HAEMOKINESIS®

THINK.BLOOD.INNOVATE

BASIC IMMUNOHAEMATOLOGY TRAINING

BASIC IMMUNOLOGY:
ANTIBODIES/ANTIGENS
BLOOD GROUPS
THE PRINCIPLE OF THE GEL TECHNOLOGY
COMMON IMMUNOHAEMATOLOGY TESTS

BASIC IMMUNOLOGY

The immune system comprises a series of mechanisms responsible for distinguishing ‘self’ from ‘non-self’, and for mounting defence against infectious agents and other foreign substances.

The Immune response

Historically, the immune system was separated into two branches: **Humoral immunity**, for which the protective function of immunization could be found in the humor (cell-free bodily fluid or serum) and **Cellular immunity**, for which the protective function of immunization was associated with cells. T cells provide protection against different pathogens.

BASIC IMMUNOLOGY

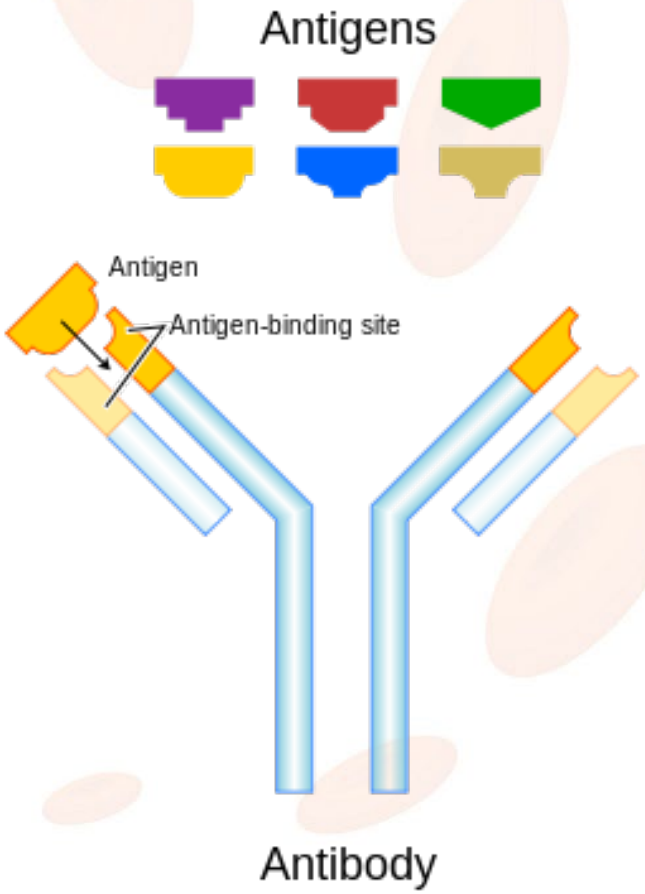
Humoral Component:

- Essentially comprised of antibody proteins and the complement system.
- Immunohaematology is predominately concerned with this branch of the immune system.

BASIC IMMUNOLOGY

Antigen: any substance that causes the immune system to mount a response by producing antibodies against it.

Antibody: a specific protein produced by the immune system when it detects a foreign substance or antigen.



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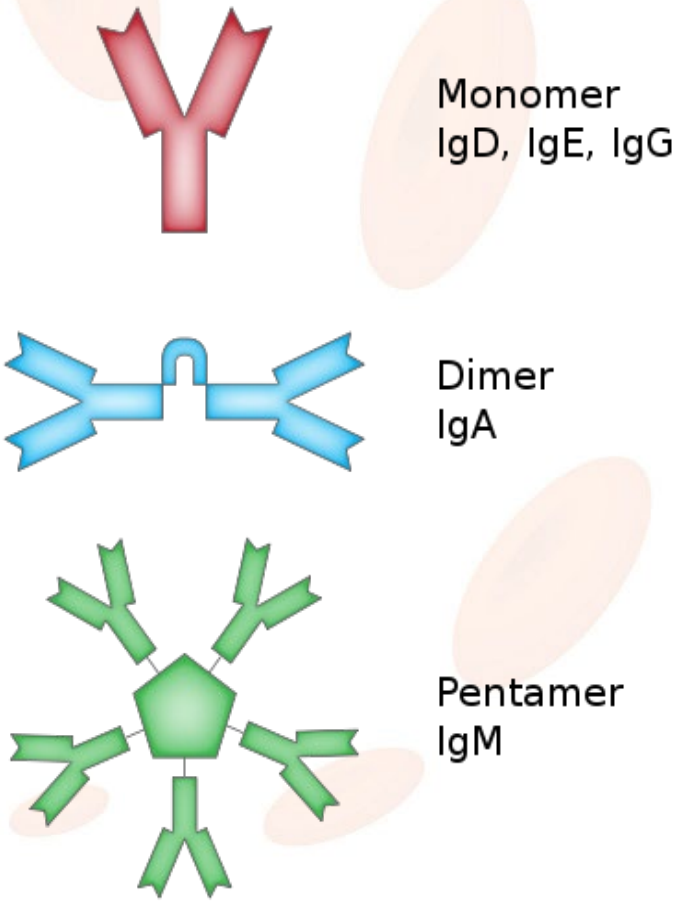
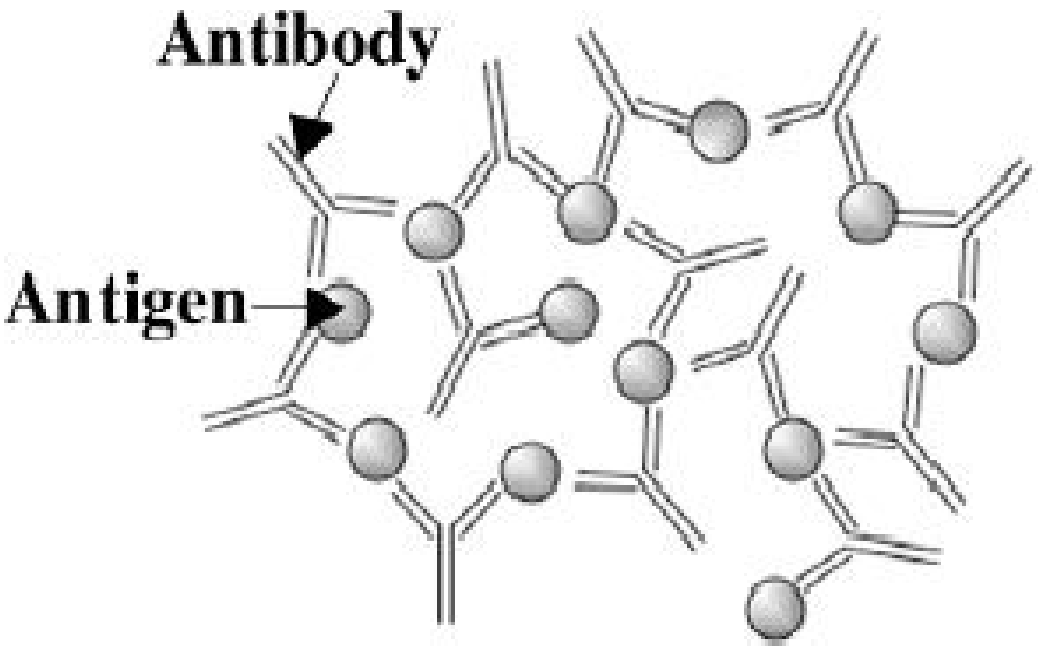
BASIC IMMUNOLOGY

Antibody Sub-classes



BASIC IMMUNOLOGY

It is the IgG and IgM antibodies and their reactions with red blood cell antigens that form the basis of blood banking.



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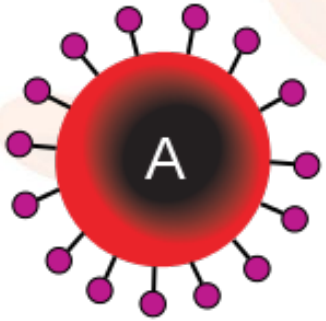
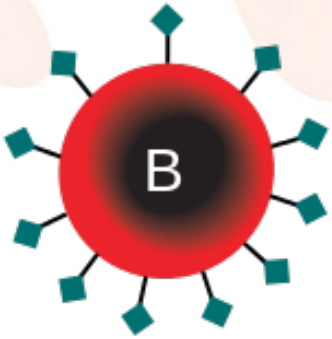
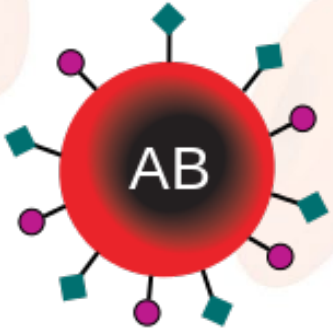
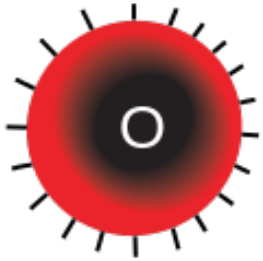
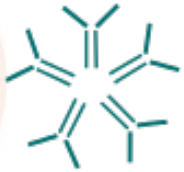
THE ABO BLOOD GROUP SYSTEM

The first human blood group antigen system to be discovered and remains the single most important system in transfusion.

