NHS Blood and Transplant

Antibody Screening, Identification & Crossmatch

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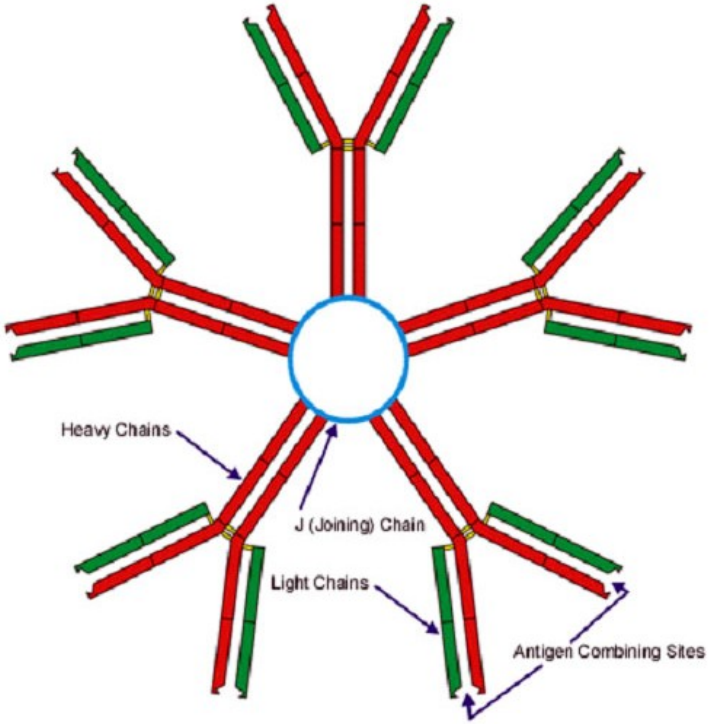
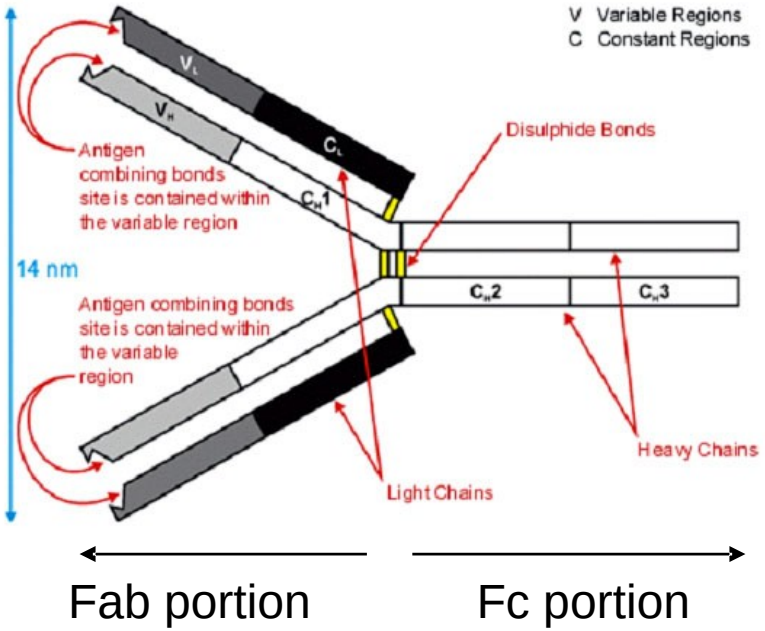
NHS Blood and Transplant

Red Cell Antibodies

- Produced by the immune system
- Created in response to a foreign antigen
- Protein (Immunoglobulins) in serum/plasma
- Red cell antibodies are either:
  - IgG, 2 binding site
  - IgM, 10 binding sites
- Specific binding of antigen to antibody





IgM molecule

IgG molecule

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Antibodies can be ...

- Naturally occurring
  - Anti-A
  - Anti-B

Or

- Immune - examples include:
  - Anti-D
  - Anti-K

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Function of antibodies

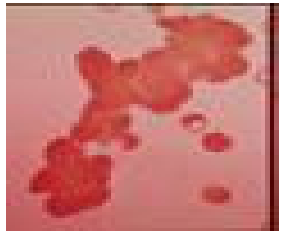
To remove foreign antigens from circulation

In-vivo destruction of red cells:

- Extravascular haemolysis, or
- Intravascular haemolysis

In-vitro destruction of red cells:

- Agglutination
- Lysis



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Antibodies are important when...

