Fetal Genotyping

NHS Blood and Transplant

Optimising antenatal care

Erika Rutherford
Business Development Manager

Caring Expert Quality

Fetal Genotyping

NHS Blood and Transplant

- Background
- Science
- Accuracy
- Ethics & benefits
- Project set up
- Contact details
- Any questions

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Fetal Genotyping: Why?

NHS Blood and Transplant

- Optimising antenatal care
- Closely monitor women with maternal alloantibodies against fetal red cell surface antigens that the she lacks
- Preventing Haemolytic Disease of the Fetus and Newborn (HDFN)
- D, c, C, E, K (and others – rare)

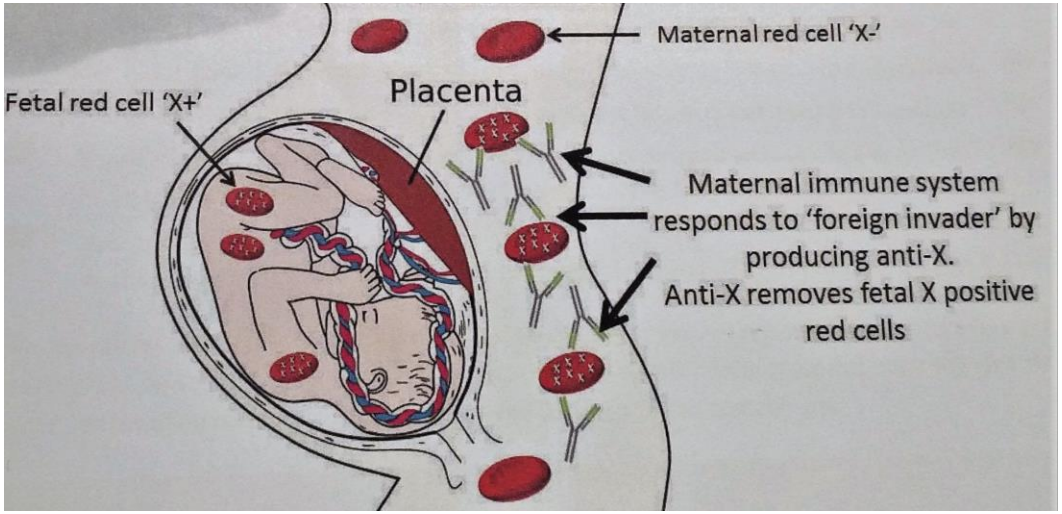

Image: Qureshi, R (2015) *Introduction to Transfusion Science Practice*, British Blood Transfusion Society, 6th Edition.

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Background

NHS Blood and Transplant

Alloimmunised women

1994: Fetal blood group genotyping introduced
DNA from amniocytes or chorionic villi

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

2001: Fetal D typing
non invasive prenatal testing (NIPT) from maternal blood which contains cell free fetal DNA (cffDNA)

Later extended to K, C, c, E

- No risk to the pregnancy

Standard care in England

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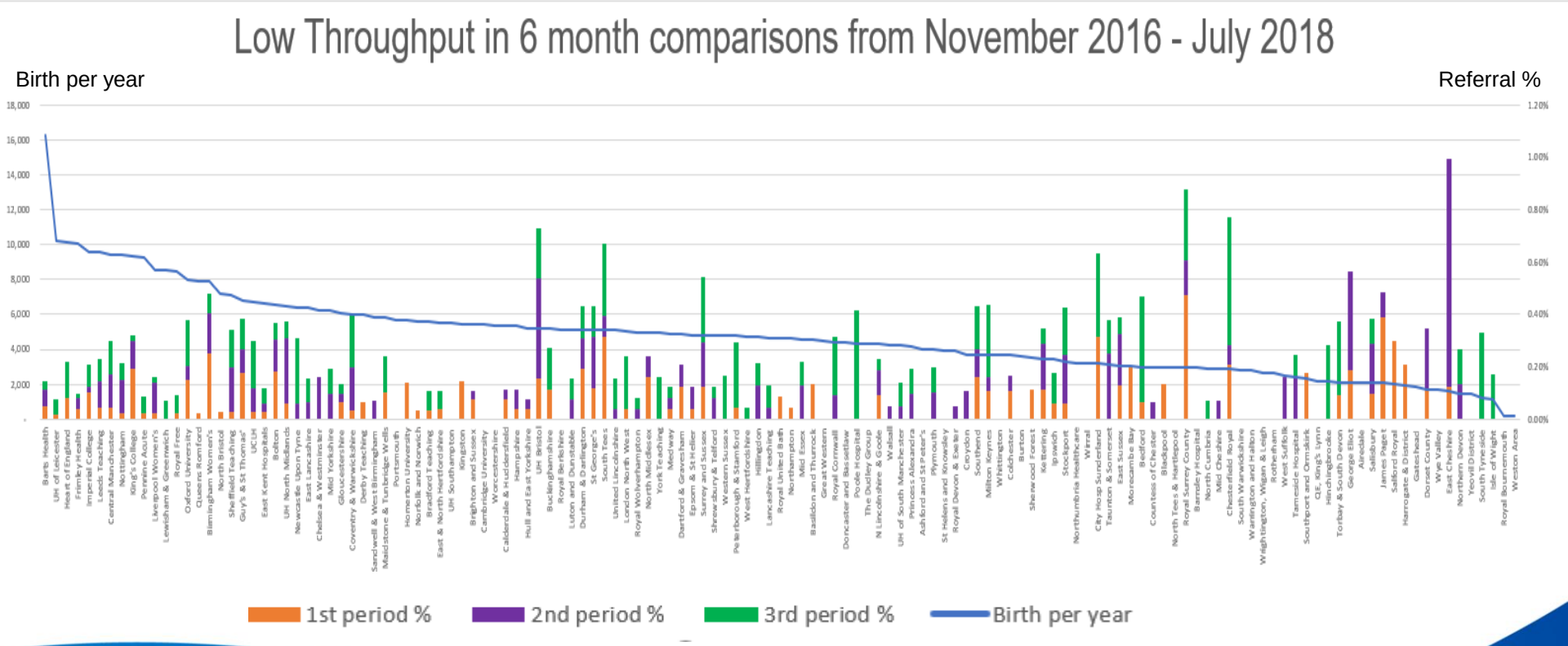
Referrals rate for fetal genotyping

NHS Blood and Transplant

Although standard of care in England for women with antibodies,
Not all NHS Trusts have implemented best practice



