Shine
SCIENTIFIC AND
CLINICAL DEVELOPMENT

NHS
Blood and Transplant

Serology Overview

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NHS
Blood and Transplant

Aims of session

To provide an overview of the following:

- Antigens and antibodies
- ABO blood group system
- Rh blood group system
- Other clinically significant blood group systems

Blood Group Antigens and Blood Group Antibodies

NHS Blood and Transplant

- **Antigens are part of the surface of cells**
  - Red blood cells, white blood cells and platelets all have antigens
- **Antibodies are protein molecules - called immunoglobulins (Ig)**
  - Usually of the immunoglobulin classes: IgG and IgM
  - Found in the plasma
  - Produced by the immune system following exposure to a foreign antigen
- Reactions to blood usually occurs when the antigen on the cells reacts with an antibody in the plasma

Blood Group Antigens

NHS Blood and Transplant

- There are 30 known blood group systems
- Most clinically important are the ABO and Rh antigens
- Antigens in transfused blood can stimulate a patient to produce an antibody but only if the patient lacks the antigen themselves.
- The frequency of antibody production is very low but increases the more transfusions that are given

Antibodies are Stimulated by:

- Blood transfusion
  - i.e. blood carrying antigens foreign to the patient
- Pregnancy
  - Foetal antigen entering maternal circulation during pregnancy or at birth stimulating antibody in mother
- Environmental factors
  - (ie naturally acquired as with anti-A and anti-B)

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Antibody - Antigen Reactions

IN VIVO (*in the body)*

- leads to the destruction of the cell either:
  - directly when the cell breaks up in the blood stream (intravascular)
  - indirectly where liver and spleen remove antibody coated cells (extravascular)

IN VITRO *(in the lab)*

- reactions are normally seen as agglutination
- tests in blood transfusion utilise the specific nature of the antibody-antigen reaction

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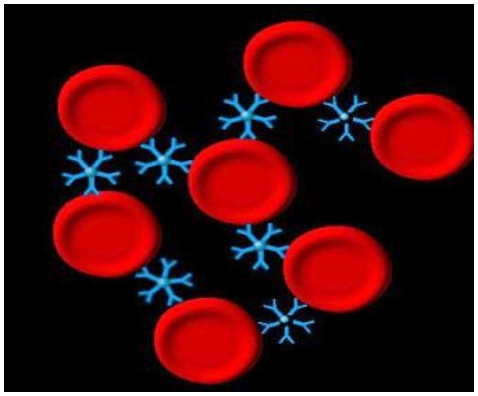
Agglutination

- Agglutination is the clumping together of red cells into visible agglutinates by antigen-antibody reactions

- Agglutination results from antibody cross-linking with the antigens



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Agglutination

As the antigen-antibody reaction is specific, agglutination can identify:-

The presence of a red cell antigen,

- i.e. ‘blood grouping’

The presence of an antibody in the plasma,

- i.e. ‘antibody screening/identification’

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The ABO Blood Group System

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The ABO Blood Group System

- Discovered by Karl Landsteiner in 1900
- Inheritance of either A, B or O gene from each parent, giving four possible groups

  (i.e. A, B, AB and O)
- A and B genes are co-dominant

ABO Inheritance

NHS Blood and Transplant

Parents group:	A	x	B
Genotype:	*A O*		*B O*

Possible childrens' genotype:

Group:

i.e. **each group has 25% chance of production**

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The ABO Blood Group System

Landsteiner's Law

When an individual lacks the A or B antigen the corresponding antibody is produced in their plasma

