

Fetal Genotyping



Blood and Transplant

Optimising antenatal care



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Caring Expert Quality

Fetal Genotyping

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

Fetal Genotyping: Why?

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

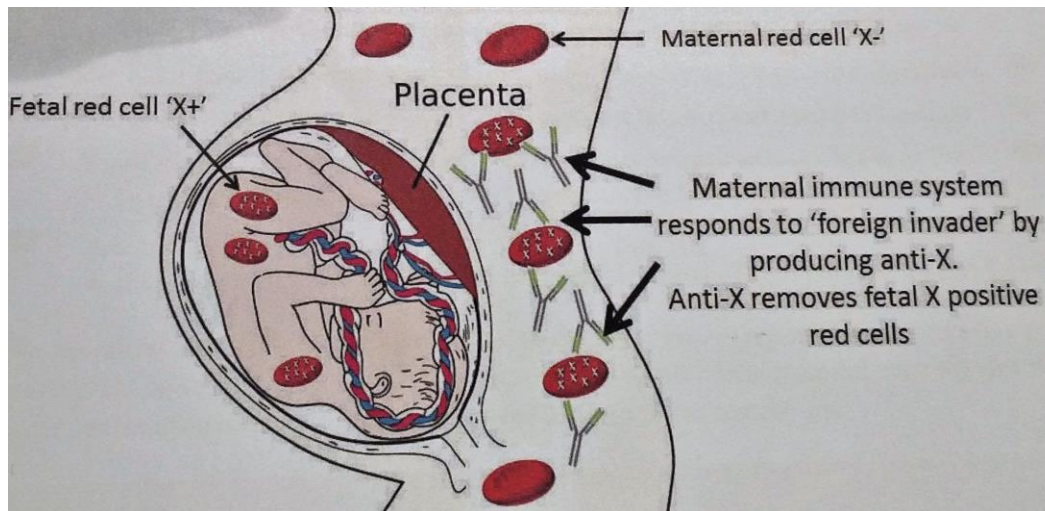


Image: Qureshi, R (2015) *Introduction to Transfusion Science Practice*, British Blood Transfusion Society, 6th Edition.