Background

NHS Blood and Transplant

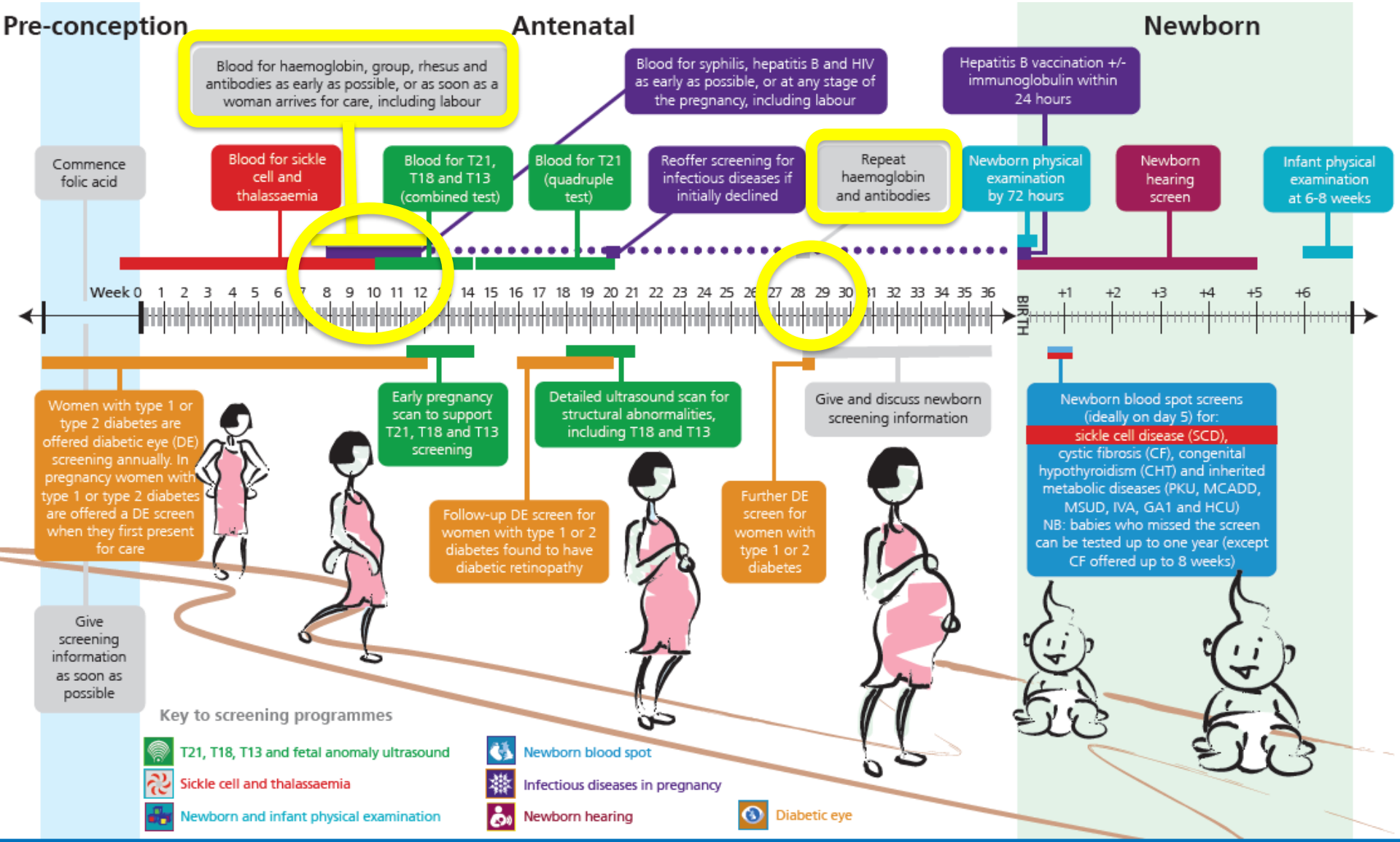
Fetal *RHD* screen

2002: NICE – recommended prophylactic anti-D IG
and endorsed studies into high throughput NIPT for cffDNA
2003: IBGRL developed fetal *RHD* screening test – Dr Kirstin Finning
2006: NIHR studies – established gestational age – from 11^{+2} weeks
2013: Piloted in 3 hospitals

2015: Introduced as a routine screening test
2016: NICE recommendation published - cost effective test
2017: Request to work with NICE on research recommendations

Maternity Care Pathway

NHS Blood and Transplant



Antenatal and Newborn Screening Timeline - optimum times for testing

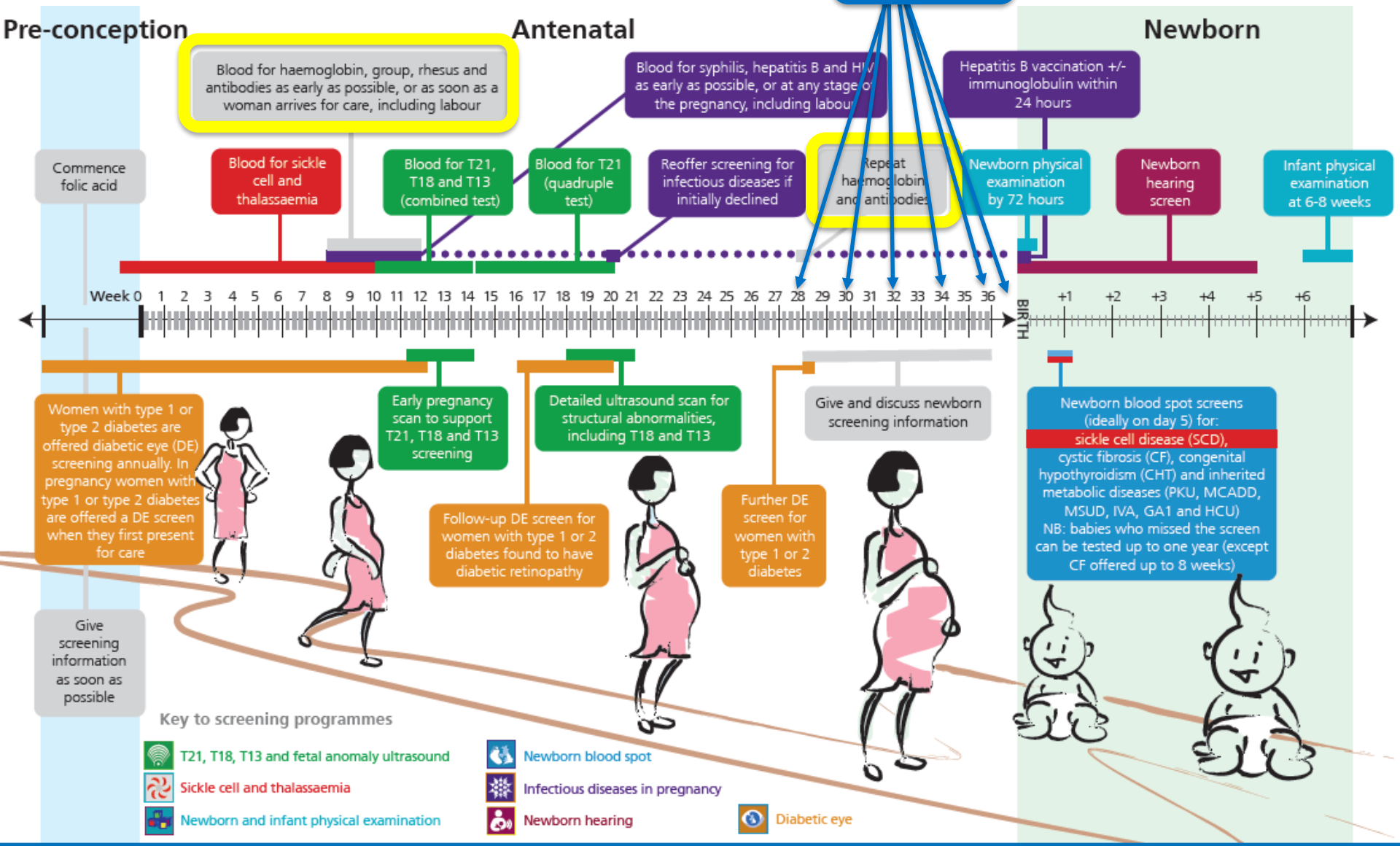
Version 8.1, March 2016, Gateway ref: 2014696, Public Health England leads the NHS Screening Programmes