In the transfusion medicine laboratory it is essential to determine an individual's blood group in order to ensure a compatible product is provided.

Almost all normal, healthy people older than three months of age have **naturally occurring** antibodies to the ABO antigens they lack.

These antibodies (generally IgM) can readily lead to **life-threatening** haemolysis if the incorrect blood group is transfused.

	Group A	Group B	Group AB	Group O
Red blood cell type				
Antibodies in Plasma	 Anti-B	 Anti-A	None	 Anti-A and Anti-B
Antigens in Red Blood Cell	 A antigen	 B antigen	 A and B antigens	None

The ABO Blood Group Forward and Reverse

Blood Group	Anti-A Reagent	Anti-B Reagent	A cells Reagent	B cells Reagent
A	+	0	0	+
B	0	+	+	0
O	0	0	+	+
AB	+	+	0	0

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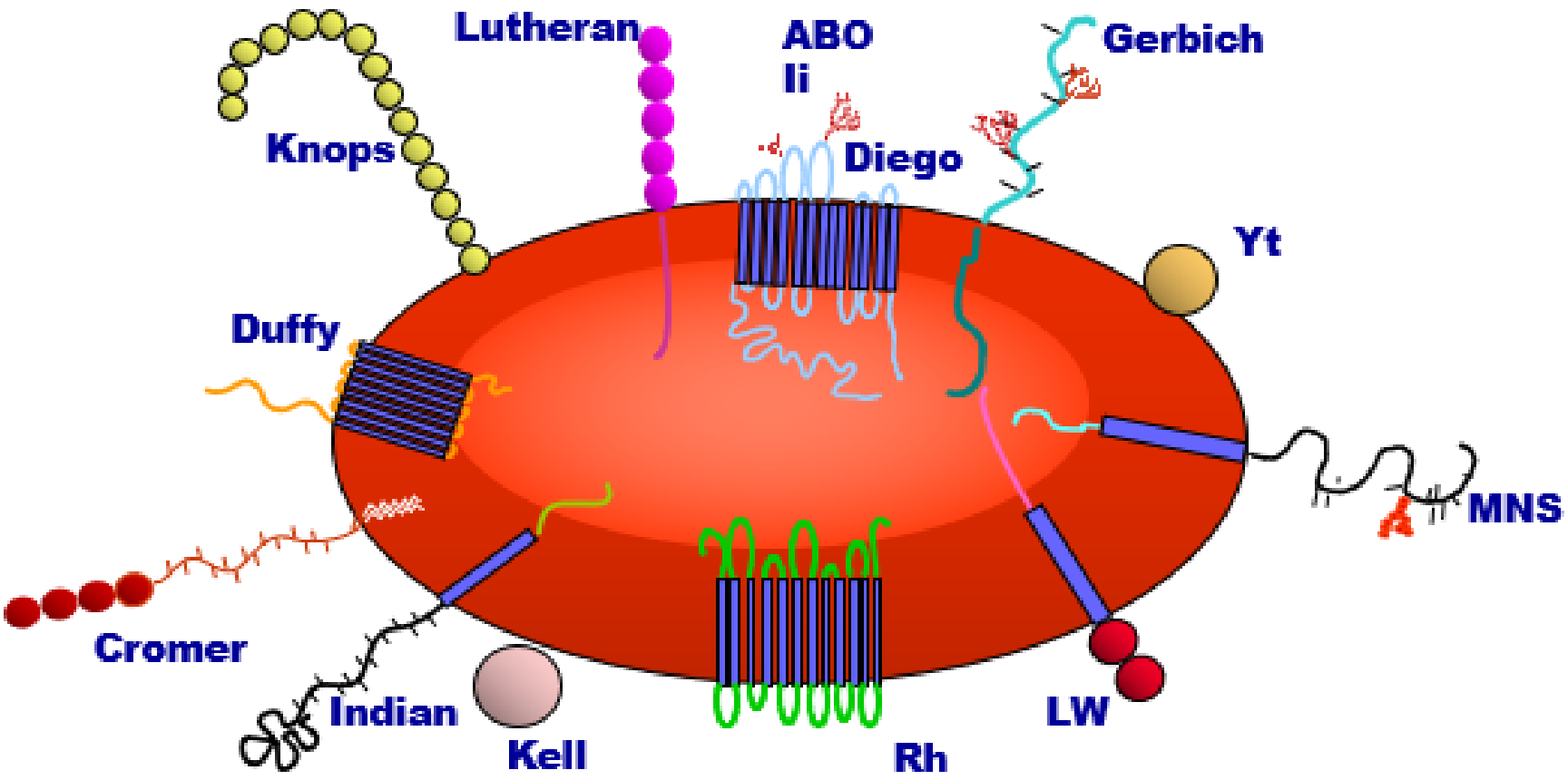
The Rh System

- Comprises Antigens D, C, c, E, e
- Rh D is the most important as it is **highly** immunogenic - 40% of exposures likely to produce anti-D
- Antigen D is present on the red cell, then Rh Positive e.g. A Pos
- Antigen D is absent on the red cell, then Rh Negative e.g. O Neg

Variations of D

- Some individuals have weakened or variant expressions of D antigen
- **Patients** with ? D variant or D weak treated as Rh negative
- **Donors** with ? D variant or D weak treated as Rh Positive.
- Anti-D Clones differ for patient (VI-) and donor (VI+) testing

Other Red Cell Antigen Groups



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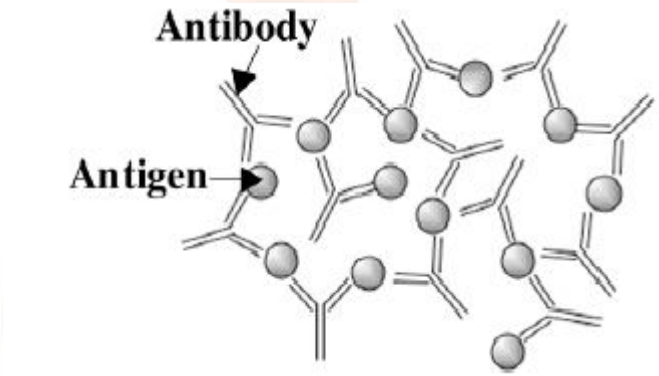
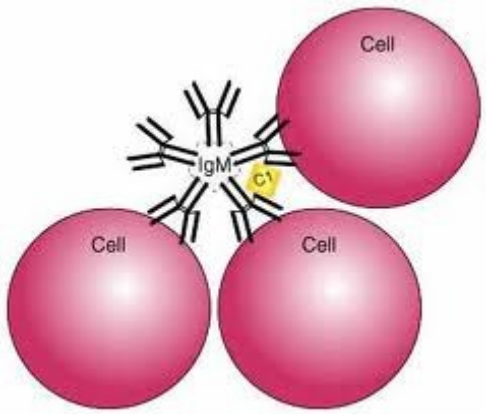
Gel Technology





In the case of red cell antigens and antibodies, these interactions cause a phenomenon known as HAEMAGGLUTINATION or clumping of the red cells. We are able to visualise this reaction between the antibody and the antigen.