ABO Antigens/Antibodies

NHS Blood and Transplant

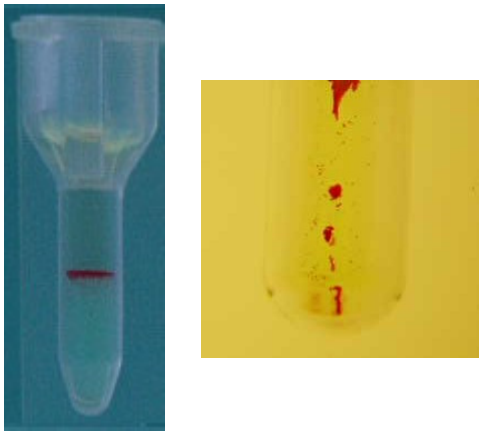
Red Cell Type	Antibody Present
O	
A	
B	
AB	

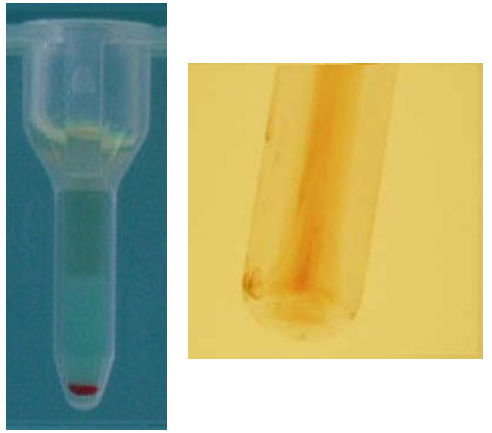
As a result of this ‘natural’ presence of A and B antibodies (97% of the population), it is the most clinically important blood group system with regard to transfusion

Blood Grouping

NHS Blood and Transplant

- The patients red cells and plasma are both tested
- Test patients red cells with
  - anti-A, anti-B and anti-D
    - agglutination shows that particular antigen is on the red cells
    - no agglutination shows the antigen is absent
- Test patients plasma with
  - A cells and B cells
    - agglutination shows that particular antibody is in the plasma or serum
    - no agglutination shows the antibody is absent





Front group
patients cells

Reverse group
(patients plasma)

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ABO Grouping

Anti-A 	Anti-B 	Ctrl	A1 cells	B cells	O cells	Grp
		Neg				
		Neg				
		Neg				
		Neg				

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Clinical Significance of the ABO Blood Group System

Significance

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" ABO grouping is the most important serological test performed pre-transfusion" (Red Book)

"ABO grouping is the single most important serological test performed on pre-transfusion samples and the sensitivity and security of testing systems MUST NOT be compromised"(BCSH)

NHS Blood and Transplant

Why Clinically Significant?

- A and B antigens very common (55% UK)
- ABO blood group antibodies very common (97% UK)
- High risk of A or B cells being transfused into someone with the antibody in a random situation
- ABO antibodies can activate complement causing INTRAVASCULAR HAEMOLYSIS

ABO COMPATIBILITY

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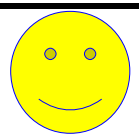
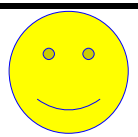
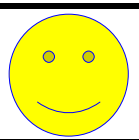
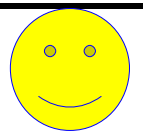
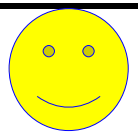
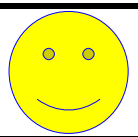
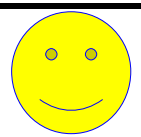
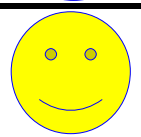
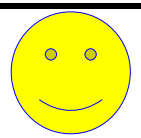
Donor Red Cells Group	Recipient Blood Group O	A	B	AB
O				
A				
B				
AB				

•Compatible: patient survives

•Incompatible: potentially fatal

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Rh Blood Group System

History

NHS Blood and Transplant

1939

Levine and Stetson describe an antibody in a woman who had given birth to a stillborn baby, then had a haemolytic transfusion reaction after a transfusion from her husband

1940

Landsteiner and Weiner immunised guinea pigs and rabbits with blood from the Rhesus monkey

The antibody they obtained, agglutinated the red cells of the Rhesus monkey AND 85% of red cells from a panel of samples from white New York donors

Is this the same antibody?