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

Maternity Care Pathway

Quantifications

NHS Blood and Transplant



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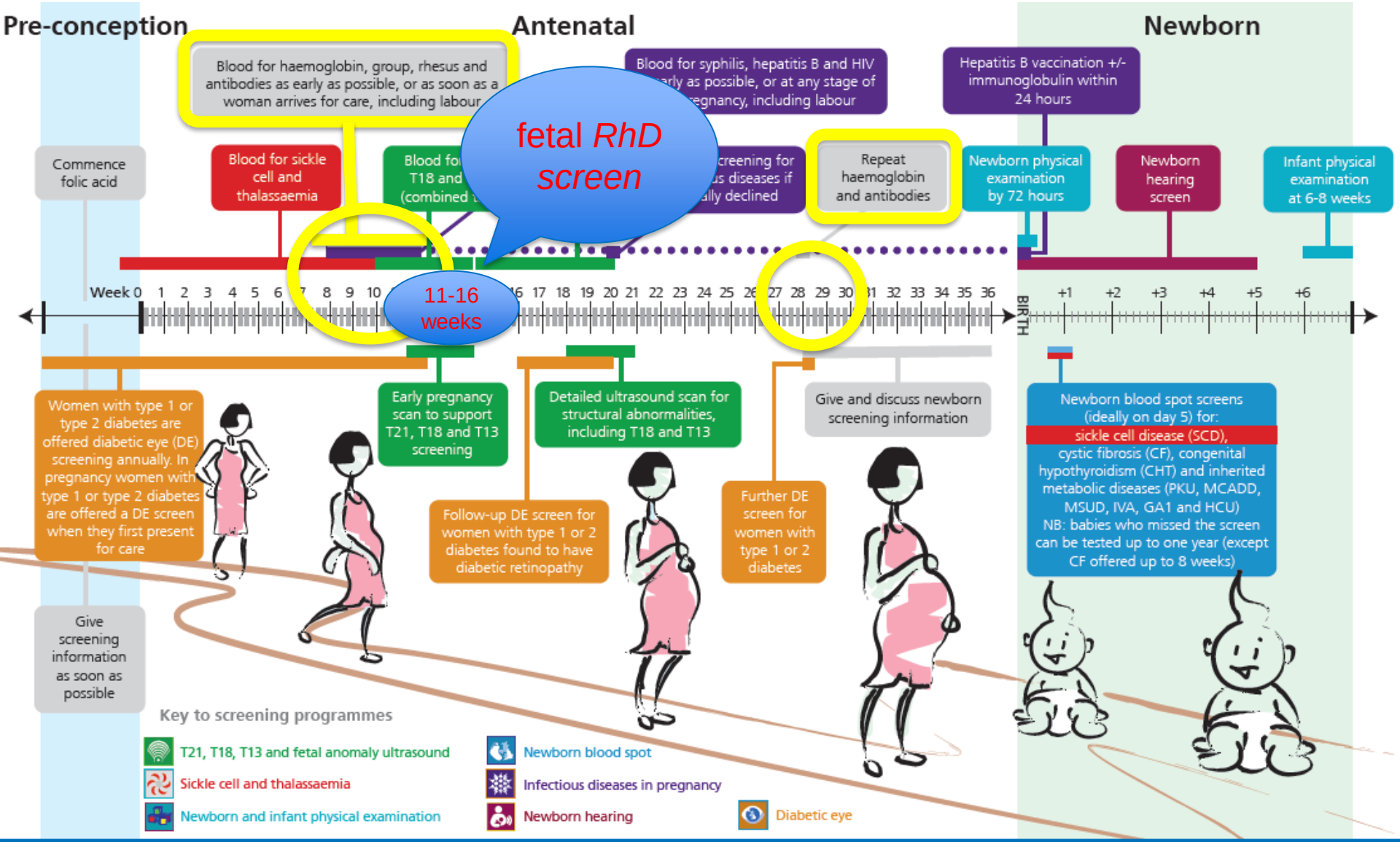
Maternity Care Pathway

NHS Blood and Transplant



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Maternity Care Pathway

Quantifications

NHS Blood and Transplant

Pre-conception | Antenatal | Newborn

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

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

Commence folic acid

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

Newborn physical examination by 72 hours

Newborn hearing screen

Infant physical examination at 6-8 weeks

Week 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 | +1 +2 +3 +4 +5 +6

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

Early pregnancy scan to support T21, T18 and T13 screening

Detailed ultrasound scan for structural abnormalities, including T18 and T13

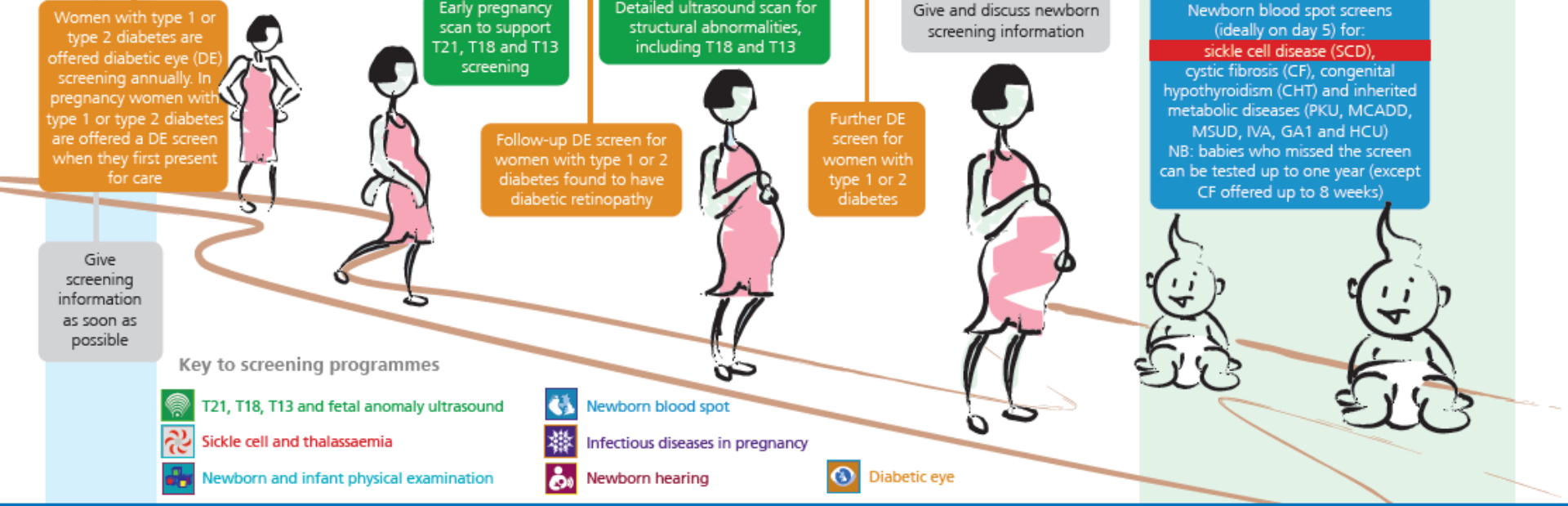
Follow-up DE screen for women with type 1 or 2 diabetes found to have diabetic retinopathy

Further DE screen for women with type 1 or 2 diabetes

Give and discuss newborn screening information

Newborn blood spot screens (ideally on day 5) for:
sickle cell disease (SCD),
cystic fibrosis (CF), congenital hypothyroidism (CHT) and inherited metabolic diseases (PKU, MCADD, MSUD, IVA, GA1 and HCU)
NB: babies who missed the screen can be tested up to one year (except CF offered up to 8 weeks)