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The principle of gel technology

- The gel acts as a sieve or density gradient
- Antibodies added to the gel supernatant agglutinate the red cells
- Under specified centrifugal conditions:
  - Large agglutinates remain on or near the interface of the gel/supernatant
  - Smaller agglutinates pass down the column of gel depending on size
  - Non- agglutinated cells pass to the bottom of the gel

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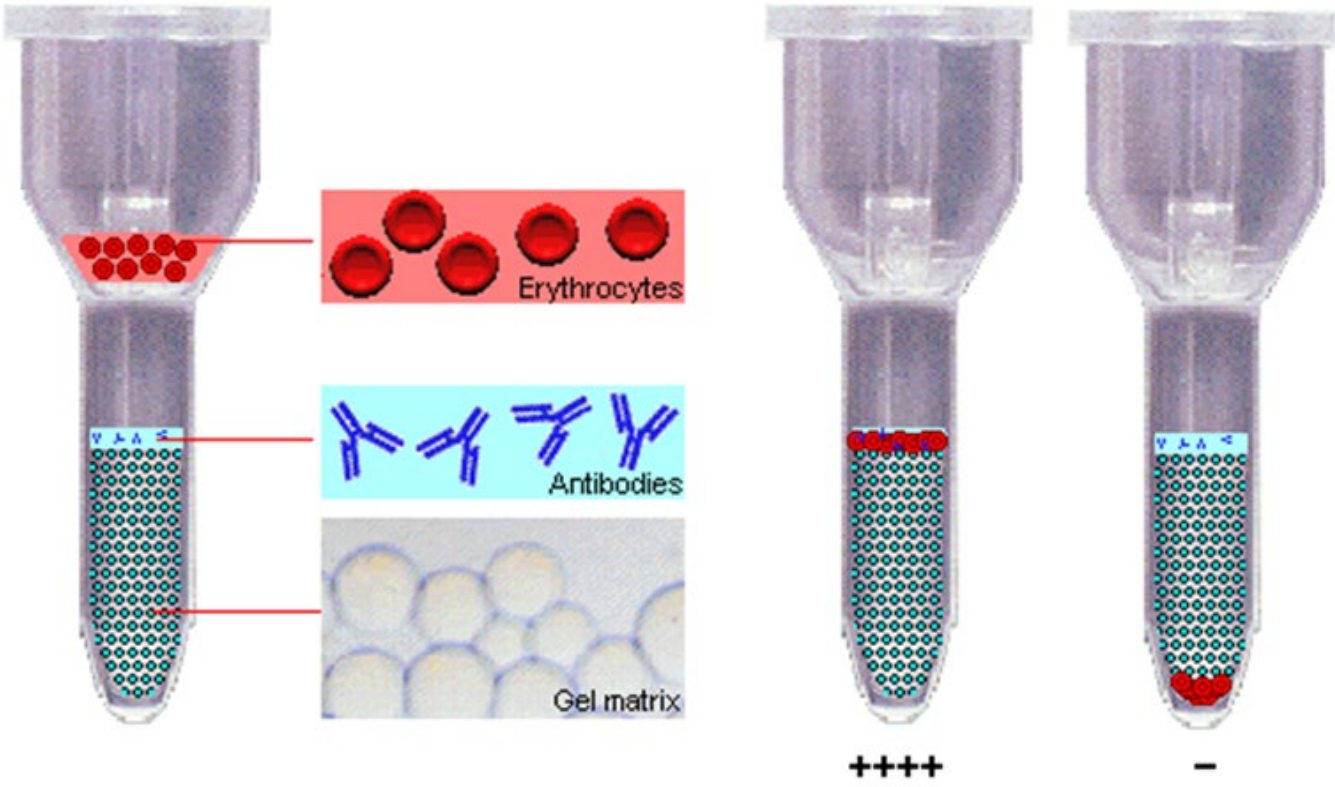
Principle of the Gel Test



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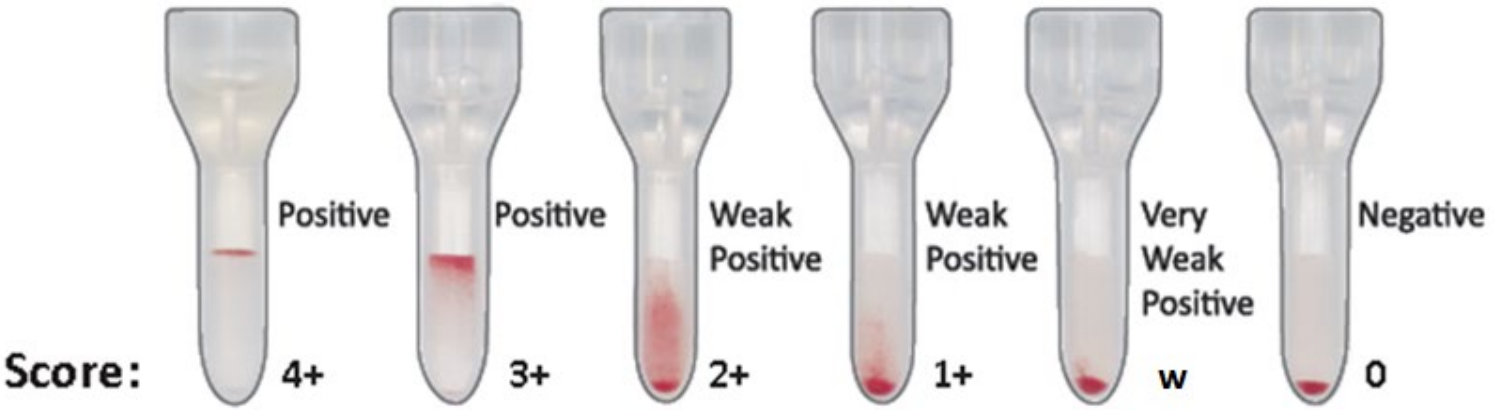
Reaction Scoring



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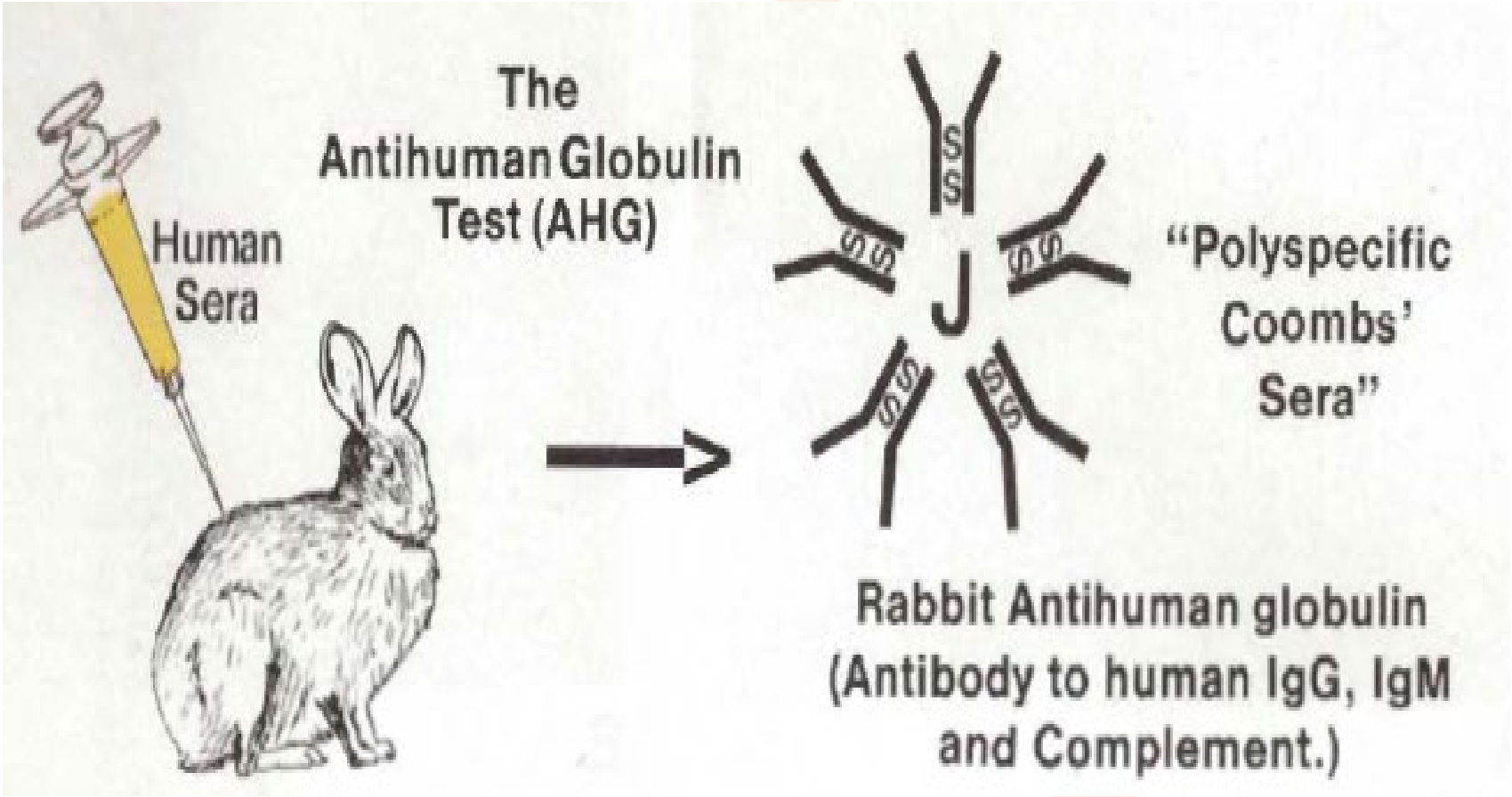
THE AHG TEST

