Blood Groups and Antibodies, Transfusion and Pregnancy

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To cover:

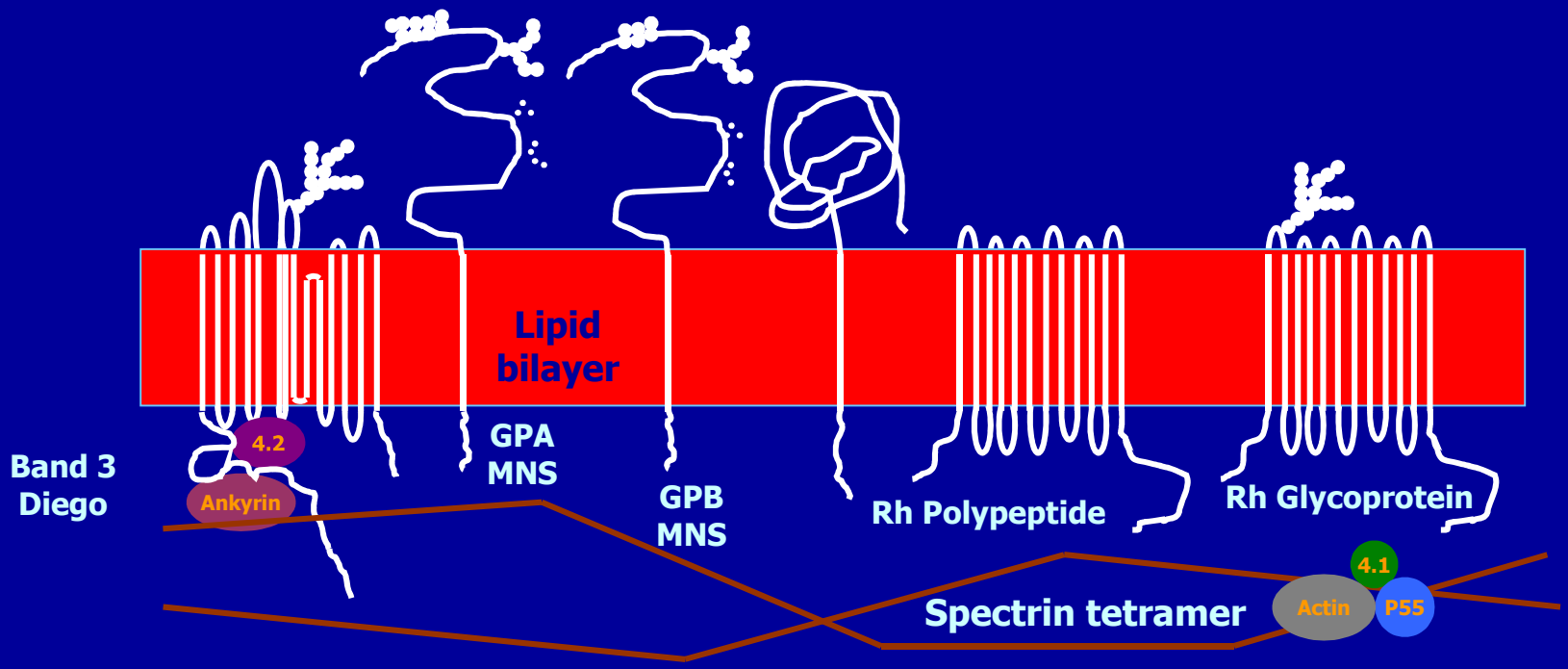
- What is a red cell antigen?
- What is a red cell antibody?
- Haemolytic Disease of the Fetus and Newborn
  - Serological testing through pregnancies
  - Finding and monitoring the ‘at risk’ pregnancies
  - Use of antenatal anti-D prophylaxis
  - Difficult interpretations and working together
  - Molecular testing – diagnostic testing v screening
  - Post delivery testing

An Antigen

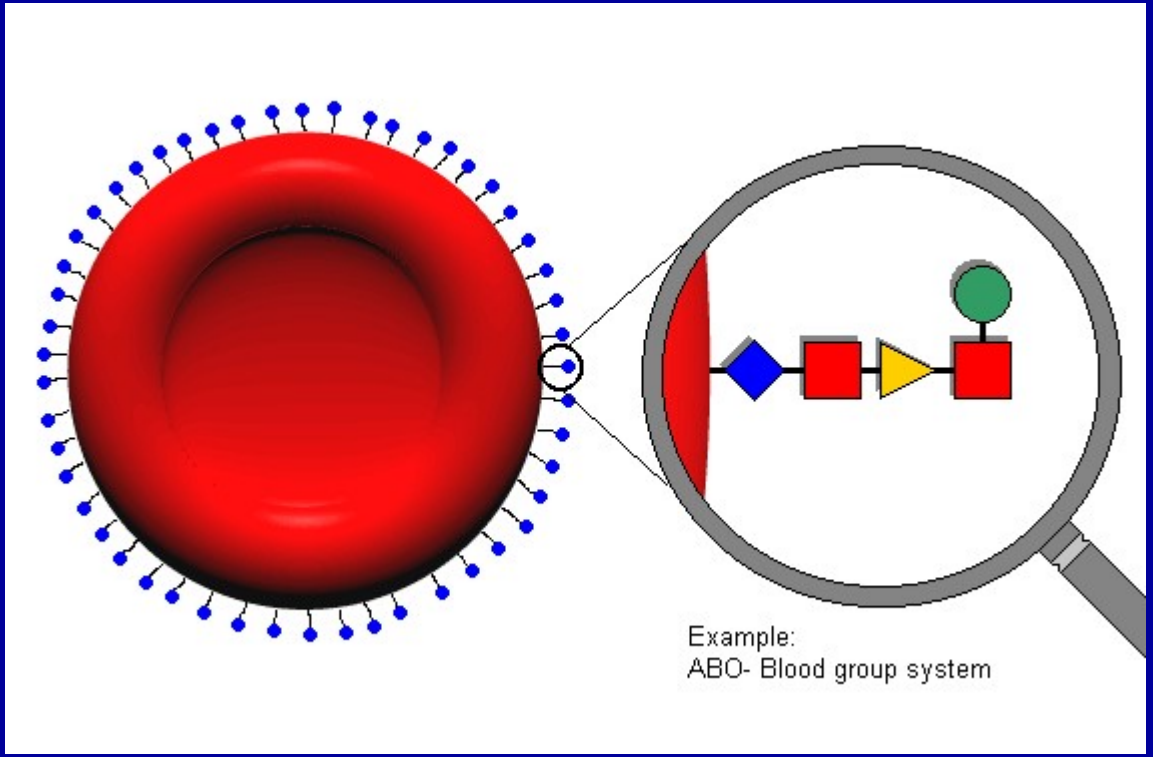
- An antigen can be defined as **a substance** that, when introduced into the circulation of an individual lacking that antigen, can **stimulate the production of a specific antibody**.

