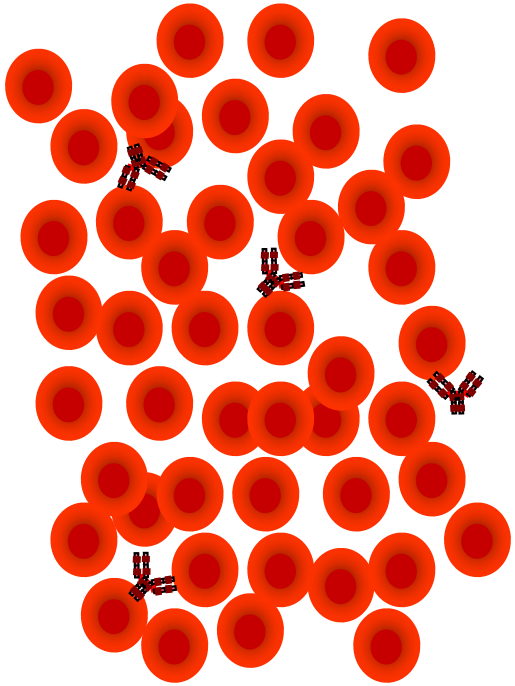


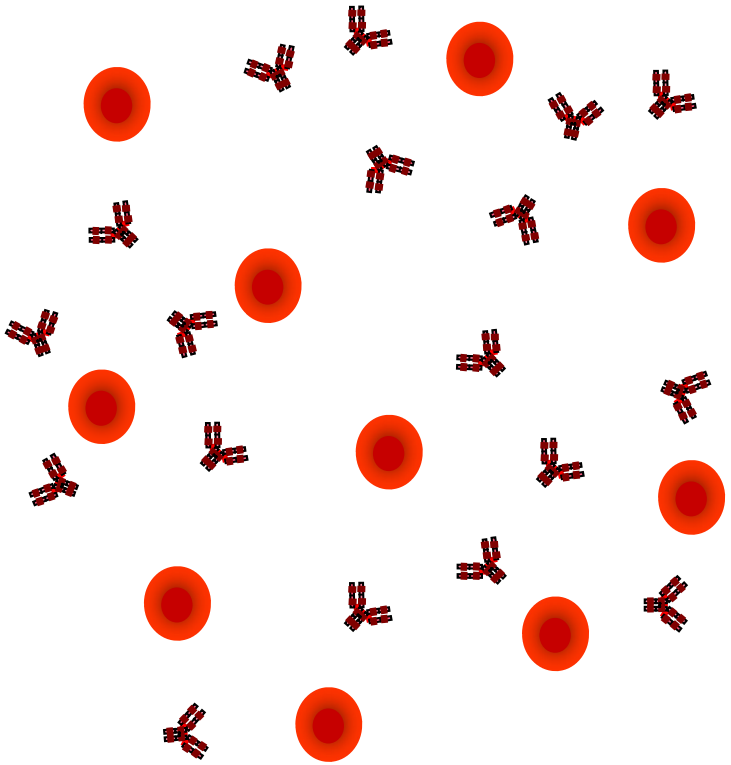
Y = AHG



pH

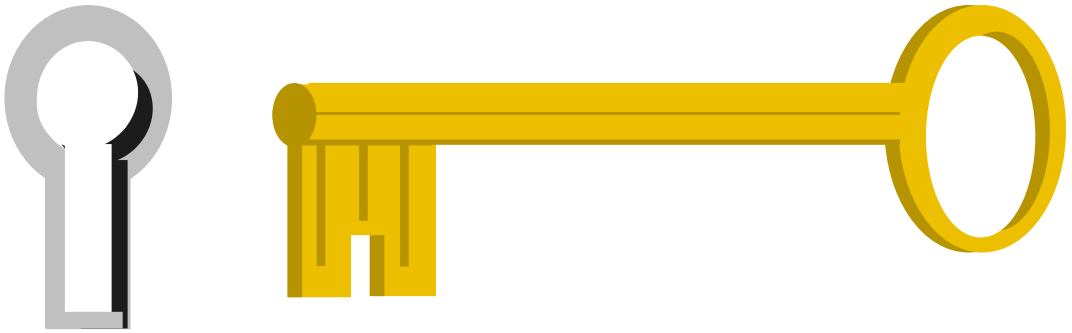
Antigen/antibody concentration





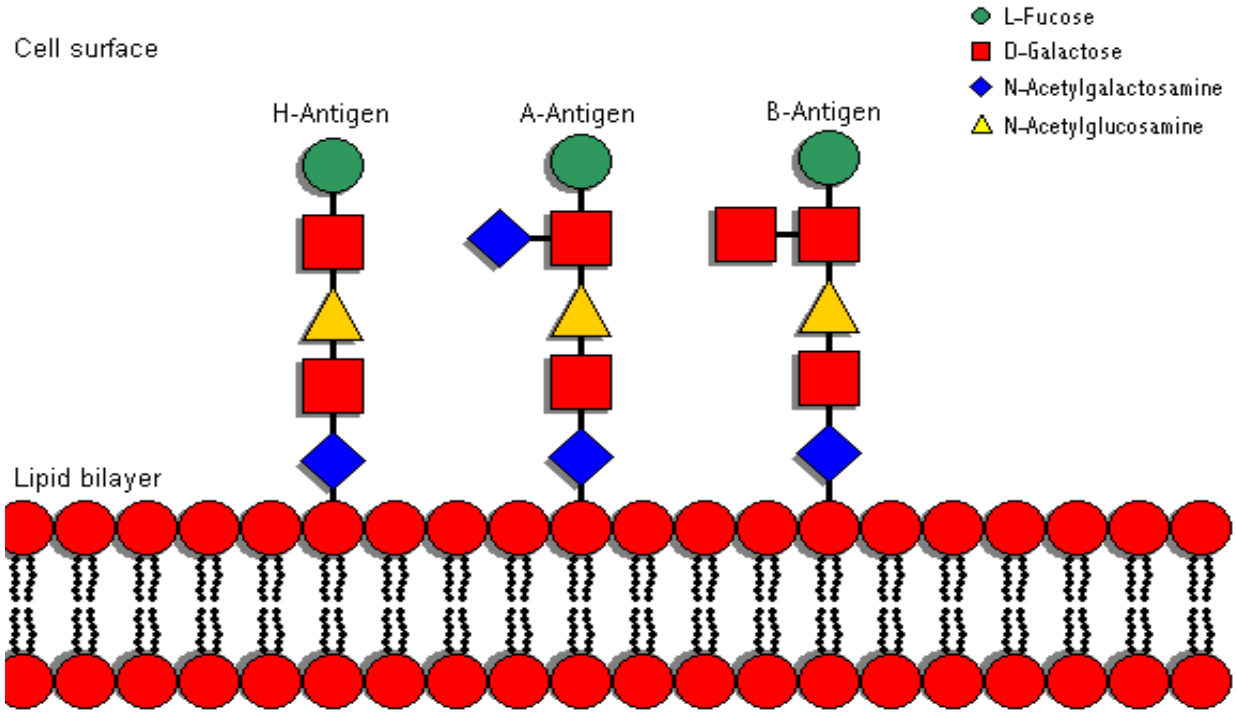
Antibody affinity

- It is the sum of the attractive and repulsive forces operating between the antigenic determinant and the combining site of the antibody



ABO groups – why are they so special?

ABO antigens



ABO groups

Red cells (antigens)	Plasma (antibodies)
A	Anti-B
B	Anti-A
O	Anti-A and Anti-B
AB	No ABO antibodies

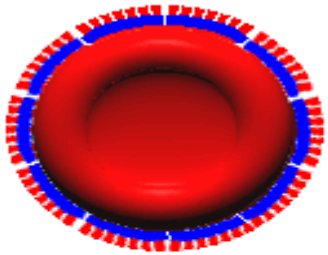
Routine ABO Typing					
Reaction of Cells Tested With		**Red Cell ABO Group**	**Reaction of Serum Tested Against**		**Reverse ABO Group**
Anti-A	Anti-B		A_1 Cells	B Cells	
0	0	**O**	+	+	**O**
+	0	**A**	0	+	**A**
0	+	**B**	+	0	**B**
+	+	**AB**	0	0	**AB**

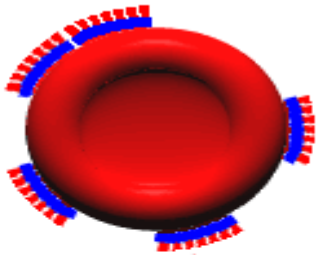
D typing – it's complicated!