AHG Test = Anti-Human Globulin test

The AHG test is also known as the *Coombs test*

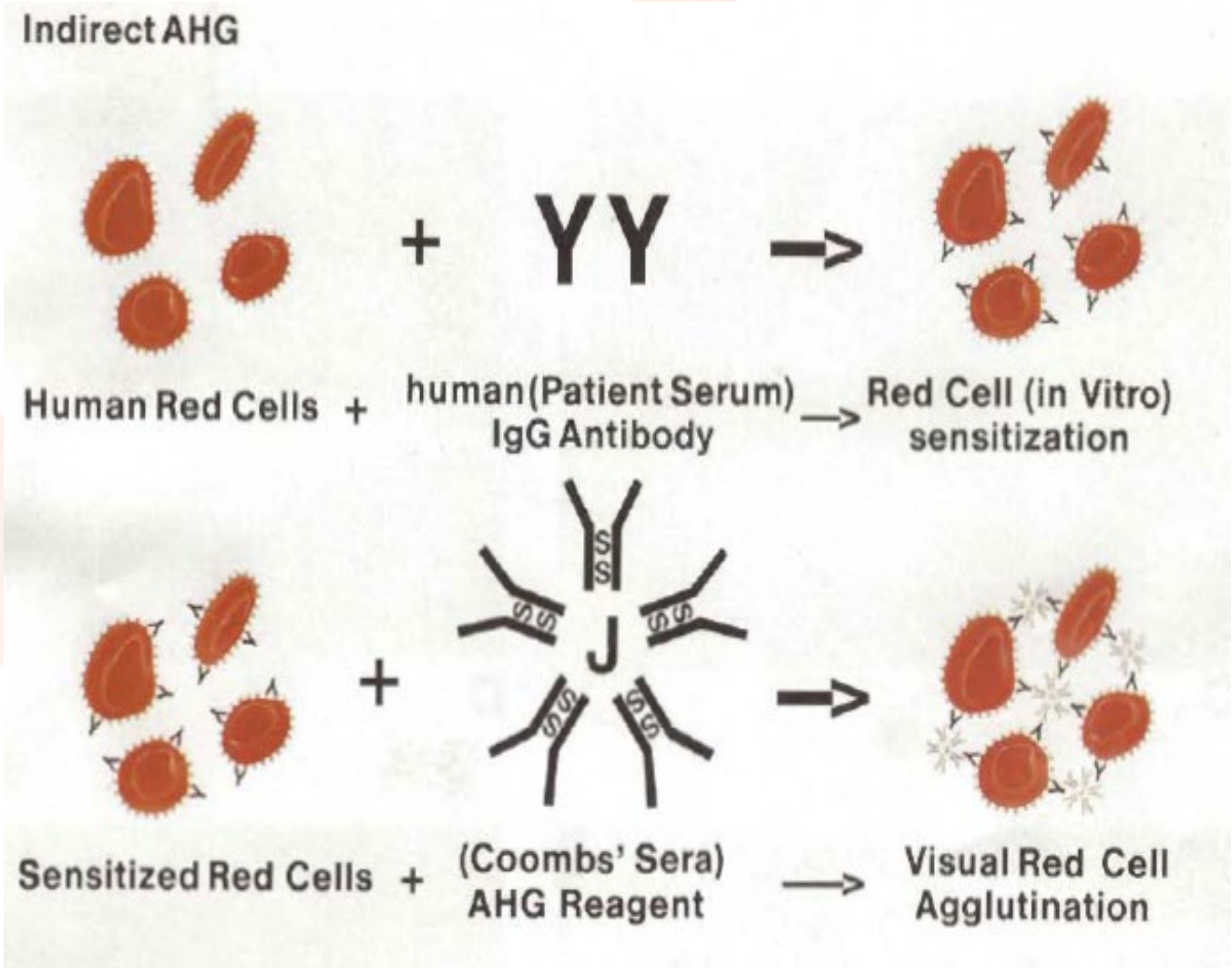
Incubation of cells with plasma promotes sensitization – Ab/Ag attachment.

AHG reagent enhances crosslinking of Antibody bound to RBCs.



Production of the AHG reagent

THE INDIRECT AHG TEST



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THE INDIRECT AHG TEST

The IAHG test can be used to detect either red cell antigens or red cell antibodies.

The IAHG test is utilised in the laboratory for numerous tests:

- Antibody detection
- Antibody identification
- Compatibility testing (Crossmatching)
- Antigen typing
- Antibody titration

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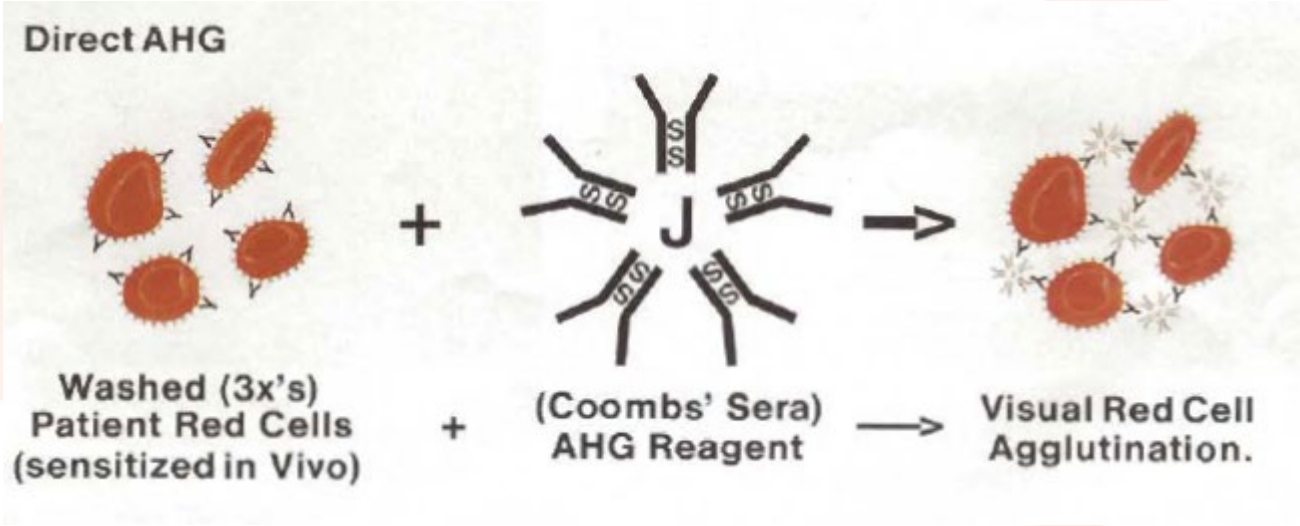
THE DIRECT AHG TEST