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

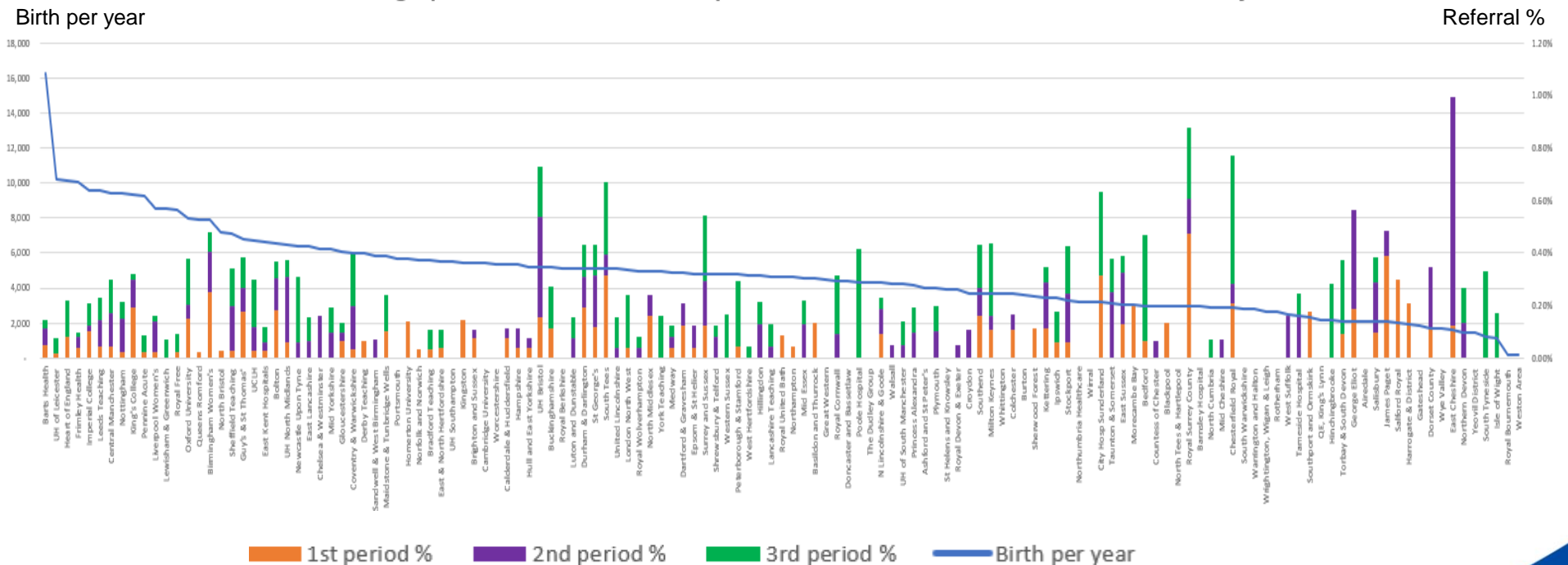
Referrals rate for fetal genotyping



Blood and Transplant

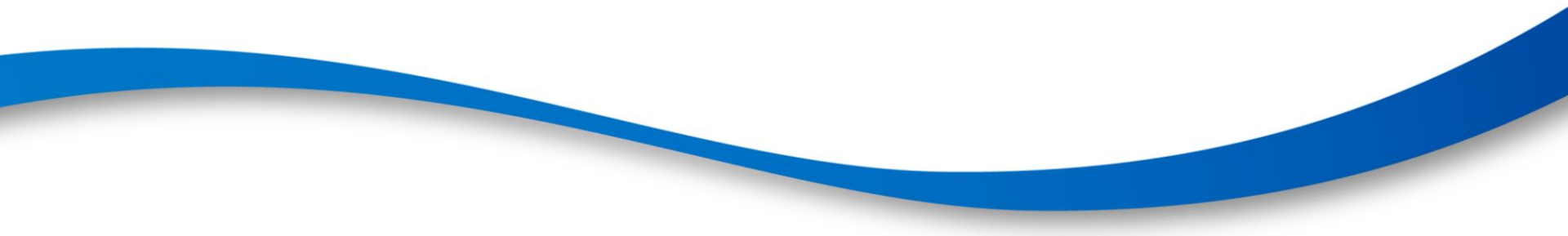
Although standard of care in England for women with antibodies,
Not all NHS Trusts have implemented best practice

