Fetal Genotyping Testing

NHS Blood and Transplant

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Fetal Genotyping

NHS Blood and Transplant

- Background
- Science
- NHSBT offer
- Ethics
- Accuracy
- Benefits
- Any questions

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Fetal Genotyping: Why?

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

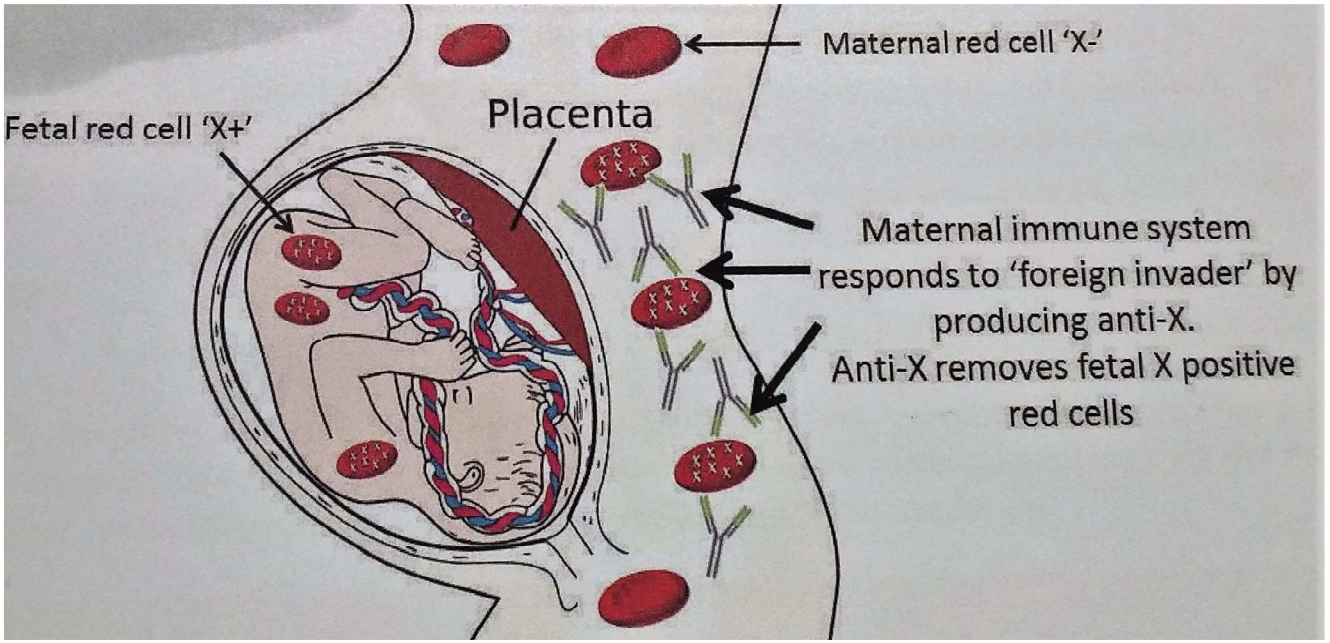

Image: Qureshi, R (2015) *Introduction to Transfusion Science Practice*, British Blood Transfusion Society, 6th Edition.