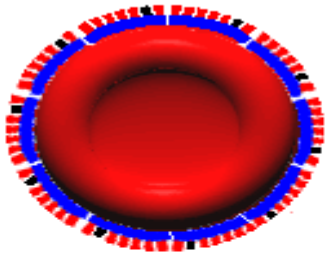
The D antigen

- **Most individuals are D positive or D negative**
- **An individual may have a weak D antigen (previously known as D^u)**
- **An individual may have a partial D antigen (previously known as a $D^{variant}$)**

RhD

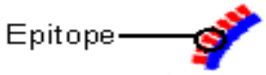


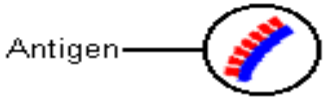




	Normal D-Antigen	D-weak	D-Variant
Epitope:	Normal	Normal	Mutated
Antigen frequency:	Normal	Reduced	Normal or reduced

Legend:



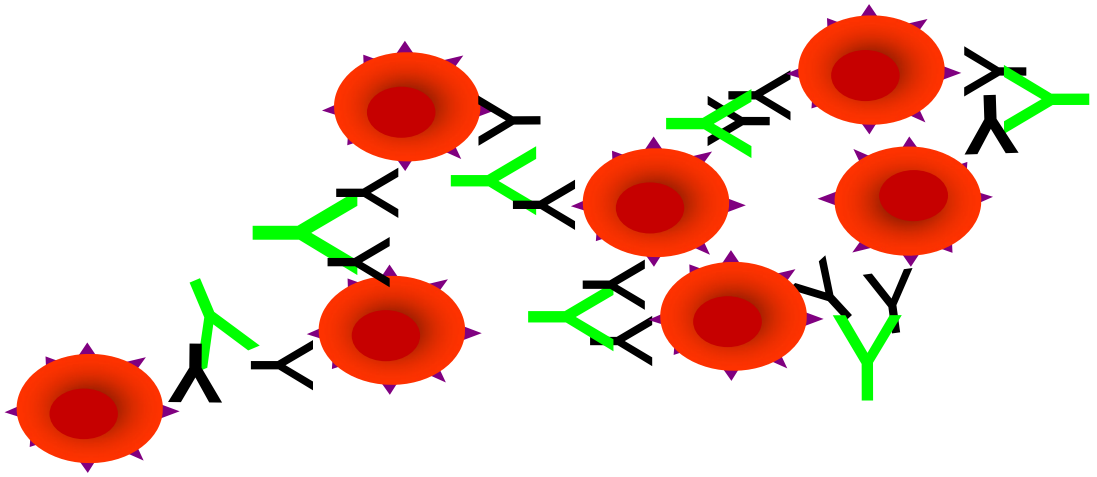


The antibody screen

- The antibody screen is a serological technique designed to detect the presence of any clinically significant antibodies (excluding ABO) present in a sample.
- A clinically significant antibody is able to bind to its corresponding antigen in vivo, sensitising the red cell. It may initiate activation of complement, and is generally associated with shortened survival of transfused red cells and/or haemolytic disease of the newborn/fetus.