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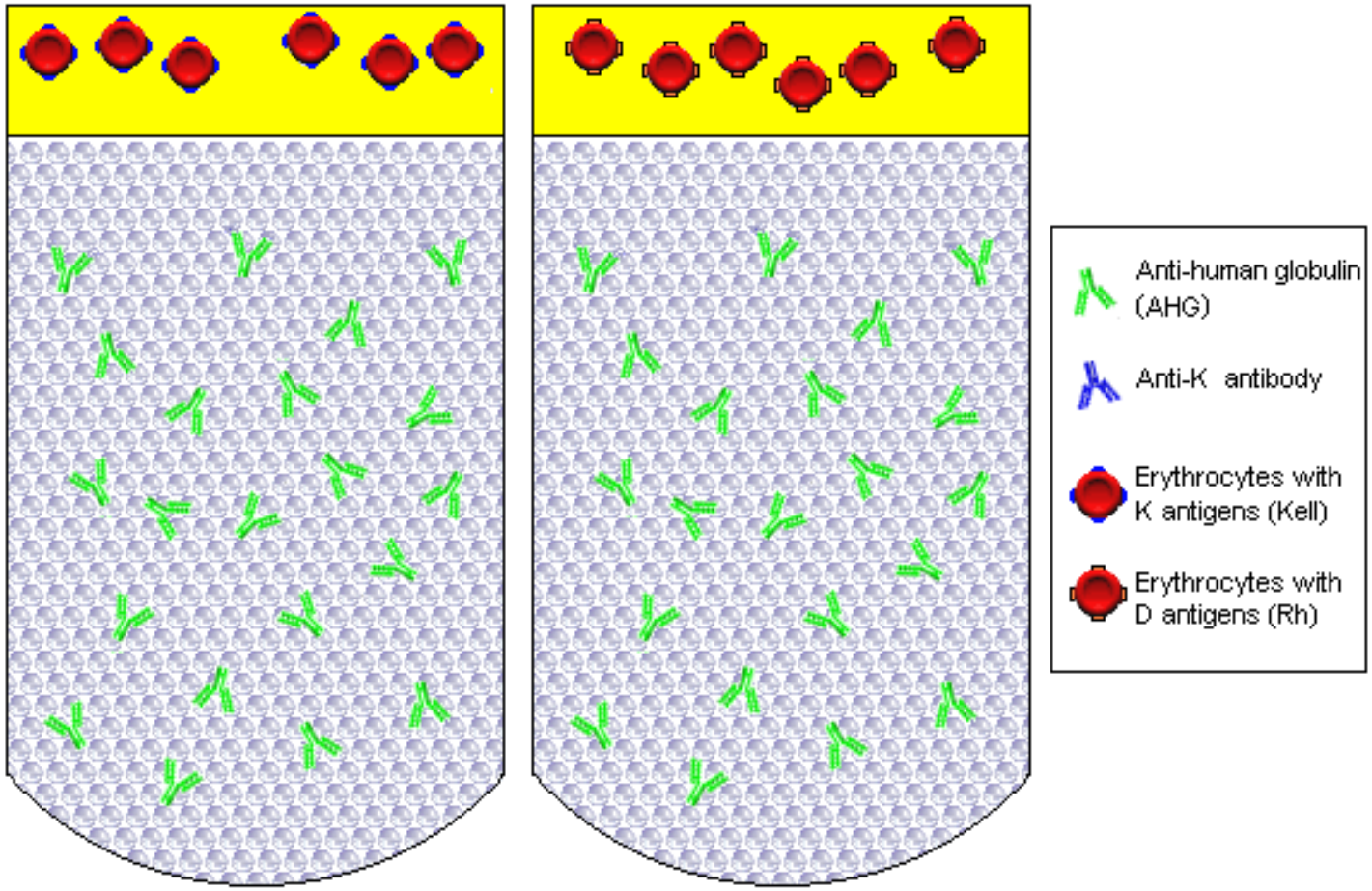
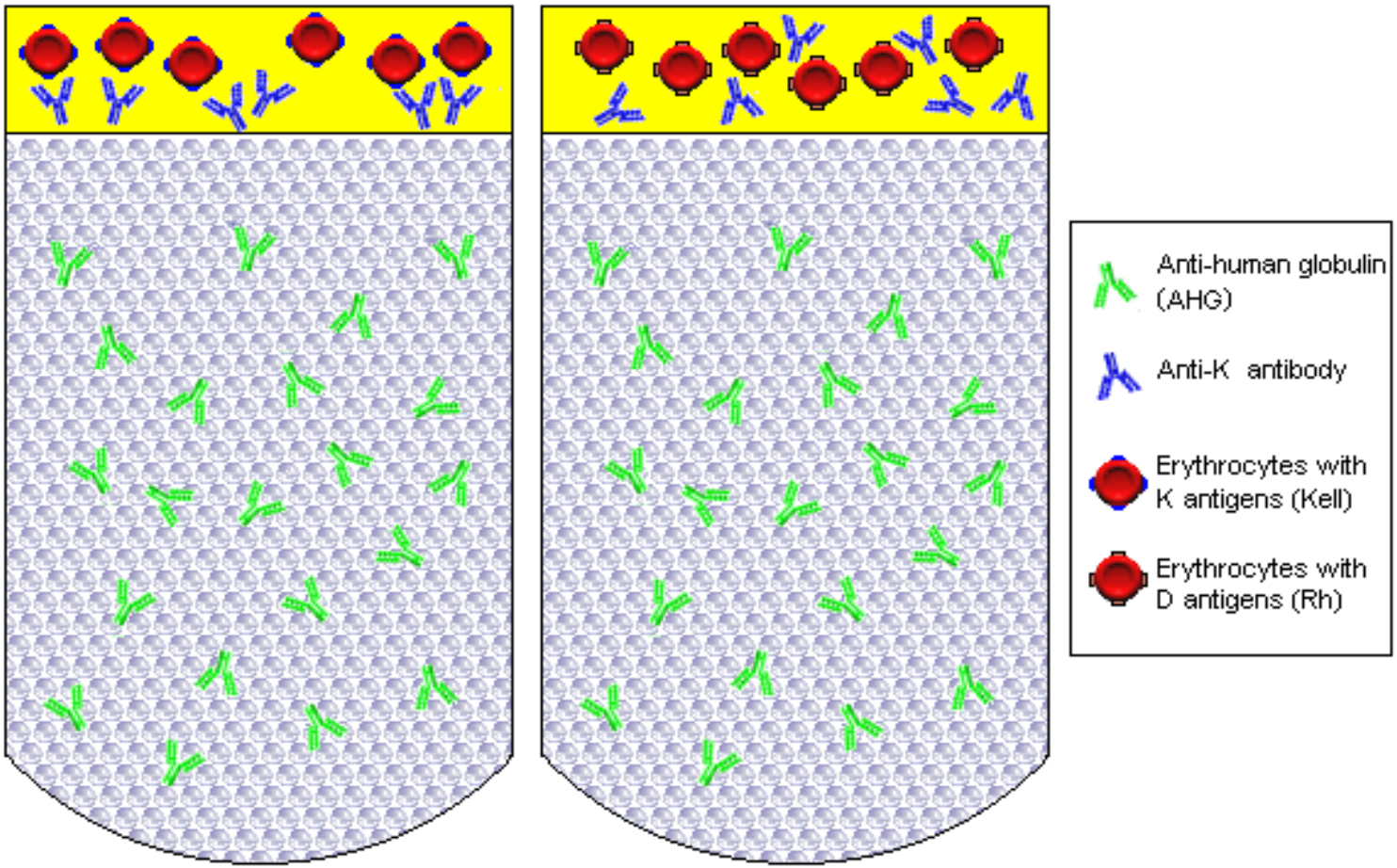
THE DIRECT AHG TEST

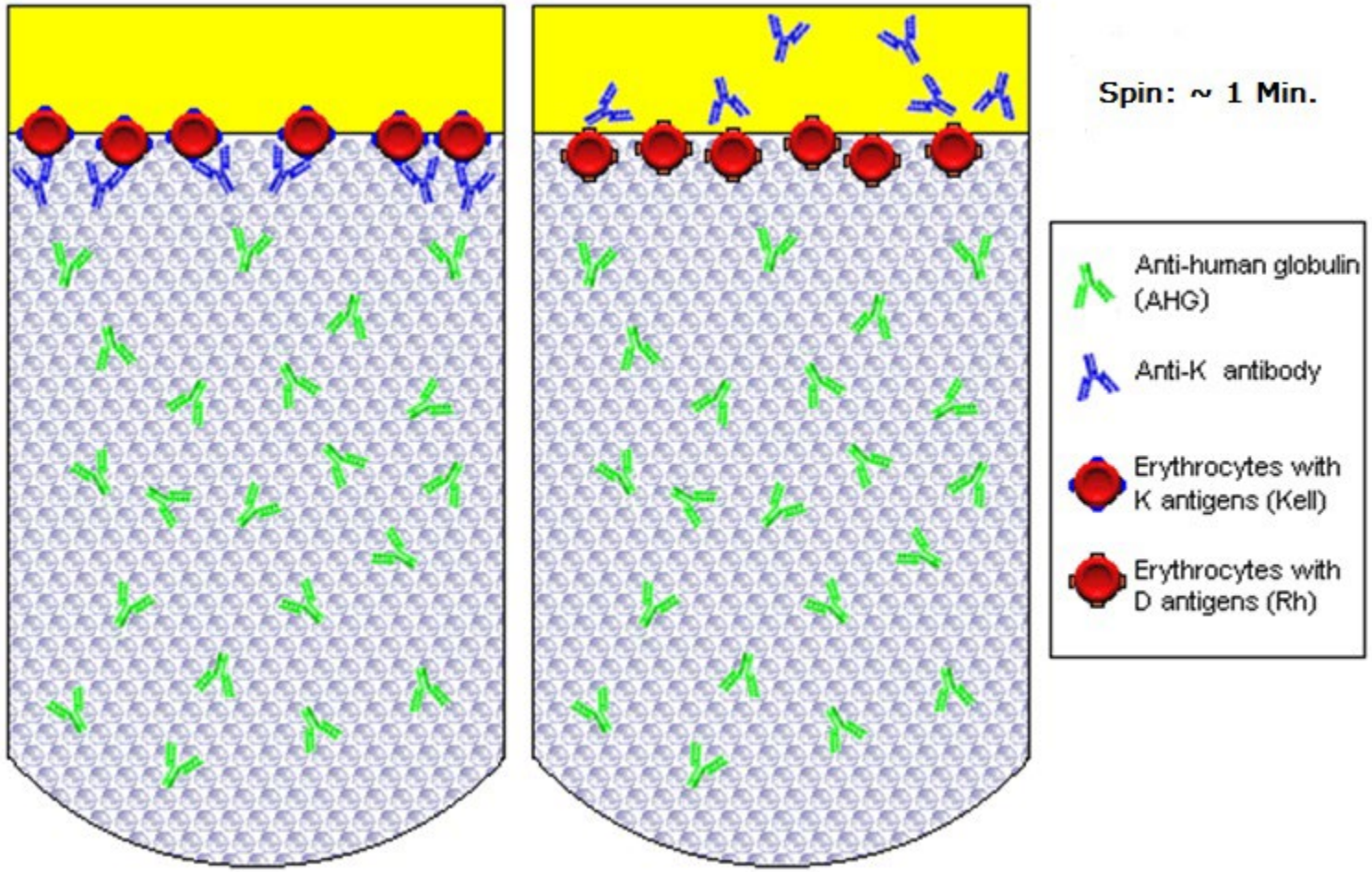
The DAT is designed to demonstrate the presence of an antibody or complement, or both, that has caused in vivo sensitisation of the red blood cells.