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Maternity Care Pathway

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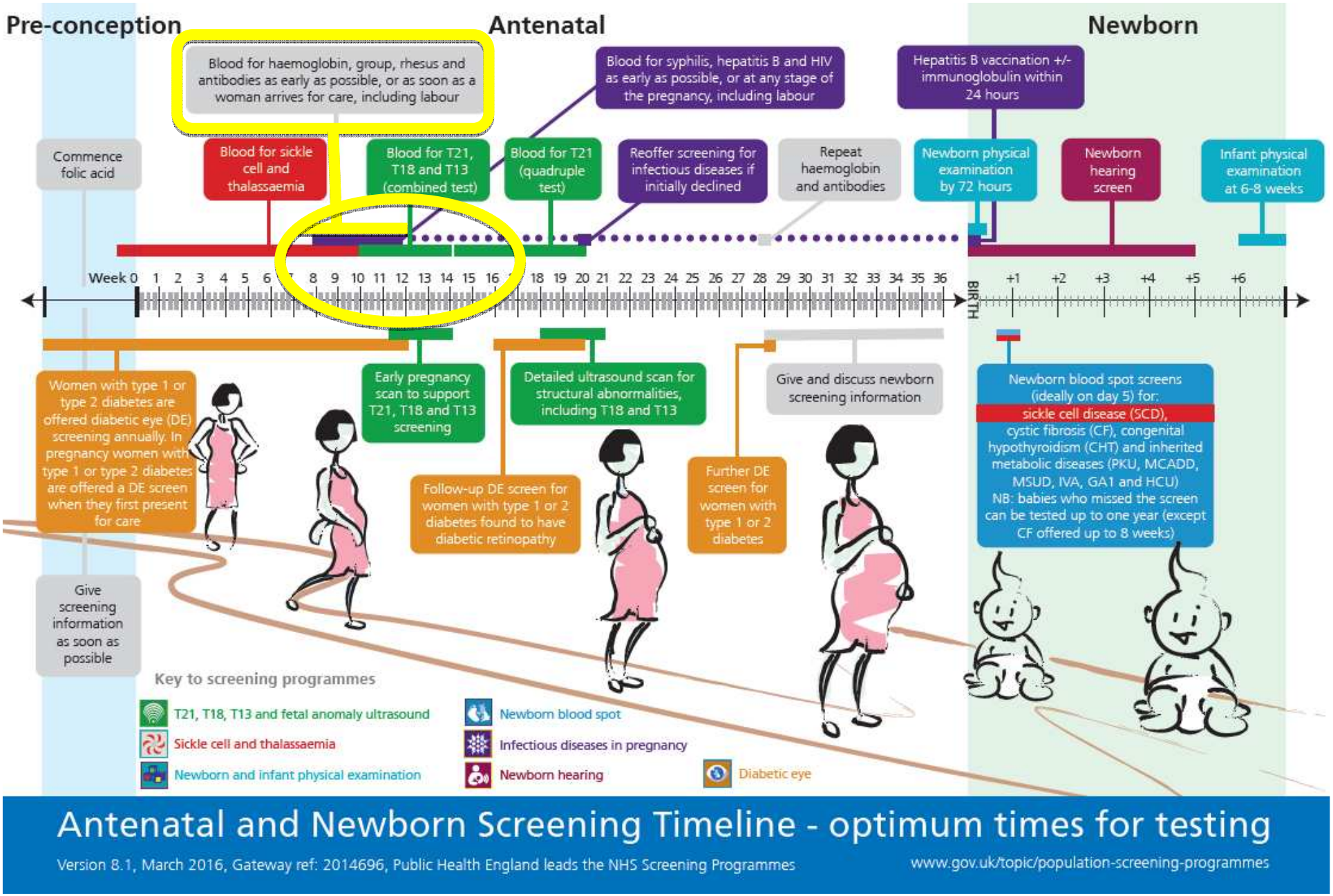