- A patient needs a transfusion

Or

- During pregnancy

Red Cell Antigens

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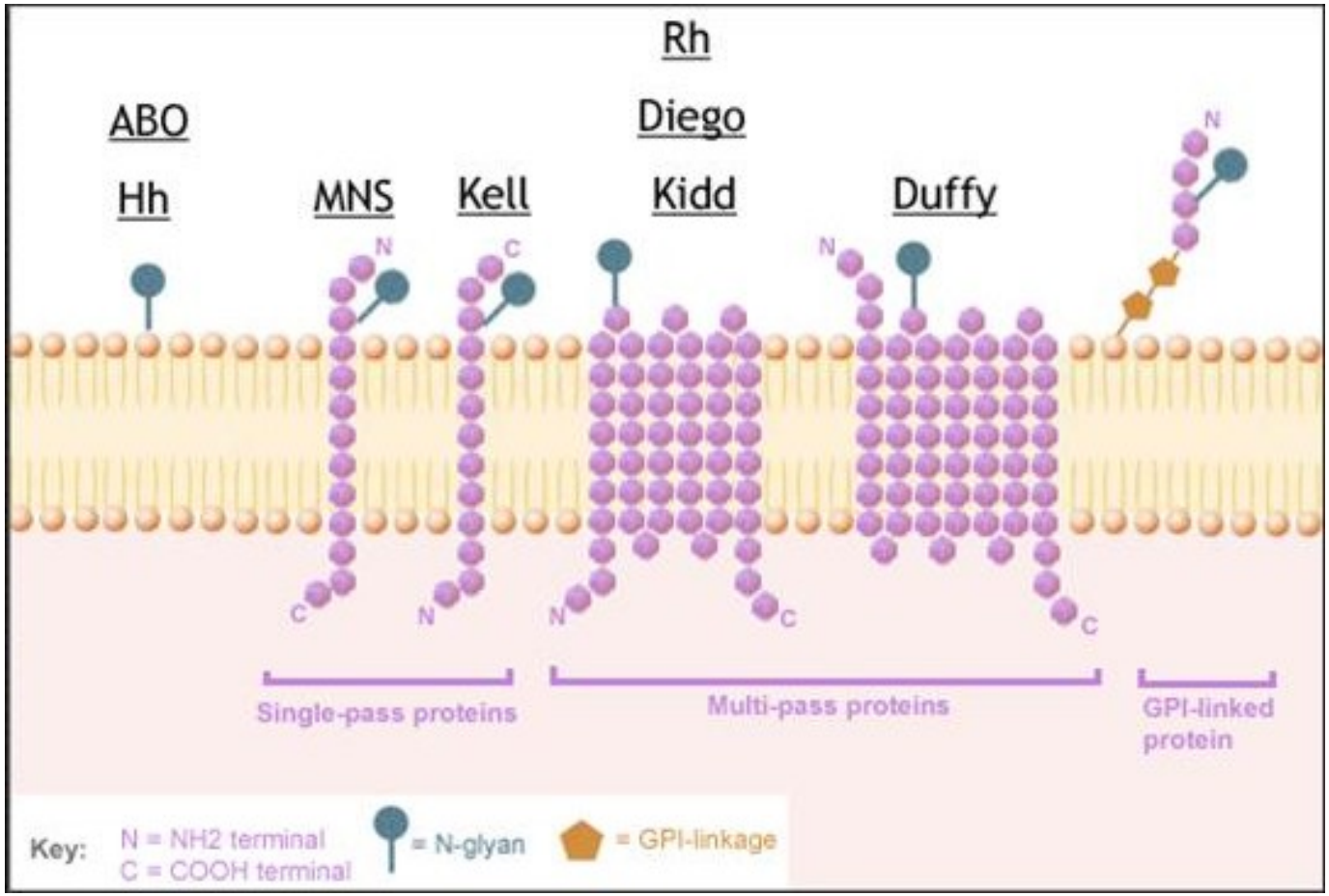

http://www.ncbi.nlm.nih.gov/books/bookres.fcgi/rbcantigen/imagemap.gif

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To prevent serious complications we must ....

- Prevent ABO incompatibility
- Detect alloantibodies in patient's plasma/serum, particularly IgG antibodies

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Guidelines! Which ones?

- UK Guidelines for the Blood Transfusion Service (red book)
- Guidelines for (pre-transfusion) compatibility procedures in blood transfusion laboratories 2004
- Guidelines for blood grouping and red cell antibody testing during pregnancy
- The specification and use of information technology (IT) systems in blood transfusion practice (2006)- Electronic issue

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Why do we use them?

They are

- Recommendations
- Advice on Best Practice

Are not

- Compulsory by Law

But

- Need a good reason to deviate
- Would be used in a court of Law as minimum standard

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The request form and sample

3 identifiers please + date taken + signature of person taking sample

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Antibody Detection
First step: Antibody Screen

Aim of screening procedure

- To detect all clinically significant blood group alloantibodies
- Immune IgG antibodies optimally active at 37ºC
- IgM antibodies which are active at or near 37ºC
- Should also detect autoantibodies active at 37ºC

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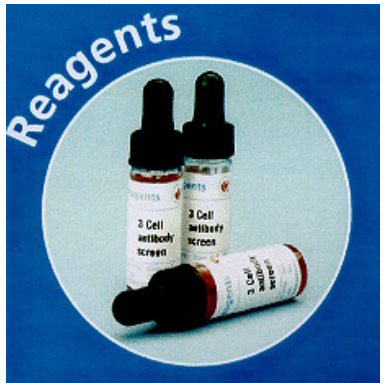
Antibody Screening

- Test the patient's plasma/serum against either a
- 2 cell panel

  or
- 3 cell panel



- Cells are specially selected fully phenotyped "Reagent Screening Red Cells"

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Reagent screening cells must:

Be capable of reacting with all clinically significant antibodies in a patient sample

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Specification:

- Must be group O
- Should not be pooled
- All antigens must have homozygous expression, for all clinically significant antibodies
- Antigen profile always follows the same format

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FORM FRM/DDR/RE/004/04 Approved: 14 December 07

2 Cell Screen Profile PR101 & 102

CE 0843

IVD

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NBS REAGENTS LIVERPOOL

Product	Lot No.	Product	Lot No.	Expiry
Alsevers	R101 3280	CellStab	R102 3280	2009.05.14

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 _ http://hospital.blood.co.uk/hospitals/diagnostic_services/reagents/index.asp#Pro

	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	
2	R_2R_2	0	+	+	+	0	0	+	0	0	+	+	+	+	0	0	+	0	+	0	+	

3 cell screening panel usually Orr or may be $R_1{}^W R_1$.
3 cell panel can also include a Kp(a+) cell

(Template Version 01/04)

Cross-Referenced in Primary Document: SOP/DDR/RE/001 Page 1 of 1

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Antigen Expression

Antigens that must be expressed:-

Cc D Ee, Kk, Fya Fyb, Jka Jkb, Ss, MN, Lea

(P1 and Leb expressed)

Must have homozygous expression:-

D, C, c, E, e, (R_1R_1 and R_2R_2)

Jk^a, Jk^b, S, s, Fy^a, Fy^b

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Antibody screening

IAT – Indirect antiglobulin test

- Positive antibody screen indicates the presence of an antibody
- Follow-up with antibody identification process