Give screening information as soon as possible



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

Antenatal and Newborn Screening Timeline - optimum times for testing

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Laboratory tests

NHS Blood and Transplant

Quantification for D and c

reliable indication for HDFN when increase is observed

Titres for C, E and other antibodies

indication for HDFN when increase is observed

Titre for K

Unreliable indication for HDFN

Fetal genotyping

Determines which pregnancies are at risk of HDFN and need close monitoring

Clinical

Doppler scan

Monitoring & confirmation of HDFN severity

Intervention

Exchange transfusion - intrauterine or post natal

Maternity Care Pathway

Quantifications
Titres
Doppler scans

NHS Blood and Transplant

Pre-conception

Commence folic acid

Give screening information as soon as possible

Antenatal

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Newborn

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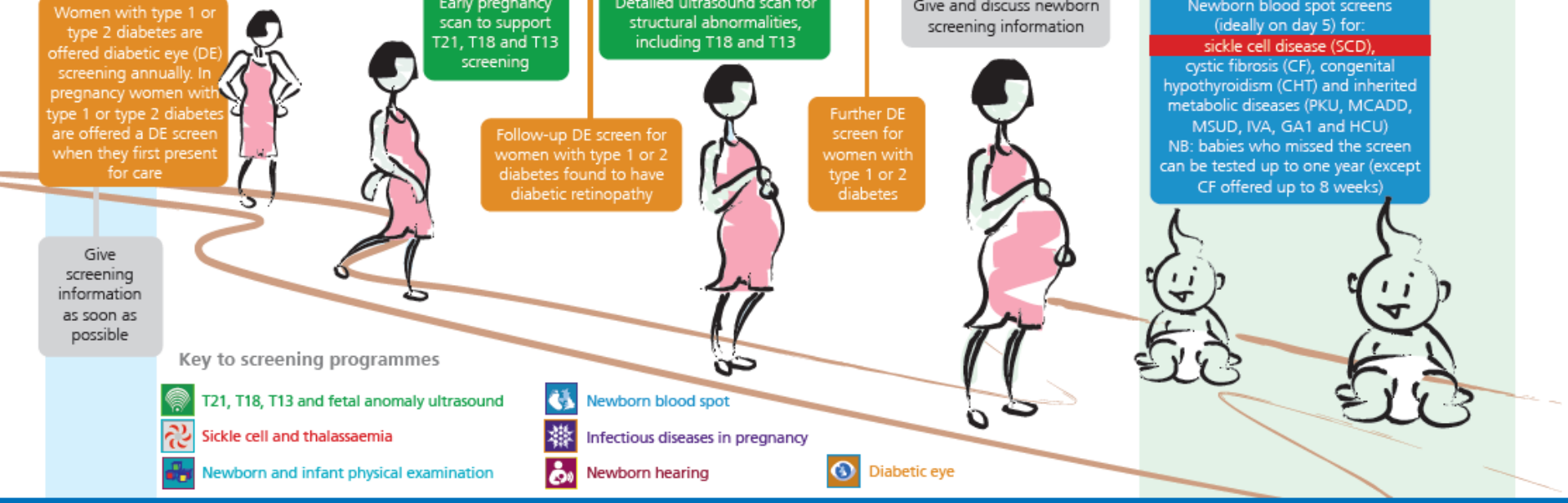

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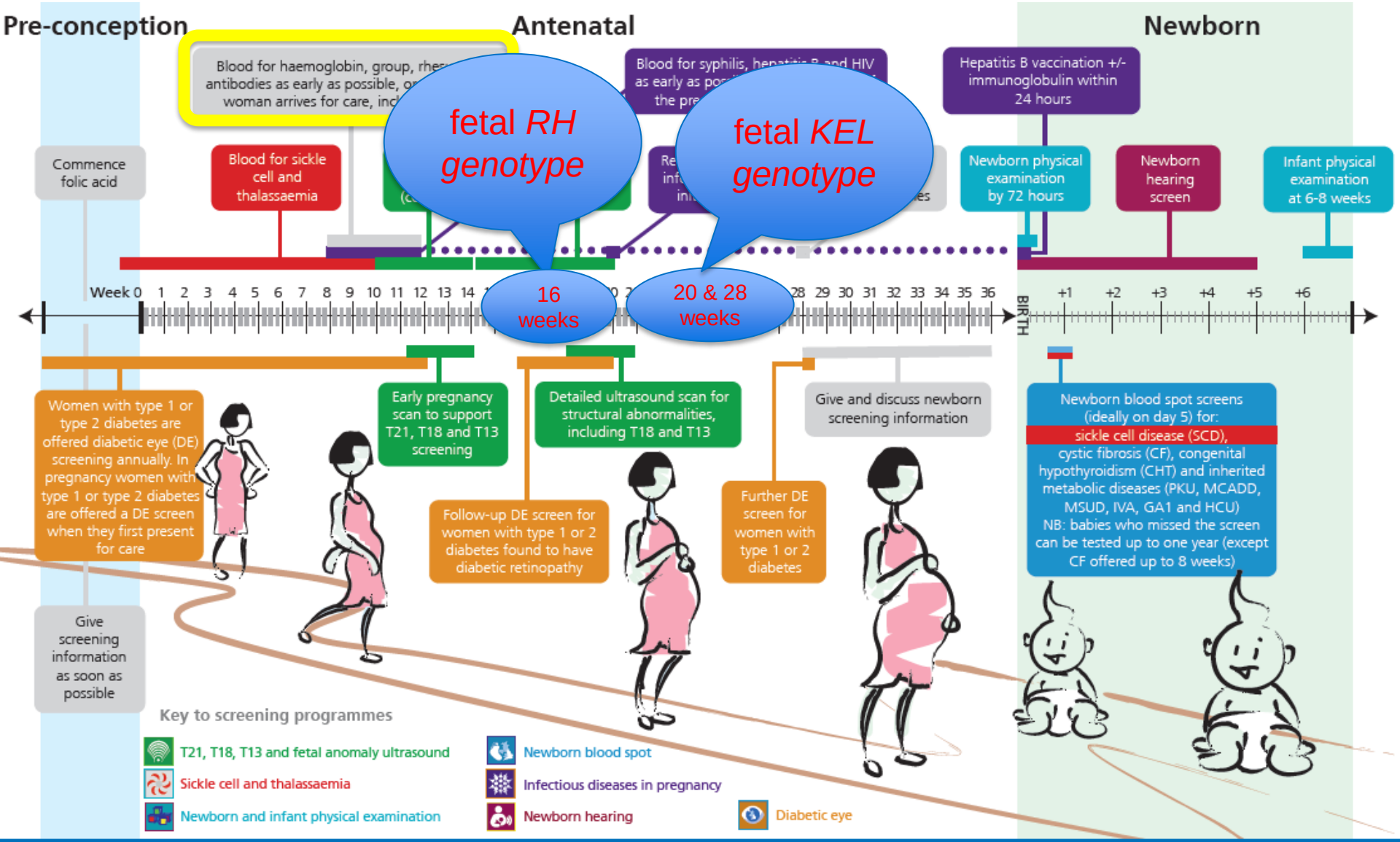
Maternity Care Pathway

