Fetal Genotyping Testing

IBGRL Filton

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Fetal Genotyping

- Background
- Science
- NHSBT offer
- Ethics
- Accuracy
- Benefits
- Any questions
Fetal Genotyping: Why?

- HDFN – maternal alloantibodies against fetal red cell surface antigens that the mother lacks
- D, c, C, E, K antigens (and others – rare)

Maternity Care Pathway

Pre-conception

Commence folic acid

Blood for haemoglobin, group, rhesus and antibodies as early as possible, or as soon as a women arrives for care, including labour

Women with type 1 or type 2 diabetes are offered diabetic eye (DE) screening annually. In pregnancy, women with type 1 or type 2 diabetes are offered a DE screen when they first present for care

Give screening information as soon as possible

Antenatal

Blood for sickle cell and thalassaemia

Blood for T21, T18 and T13 (combined test)

Blood for T21 (quadruple test)

Reoffer screening for infectious diseases if initially declined

Repeat haemoglobin and antibodies

Give and discuss newborn screening information

Antenatal and Newborn Screening Timeline - optimum times for testing

Version 8.1, March 2016, Gateway ref: 2014696, Public Health England leads the NHS Screening Programmes

www.gov.uk/topic/population-screening-programmes

Blood for syphilis, hepatitis B and HIV as early as possible, or at any stage of the pregnancy, including labour

Hepatitis B vaccination +/- immunoglobulin within 24 hours

Newborn physical examination by 72 hours

Newborn hearing screen

Newborn physical examination at 6-8 weeks

Newborn blood spot screen (usually on day 3) for sickle cell disease (SCD), cystic fibrosis (CF), congenital hypothyroidism (CHT) and inherited metabolic diseases (PKU, MCADD, MSUD, MMA, GA1 and HCU)

Newborn: babies who missed the screen can be tested up to one year (except CF offered up to 8 weeks)

Key to screening programmes

T21, T18, T13 and fetal anomaly ultrasound

Sickle cell and thalassaemia

Newborn and infant physical examination

Newborn blood spot

Infectious diseases in pregnancy

Newborn hearing

Diabetic eye
Rh and Ab Screening

- **RhD Pos**
  - No further action

- **RhD Neg**
  - **No Abs**
    - Repeat at 28 weeks
  - **Abs Detected**
    - Identify/quantify/genotype
      - **RHD genotyping screening test**

- **RhD Pos fetus**
  - Prophylactic Anti-D

- **RhD Neg fetus**
  - Consent to withhold Anti-D Ig
    - No Anti-D Ig

- **Antigen Pos**
  - At risk of HDFN
    - Appropriate management required

- **Antigen Neg**
  - Not at risk of HDFN
    - No intervention required
Background: Timeline

Alloimmunised women
1994: Fetal blood group genotyping introduced

2001: Fetal D typing on cffDNA
Later extended to K, C, c, E blood groups

Standard care in England

RhD Neg Women

2002: NICE studies into the feasibility of mass antenatal testing for fetal blood group by analysis of fetal DNA in maternal plasma

2013/14: Fetal RHD service pilot with North Bristol, U.H.B and Weston hospital

2015: Introduction as routine screening test
NICE guidance for high-throughput non-invasive prenatal testing for fetal RHD genotype was published on the 9th November 2016

*Recommendation:*
*High-throughput non-invasive prenatal testing (NIPT) for fetal RHD genotype is recommended as a cost-effective option to guide antenatal prophylaxis with anti-D immunoglobulin.......

You can find further information on the NICE website

https://www.nice.org.uk/guidance/dg25
Sources of fetal DNA

Before 2001: DNA from amniocytes or chorionic villi

Amniocentesis:
- 0.5-1.0% risk of spontaneous abortion
- 20% risk of transplacental haemorrhage

CVS: similar risks
Cell free fetal DNA from maternal plasma

Maternal plasma: Excellent source of fetal DNA for fetal genotyping

10–20 weeks:

85-90% maternal DNA
E.g No $RHD$ present (mother D-neg)

10-15% cell-free fetal DNA (Range = 3 - 30%)
E.g $RHD$ present if fetus D-pos
No $RHD$ if fetus D-neg

>21 weeks: increases by ~1% per week
Testing: Gestation

**Alloimmunised women:**
Rh: 16 weeks gestation
K: 20 weeks gestation

**RhD neg women:**
2006-11 High throughput fetal \( RHD \) testing trials at different stages of gestation (NIHR study)

Highly accurate from 11\(^{+2} \) weeks gestation
RHD genotyping tests detect presence or absence of RHD gene

RhD+ and D- blood groups

Noninvasive prenatal diagnosis of fetal blood group phenotypes: current practice and future prospects
Geoff Daniels, Kirstin Finning, Pete Martin, *Prenatal Diagnosis* 2009
RhD-neg phenotypes: African origin

66% have $RHD\psi$

Other SNPs in exon 5

37 bp insertion

nonsense TAT $\rightarrow$ TAG

Tyr stop

15% have $RHD-CE-D_s (4,7 e 8)$

Other SNPs in exon 5
# Testing: What’s involved?