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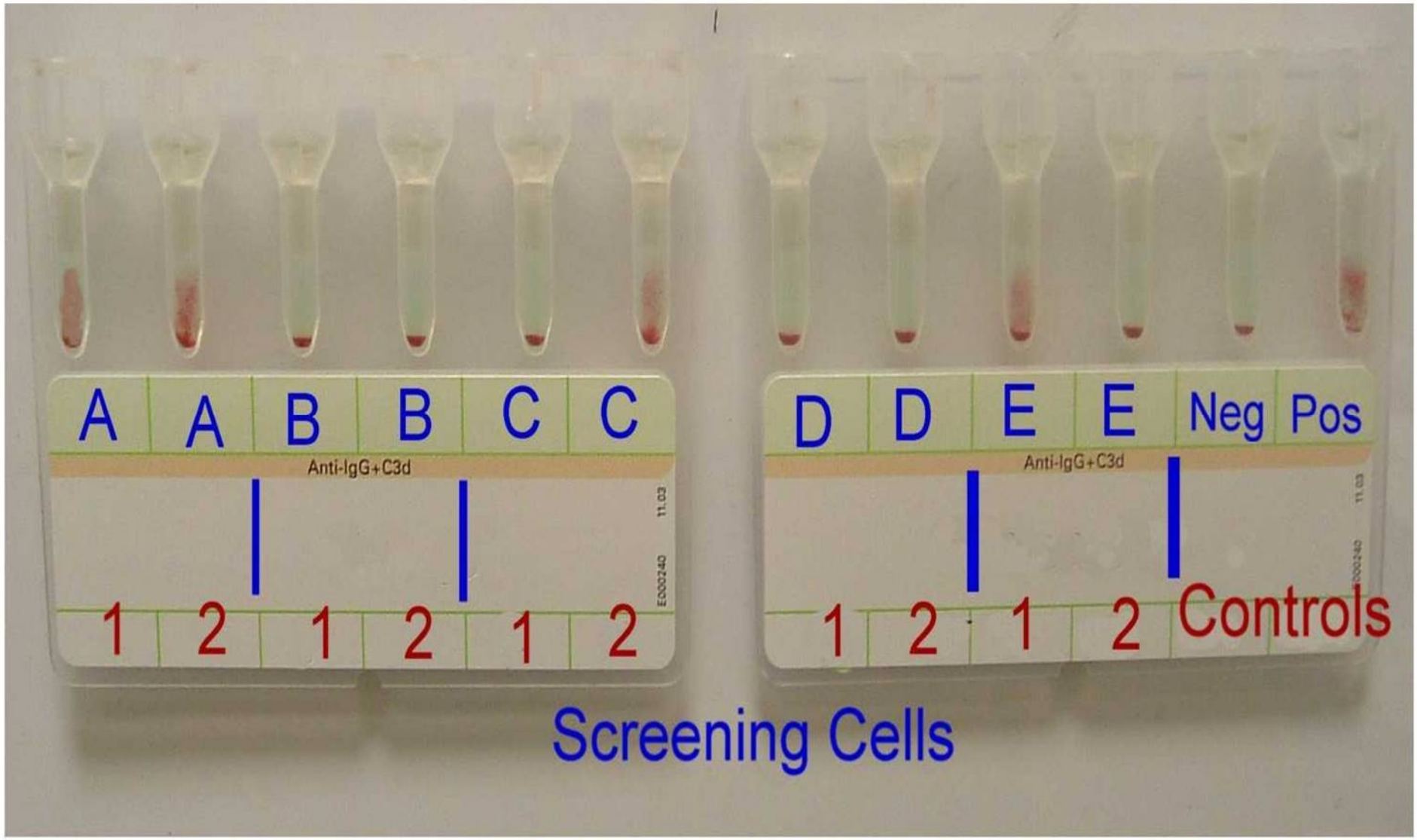

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Antibody screening results

Pat A		Pat B		Pat C		Pat D		Pat E		Neg	Pos
+	+	0	0	0	+	0	0	+	0	0	+
Cell 1	Cell 2	Cell 1	Cell 2	Cell 1	Cell 2	Cell 1	Cell 2	Cell 1	Cell 2	Controls	

Patients A, C and E antibodies detected

Patients B and D no antibodies detected

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Results:

- Negative reaction with reagent cells indicates the absence of any clinically significant antibodies in the patient sample
- Positive reaction with one or more reagent cells indicates the presence of a red cell antibody
- Need to identify the specificity of the antibody and determine its clinical significance.

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POSITIVE ANTIBODY SCREEN

does not

- Show Antibody Specificity
- Indicate clinical significance with respect to
  - Transfusion
    - is antigen negative blood required
  - Haemolytic disease of the newborn (HDN)
    - Is it able to cause HDN

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ANTIBODY SIGNIFICANCE

- Identify the antibody and assess it's clinical significance
- Is the antibody IgG – capable of red cell destruction / placental transfer
- Is the antibody IgM – usually not significant - active primarily at 18°C
- Isoagglutinins Anti-A and Anti-B are significant

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Antibody Identification

If an antibody is detected we must:-

- Identify the antibody
- Assess its clinical significance
  - For transfusion
  - In pregnancy

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How to Identify an Antibody

- Compare pattern of reactions with each reagent cell of ID panel with the pattern of antigens on the reagent cells
- Matching pattern will identify the antibody
- Need to know how the antibody reacts