Low Throughput in 6 month comparisons from November 2016 - July 2018

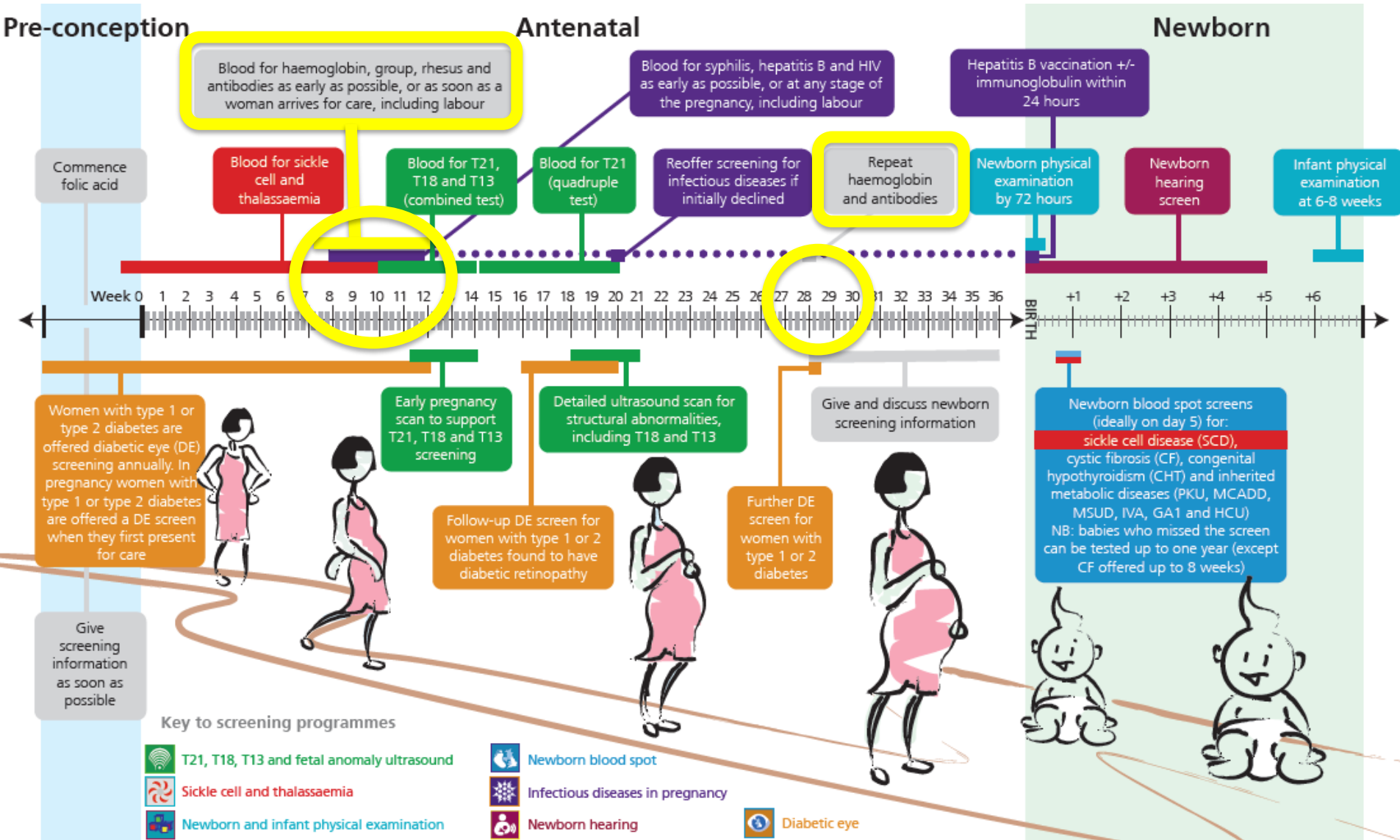


Fetal *RHD* screen

- 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



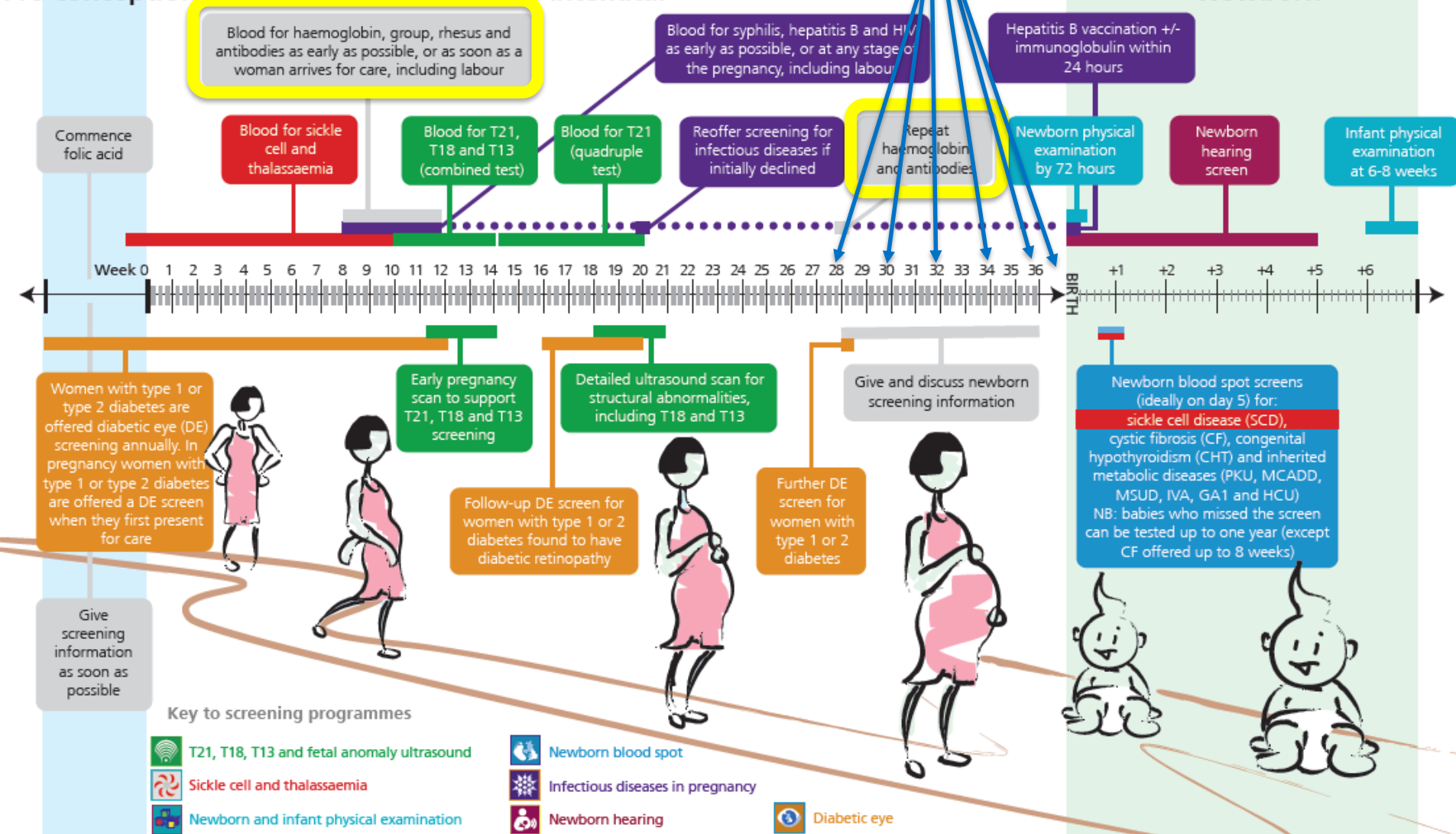
Antenatal and Newborn Screening Timeline - optimum times for testing