<table>
<thead>
<tr>
<th>RhD negative women</th>
<th>Alloimmunised women</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>RHD</em> exons 5 &amp; 7 are targeted in triplicate as a multiplex (same wells), Automated extraction, Real-time Quantitative PCR</td>
<td><em>RHD</em> exons 4, 5, 7, 10</td>
</tr>
<tr>
<td>Exon 5 will not amplify <em>RHDΨ</em></td>
<td>Manual extraction, Real-time Quantitative PCR</td>
</tr>
<tr>
<td>Confirmation of successful DNA extraction (not fetal-specific) by single amplification of control gene (<em>CCR5</em>)</td>
<td>Only exons 7 &amp; 10 amplify <em>RHDΨ</em>, <em>RHD-CE-Ds</em>, <em>RHD</em>DVI</td>
</tr>
</tbody>
</table>
DNA extraction & qPCR

DNA is extracted robotically and amplified by real-time PCR.

CCR5 used to confirm successful extraction
## Sensitivity & Specificity

<table>
<thead>
<tr>
<th>Result</th>
<th><em>RHD Screening Test</em> (High sensitivity)</th>
<th><em>RHD Diagnostic Test</em> (High specificity &amp; sensitivity)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>False Positive</strong></td>
<td>Unnecessary anti-D Ig administered</td>
<td>-Regular assessment</td>
</tr>
<tr>
<td><em>(Fetus D neg, called D pos)</em></td>
<td></td>
<td>-Could lead to increased monitoring and possibly invasive testing</td>
</tr>
<tr>
<td><strong>False Negative</strong></td>
<td>-No anti-D Ig received</td>
<td>-Pregnancy not managed appropriately</td>
</tr>
<tr>
<td><em>(Fetus D pos, called D neg)</em></td>
<td>-May become alloimmunised</td>
<td>-Fetal anaemia may not be detected</td>
</tr>
<tr>
<td></td>
<td>-Risk of HDFN in future pregnancies</td>
<td>-HDFN</td>
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<tr>
<td></td>
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<td>-Fetal death/morbidity</td>
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</tbody>
</table>

Sensitivity: True positives are identified as such

Specificity: True negatives are identified as such
Alloimmunised women, IBGRL

• Offer RhD/C/c/E and K fetal genotyping nationally and internationally; Canada, South Africa, Pakistan, Israel, Greece, Ireland, Spain and others

• Numbers tested: April 2016 – present = 503 samples

• Rely on cord blood results from hospitals to determine accuracy
RHD Screening programme

Ethics

Anti-D Ig is and exceptionally safe product

Risks:
- human derived pooled product
- unknown agents (prion) to be considered
- allergic reactions
- efficacy – 0.35% when given at the correct time
- limited availability

Both the difficulty in availability and the theoretical risk mean it should be only used when required
99.9% for RhD pos and neg predictions

Inconclusive results – 77-80% of these will have RhD pos babies, recommendation to give anti-D Ig

**Caucasian population distribution:**
15% of mothers are RhD negative, of these 38% - 40% carry RhD negative babies
Benefits

Elimination of donor exposure for RhD negative women expecting RhD negative babies.

Only giving anti-D Ig to those women who need it.

Samples will be taken at the time when women attend the clinic for other routine tests.

Clinicians can focus on women who expect RhD positive babies.

Reduce concerns over supply of anti-D or risks associated with this product.
RHD Screening programme

Middlesbrough
Harrogate
Leeds
Sheffield
West Suffolk
University Hospital Bristol
North Bristol Trust
Oxford
Watford
Hillingdon
The Birth Company Ltd
West Middlesex
Weston-Super-Mare
West Hertfordshire
Barts Health NHS Trust
Chelsea and Westminster
Taunton
Yeovil
Southampton
Portsmouth
How will it work in your lab?

EDTA blood sample from 11+2 weeks at a routine antenatal appointment

Send to local pathology lab who will forward them to NHSBT

Electronic report within <14 days via Sp-ICE
What do we offer

We offer:
Competitive price

Which includes:
NHSBT transport
Address labels
Request form
Patient Leaflet
User Guide
Electronic report
Help with Business plan
Calculation spreadsheet &
Maternity Pathways
With thanks to the Molecular Diagnostics team
Any questions