- Red cell antigens

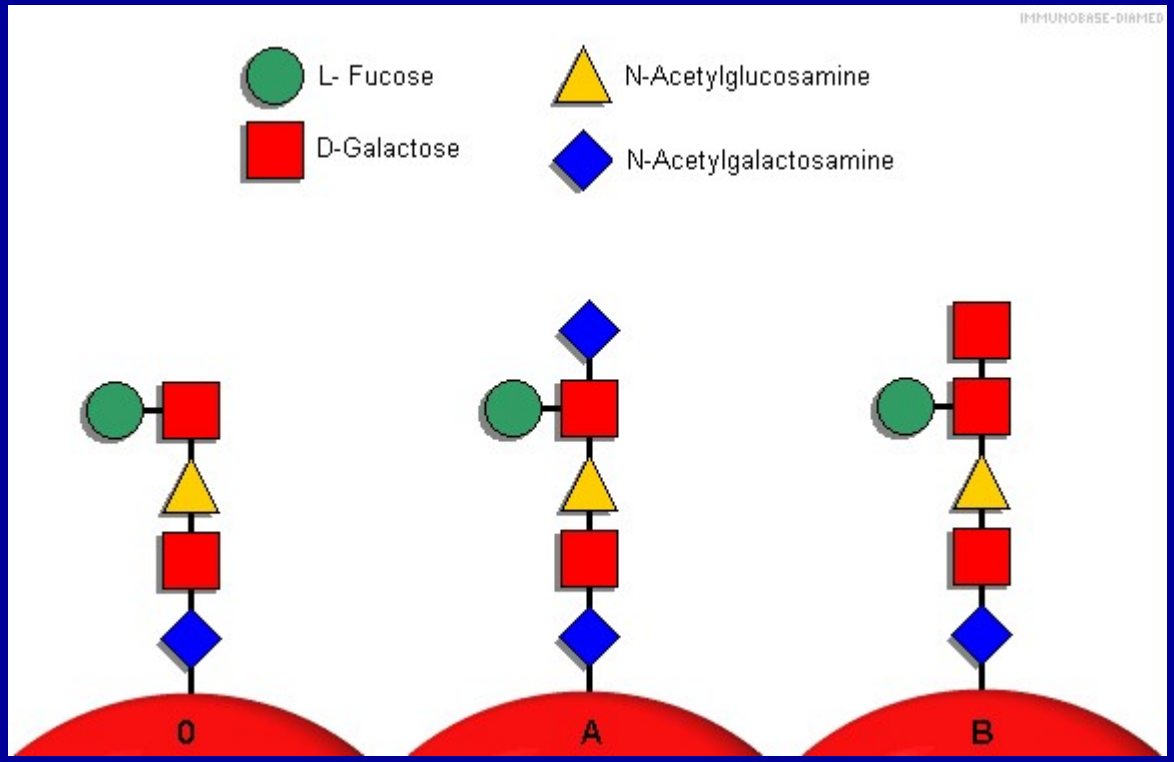
Blood Group Antigens



ABO Antigens



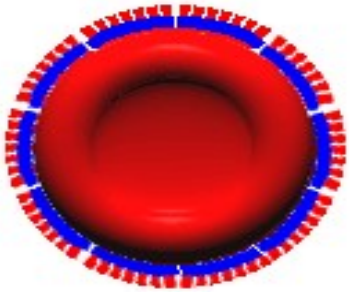
A Close Up

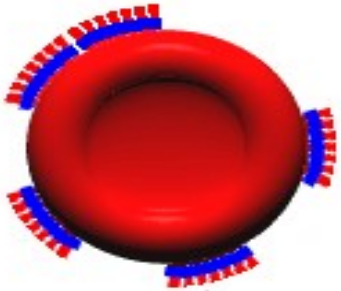

L- Fucose
D-Galactose
N-Acetylglucosamine
N-Acetylgalactosamine
0
A
B

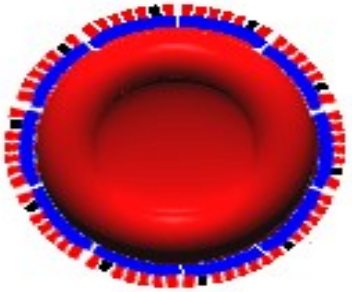
The D Antigen