  i.e. IAT, enzymes, 37ºC, 16ºC

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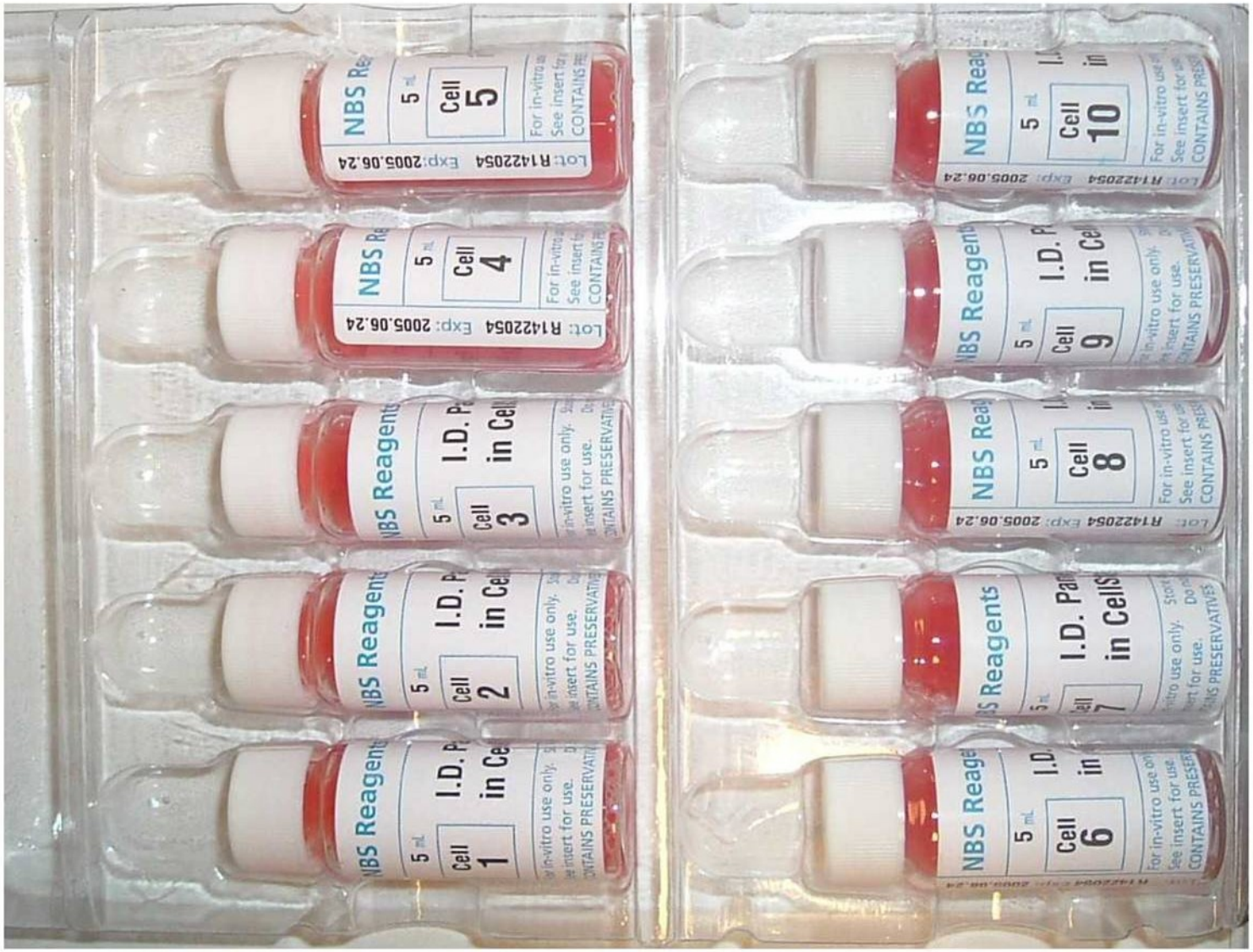
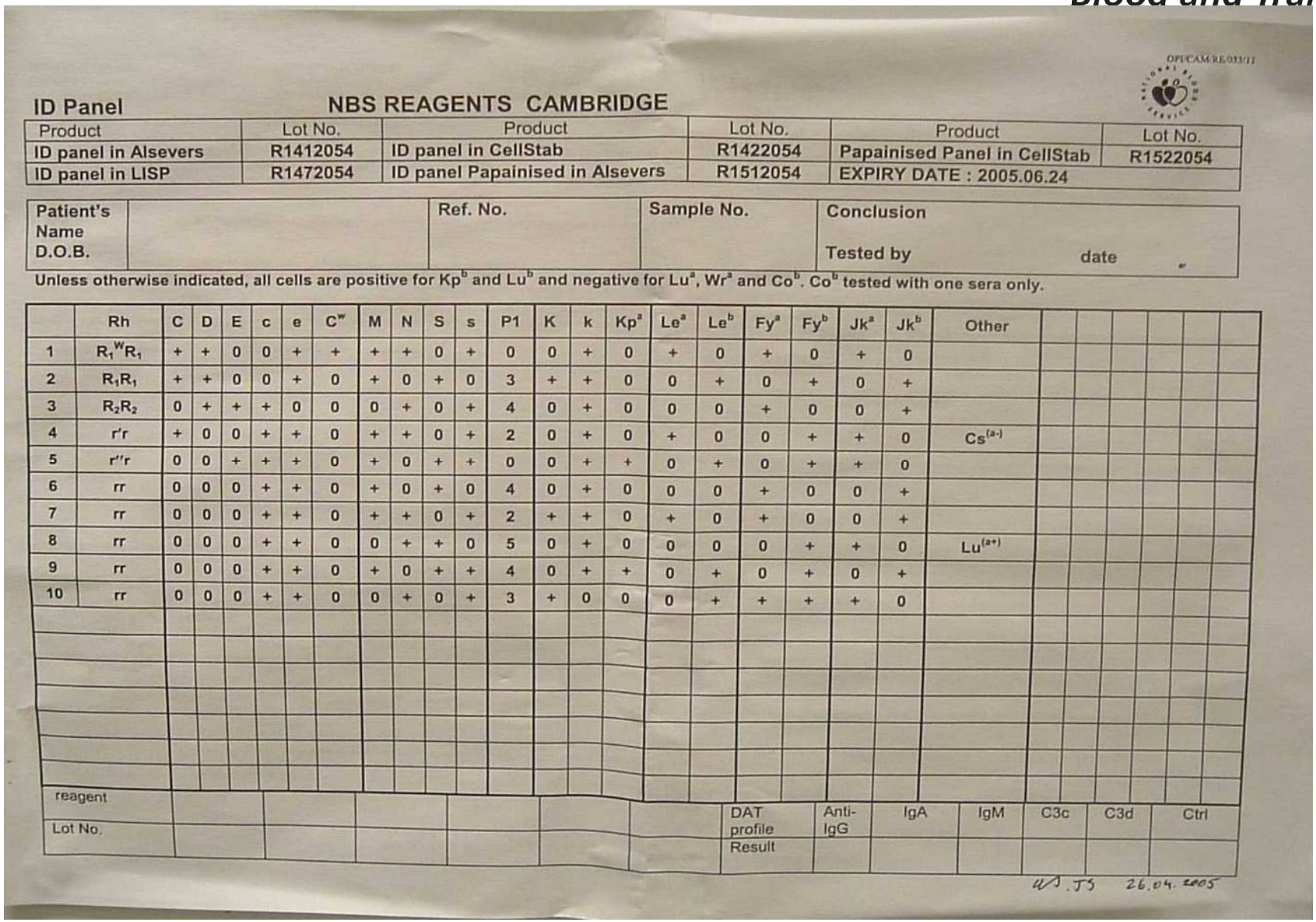