Maternity Care Pathway

Pre-conception

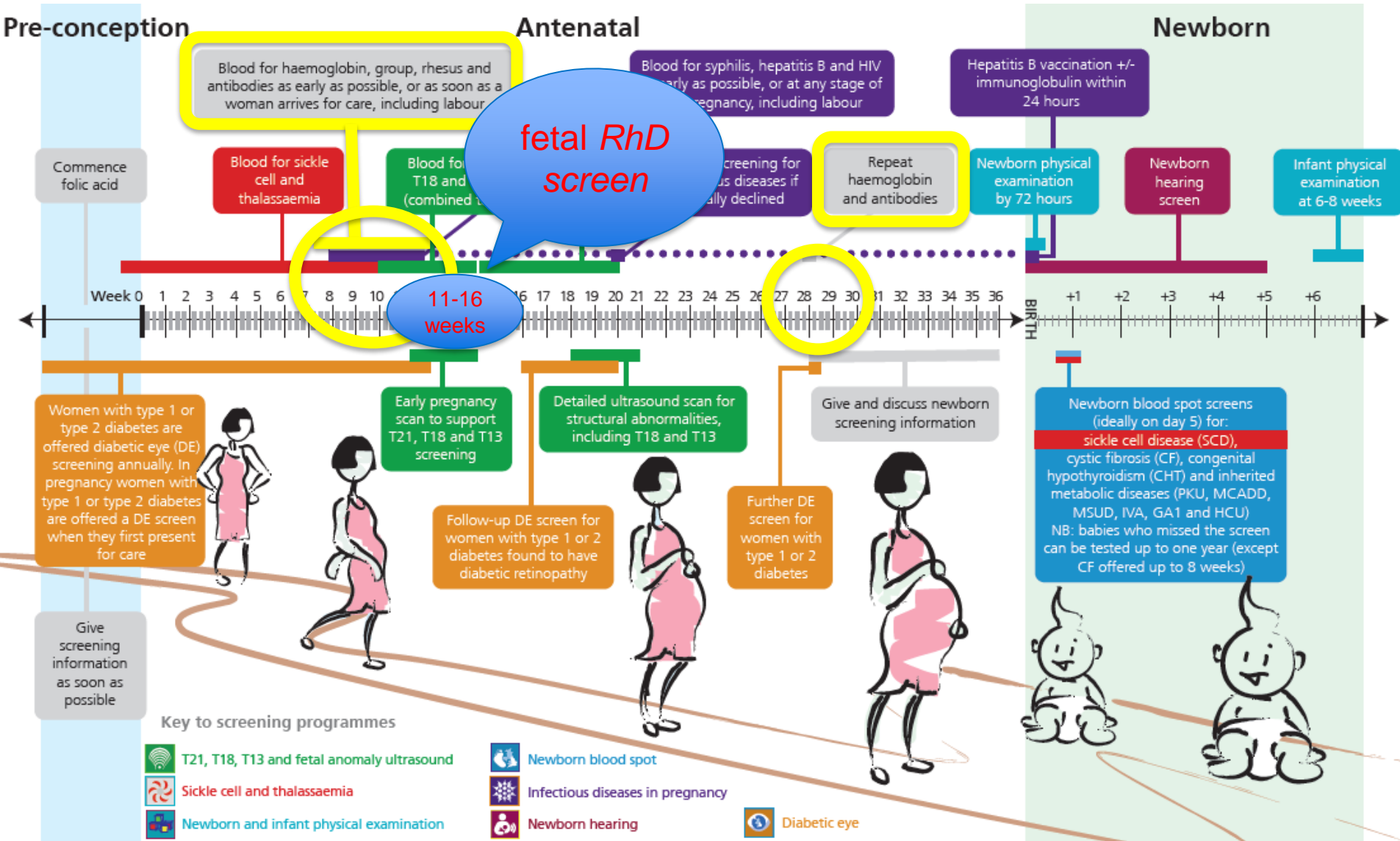
Antenatal

Newborn



Antenatal and Newborn Screening Timeline - optimum times for testing