What's in a name?

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In 1940 they appeared to be the same antibody so it was called:-

..... **Rhesus System**

Now known as Rh

BUT now

- Antigens are known to be genetically different

Human derived blood group -chromosome 1- **Rh**

Rabbit/ monkey derived - chromosome 19 - LW

The Rh Blood Group System

5 Main antigens

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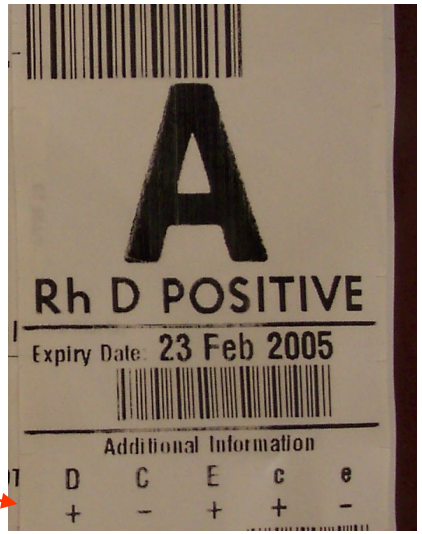
- Most important antigen is called D.
- People with D antigen are RhD positive (85% of UK)
- People who do not produce any D antigen are RhD negative (15%)

- The other 4 main Rh antigens are known as C and c & E and e
  - shown on the Blood Pack label

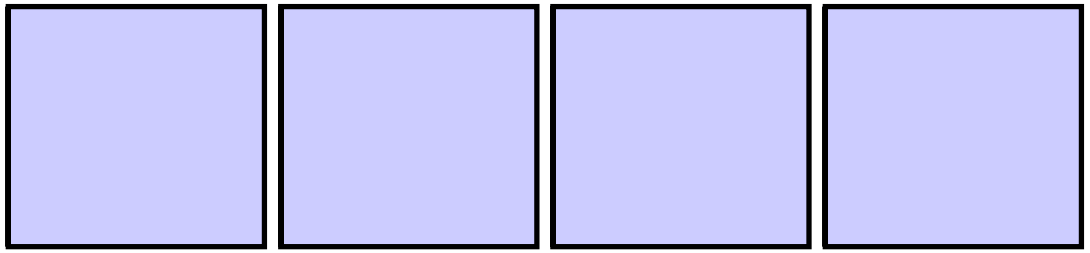


Inheritance (of *D*)

NHS Blood and Transplant

Parents: Dd x Dd

Children:



- Rh D positive = inheritance of the D gene / antigen
- Rh D negative = non inheritance of the D gene

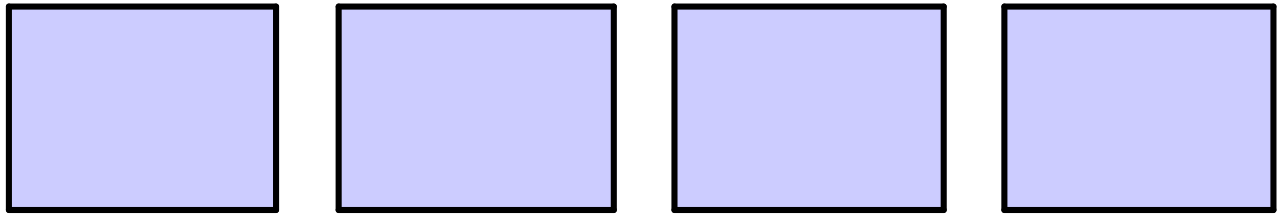
- Absence of D gene or antigen is identified by d