Red Cell Immunohaematology



ID Panel — NBS REAGENTS CAMBRIDGE

OPI/CAM/RE/033/11

Product	Lot No.	Product	Lot No.	Product	Lot No.
ID panel in Alsevers	R1412054	ID panel in CellStab	R1422054	Papainised Panel in CellStab	R1522054
ID panel in LISP	R1472054	ID panel Papainised in Alsevers	R1512054	EXPIRY DATE : 2005.06.24	

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

Unless otherwise indicated, all cells are positive for Kp^b and Lu^b and negative for Lu^a, Wr^a and Co^b. Co^b tested with one sera only.

	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	$R_1^wR_1$	+	+	0	0	+	+	+	+	0	+	0	0	+	0	+	0	+	0	+	0	
2	R_1R_1	+	+	0	0	+	0	+	0	+	0	3	+	+	0	0	+	0	+	0	+	
3	R_2R_2	0	+	+	+	0	0	0	+	0	+	4	0	+	0	0	0	+	0	0	+	
4	r'r	+	0	0	+	+	0	+	+	0	+	2	0	+	0	+	0	0	+	+	0	$Cs^{(a-)}$
5	r''r	0	0	+	+	+	0	+	0	+	+	0	0	+	+	0	+	0	+	+	0	
6	rr	0	0	0	+	+	0	+	0	+	0	4	0	+	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	5	0	+	0	0	0	0	+	+	0	$Lu^{(a+)}$
9	rr	0	0	0	+	+	0	+	0	+	+	4	0	+	+	0	+	0	+	0	+	
10	rr	0	0	0	+	+	0	0	+	0	+	3	+	0	0	0	+	+	+	+	0	

reagent							DAT profile	Anti-IgG	IgA	IgM	C3c	C3d	Ctrl
Lot No.							Result						

WS. JS 26.04.2005

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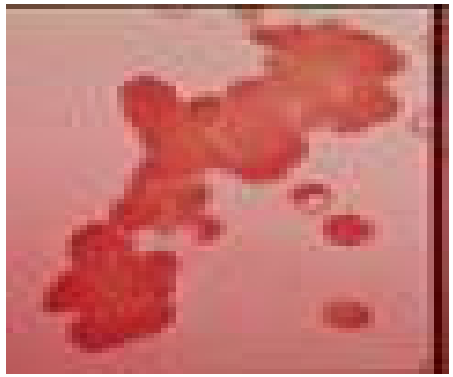
Indirect Antiglobulin Test

- Used to detect IgG antibodies
- Anti-human globulin bridges gap between two sensitised cells
- Results in agglutination



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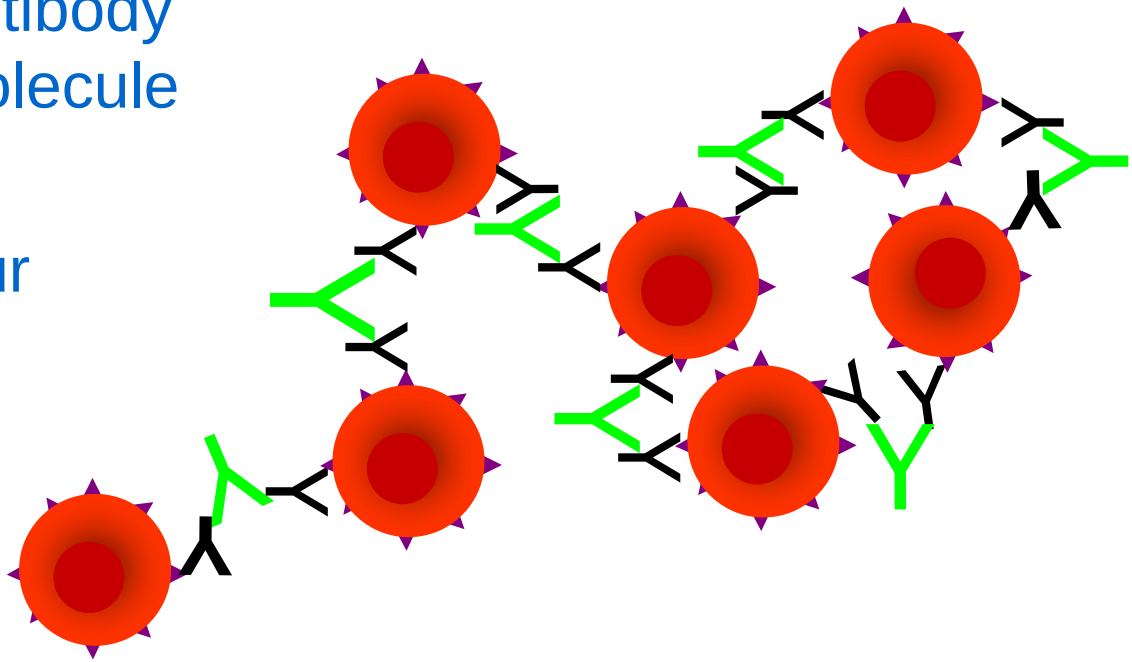
Indirect Antiglobulin Test

- Extend antibody molecules by using a ‘bridge’
- This molecule is an antibody to the IgG antibody molecule
- Anti Human Globulin
- Agglutination can occur



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DiaMed reactions and grading

A **negative** reaction is characterized by unagglutinated red cells forming a well-delineated pellet at the bottom of the microtube.