Maternity Care Pathway



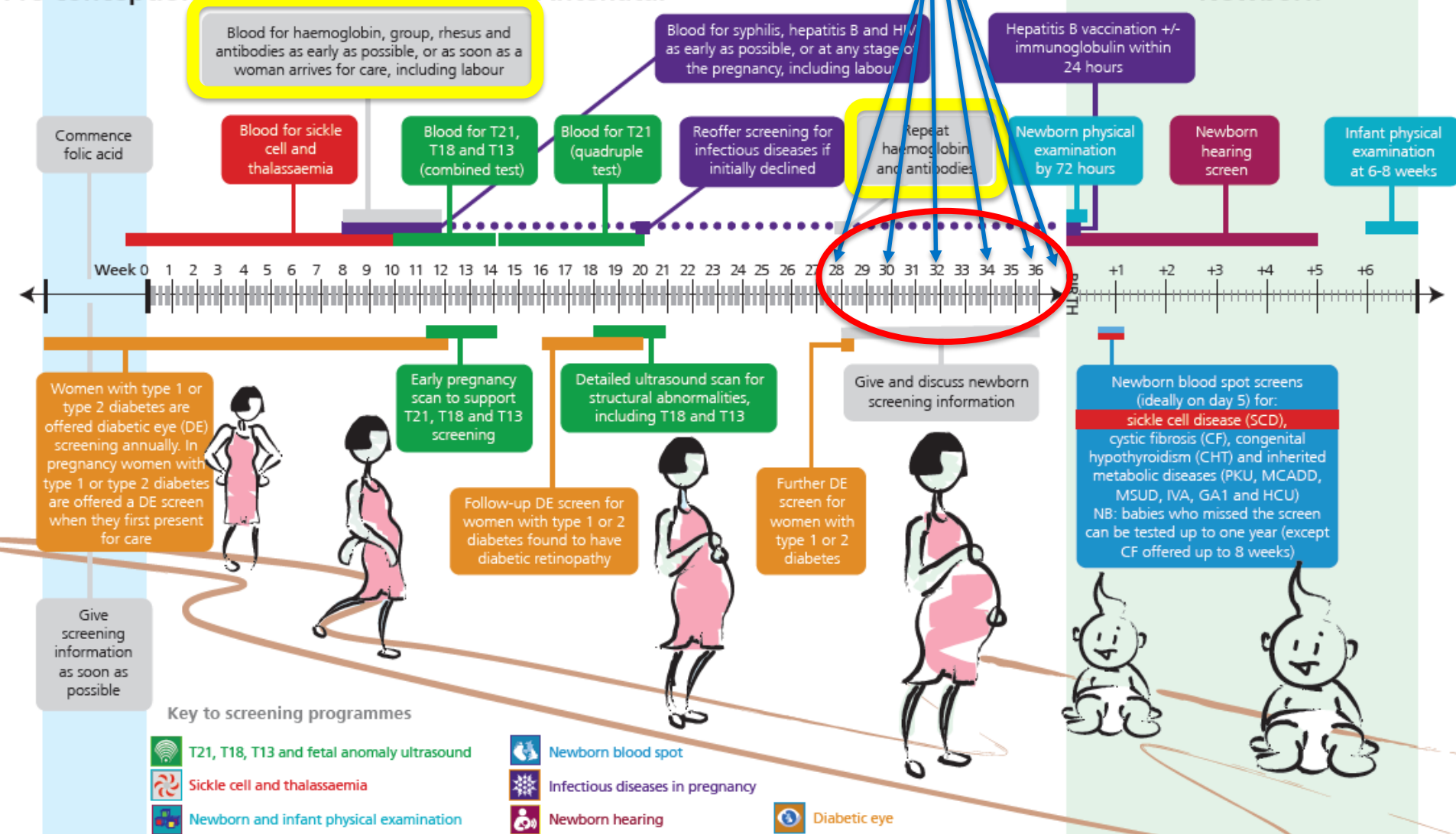
Antenatal and Newborn Screening Timeline - optimum times for testing