- 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

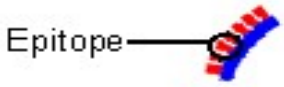


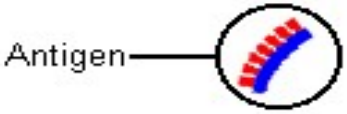




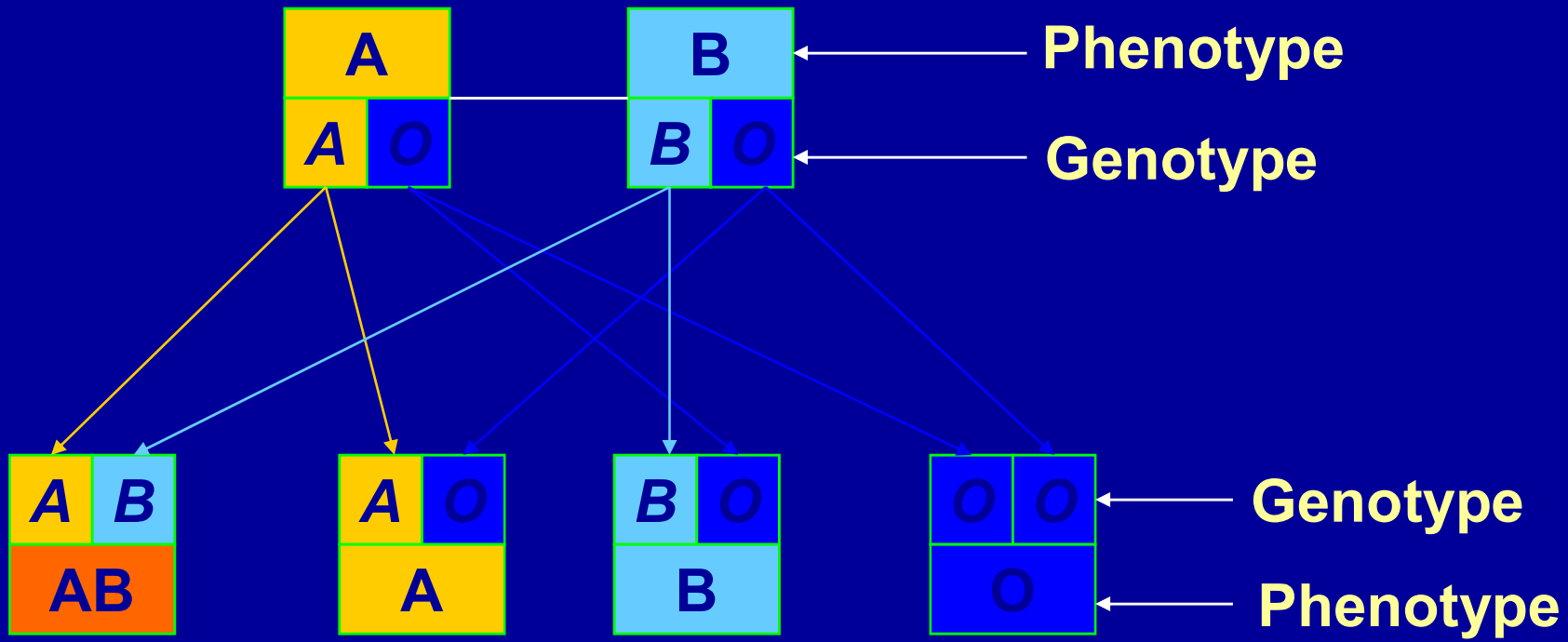
	Normal D-Antigen	D-weak	D-Variant
Epitope:	Normal	Normal	Mutated
Antigen frequency:	Normal	Reduced	Normal or reduced

Legend:

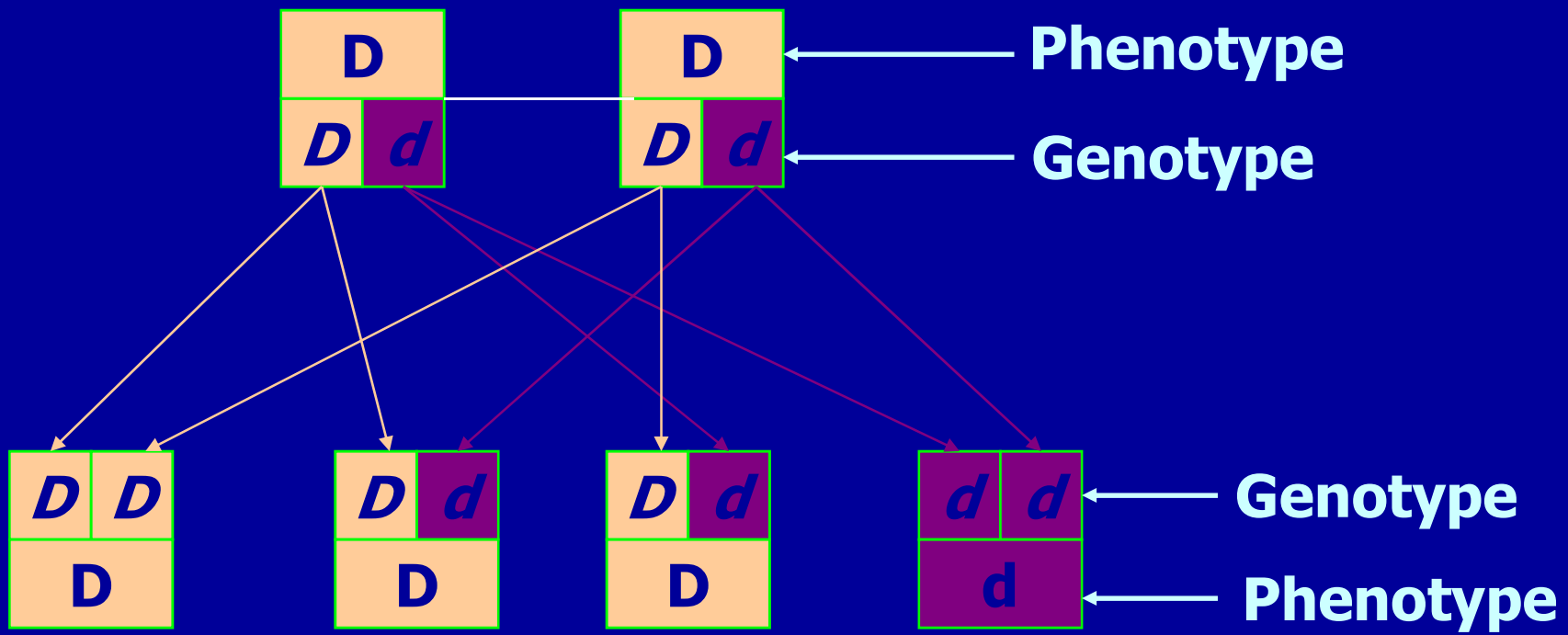




Inheritance



Genetics


D
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Genotype
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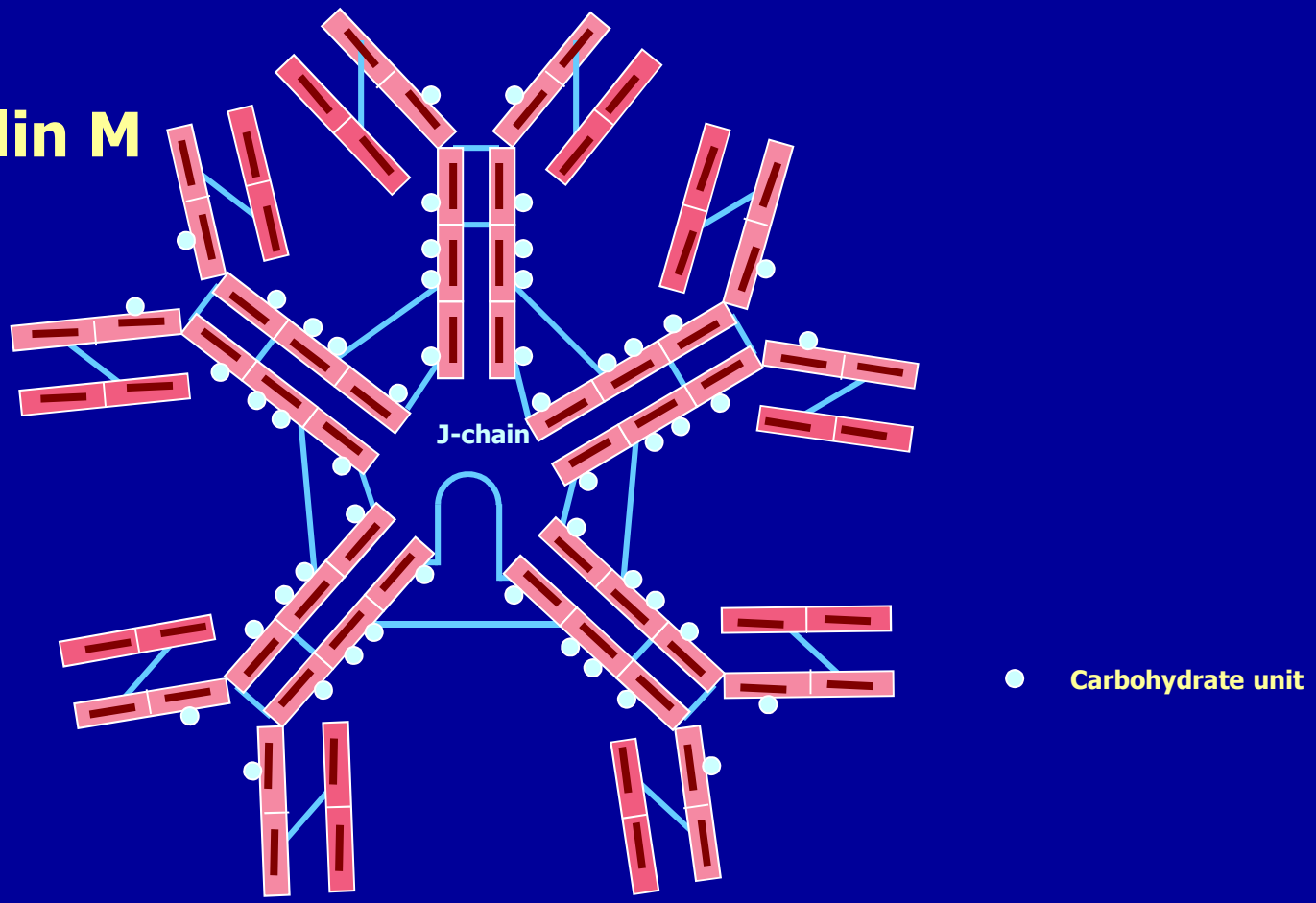
D d
D
d d
d
Genotype
Phenotype