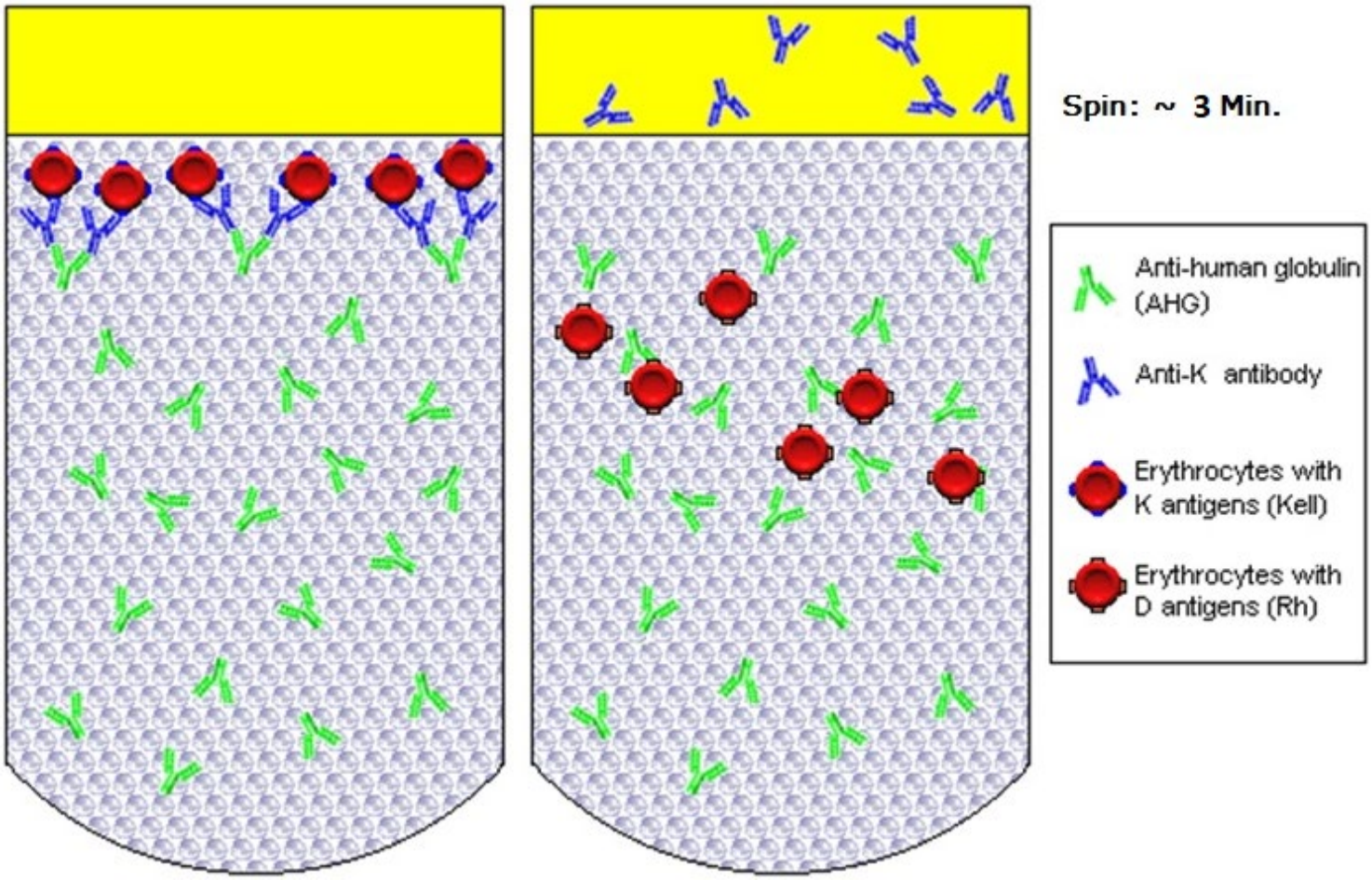
The DAT is useful in investigating the following disorders:

- Autoimmune haemolytic anaemia (AIHA)
- Haemolytic disease of the newborn (HDN)
- Haemolytic transfusion reactions
- Drug-induced haemolytic anaemia