Antenatal and Newborn Screening Timeline - optimum times for testing

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

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

Rh and Ab Screening

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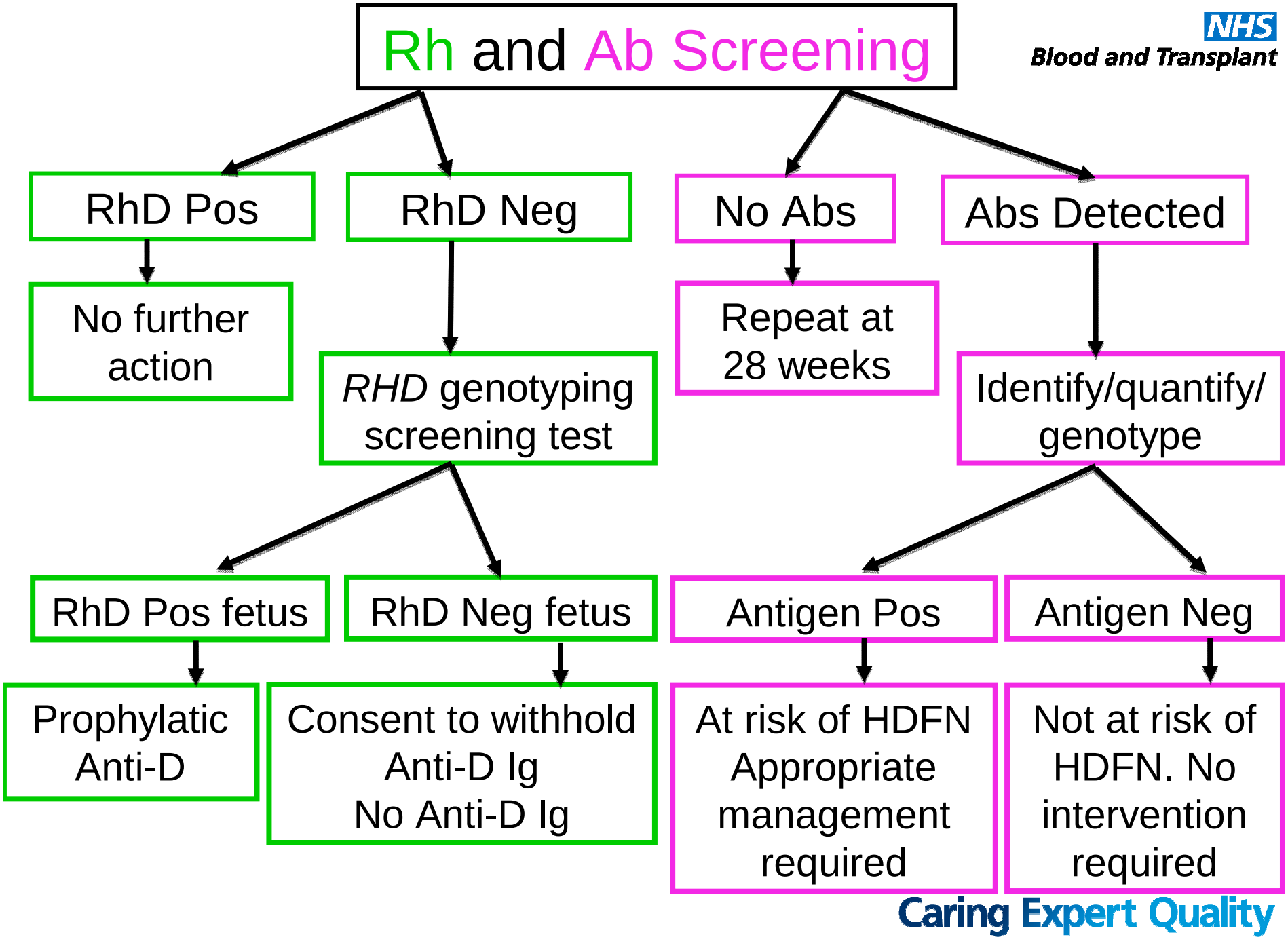

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Background:Timeline

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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 Recommendation

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2016

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

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Sources of fetal DNA

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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

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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

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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

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RHD genotyping tests detect presence or absence of *RHD gene*