REMEMBER d gene or d antigen DOES NOT exist

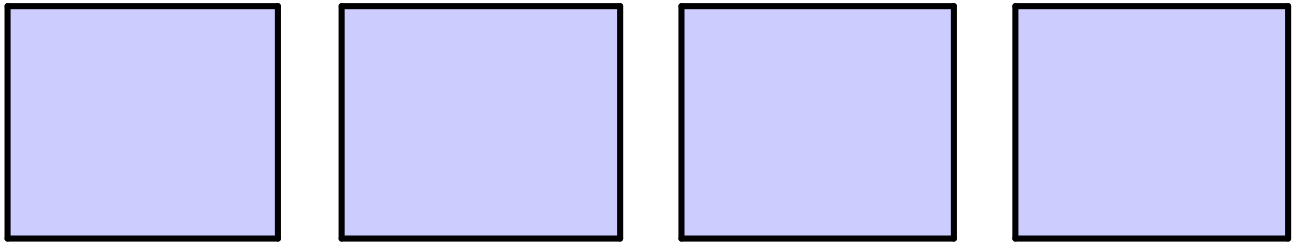
Inheritance (of *D*)

NHS Blood and Transplant

Parents: Dd x dd

Children: 

Parents: DD x dd

Children: 

Inheritance (of *CE*)

NHS Blood and Transplant

Inheritance of *Rh CE* gene gives one of 4 different protein options:-

Ce

cE

ce

CE

These are inserted into the red cell membrane in the presence or absence of the D antigen.

Inheritance (of *CE*)

NHS Blood and Transplant

CE gene	*D*	Shorthand	*d*	Shorthand
Ce	CDe	R_1	Cde	r’
cE	cDE	R_2	cdE	r”
ce	cDe	R_o	cde	r
CE	CDE	R_z	CdE	r^y

One haplotype is inherited from each parent to give the genotype

Inheritance (of *CE*)

NHS Blood and Transplant

Parents: CDe / cde x cde / cde

R_1 r r r

Children:

The Rh Blood Group System

NHS Blood and Transplant

- The D antigen is very immunogenic
- D negative patients should be given
  - D negative blood
  - usually also C neg and E neg (shorthand = rr)
- In most circumstances, the other Rh antigens, C, c, E and e are not matched for

  unless the patient has produced an antibody
- The next most commonly produced Rh antibody is anti-c

Rh typing - D

NHS Blood and Transplant

- Most clinically significant antigen in Rh system
- Most important after ABO
- Must be tested in duplicate with potent IgM monoclonal Anti D
- Or tested each time and compared to historical result
- Patient / Donor classified as D pos or D neg

Clinical Significance

NHS Blood and Transplant

Rh antibodies are clinically significant – Second only to ABO

Transfusion

D antigen is very immunogenic and anti-D is easily stimulated - PREVENTION!

All Rh antibodies are capable of causing severe transfusion reaction- ANTIBODY DETECTION

- **Pregnancy**

  Rh antibodies are usually IgG and can cause haemolytic disease of the newborn.

  Anti-D is still most common cause of **severe** HDN

Other Blood Group Systems

- 30 different known blood group systems
- Of these 9 are ‘major’ systems
- ABO and Rh most clinically significant
- But... the others are capable (if infrequently) of causing clinically significant antibody stimulation

Other Blood Group Systems

- In each system there are usually a minimum of 2 possible antigens
- The different antigens within a system arise because of variations at the level of the genes.
- These variations give slight differences to the protein produced by the gene
- And these are detected by antibodies

Nomenclature of blood group systems

- Different capital letters:
  - A and B (ABO) also M and N (MNS)
- Capital letter plus subscript number
  - P_1 and P_2 (P1) also A_1 and A_2 (ABO)
- Capital and lower case
  - K and k (Kell) also S and s (MNS), C and c (Rh)
- Abbreviation of name with superscript a/b
  - Fy^a and Fy^b (Duffy) also Jk^a and Jk^b (Kidd)
- Also use of non-subscript numbers (usually reserved for rare types)
  - Fy3, Jk3

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Clinical Significance of blood group systems

- Antibodies are usually produced in absence of antigen
- Some antibodies are more clinically significant than others i.e. do more harm when encounter antigen in vivo
- ABO, Rh, Kell, Duffy and Kidd are most clinically significant.
- MNS are sometimes clinically significant
- P1, Lutheran and Lewis are less so.