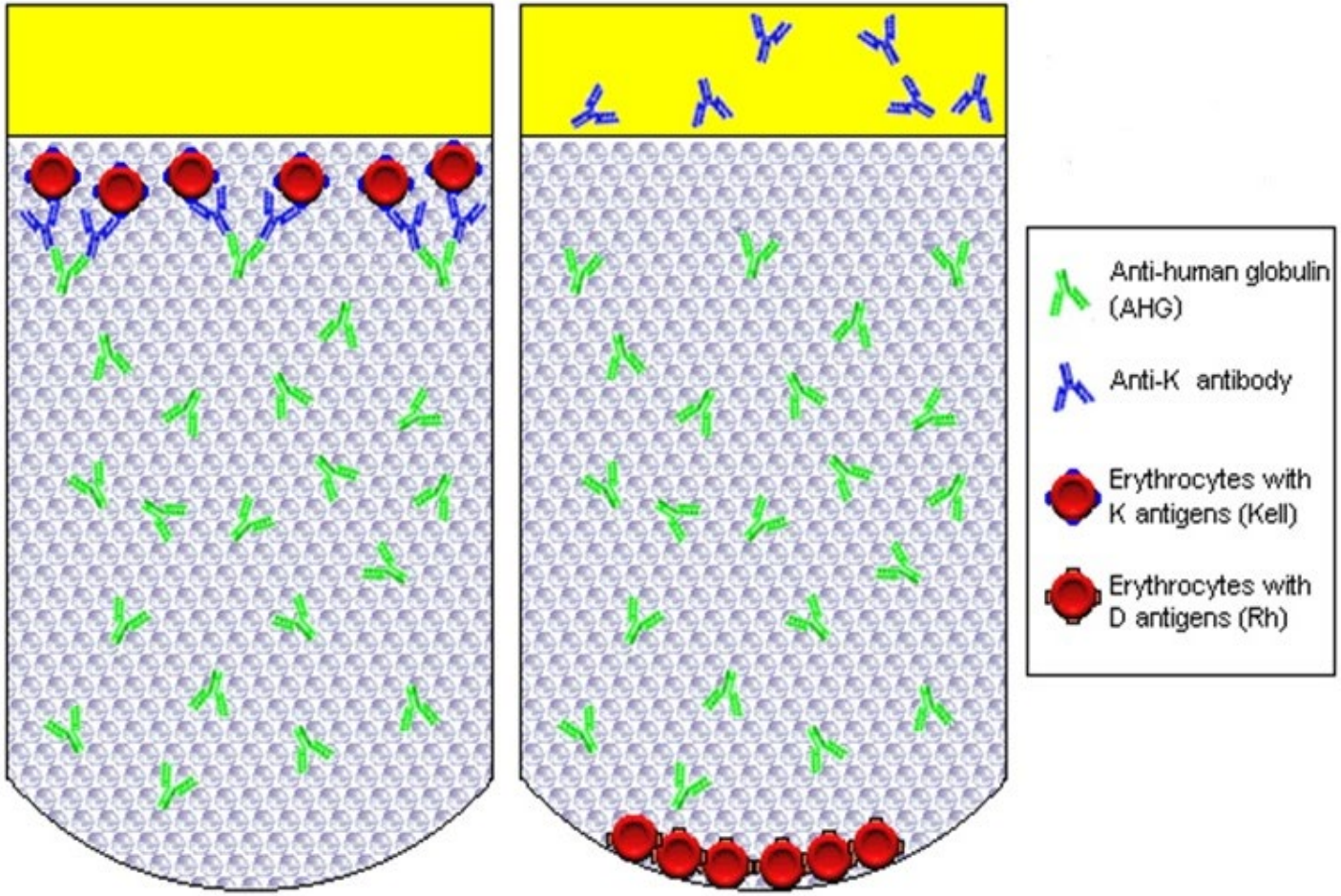








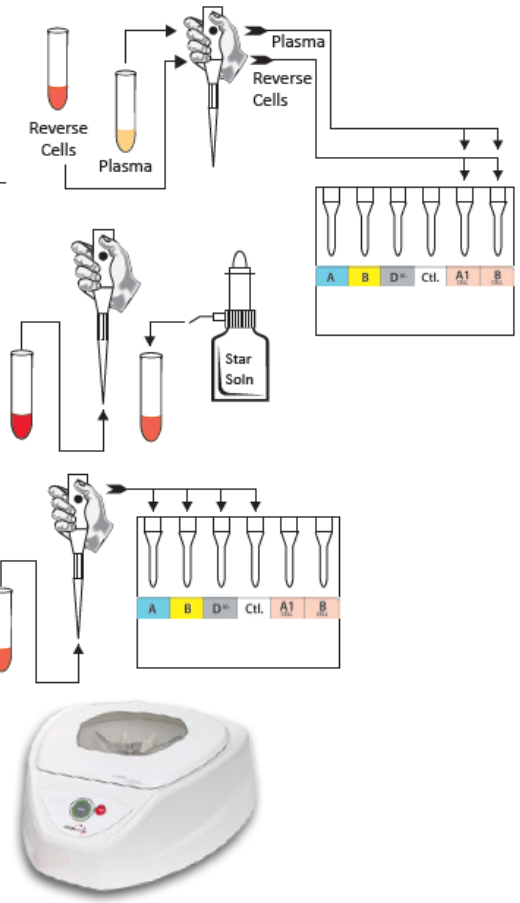
HAEMOKINESIS



Group & Reverse

Cards: **ABO/D + Reverse for Patients or Donors**
Cells: **Reverse A1 + B Cell**

1. Label card
2. Add 50µL of Reverse cells A1 & B (0.8% v/v) to wells A1 cell & B cell respectively
3. Add 25µL plasma to both A1 & B wells
4. Prepare 0.8% v/v suspension of sample red cells in Star Solution (1.0 mL Star Solution + 10µL packed red cells)
5. Add 50µL of the 0.8% v/v suspension to wells A, B, D, Ctl.
6. Spin 5.5 minutes in STARGEL10 Centrifuge
7. Read, record and interpret results



Most commonly used grouping card. Used for pre-transfusion and antenatal testing.

Forward grouping tests patient cells against reagent Anti-A, Anti-B, and Anti-D.

Reverse grouping tests patient plasma against reagent A cells and B cells.

Combined reaction pattern used for interpretation.

HAEMOKINESIS

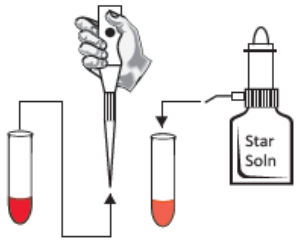
Group Check

GROUP CHECK TEST

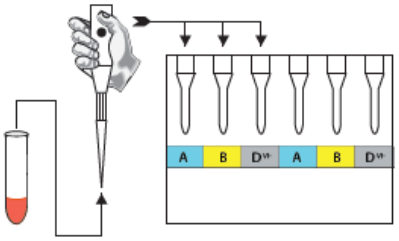
Cards: **Group Check for Patients or Donors**

1. Label card

2. Prepare 0.8% v/v suspension of sample red cells in Star Solution (1.0 mL Star Solution + 10µL packed red cells)



3. Add 50µL of the 0.8% v/v cell suspension to wells A, B and D



4. Spin 5.5 minutes in STARGEL10 Centrifuge

5. Read, record and interpret results

Used for first presentation of patient to confirm ABO/D typing. Different anti-A, anti-B and Anti-D to the Group and Reverse card.

HAEMOKINESIS

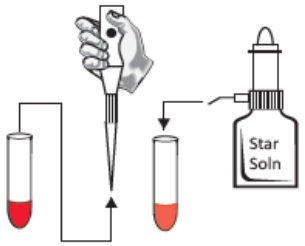
Newborn & Rh/Kell

NEWBORN AND RH/KELL

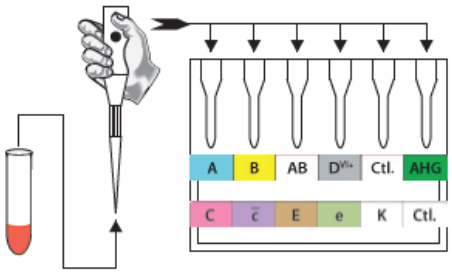
Cards: **Newborn / Rh/Kell**

1. Label card

2. Prepare 0.8% v/v suspension of sample red cells in Star Solution (1.0 mL Star Solution + 10µL packed red cells)



3. Add 50µL of the 0.8% v/v suspension to all six wells of the card



4. Spin 5.5 minutes in STARGEL10 Centrifuge

5. Read, record and interpret results

Babies of Rh(D) Negative Mothers.

Mother of Rh(D) Positive baby will require Anti-D injection to mop up Rh(D) Positive cells that may have crossed into her circulation at delivery.
Newborn card has two Anti-D antibodies as even incomplete D antigen can stimulate a response.

Also includes Direct Coombs to test for sensitization of babies cells.
HDN – haemolytic disease of the Newborn.

Beside ABO/Rh(D), most common antigen test is Rh antigens C,c,E,e and Kell.

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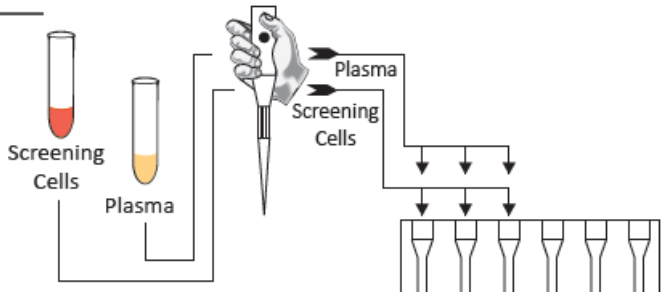
Antibody Screens

ANTIBODY SCREENING

Cards: **AHG / Anti-IgG / Neutral**
Cells: **Pool Cell / 2 Cell Screen / 3 Cell Screen**

1. Label card
2. Add 50µL of screening cell (0.8% v/v) to appropriate well
3. Add 25µL plasma to each well
4. a) AHG/IgG: Incubate 5 minutes at 38.5°C
   OR
   b)Neutral: Incubate 30 minutes at 2-8°C
5. Spin 5.5 minutes in STARGEL10 Centrifuge
6. Read, record and interpret results



Pre-transfusion and antenatal testing.

Screening cells are Group O, so will not react with ABO antibodies.

Rh(D) Negative mothers have repeat screens through pregnancy.

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Antibody Screens

For Positive Antibody Screen tests, reaction pattern from the screening cells can give some indication of the Antibody specificity, but is not conclusive.

=> Antibody Identification Panel is the next step.