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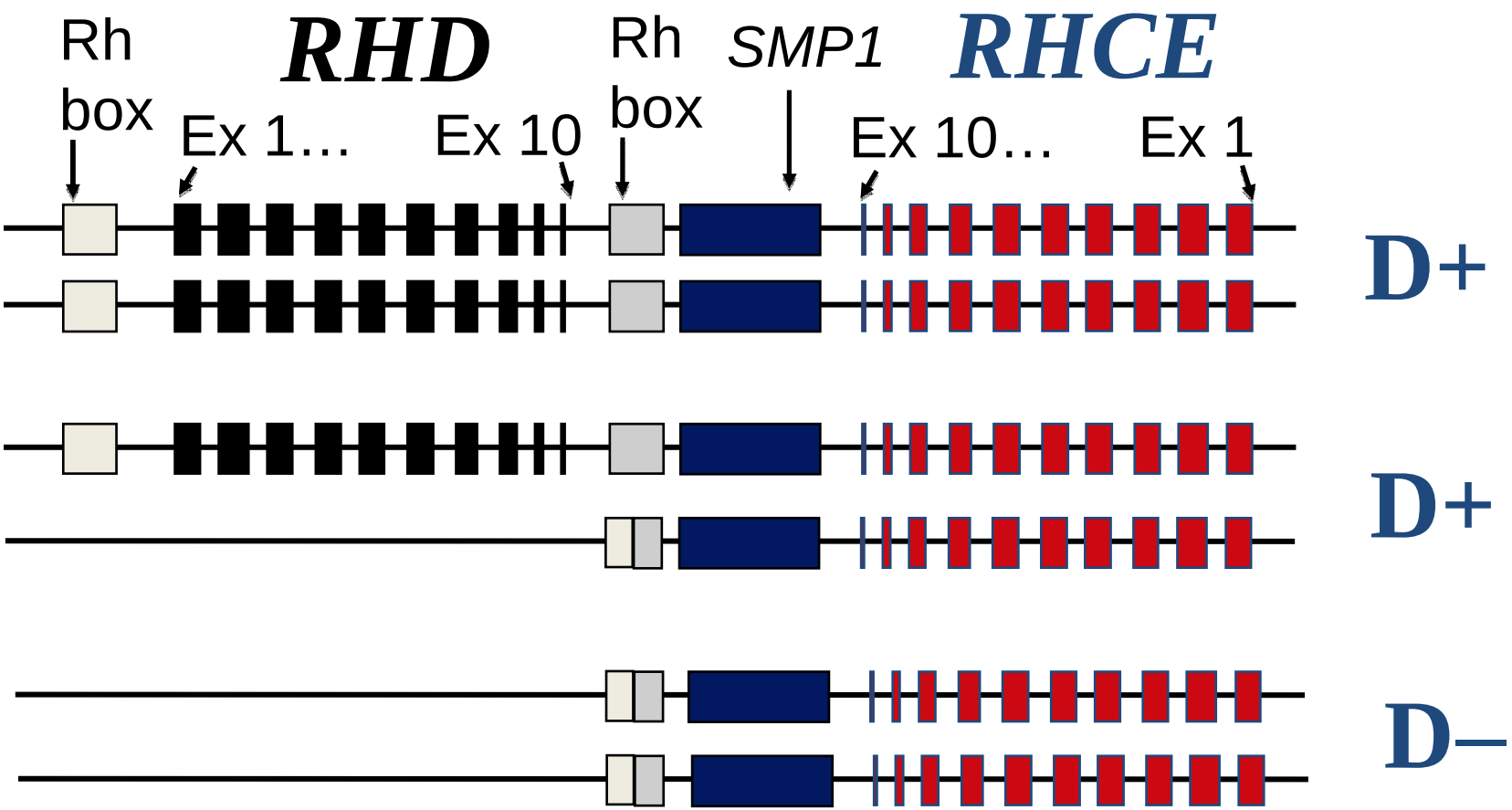
RhD+ and D- blood groups