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

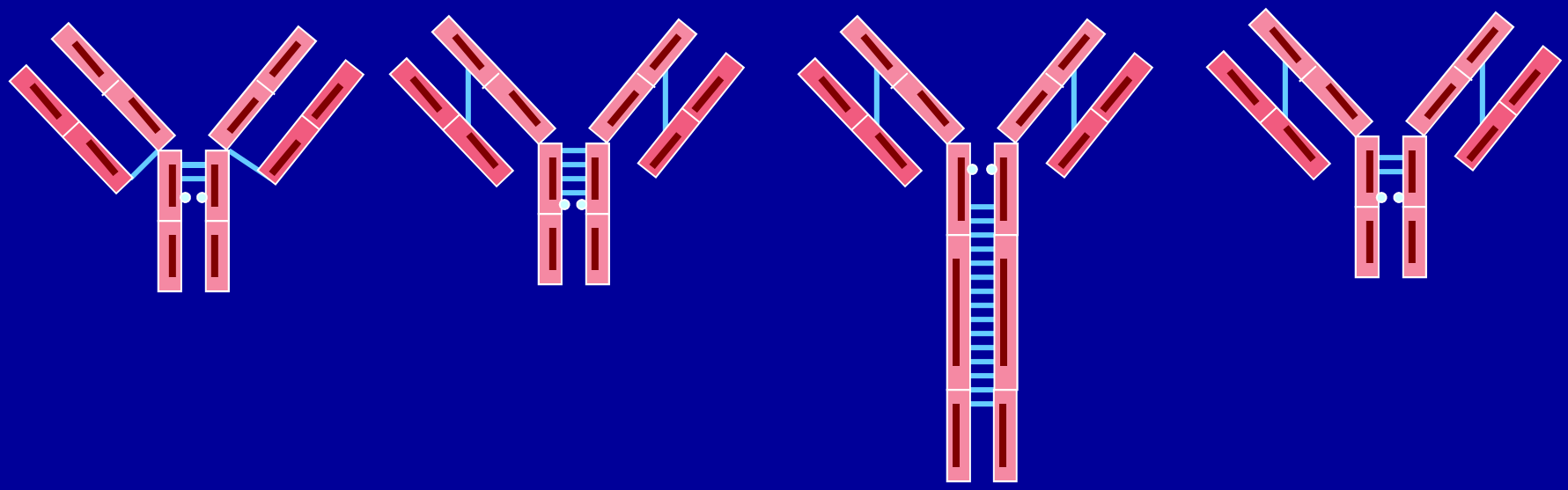
Antibodies - IgM

Immunoglobulin M



Antibodies - IgG

Immunoglobulin IgG subclasses



IgG1 **IgG2** **IgG3** **IgG4**

Red Cell Antibodies

Produced when exposed to foreign **blood**:

- Previous transfusion of blood/components
- Fetal maternal haemorrhage

ABO System

Red Cells (Antigens)	Plasma (Antibodies)
• A	• Anti-B
• B	• Anti-A
• O	• Anti-A,B
• AB	• None

Haemolytic Disease of the Fetus and Newborn (HDFN)

- Is a condition in which the lifespan of the infant's red cells is shortened by the action of specific antibodies derived from the mother by placental transfer.
- Anaemia, jaundice, liver damage, kernicterus, IUD

Serological Testing During Pregnancy

Purpose:

- Identify RhD negative individuals so that appropriate anti-D prophylaxis can be given to prevent HDFN due to anti-D
- To identify those at risk of HDFN
- To predict the severity of the HDFN to plan treatment

Maternal Monitoring

- Booking bloods
  - ABO, D type and antibody screen
- Repeat test at 28 weeks
  - Confirm ABO and D type, repeat antibody screen
- If antibodies detected
  - Identify and monitor, regime dependent upon antibody

The Big Three

- Anti-D, anti-c and anti-K
- Test monthly up to 28 weeks
- Test every 2 weeks up to delivery
- Anti-D and anti-c are quantitated against a National Standard with results in IU/mL
- Anti-K is titrated
- Current sample is tested in parallel with previous sample to accurately identify changes in antibody level

NHSBT Report 1

Red Cell Immunohaematology

NHS
Blood and Transplant

HOSPITAL TRANSFUSION LABORATORY
NORFOLK & NORWICH UNIVERSITY
HOSPITAL
COLNEY LANE
COLNEY
NORWICH
NORFOLK
NR4 7UY

Patient:

DoB:
NHS No:
Hospital No:
Address:

Sample No:

NHSBT No:

Date Sampled:

Date Received:

Date Reported:

Hosp Samp ID:

Charge Code: D030

Primary Requesting Clinician:
X0000001 HEAD OF BLOOD TRANSFUSION

EDD: 17-Oct-2016
Gestation: 10 weeks at sampling

O RhD negative

Red Cell Antibody Results

Type	Specificity	Technique	Quantification IU/mL or Titre		Sample Type
Allo	Anti-D	IAT	Quant	4.6	Plasma
Allo	Anti-C	IAT			Plasma

ANTIBODY AND CLINICAL SIGNIFICANCE
There is a moderate risk of haemolytic disease of the fetus and newborn when the maternal anti-D level is between 4-15 IU/mL.

The risk of haemolytic disease of the fetus and newborn may increase if gestation proceeds beyond term.

CLINICAL ADVICE
Refer to / continue to monitor by a fetal medicine specialist.

REPEAT SAMPLING
Please send further sample as soon as possible for further anti-G investigation.

FURTHER ACTIONS
At delivery a cord DAT should be performed and, if positive, the baby's Hb and bilirubin monitored.

BLOOD SELECTION
Select ABO compatible D- C- E- K compatible red cell units for crossmatching by IAT.

OTHER
Guidelines recommend a current paternal phenotype is performed as it may provide useful information.
This case was discussed with Vanessa on 06/04/2016 at 16:02.