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Antenatal and Newborn Screening Timeline - optimum times for testing

Laboratory tests

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

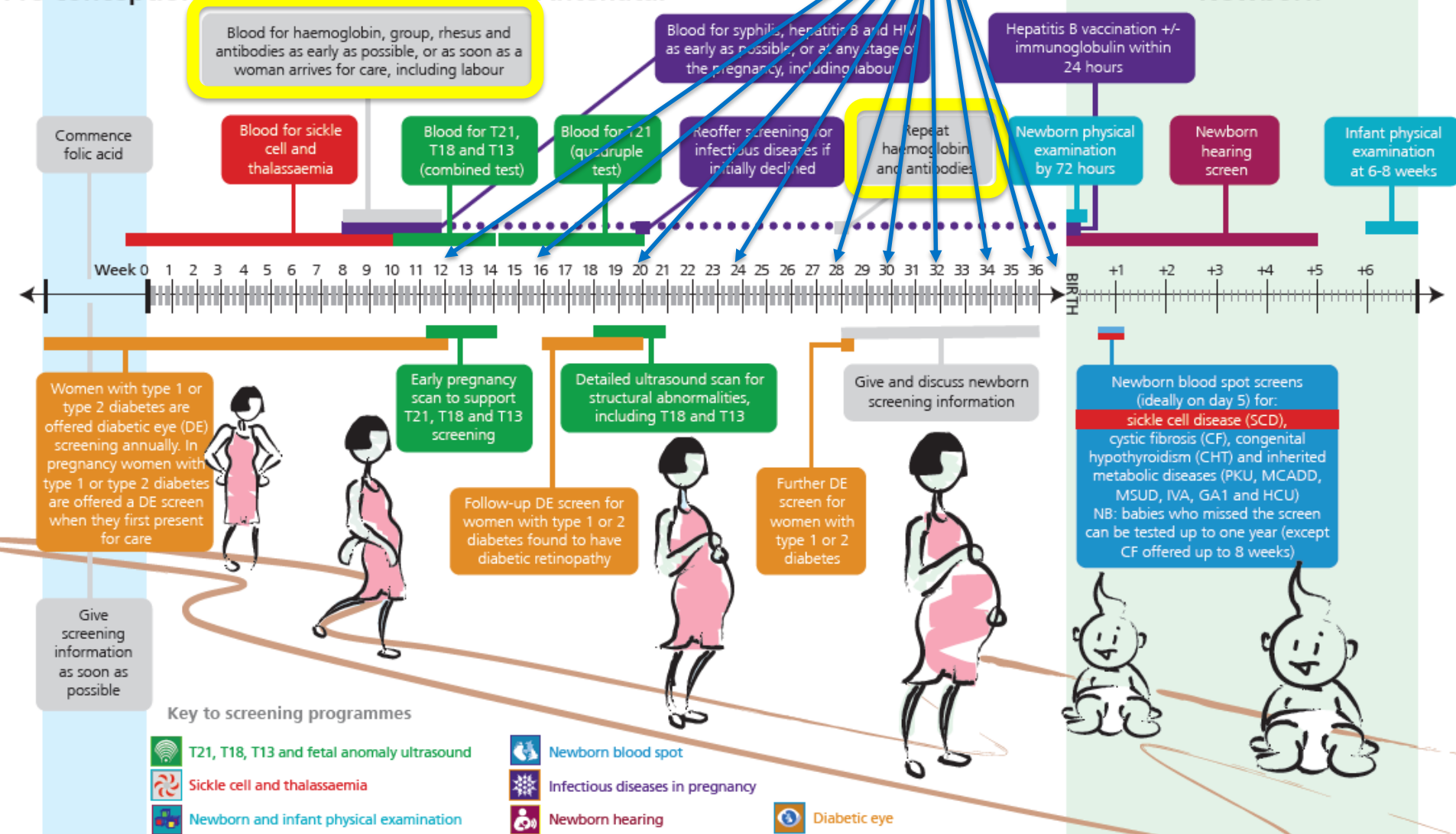
Exchange transfusion - intrauterine or post natal