Indirect antiglobulin test (IAT)

- **Clinically significant antibodies in vitro are detected by the indirect antiglobulin technique (IAT) at 37°C**



Antibody screening cells must express the following antigens:

- C, c, D, E, e,
- K, k,
- Fy^a, Fy^b,
- Jk^a, Jk^b,
- S, s, M, N,
- P_1,
- Le^a

FORM FRM823/2

Effective: 18/03/13

2 Cell Screen Profile PR101, PR102 & PR103

CE 0843

IVD

NHS Blood and Transplant

Started 8/5/13

NHSBT REAGENTS

Product	Lot No.	Product	Lot No.
Alsevers	R101 3388	CellStab	R102 3388
CellMedia	R103 3388	EXPIRY DATE: 2013.06.06	

Unless otherwise indicated, all cells are positive for Kp^b and Lu^b and negative for Wr^a , Lu^a and Co^b
Instructions for use can be found at www.blood.co.uk/reagents

Red Cell Reagent Validation
Validated by: HA
Date: 30 / 04 / 2013
Type of Cells: ScreenCells cellstab
Recorded on worksheet: ✓

	Rh	C	D	E	c	e	C^w	M	N	S	s	P1	K	k	Kp^a	Le^a	Le^b	Fy^a	Fy^b	Jk^a	Jk^b	Other
1	R_1R_1	+	+	0	0	+	0	+	+	+	0	0	0	+	0	+	0	+	0	+	0	-
2	R_2R_2	0	+	+	+	0	0	+	0	0	+	+	+	+	0	0	+	0	+	0	+	

Cross-Referenced in Primary Document: SOP883

(Template Version 01/04)

Antibody Identification

- Once an antibody has been detected it should be identified as quickly as possible to ascertain its potential clinical significance
- When an antibody is detected in the antibody screen, crossmatch compatible blood should not be issued until:
  - The antibody has been positively identified

  and
  - If the antibody is clinically significant, units phenotyped and found antigen negative for the appropriate antibody have been obtained

FORM FRM783/2

Effective: 20/03/13

ID Panel Profile

CE

IVD

NHS Blood and Transplant

NBS REAGENTS **Panel 2**

Product	Lot No.	Product	Lot No.	Product	Lot No.
ID Panel in Alsevers	R141 3273	ID Panel Papainised in Alsevers	R15n/a	ID Panel in LISP	R14n/a
ID Panel in CellStab	R142 3273	ID Panel Papainised in CellStab	R152 3273	Expiry Date : 2013.06.20	
ID Panel in CellMedia	R16n/a	ID Panel Papainised in CellMedia	R172 3273		

Patient's Name		Ref. No.	Sample No.	Conclusion
D.O.B.				Tested by Date

Unless otherwise indicated, all cells are positive for Kp^b and Lu^b and negative for Wr^a and Co^b.

Instructions for use can be found at http://www.blood.co.uk/reagents

	Rh	C	D	E	c	e	C^w	M	N	S	s	P1	Lu^a	K	k	Kp^a	Le^a	Le^b	Fy^a	Fy^b	Jk^a	Jk^b	Other
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	+	2	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	+	2	0	0	+	0	+	0	+	+	+	0	
5	r''r	0	0	+	+	+	0	0	+	0	+	4	0	0	+	0	+	0	+	0	+	0	Cob+
6	rr	0	0	0	+	+	0	+	0	+	0	0	0	+	0	0	0	0	+	0	+	0	
7	rr	0	0	0	+	+	0	0	+	+	0	3	+	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	0	+	1	0	0	+	0	0	+	0	+	+	0	Cob+
10	rr	0	0	0	+	+	0	0	+	0	+	2	+	0	+	0	0	+	+	0	0	+	

Reagent							DAT Profile	Anti-IgG	IgA	IgM	C3c	C3d	Ctrl
Lot No.							Result						

Cross-Referenced in Primary Document: SOP883

(Template Version 01/04)

Antibody specificity

- **Current pre-transfusion guidelines state:**

  'The specificity of an antibody should only be assigned when it is reactive with at least two examples of reagent red cells carrying the antigen and non-reactive with at least two examples of reagent red cells lacking the antigen'

- **In order to meet this criteria more than one identification panel may be necessary**

Antibody exclusion

- It is essential when one antibody has been identified, that the potential presence of another masked antibody has not been overlooked

- Antibody exclusion is an important part of antibody investigative work and should always be performed