NHS Blood and Transplant



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Maternity Care Pathway

Quantifications
Titres
Doppler scans

NHS
Blood and Transplant

Pre-conception | Antenatal | Newborn

Commence folic acid

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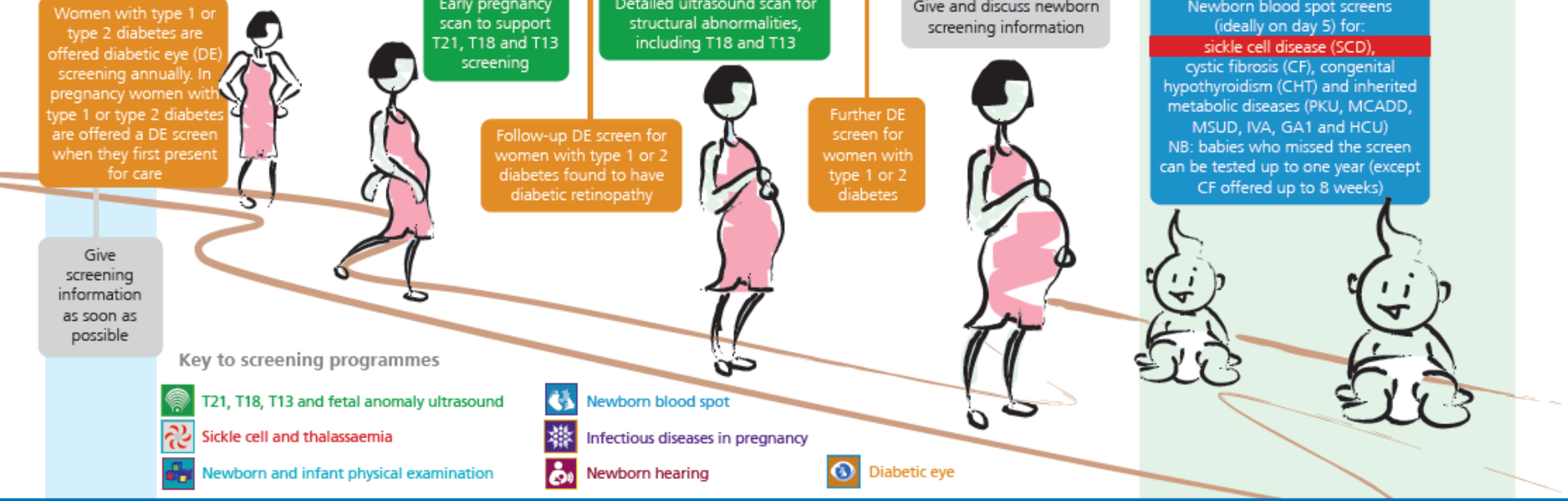

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www.gov.uk/topic/population-screening-programmes

Rh and Ab Screening

NHS Blood and Transplant

- D positive
  - No antibodies repeat at 28 weeks
- D negative
  - No D or G antibodies
    - fetal *RHD* screen
      - fetus D pos
        - prophylactic anti-D
      - fetus D neg
        - No prophylactic anti-D required
- no antibodies
  - repeat at 28 weeks
- antibodies detected
  - identify/quantify/ genotype
    - fetus antigen positive
      - at risk of HDFN appropriate management required
    - fetus antigen negative
      - **not** at risk of HDFN
        minimal monitoring recommended

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Cell free fetal DNA from maternal plasma

NHS Blood and Transplant

Excellent source of fetal DNA for genotyping
where the fetus is positive for a gene the mother does not have

10–20 weeks:

85-90% is maternal DNA
but antigen-negative for the antibody she has

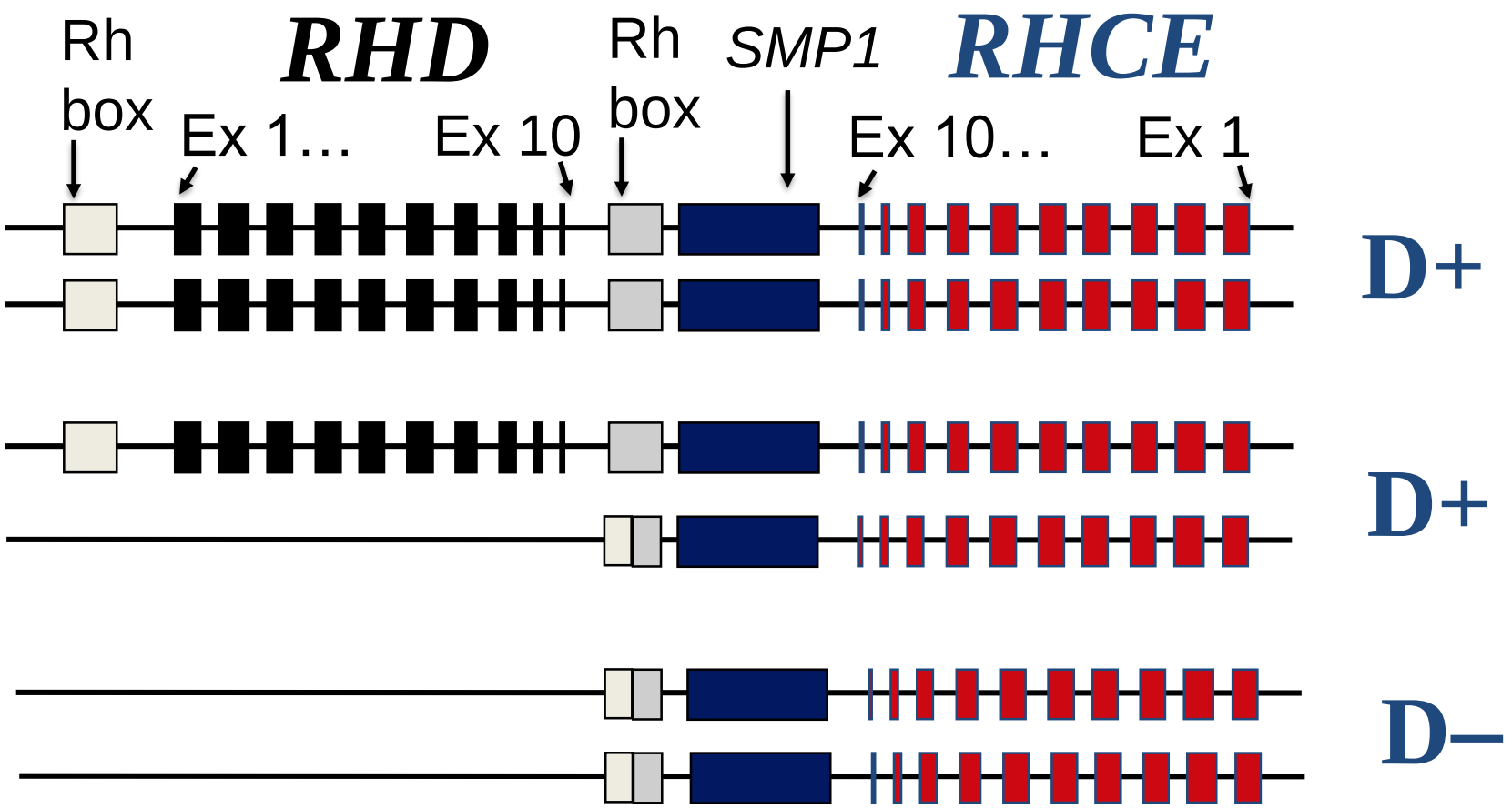
10-15% cell-free fetal DNA (Range = 3 - 30%)
fetal *D/C/c/E/K* present if **fetus antigen-positive**
NO fetal *D/C/c/E/K* if **fetus antigen-negative**

>21 weeks: increases by ~1% per week

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

NHS Blood and Transplant

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

Testing: What's involved?