Maternity Care Pathway

Pre-conception

Antenatal

Newborn



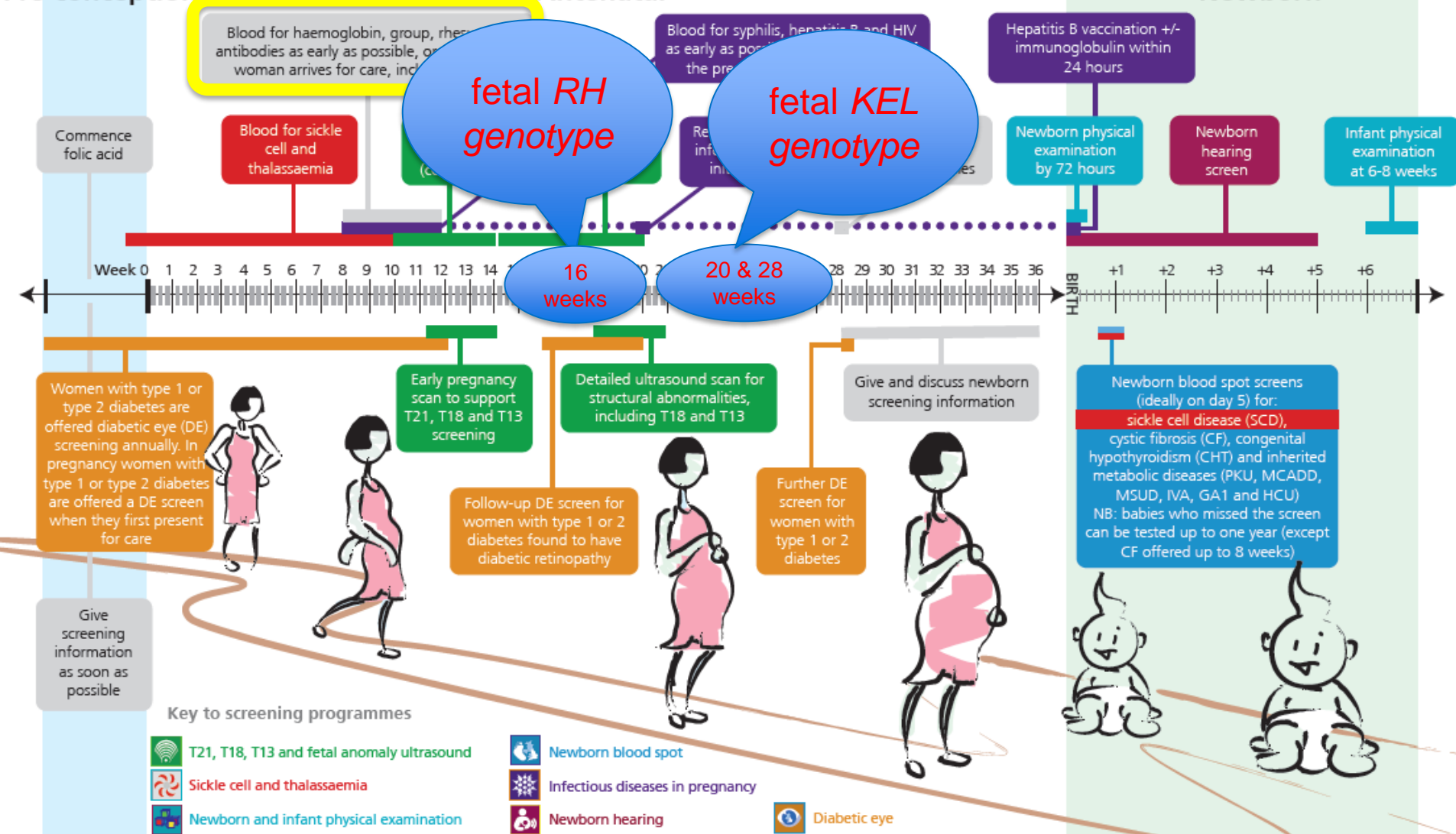
Antenatal and Newborn Screening Timeline - optimum times for testing