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

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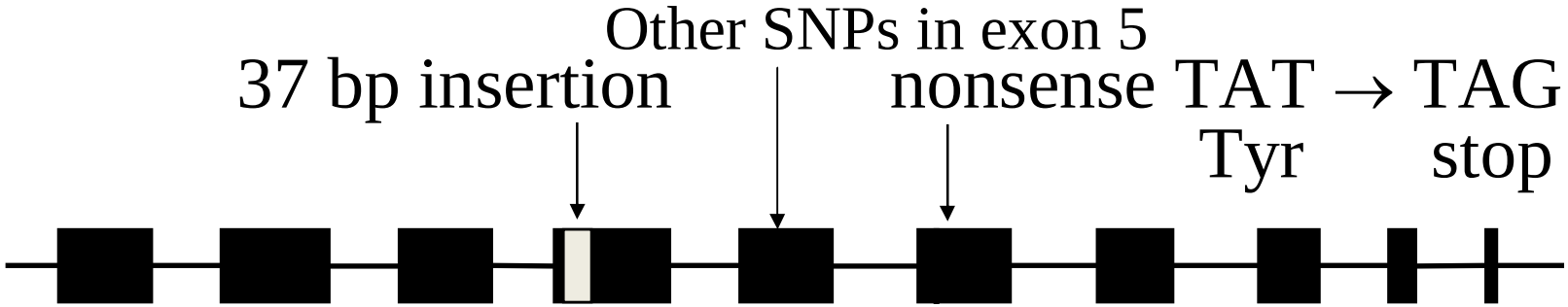
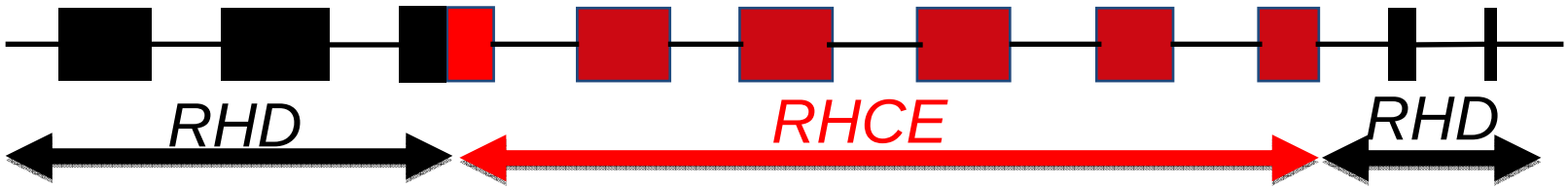
66% have *RHD Ψ*



15% have *RHD-CE-D^s (4-7 e 8)*



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Testing: What's involved?

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RhD negative women

RHD exons 5 & 7 are targeted in triplicate as a multiplex (same wells),

Automated extraction, Real-time Quantitative PCR

Exon 5 will not amplify *RHDΨ*

Confirmation of successful DNA extraction (not fetal-specific) by single amplification of control gene (*CCR5*)

Alloimmunised women

RHD exons 4, 5, 7, 10

Manual extraction, Real-time Quantitative PCR

Only exons 7 & 10 amplify *RHD*Ψ, RHD-CE-Ds*, *RHD*DVI*

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DNA extraction & qPCR

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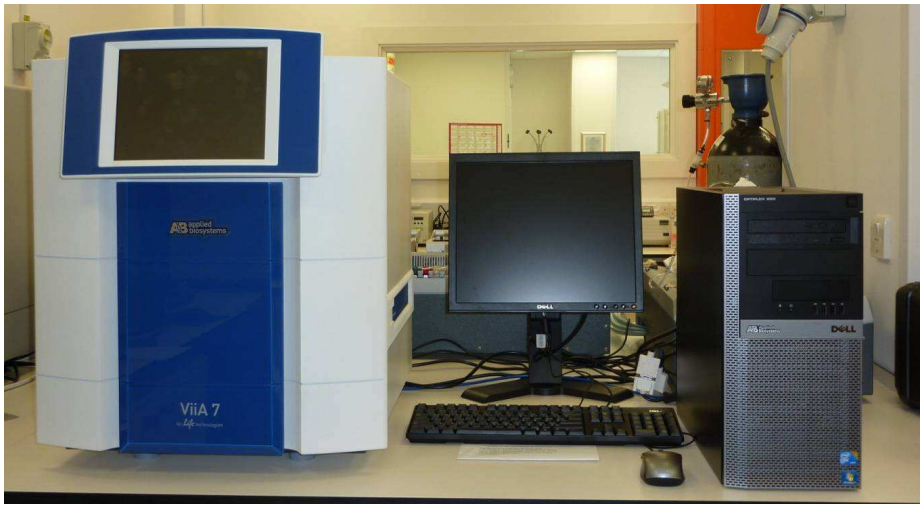