NHS Blood and Transplant

D 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

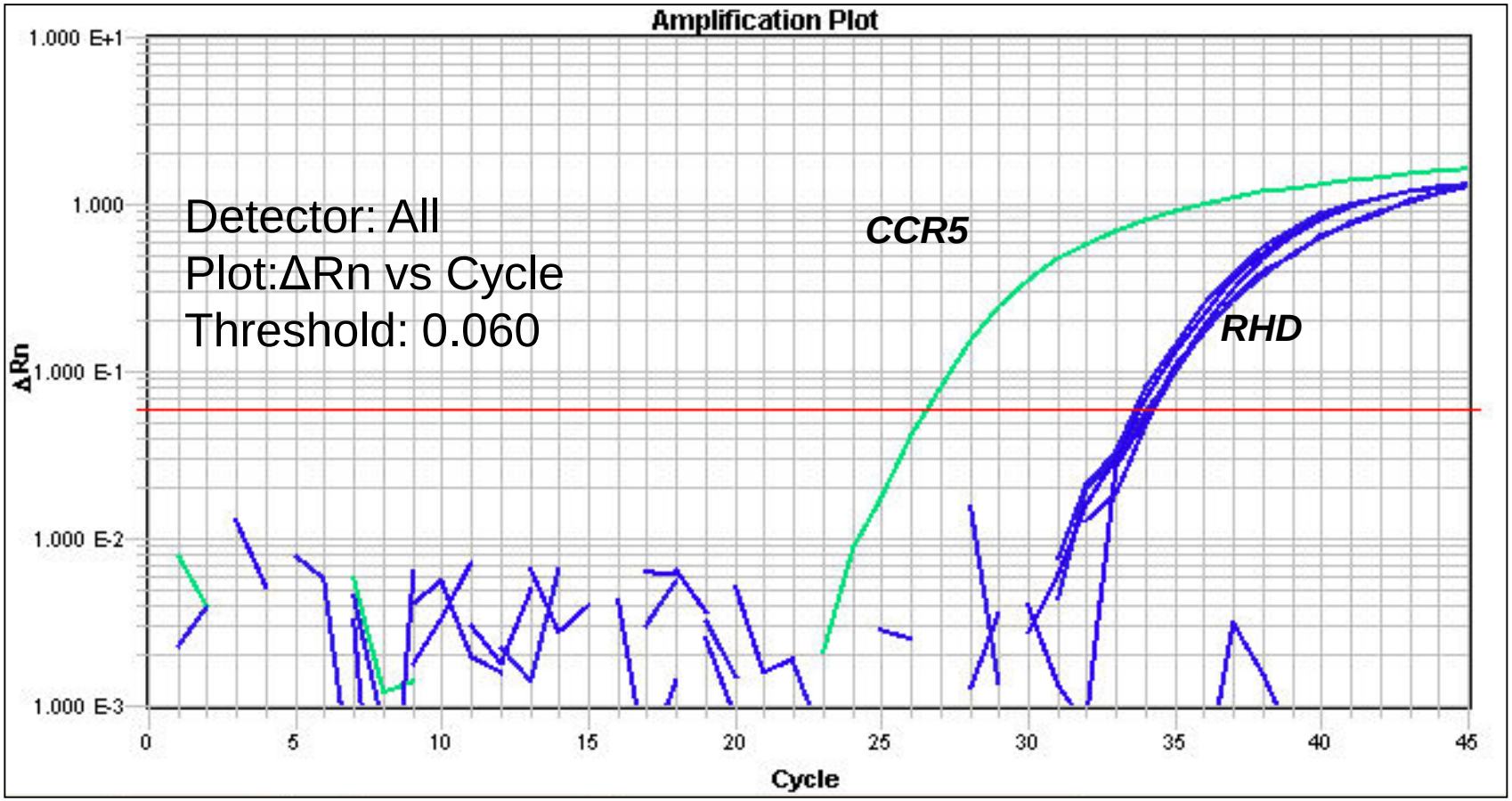
Only exons 7 & 10 amplify *RHD*Ψ, *RHD-CE-Ds*, *RHD*DVI*

Fetal *RHD* screen

NHS Blood and Transplant

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

CCR5 used to confirm successful extraction



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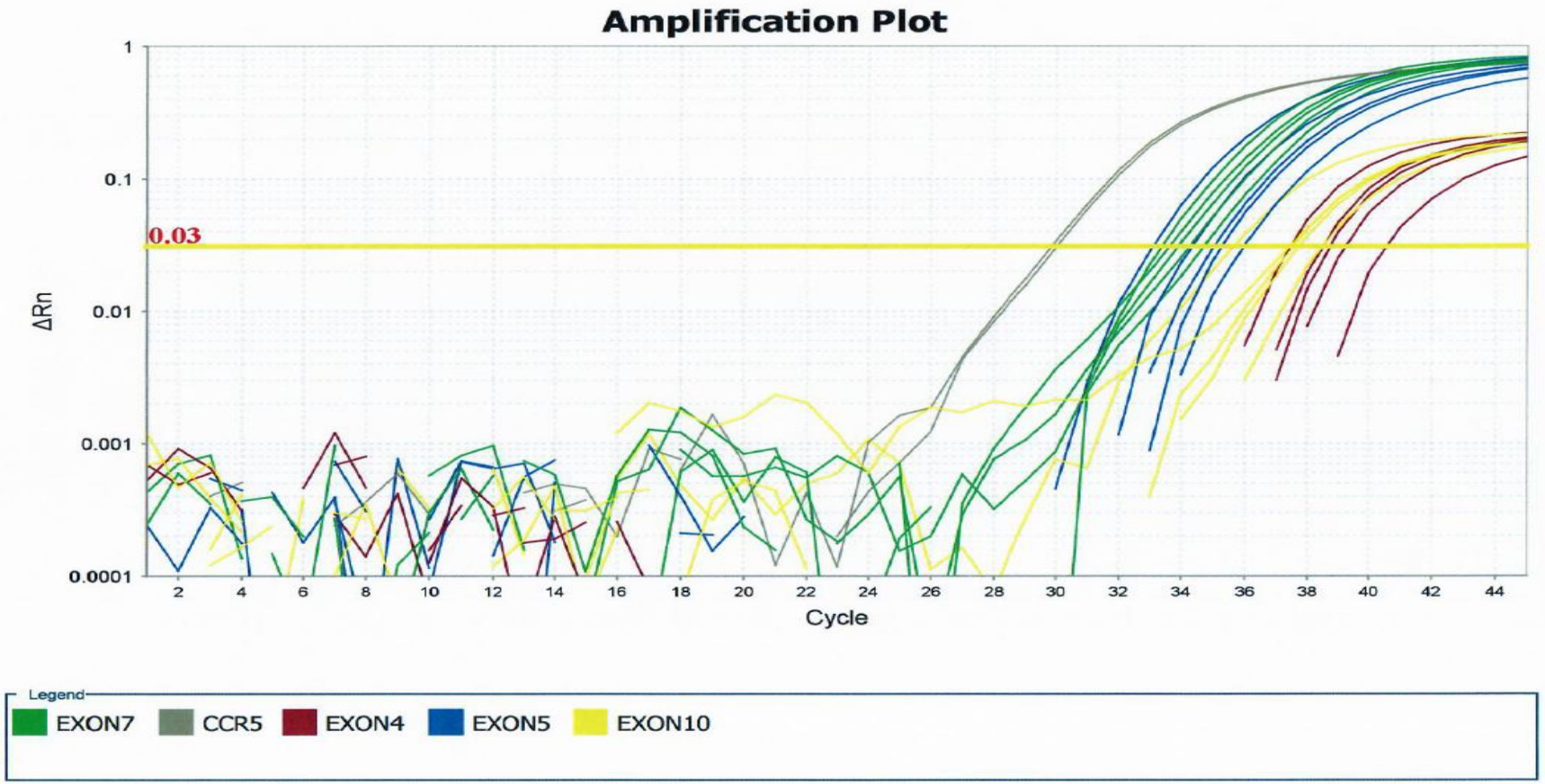
Fetal genotype diagnostic

