Blood Groups and Antibodies, Transfusion and Pregnancy

Debbie Asher
EPA Network Transfusion Laboratory Manager
To cover:

• What is a red cell antigen?
• What is a red cell antibody?
• Haemolytic Disease of the Fetus and Newborn
  – Monitoring pregnancies
  – Preventing HDFN, particularly through antenatal anti-D prophylaxis
  – Predicting outcomes
  – Difficult interpretations and working together
• The Direct Antiglobulin Test (DAT/DCT)
• The future?
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

- Band 3
- Diego
- Ankyrin
- GPA
- MNS
- GPB
- MNS
- Rh Polypeptide
- Rh Glycoprotein
- Lipid bilayer
- Spectrin tetramer
- Actin
- PS
- 4.1
- 4.2
ABO Antigens

Example:
ABO - Blood group system
A Close Up

- L-Fucose
- D-Galactose
- N-Acetylglucosamine
- N-Acetyl/galactosamine

Diagram with structures labeled as C, A, and B.
The D Antigen

• Most individuals are D positive or D negative.

• An individual may have a weak D antigen (previously known as D\textsuperscript{u}).

• An individual may have a partial D antigen (previously known as a D\textsuperscript{variant}).
RhD

Normal D-Antigen

D-weak

D-Variant

Epitope: Normal
Antigen frequency: Normal

Epitope: Normal
Antigen frequency: Reduced

Epitope: Mutated
Antigen frequency: Normal or reduced

Legend: Epitope → Antigen
Inheritance

Phenotype

Genotype

A
A
O
B
B
O

A
A
O
B
B
O

A
B
AB

A
A
O
B
B
O

A
B
AB

O
O
O

Phenotype

Genotype
Genetics

Phenotype

Genotype

Genetics
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

J-chain

Carbohydrate unit
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

<table>
<thead>
<tr>
<th>Red Cells (Antigens)</th>
<th>Plasma (Antibodies)</th>
</tr>
</thead>
<tbody>
<tr>
<td>• A</td>
<td>• Anti-B</td>
</tr>
<tr>
<td>• B</td>
<td>• Anti-A</td>
</tr>
<tr>
<td>• O</td>
<td>• Anti-A,B</td>
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<tr>
<td>• AB</td>
<td>• None</td>
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Haemolytic Disease of the Fetus and Newborn

• 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**

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<thead>
<tr>
<th>Patient:</th>
<th>Sample No:</th>
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**Blood and Transplant**

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<tr>
<th>Sample No:</th>
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**Hospital Transfusion Laboratory**

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**Address:**

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**Primary Requesting Clinician:**

<table>
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<th>Requesting Clinician:</th>
<th>EDD:</th>
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<tr>
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<td>17-Oct-2016</td>
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**Gestation:**

<table>
<thead>
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<th>Gestation:</th>
<th>10 weeks at sampling</th>
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**O RhD negative**

<table>
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<tr>
<th>Red Cell Antibody Results</th>
<th>Type</th>
<th>Specificity</th>
<th>Technique</th>
<th>Quantification (IU/mL)</th>
<th>Sample Type</th>
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<tbody>
<tr>
<td></td>
<td>Anti-D</td>
<td>IAT</td>
<td>Quant</td>
<td>4.6</td>
<td>Plasma</td>
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<tr>
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<td>Anti-C</td>
<td>IAT</td>
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<td>Plasma</td>
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**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-D 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 09/04/2016 at 15:02.
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
**Red Cell Immunohaematology**

**Blood and Transplant**

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**Primary Requesting Clinician:**

X0000001 HEAD OF BLOOD TRANSFUSION

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<th>EDD:</th>
<th>Gestation:</th>
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<td>03-Sep-2016</td>
<td>8 weeks at sampling</td>
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**A RhD positive C+c+E-e+K-**

**Red Cell Phenotype:** M-

### Red Cell Antibody Results

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<tr>
<th>Type</th>
<th>Specificity</th>
<th>Technique</th>
<th>Quantification IU/mL or Titre</th>
<th>Sample Type</th>
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**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
Cell free fetal DNA (cffDNA) testing 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)