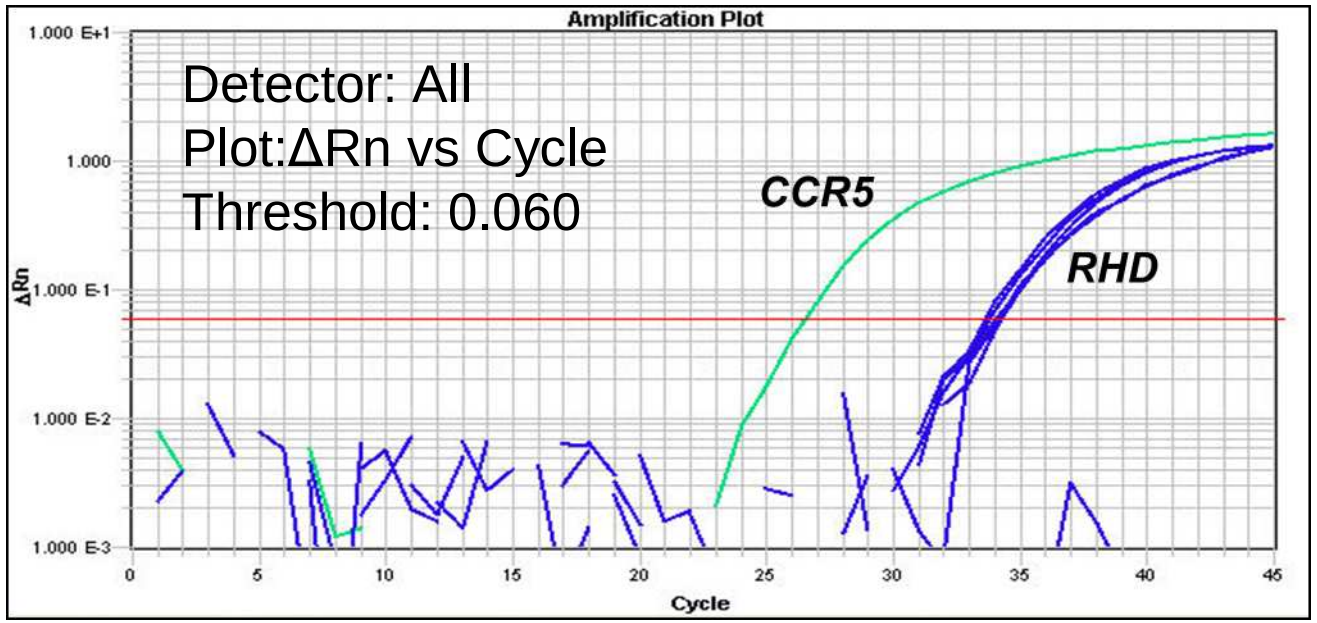

DNA is extracted robotically and amplified by real-time PCR.