NHS Blood and Transplant



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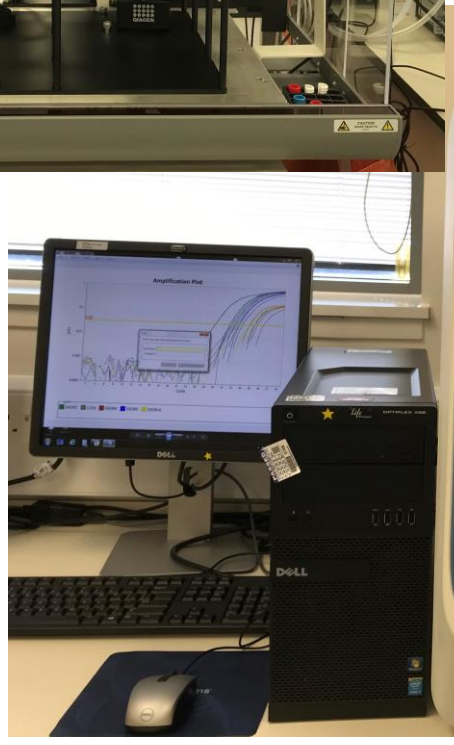
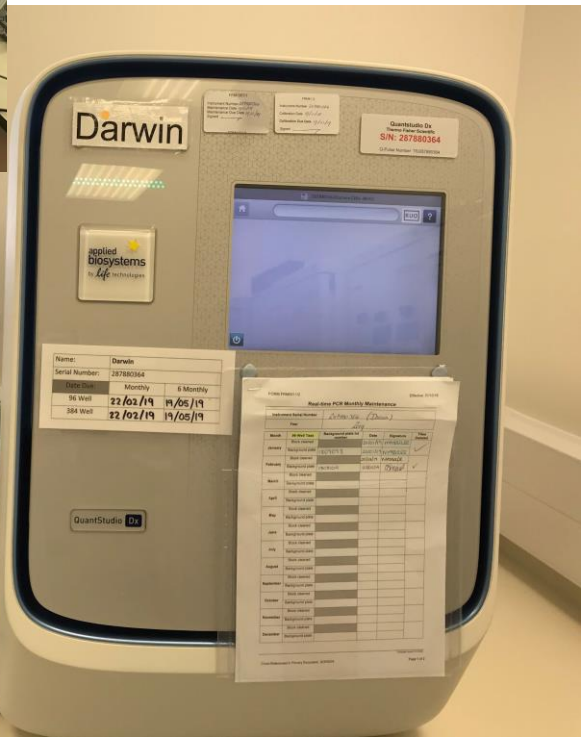
DNA extraction & qPCR

NHS
Blood and Transplant





Sensitivity & Specificity

NHS Blood and Transplant

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 invasive testing (fetal blood sampling)
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

Relying on cord blood results from hospitals to determine accuracy

Accuracy

NHS Blood and Transplant

Fetal *RHD* screen

≤0.1% for false negative predictions

Fetal D genotype

sensitivity of 99.8% and specificity of 99.2%
2514 tests – 5 false pos / 2 false neg

Fetal C, c, E genotype

we have not been informed of any false results

Fetal K genotype

≤0.5% for false negative predictions

https://ibgrl.blood.co.uk/services/molecular-diagnostics/

Ethics and benefits

NHS Blood and Transplant

- Clinicians can focus on women with an antigen positive fetus
- Mothers with an antigen negative fetus can relax and enjoy their pregnancy with minimal monitoring
- Overall it saves cost and time for those mothers who do not need:
    - repeated clinic attendance
    - doppler scans
    - referrals for antibody quantification and titres

Ethics (fetal RHD screen)

NHS Blood and Transplant

Anti-D Ig is an exceptionally safe product

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

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

Sample requirements:

Fetal genotype diagnostic test for alloimmunised women:

Rh:16 weeks gestation
K: 20 weeks gestation
repeat at 28 weeks if K negative

Sample volume:
16mL EDTA per genotype

Reaching Filton within:
Rh: 3 days from venepuncture
K: 2 days from venepuncture
By 1st class post

Fetal *RHD* screen for RhD neg women without D&G antibodies:

From 11^{+2} weeks gestation

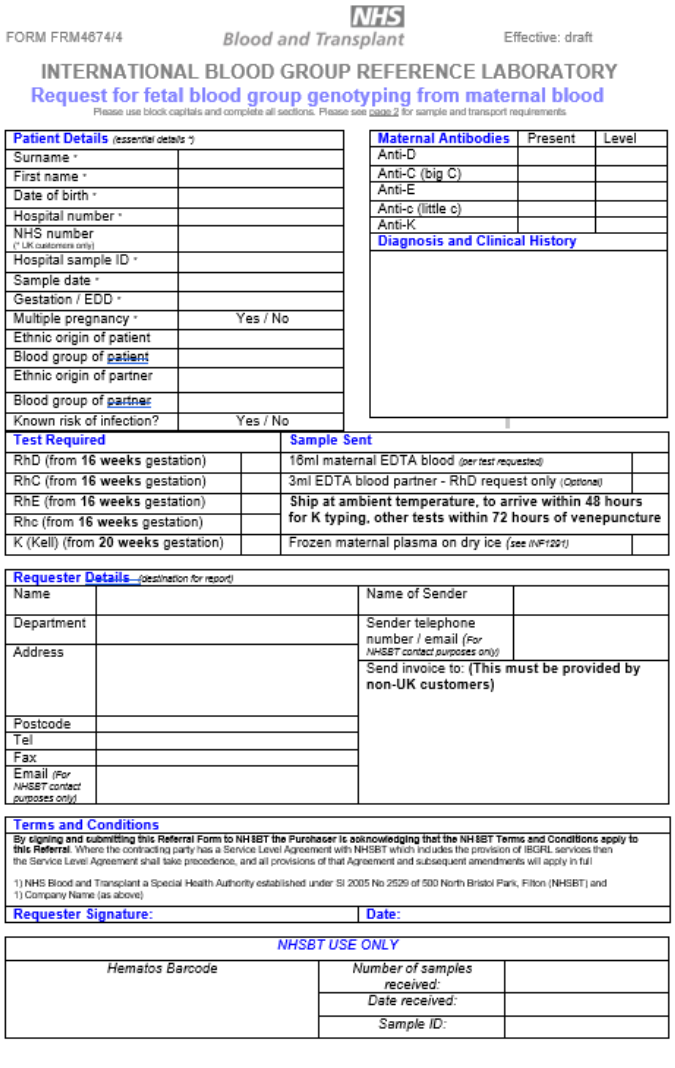
Sample volume:
6mL EDTA

Reaching Filton within:
7 days from venepuncture
via NHSBT transport

Referral forms & address labels

NHS Blood and Transplant

Fetal genotyping for alloimmunised women



FORM FRM4674/4 — NHS Blood and Transplant — Effective: draft

INTERNATIONAL BLOOD GROUP REFERENCE LABORATORY

Request for fetal blood group genotyping from maternal blood

Please use block capitals and complete all sections. Please see page 2 for sample and transport requirements

Patient Details (essential details *)	
Surname *	
First name *	
Date of birth *	
Hospital number *	
NHS number (* UK customers only)	
Hospital sample ID *	
Sample date *	
Gestation / EDD *	
Multiple pregnancy *	Yes / No
Ethnic origin of patient	
Blood group of patient	
Ethnic origin of partner	
Blood group of partner	
Known risk of infection?	Yes / No

Maternal Antibodies	Present	Level
Anti-D		
Anti-C (big C)		
Anti-E		
Anti-c (little c)		
Anti-K		

Diagnosis and Clinical History

Test Required		Sample Sent	
RhD (from **16 weeks** gestation)		16ml maternal EDTA blood *(per test requested)*	
RhC (from **16 weeks** gestation)		3ml EDTA blood partner - RhD request only *(Optional)*	
RhE (from **16 weeks** gestation)		**Ship at ambient temperature, to arrive within 48 hours for K typing, other tests within 72 hours of venepuncture**	
Rhc (from **16 weeks** gestation)			
K (Kell) (from **20 weeks** gestation)		Frozen maternal plasma on dry ice *(see INF1291)*	

Requester Details *(destination for report)*

Name		Name of Sender	
Department		Sender telephone number / email *(For NHSBT contact purposes only)*	
Address		Send invoice to: **(This must be provided by non-UK customers)**	
Postcode			
Tel			
Fax			
Email *(For NHSBT contact purposes only)*			

Terms and Conditions

By signing and submitting this Referral Form to NHSBT the Purchaser is acknowledging that the NHSBT Terms and Conditions apply to this Referral. Where the contracting party has a Service Level Agreement with NHSBT which includes the provision of IBGRL services then the Service Level Agreement shall take precedence, and all provisions of that Agreement and subsequent amendments will apply in full

1) NHS Blood and Transplant a Special Health Authority established under SI 2005 No 2529 of 500 North Bristol Park, Filton (NHSBT) and
1) Company Name (as above)

Requester Signature: **Date:**

NHSBT USE ONLY

Hematos Barcode	*Number of samples received:*	
	Date received:	
	Sample ID:	

Page 1 of 2

Send by 1st class post

Please use these labels for IBGRL Molecular Diagnostic samples – **NOT** for fetal *RHD* screening test

FAO: IBGRL Molecular Diagnostics
NHS Blood and Transplant - Filton
500 North Bristol Park, Northway
Filton, Bristol, UK
BS34 7QH

to arrive within – 2 – 3 – 7 days
please circle transfer time

Referring Hospital..... Date.....