At booking
All pregnant women
ABO + RhD* typing
Antibody screen

Clinically significant antibody screen positive (see 6.2)

Anti-D, -c or -K antibodies**
Test monthly until 28 weeks
See Appendix 3

From 28 weeks:
Test 2 weekly until delivery
See Appendix 3

All other clinically significant antibodies (see 6.2)
Consider paternal/fetal genotyping for corresponding antigen(s) (see 6.4)

Repeat testing at 28 weeks
Repeat antibody screen at 28 weeks

Cord blood for:
DAT, Hb, bilirubin

No clinically significant antibodies

No antibodies
No further action

Clinically significant antibodies
Management Algorithm for Pregnancies Complicated by anti-D, anti-K or anti-c
(RCOG Greentop guideline 65, 2014)

Legend:
Ab – antibody
BiP – bilirubin
DAT – direct antiglobulin test
fDNA – free fetal DNA
Hb – haemoglobin
HDFN – haemolytic disease of the fetus and newborn
IUT – intratartal transfusion
MCA Doppler – middle cerebral artery Doppler
Mod – moderate
Pos/Neg – positive/negative
US – ultrasound

1. Anti-K antibodies
   Titre Ab
   Or
   Non-invasive prenatal diagnosis
   Test father
   K Pos
   K Neg
   Heterozygous
   Homozygous
   fDNA
   K Pos
   K Neg
   HDFN

2. Anti-D antibodies
   Quantitate Ab level
   4–15 iu/ml mod risk HDFN
   > 15 iu/ml severe risk HDFN
   Or
   Non-invasive prenatal diagnosis
   Test father
   D Pos
   D Neg
   Heterozygous
   Homozygous
   fDNA
   D Pos
   D Neg

3. Anti-c antibodies
   Quantitate Ab level
   7.5–20 iu/ml mod risk HDFN
   > 20 iu/ml severe risk HDFN
   Or
   Non-invasive prenatal diagnosis
   Test father
   c Pos
   c Neg
   Heterozygous
   Homozygous
   fDNA
   c Pos
   c Neg

FETUS AT RISK OF HDFN

- Refer to specialist unit
- Fetus US, MCA Doppler
- IUT if needed
- Deliver at 37 weeks
- Cord blood Hb, BiP, DAT
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 an 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
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: 
Sample No:

DoB: 
NHS No:

NHSBT No: 
Address:

Date Sampled:

Hosp Samp ID:

Date Received:

Charge Code: 0000

Date Reported:

Primary Requesting Clinician:

ERD: 25-Apr-2016
Gestaton: 31 weeks at sampling

A RhD negative C-c+e-e+K-

<table>
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<tr>
<th>Red Cell Antibody Results</th>
<th>Type</th>
<th>Specificity</th>
<th>Technique</th>
<th>Quantification IU/mL or Titre</th>
<th>Sample Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not specified</td>
<td>Anti-D</td>
<td></td>
<td>Bio-Rad IAT</td>
<td>Quant</td>
<td>Plasma</td>
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</table>

Request form states 1500 IU anti-D Ig was given on 05/02/2016.

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
No further samples are required by NHSBT for reassessment in this pregnancy.

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

OTHER
An antibody card has not been supplied.
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.
Direct Antiglobulin Test (Direct Coombs Test)

- A test performed on the cord/baby’s sample soon after birth
- The test to see whether an antibody is attached to an antigen on red cells (in HDFN that is maternal antibody on baby’s red cells)
- Under what circumstances should a DAT be tested?
The Future?

- Cell free fetal DNA (cffDNA) testing to guide anti-D prophylaxis in non-immunised D negative women
  - 40% of D negative women (40,000 per annum in the UK) are given anti-D prophylaxis unnecessarily.
  - Routine fetal RHD typing for D negative women now provided by IBGRL but not routinely implemented.
  - NICE will assess the clinical and cost effectiveness and make recommendations