A **1+** reaction is characterized by red cell agglutinates predominantly observed in the lower half of the gel column. Unagglutinated cells form a pellet in the bottom of the microtube.

A **2+** reaction is characterized by red cell agglutinates dispersed throughout the length of the gel column. Few agglutinates may be observed in the bottom of the microtube.

A **3+** reaction is characterized by the majority of red cell agglutinates trapped in the upper half of the gel column.

A **4+** reaction is characterized by a solid band of red cell agglutinates on top of the gel. A few agglutinates may filter into the gel but remain near the predominant band.

A **mixed cell** reaction is characterized by a band of red cell agglutinates on top of the gel, accompanied by a pellet of unagglutinated cells at the bottom of the microtube.

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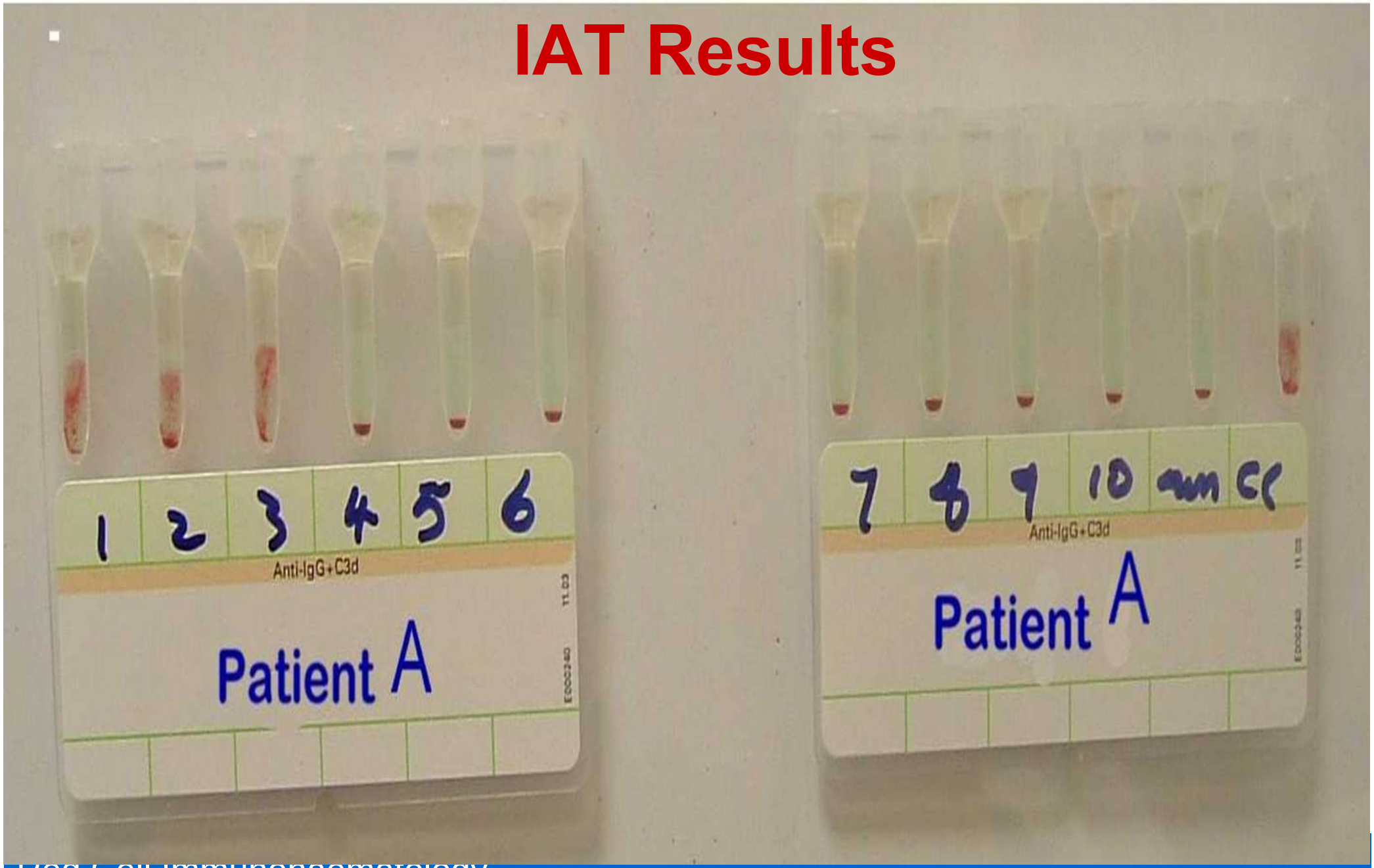
Confirming the specificity

- Reacts with at least 2 cells positive for the antigen
- Not react with at least 2 cells negative for the antigen
  - These cells should cover all other clinically significant antigens between them, to exclude the presence of other antibodies.
  - These cells should have homozygous expression for the clinically significant antigens
- Patient red cells should be phenotyped for the antigen, for an alloantibody it will be negative.