NHS Blood and Transplant

Kell Blood Group System

- Two main antigens: K (Kell) and k (Cellano) which are very “immunogenic”
- After Rh antibodies anti-K is most common
- Relatively easy to find K negative blood
- 91% population are K neg
- 0.2% population are k negative (KK). Anti-k not common but difficult to find k negative blood

RBC reaction with		Phenotype	Genotype	Approx. frequency: white UK	black US
Anti-K	Anti-k				
+	-	K+k-	*KK*	0.2%	rare
+	+	K+k+	*Kk*	8.8%	2%
-	+	K-k+	*kk*	91%	98%

Duffy Blood Group System

NHS Blood and Transplant

- Two main antigens Fy^a and Fy^b
- Anti-Fy^a is a fairly common antibody. If a patient has anti-Fy^a, transfuse with Fy(a-) blood (33%).

RBC reaction with Anti-Fy^a	RBC reaction with Anti-Fy^b	Phenotype	Genotype	Approx. frequency: white UK	Approx. frequency: black US
+	-	Fy(a+b-)	Fy^aFy^a	20%	9%
+	+	Fy(a+b+)	Fy^aFy^b	47%	1%
-	+	Fy(a-b+)	Fy^bFy^b	33%	22%
-	-	Fy(a-b-)	FyFy	0%	68%

- The Fy(a-b-) blood type confers resistance to malarial parasite: Plasmodium vivax.
- High % of populations of endemic malarial areas eg West African origin are Fy(a-b-)

The Kidd Blood Group System

NHS Blood and Transplant

- Two antigens: Jk^a and Jk^b
- Both anti-Jk^a and anti-Jk^b are relatively rare. Not difficult to find blood
  - Jk(a-) = 24%, Jk(b-) = 26%

RBC reaction with Anti-Jk^a	RBC reaction with Anti-Jk^b	Phenotype	Genotype	Approx. frequency: white	Approx. frequency: black
+	-	Jk(a+b-)	Jk^aJk^a	26%	51%
+	+	Jk(a+b+)	Jk^aJk^b	50%	41%
-	+	Jk(a-b+)	Jk^bJk^b	24%	8%

- Both Jk^a and Jk^b antigens show dosage - much less antigen is detectable on single dose than double dose cells
- Levels of antibody may be so low as to be undetectable but can still cause transfusion problems if not detected.

MNS Blood Group System

- Originally thought to be 2 separate systems: MN and Ss
- M/N and S/s now known to be proteins produced by 2 very close genes
- Carbohydrate groups on the end of these proteins are responsible for giving all red cells negative charge which stops them sticking together in blood stream

Compare and Contrast

NHS Blood and Transplant

MN	Ss
Anti-M and Anti-N are rarely clinically significant	Anti-S and Anti-s can be clinically significant
Both antibodies do not usually act above room temperature (M)	Both antibodies may be active at blood temperature (37^{o}C)
Relatively easy to find compatible blood M negative = 22% N negative = 28%	Both antibodies not very common S negative = 45% s negative = 11%

Blood provision

NHS Blood and Transplant

- If patient has blood group antibody which is classed as **clinically significant** then we must provide blood that is negative for the antigen

- If patient has blood group antibody which is NOT classed as clinically significant then we provide blood that has been crossmatched by IAT @ 37^{o}C and found negative

NHS
Blood and Transplant

Blood provision

- If patient has blood group antibody which is classed as clinically significant then we must provide blood that is negative for the antigen
- Anti-c :provide
- Anti-K :provide
- Anti-P_1 :provide
- Anti-Fy^a :provide
- Anti-M :provide