The Others

- Tested at booking and 28 weeks
- In general a titre of >32 may possibly cause HDFN
- A steep increase in titre between the two samples is worrying and may lead to further monitoring

NHSBT Report 2

Red Cell Immunohaematology

NHS
Blood and Transplant

HOSPITAL TRANSFUSION LABORATORY
NORFOLK & NORWICH UNIVERSITY
HOSPITAL
COLNEY LANE
COLNEY
NORWICH
NORFOLK
NR4 7UY

Patient

DoB:
NHS No:
Hospital No:
Address:

Sample No:

NHSBT No:

Date Sampled:

Date Received:

Date Reported:

Hosp Samp ID:

Charge Code: D030

Primary Requesting Clinician:
X0000001 HEAD OF BLOOD TRANSFUSION

EDD: 03-Sep-2016
Gestation: 8 weeks at sampling

A RhD positive C+c+E-e+K-

Red Cell Phenotype: M-

Red Cell Antibody Results

Type	Specificity	Technique	Quantification IU/mL or Titre		Sample Type
Allo	Anti-M	Bio-Rad IAT	Titre	Neat	Plasma

ANTIBODY AND CLINICAL SIGNIFICANCE
The risk of haemolytic disease of the fetus and newborn is low as the titre is less than 32.

CLINICAL ADVICE
This woman should be in the care of a hospital obstetric unit.

REPEAT SAMPLING
If gestation is less than 28 weeks, guidelines recommend repeat testing at 28 weeks gestation.

FURTHER ACTIONS
At delivery a cord DAT should be performed and, if positive, the baby's Hb and bilirubin monitored.

BLOOD SELECTION
Select ABO, D and K compatible M- red cell units for crossmatching by IAT.
An antibody card for this patient is provided.

Paternal Testing

- Determining paternal phenotype and likelihood of fetal genotype may be useful.
- Misidentification of the father needs to be acknowledged

Fetal blood group genotyping in alloimmunised pregnancies

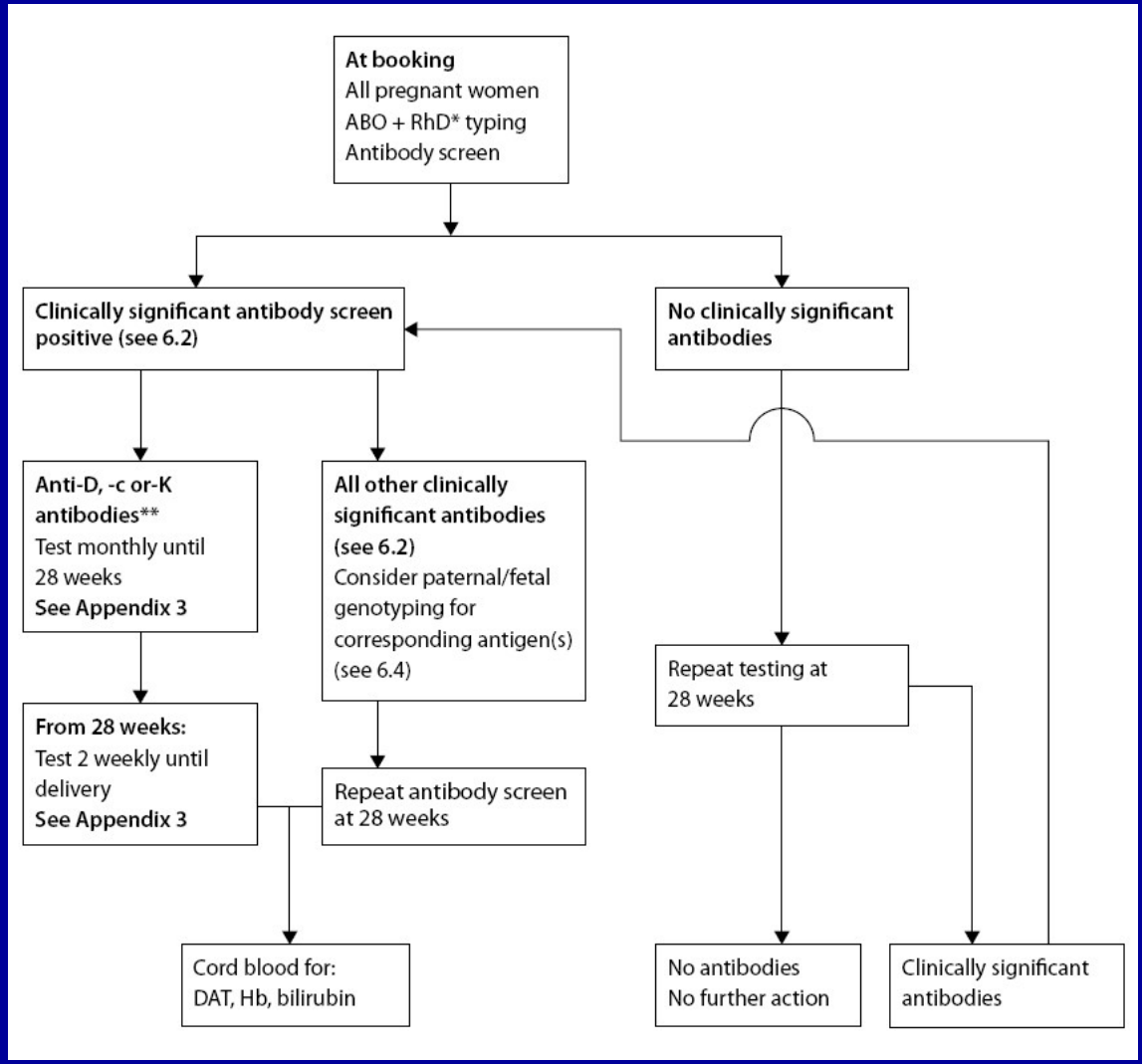
Useful when:

- Clinically significant antibody present
- History of HDFN
- Father's antigen status is unknown or he expresses the corresponding antigen

Issues:

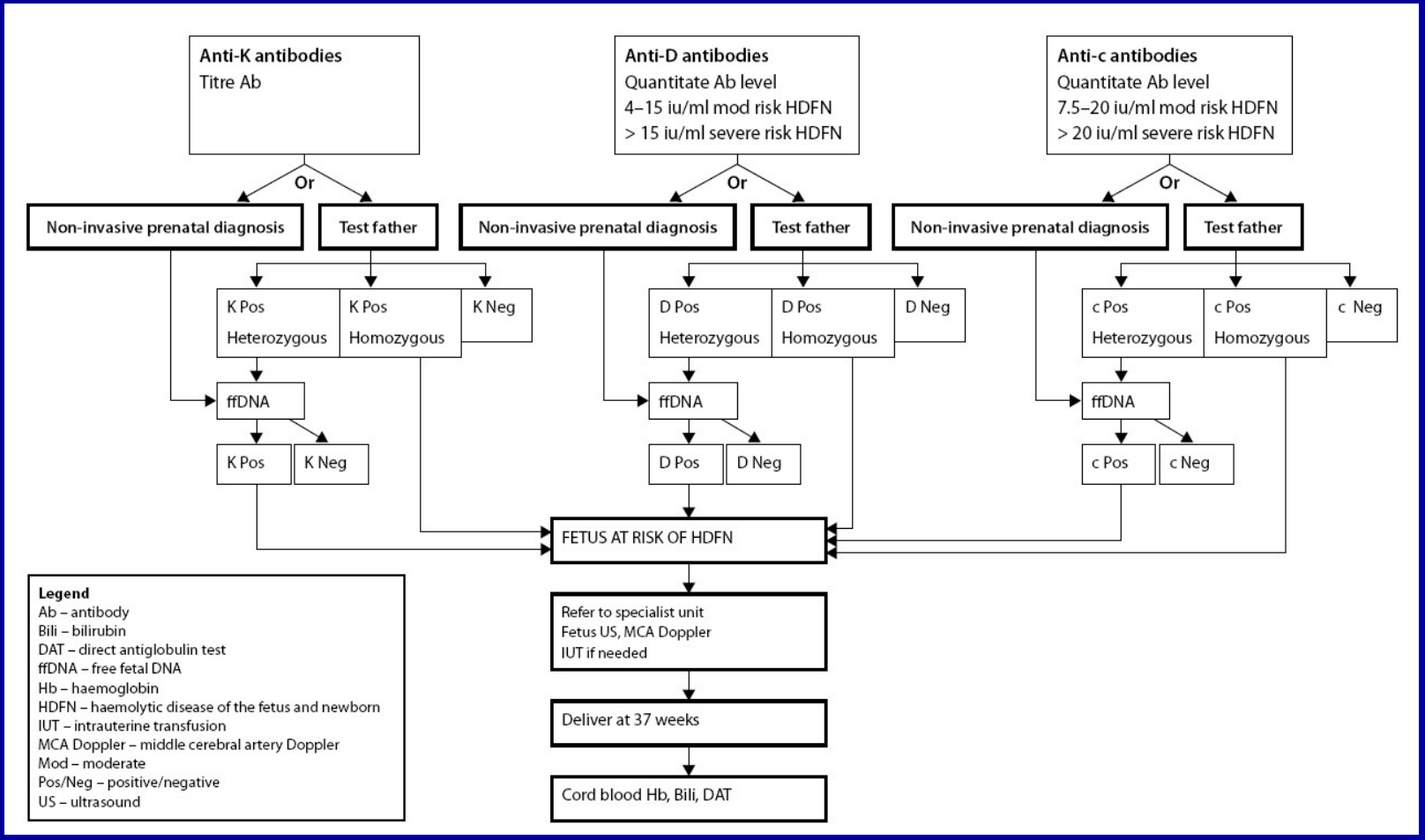
- False negative rate – not truly known (need good feedback ie testing of post delivery samples)
- Samples must not be sent too early in pregnancy

Timing and Frequency of Antibody Screening in Pregnancy (RCOG Greentop guideline 65, 2014)



Management Algorithm for Pregnancies Complicated by anti-D, anti-K or anti-c

(RCOG Greentop guideline 65, 2014)



Preventing HDFN

- Prevent production of red cell antibodies in females of child-bearing potential
  - conservative transfusion regimes
  - transfuse D negative blood to D negative females of child bearing potential
  - and K negative blood to females of child bearing potential
- Give anti-D prophylaxis

Prophylaxis Regime

Following a sensitising event:

- <20 weeks gestation 250iu
- >20 weeks gestation at least 500iu followed by a test to measure the size of the FMH

Routine antenatal anti-D prophylaxis:

- 1500iu at 28 weeks or
- 2x500iu at 28 and 34 weeks

Following delivery of a D positive baby:

- At least 500iu followed by a test to measure the size of the FMH

Difficult Interpretations

Midwives:

- Maintain a clear record of prophylactic anti-D given: dose and date.
- Inform laboratory ie must be clear on request forms
- Vital to take 28 week samples for group and antibody screen BEFORE giving routine prophylaxis

Laboratory:

- Identify and quantitate antibody
- Statement on likely significance with respect to HDFN
- Give advice on further anti-D prophylaxis based on history provided and results obtained
- Request further samples at stated times to monitor the level of antibody

Guideline for blood grouping and red cell antibody testing in pregnancy (Transfusion Medicine, 2016, 26, 246–263)