IAT Results

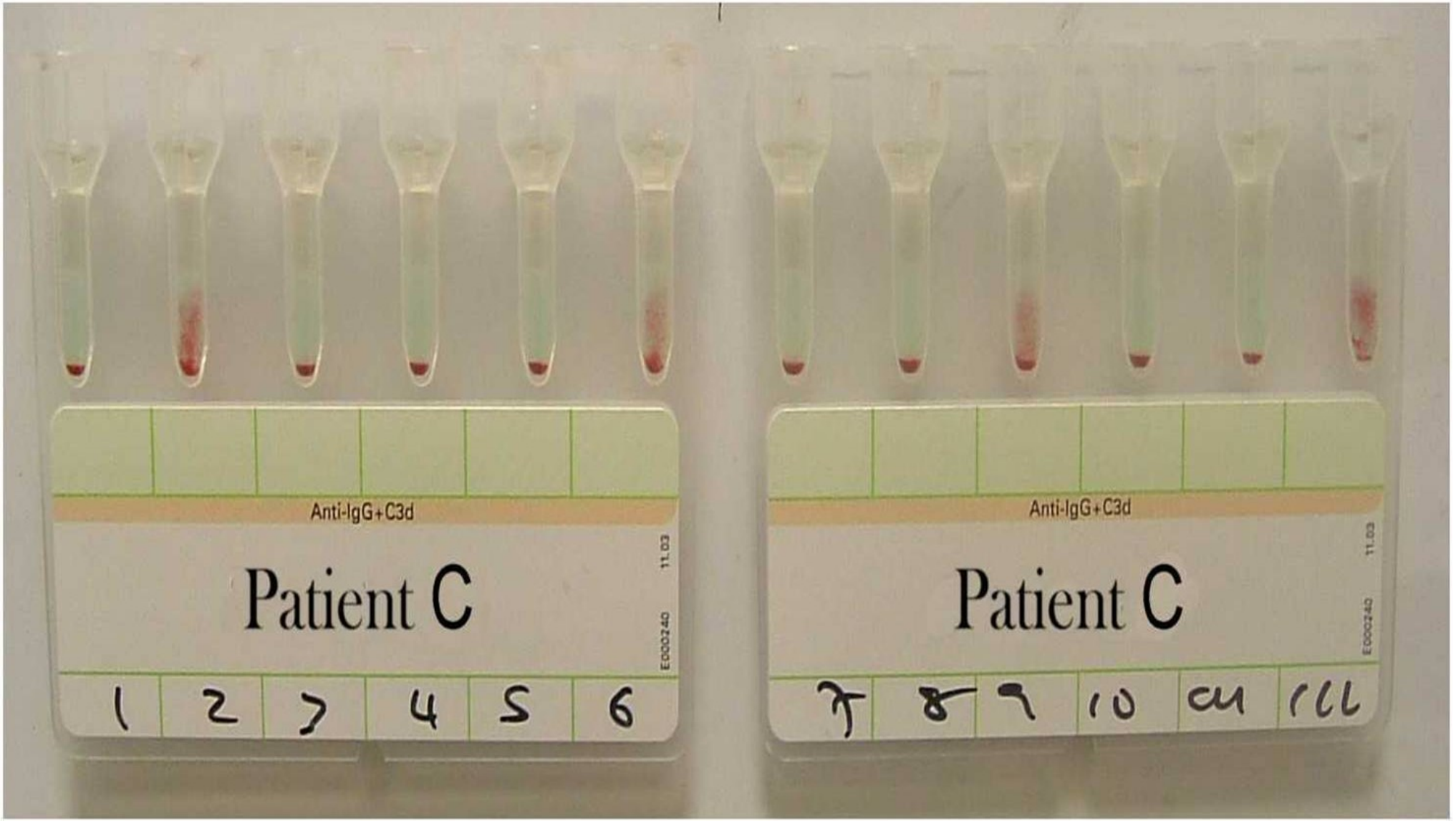

1 2 3 4 5 6
Anti-IgG+C3d
Patient A
7 8 9 10 aun cc
Anti-IgG+C3d
Patient A

Patient A

Rh	D	C	c	E	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	IAT	ENZ
$R1^wR1$	+	+	0	0	+	+	+	+	+	0	3	0	+	+	0	0	+	0	+	0	+	3	5
R1R1	+	+	0	0	+	0	+	0	+	+	5	0	0	+	0	+	0	+	0	0	+	2	5
R2R2	+	0	+	+	0	0	0	+	+	0	0	0	+	+	0	0	+	0	+	+	0	3	5
r'r	0	+	+	0	+	0	0	+	0	+	4	0	0	+	0	0	+	+	0	+	0	0	0
r''r	0	0	+	+	+	0	+	0	+	0	4	0	0	+	0	+	0	+	0	+	+	0	0
rr	0	0	+	0	+	0	+	+	0	+	0	0	0	+	0	0	+	0	+	+	0	0	0
rr	0	0	+	0	+	0	0	+	0	+	0	0	0	+	0	0	0	0	+	0	+	0	0
rr	0	0	+	0	+	0	+	0	0	+	3	0	+	+	0	+	0	+	0	+	0	0	0
rr	0	0	+	0	+	0	0	+	0	+	4	+	0	+	+	+	0	0	+	0	+	0	0
rr	0	0	+	0	+	0	+	+	+	0	0	0	0	+	0	+	0	+	0	+	0	0	0
Auto																						0	

Anti-D

IAT Results


Anti-IgG+C3d
Patient C
1 2 3 4 5 6
Anti-IgG+C3d
Patient C
7 8 9 10 CC CCC

Patient C

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Rh	D	C	c	E	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	IAT
$R1^wR1$	+	+	0	0	+	+	+	+	+	0	3	0	0	+	0	0	+	0	+	0	+	0
R1R1	+	+	0	0	+	0	+	0	+	+	5	0	+	+	0	+	0	+	0	0	+	2
R2R2	+	0	+	+	0	0	0	+	+	0	0	0	0	+	0	0	+	0	+	+	0	0
r'r	0	+	+	0	+	0	0	+	0	+	4	0	0	+	0	0	+	+	0	+	0	0
r"r	0	0	+	+	+	0	+	0	+	0	4	0	0	+	0	+	0	+	0	+	+	0
rr	0	0	+	0	+	0	+	+	0	+	0	0	+	+	0	0	+	0	+	+	0	2
rr	0	0	+	0	+	0	0	+	0	+	0	0	0	+	0	0	0	0	+	0	+	0
rr	0	0	+	0	+	0	+	0	0	+	3	0	0	+	+	+	0	+	0	+	0	0
rr	0	0	+	0	+	0	0	+	0	+	4	+	+	+	0	+	0	0	+	0	+	2
rr	0	0	+	0	+	0	+	+	+	0	0	0	0	+	0	+	0	+	0	+	0	0
Auto																						0