Diagnostic Specimen **STORE at room temperature**

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NHS Blood and Transplant - Filton
500 North Bristol Park, Northway
Filton, Bristol, UK
BS34 7QH

to arrive within – 2 – 3 – 7 days
please circle transfer time

Referring Hospital..... Date.....

Diagnostic Specimen **STORE at room temperature**

Turnaround time – 7 working days

https://nhsbtdbe.blob.core.windows.net/umbraco-assets-corp/15885/ibgrl-molecular-diagnostics-turnaround-times.pdf

Referral forms & address labels

NHS Blood and Transplant

Fetal *RHD* screen

Send via NHSBT routine transport

FAO: IBGRL – Fetal RhD Screen
NHS Blood and Transplant - Filton
500 North Bristol Park, Northway
Filton, Bristol
BS34 7QH

ROUTINE

Referring Hospital..... Date.....

Diagnostic Specimen **STORE at room temperature**

FAO: IBGRL – Fetal RhD Screen
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ROUTINE

Referring Hospital..... Date.....

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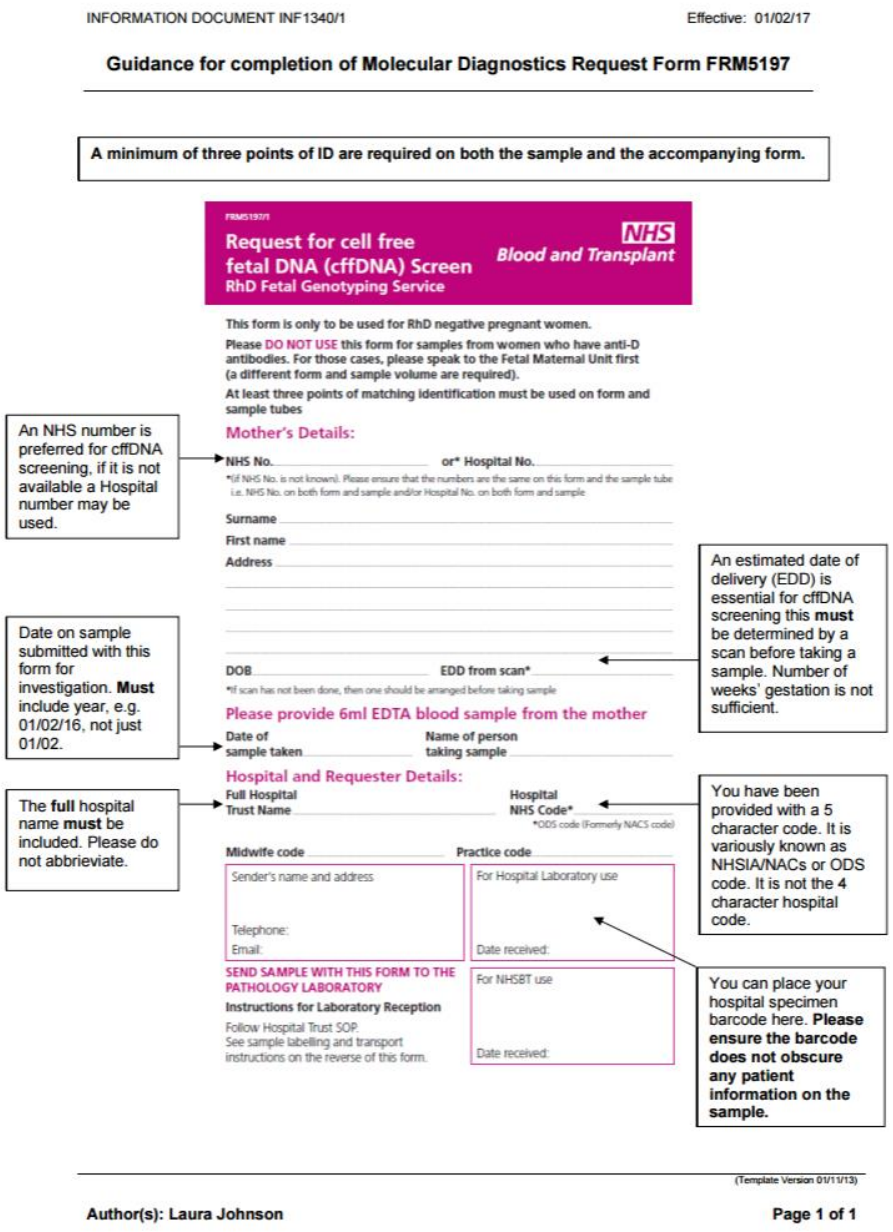

INFORMATION DOCUMENT INF1340/1 Effective: 01/02/17

Guidance for completion of Molecular Diagnostics Request Form FRM5197

A minimum of three points of ID are required on both the sample and the accompanying form.

FRM5197/1

Request for cell free fetal DNA (cffDNA) Screen
RhD Fetal Genotyping Service

NHS Blood and Transplant

This form is only to be used for RhD negative pregnant women.
Please DO NOT USE this form for samples from women who have anti-D antibodies. For those cases, please speak to the Fetal Maternal Unit first (a different form and sample volume are required).
At least three points of matching identification must be used on form and sample tubes

An NHS number is preferred for cffDNA screening, if it is not available a Hospital number may be used.

Mother's Details:

NHS No. or* Hospital No.
*(if NHS No. is not known). Please ensure that the numbers are the same on this form and the sample tube i.e. NHS No. on both form and sample and/or Hospital No. on both form and sample

Surname
First name
Address

DOB EDD from scan*
*If scan has not been done, then one should be arranged before taking sample

An estimated date of delivery (EDD) is essential for cffDNA screening this **must** be determined by a scan before taking a sample. Number of weeks' gestation is not sufficient.

Date on sample submitted with this form for investigation. **Must** include year, e.g. 01/02/16, not just 01/02.

Please provide 6ml EDTA blood sample from the mother

Date of sample taken Name of person taking sample

Hospital and Requester Details:

The **full** hospital name **must** be included. Please do not abbreviate.

Full Hospital Trust Name Hospital NHS Code*
*ODS code (Formerly NACS code)

You have been provided with a 5 character code. It is variously known as NHSIA/NACs or ODS code. It is not the 4 character hospital code.

Midwife code Practice code

Sender's name and address
Telephone:
Email:

For Hospital Laboratory use
Date received:

SEND SAMPLE WITH THIS FORM TO THE PATHOLOGY LABORATORY

Instructions for Laboratory Reception
Follow Hospital Trust SOP.
See sample labelling and transport instructions on the reverse of this form.

For NHSBT use
Date received:

You can place your hospital specimen barcode here. **Please ensure the barcode does not obscure any patient information on the sample.**

(Template Version 01/11/13)

Author(s): Laura Johnson

Page 1 of 1

Turnaround time – 10 working days

https://nhsbtdbe.blob.core.windows.net/umbraco-assets-corp/15885/ibgrl-molecular-diagnostics-turnaround-times.pdf

NHS
Blood and Transplant

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NHS
Blood and Transplant

Any questions