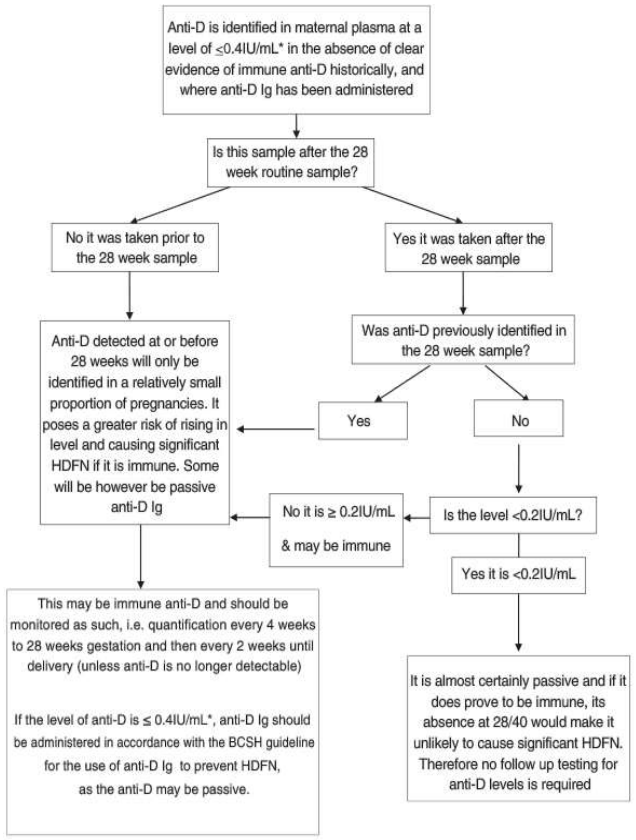


Fig. 3. Managing pregnancies when anti-D has been detected in a woman's plasma for the first time after a dose of anti-D Ig prophylaxis. *Levels of anti-D >0·4 IU mL^{-1} are assumed to be immune in origin and managed accordingly unless a large dose of anti-D Ig has been given (>3000 IU) or the sample was taken within hours of an intravenous dose of anti-D immunoglobulin, in which, case it could be passive, and anti-D Ig prophylaxis should also continue.

NHSBT Report 3

Red Cell Immunohaematology

NHS Blood and Transplant

HOSPITAL TRANSFUSION LABORATORY
NORFOLK & NORWICH UNIVERSITY
HOSPITAL
COLNEY LANE
COLNEY
NORWICH
NORFOLK
NR4 7UY

Patient:
DoB:
NHS No
Hospital
Address

Sample No:
NHSBT No:
Date Sampled: 12-Jun-2018
Date Received: 14-Jun-2018 06:16:00
Date Reported: 20-Jun-2018
Hosp Samp ID: 18.77422.7
Charge Code: 0051

Primary Requesting Clinician:
X0030001 HEAD OF BLOOD TRANSFUSION

EDD: 30-Jun-2018
Gestation: 37 weeks at sampling

AB RhD negative

Red Cell Antibody Results

Type	Specificity	Technique	Quantification IU/mL or Titre		Sample Type
Not specified	Anti-D	IAT	Quant	<0.1	Plasma

Request form states 1500IU anti-D Ig was given on 29/01/2018 and 17/03/2018.

ANTIBODY AND CLINICAL SIGNIFICANCE
From information provided the anti-D detected is probably due to recent anti-D prophylaxis.

CLINICAL ADVICE
Continue antenatal and post-natal anti-D prophylaxis.

REPEAT SAMPLING
If anti-D was NOT detected prior to administration of routine prophylaxis at 28 weeks gestation, no further samples are required.
If this cannot be ascertained, BSH Guidelines recommend repeat testing every 2 weeks until delivery.

FURTHER ACTIONS
If repeat maternal sampling was continued, at delivery, if the baby is typed as D positive, a cord DAT should be performed and, if positive, the baby's Hb and bilirubin monitored.

BLOOD SELECTION
Select ABO and K compatible D- C- E- red cell units for crossmatching by IAT.

OTHER
An antibody card has not been supplied.

Consider referring the sample for fetal DNA RHD genotyping. If genotyping confirms fetus is D negative, no further samples would be required for testing.

Actions

Midwives:

- If immune (allo) anti-D is present do NOT give prophylactic/passive anti-D
  - failed to prevent anti-D formation
  - must not give an unnecessary blood product
- If interpretation of results is in doubt give anti-D as
  - may prevent HDFN
  - anti-D is a blood product with a good safety record
- If further samples are requested send them
  - could miss catching an immune anti-D that is increasing to a dangerous level.

High throughput non-invasive prenatal testing for fetal *RHD* – Fetal *RHD* screening test

https://www.nice.org.uk/guidance/dg25

- A non-invasive fetal DNA test to predict fetal D group from 11^{+2} weeks gestation
- The test offers >99.9% negative predictive value ie fewer than 1:1000 babies will be falsely predicted to be D negative
- The test prevents unnecessary administration of anti-D. Currently 40% of women receive this human blood product when they don't need
- A cost effective option to guide antenatal prophylaxis with anti-D
- Reduces the need for laboratory tests ie quantitations, FMH estimation
- It is available from an NHS laboratory ie IBGRL, Bristol
  - Results available electronically on Sp-ICE
  - No transport costs
  - Information leaflets available

High throughput non-invasive prenatal testing for fetal *RHD* - Restrictions

- This is NOT a diagnostic test for the fetal RhD status of women who have produced immune anti-D
- This test has been designed to minimise false RhD negative results but in approx. 2% of tests the results will be incorrectly predicted to be RhD positive ie 2% of RhD negative women will receive anti-D Ig unnecessarily compared to the 40% who currently do.

Post Delivery

Maternal D type	D+	D+	D-	D-
Antibody status	None detected	Antibody detected	None detected	Antibody detected
Samples and test required	No samples	Cord sample: DAT Ag type Maternal sample: Antibody screen	Cord sample: D type Maternal sample: FMH	Cord sample: D type DAT Ag type Maternal sample: FMH Antibody screen