Anti - K

Summary

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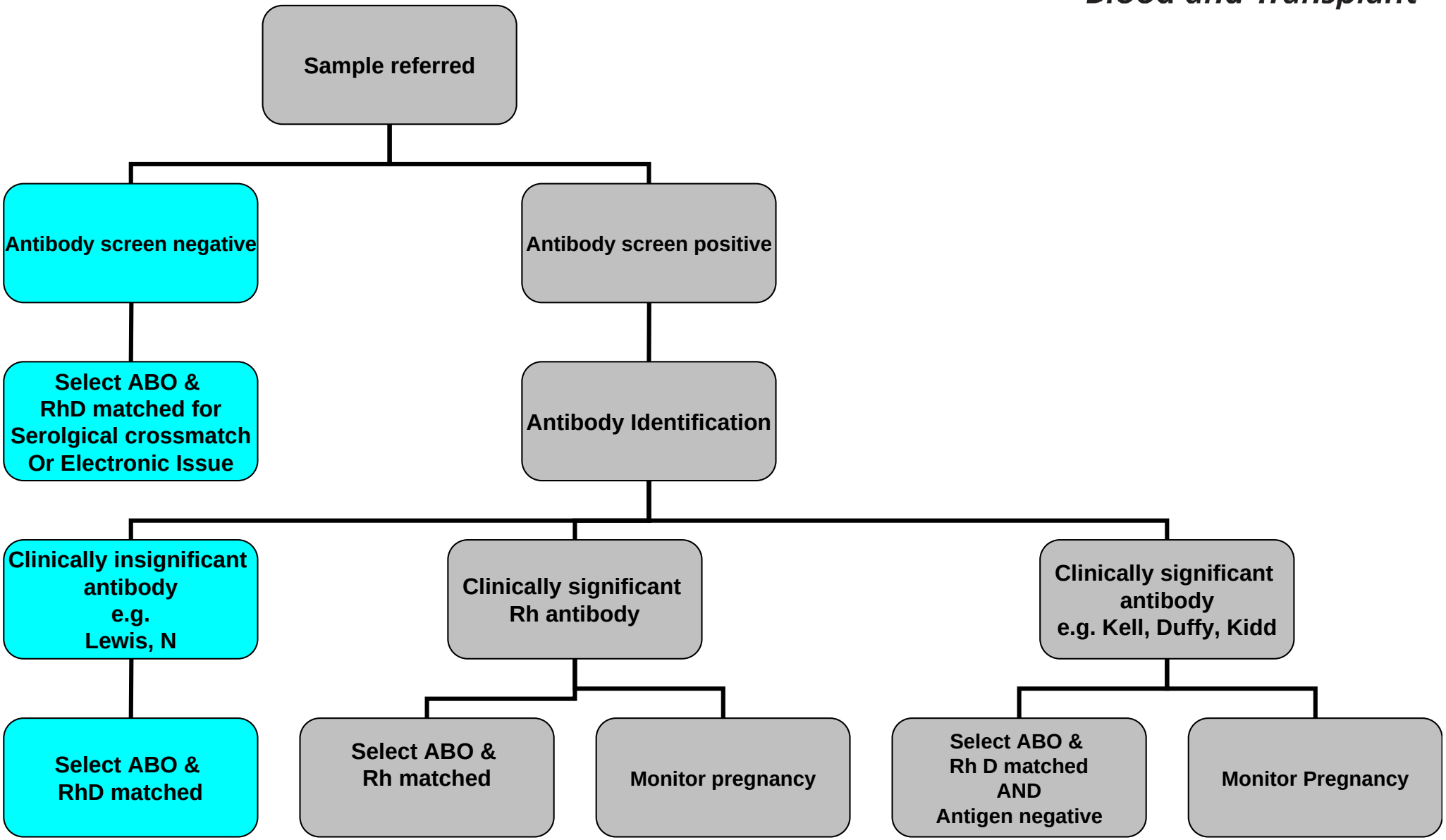

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Crossmatch - Definition

Procedure to exclude incompatibility between donor and recipient

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Crossmatch Techniques

- Serolgical - IAT or immediate spin technique if urgent

- Electronic issue – not applicable to all patients

-exceptions include post BMT / Patients with allo / auto antibodies / neonatal transfusion where maternal antibodies are present (serological crossmatch using maternal plasma)

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Selection of red cell units

- Electronic issue – ABO and RhD matched the group must be fully automated with no manual intervention and have a negative antibody screen.
- Antibodies present? Selected antigen negative units must be used for serological crossmatch.

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Patient Specific Requirements

- CMV Negative
- Irradiated
- HbS Negative
- IgA Deficient
- Paediatric products – suitable for IUT / Top Up or Red cell exchange on neonates
- Washed Red Cells

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Complex antibodies?

- Possible delay in provision of red cells
- Units may be required to be sourced from alternative NHSBT sites
- Units may be required from the Frozen Blood Bank in Liverpool – Thawing / processing time approx 6 hours + investigation time

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Sample Timing?

- Current guidelines recommend the use of 24 hour samples for patient's who have been transfused within 3-14 days - currently under review.
- Patient's who are repeatedly transfused with no change in serology or suspected transfusion reaction may be screened every 72 hours

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And finally!

- Donations must have a securely attached crossmatch label with patient full demographics / Patient group / donor group and date of crossmatch.
- A visual inspection of the unit must be performed prior to transfusion - check for leaks / haemolysis / discolouration / clots

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Acknowledgements

- NHSBT Scientific & Technical Department
- Alex Harrison Sheffield RCI