CCR5 used to confirm successful extraction

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Sensitivity & Specificity

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

Sensitivity: True positives are identified as such

Specificity: True negatives are identified as such

Alloimmunised women, IBGRL

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- 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

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RHD Screening programme Ethics

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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

Accuracy

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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

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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

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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

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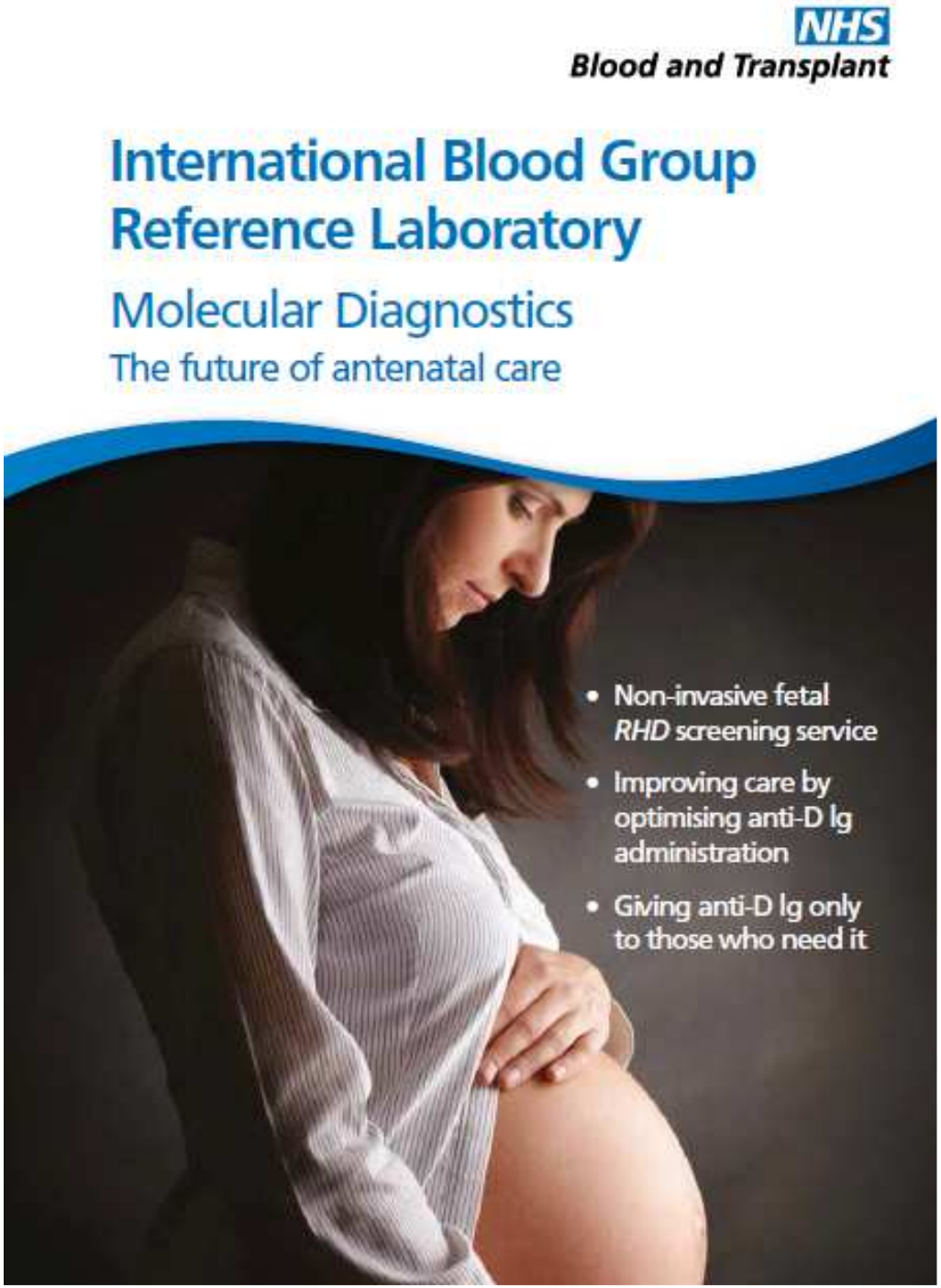
How will it work in your lab?

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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With thanks to the
Molecular Diagnostics team
Grant
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Any questions