Donor No	Rh Type	No	Rh						Kell				Duffy		Kidd		MNSs				P	Lewis		Lutheran		Co		Extra Cell Types				Special Typings	No.
			D	C	c	E	e	C^w	K	k	Kp^a	Kp^b	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s	P_1	Le^a	Le^b	Lu^a	Lu^b	Co^a	Co^b	Wr^a	Vel	Bg^a	Bg^b		
AR192	R_1R_1	1	+	+	0	0	+	0	+	+	0	+	+	+	+	0	+	0	+	0	0	+	0	0	+		0	0	NT	0	0		1
AR208	R_2R_2	2	+	0	+	+	0	0	+	+	0	+	+	0	0	+	0	+	0	+	+	0	+	0	+		0	0		0	0		2
AR182	rr	3	0	0	+	0	+	0	0	+	+	+	0	+	+	+	0	+	0	+	0	0	+	0	+		+	0	+	0	0		3

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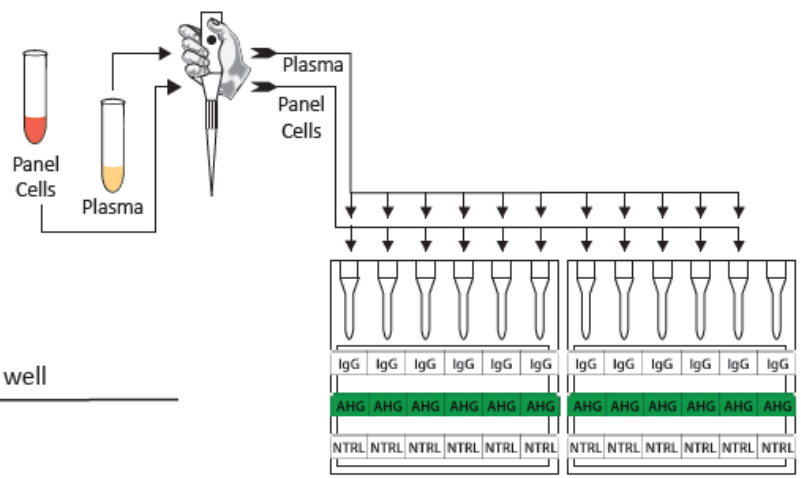
Antibody Identification Panel

Cards: **AHG / Anti-IgG / Neutral**
Cells: **Panel 11**

1. Label cards

2. Add 50µL of panel cells (0.8% v/v) to appropriate well



3. Add 25µL plasma to each well

4. a) AHG/IgG: Incubate 5 minutes at 38.5°C
OR
b) Neutral: Incubate 30 minutes at 2-8°C

5. Spin 5.5 minutes in STARGEL10 Centrifuge

6. Read, record and interpret results

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Antibody Identification Panel

Antibody specificity is derived from the reaction pattern by a process of elimination. Sometimes multiple antibodies are present, requiring different detection techniques and/or alternative panels.

Once antibodies identified from panel results, related antigen test(s) performed on patient red cells to confirm absence of antigen.

Lot No	Donor No	Rh Type	No	Rh						Kell				Duffy		Kidd		MNSs				P	Lewis		Lutheran		Co		Extra Cell Types				Cell	Results					
				D	C	c	E	e	C^w	K	k	Kp^a	Kp^b	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s	P$_1$	Le^a	Le^b	Lu^a	Lu^b	Co^a	Co^b	Wr^a	Vel	Bg^a	Bg^b							
06171.10.3	AR148	R$_1$R$_1$	1	+	+	0	0	+	0	0	+	0	+	+	0	+	0	+	0	+	0	0	0	+	0	+		0	0	+	0	0	1					
06181.10.3	AR146	R$_1$R$_1$	2	+	+	0	0	+	0	+	0	0	+	0	+	+	0	+	+	0	+	+	0	+	0	+		0	0	+	+	0	2					
06191.10.3	AR003	R$_1^w$R$_1$	3	+	+	0	0	+	+	0	+	0	+	+	+	0	+	0	+	0	+	+	+	0	0	+		0	0	+	0	0	3					
06201.10.3	AR218	R$_2$R$_2$	4	+	0	+	+	0	0	0	+	0	+	0	+	+	0	+	0	+	+	+	+	0	0	+		+	0	NT	0	0	4					
06211.10.3	AR189	R$_2$R$_2$	5	+	0	+	+	0	0	0	+	0	+	+	0	0	+	0	+	0	+	+	0	+	0	+		0	0	+	0	0	5					
06221.10.3	AR154	r'r	6	0	+	+	0	+	0	0	+	0	+	0	+	0	+	+	0	+	+	0	0	0	0	+		0	0	+	0	0	6					
06231.10.3	AR152	r"r	7	0	0	+	+	+	0	0	+	0	+	+	0	+	+	0	+	+	+	+	0	+	0	+	+	0	0	+	0	0	7					
06241.10.3	AR217	rr	8	0	0	+	0	+	0	+	+	0	+	+	+	0	+	+	0	+	0	+	0	+	0	+		0	0	NT	0	0	8					
06251.10.3	AR153	rr	9	0	0	+	0	+	0	0	+	0	+	0	+	0	+	0	+	0	+	+	0	+	+	+		+	0	+	0	0	9					
06261.10.3	AR190	rr	10	0	0	+	0	+	0	0	+	0	+	+	0	+	0	+	0	0	+	+	0	+	0	+		0	0	NT	0	0	10					
06271.10.3	AR149	rr	11	0	0	+	0	+	0	0	+	+	+	+	+	+	0	+	+	+	+	+	+	0	0	+		0	0	+	0	0	11					
	Auto																																					
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Please note: Co^a typings not done on all donations as insufficient anti-Co^a antisera available.

REGISTERED COMPANY ISO 9001 SGS

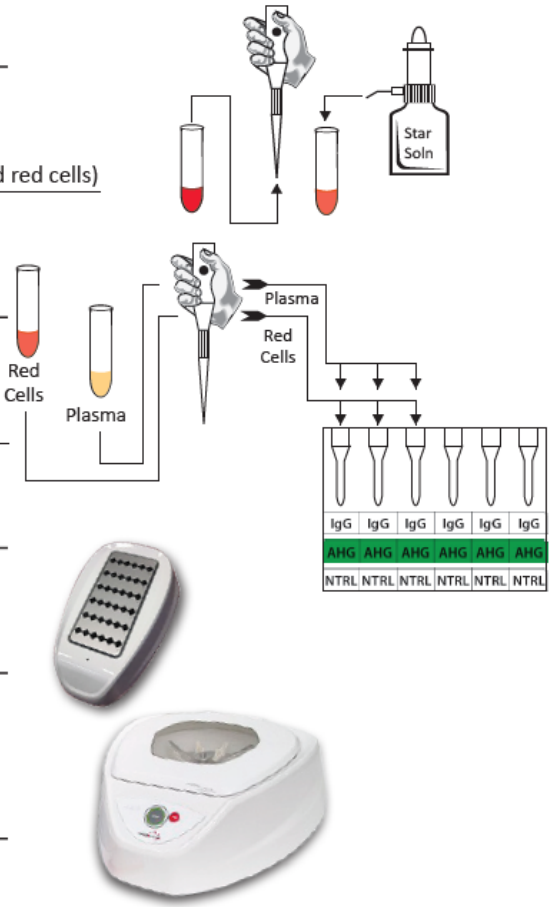
HAEMOKINESIS

Crossmatch

CROSSMATCH

Cards: **AHG / Anti-IgG / Neutral**

1. Label card
2. Prepare 0.8% v/v suspension of donor cells in Star Solution (1.0mL Star Solution + 10µL donor packed red cells)
3. Prepare Autocontrol 0.8% v/v in Star solution (1.0mL Star Solution + 10µL recipient packed red cells)
4. Add 50µL donor suspension or Autocontrol to well
5. Add 25µL recipient plasma to each well
6. AHG/IgG: Incubate 5 minutes at 38.5°C
   6b. Neutral: Incubate 15 minutes at RT
7. Spin 5.5 minutes in Stargel10 Centrifuge
8. Read, record and interpret results



Directly tests compatibility of donor
red cells with recipient plasma.

Many laboratories use ‘Computer Crossmatching’ for most patients – requires specific LIS criteria.

Full crossmatch always required if an antibody is detected or there is a history of antibodies.

HAEMOKINESIS

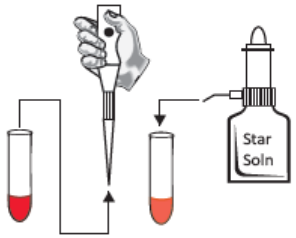
Direct Coombs

DIRECT ANTIGLOBULIN TEST

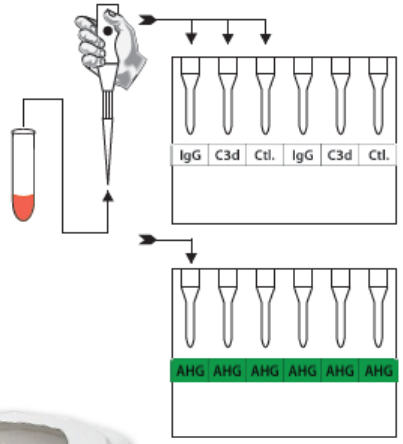
Cards: **AHG / DAT**

1. Label card

2. Prepare 0.8% v/v suspension of sample cells in Star Solution (1.0 mL Star Solution + 10µL patient red cells)



3. Add 50µL of the 0.8% v/v suspension to the wells
   a) DAT: IgG, C3d and Ctl.
   OR
   b) AHG: a single well



4. Spin 5.5 minutes in STARGEL10 Centrifuge

5. Read, record and interpret results

Tests for the presence of in vivo sensitisation of red cells.

Can be a polyspecific screen or monospecific Anti-IgG and Anti-C3d.

HAEMOKINESIS