Maternity Care Pathway

Pre-conception

Antenatal

Newborn



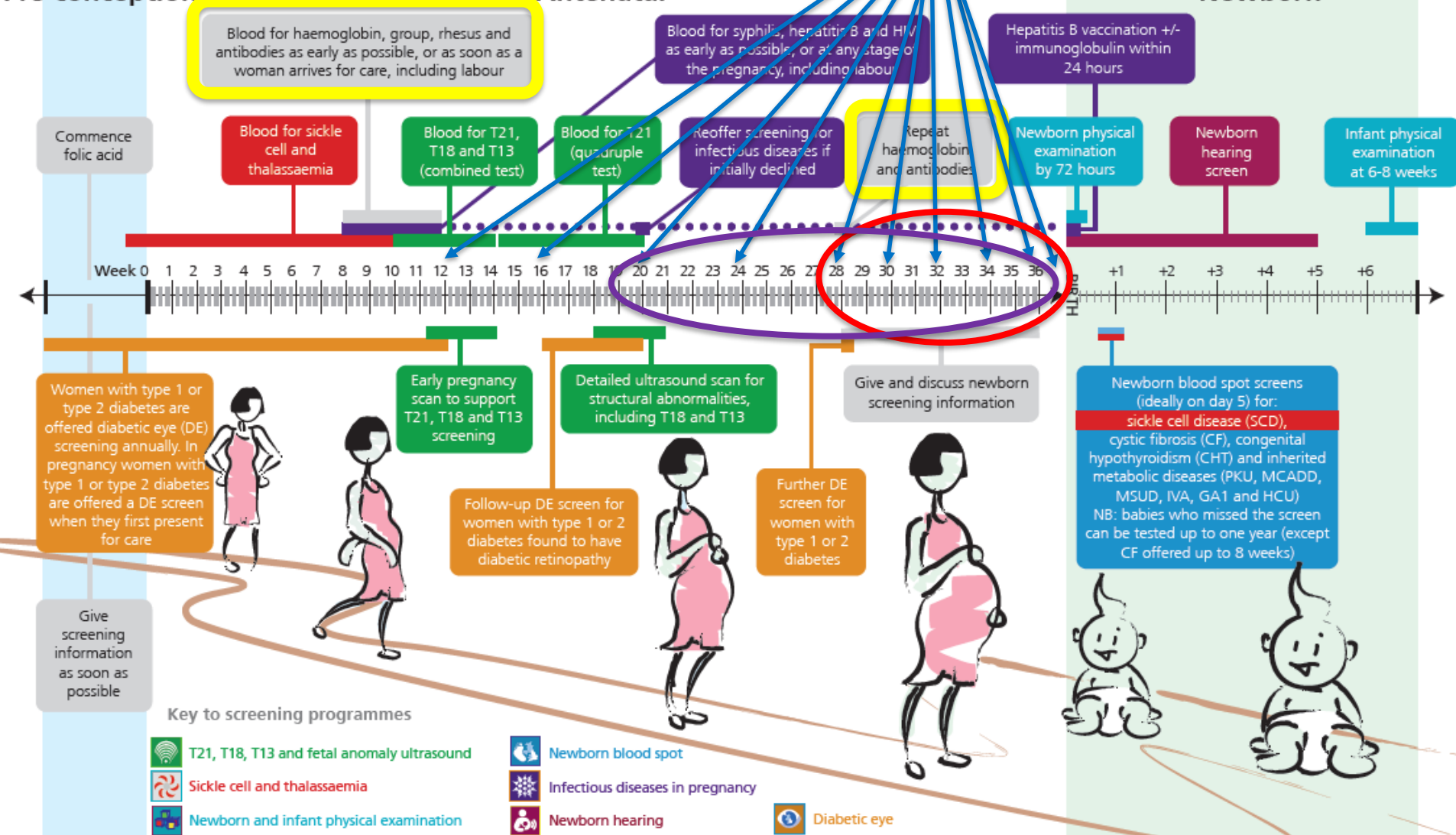
Antenatal and Newborn Screening Timeline - optimum times for testing

Maternity Care Pathway

Pre-conception

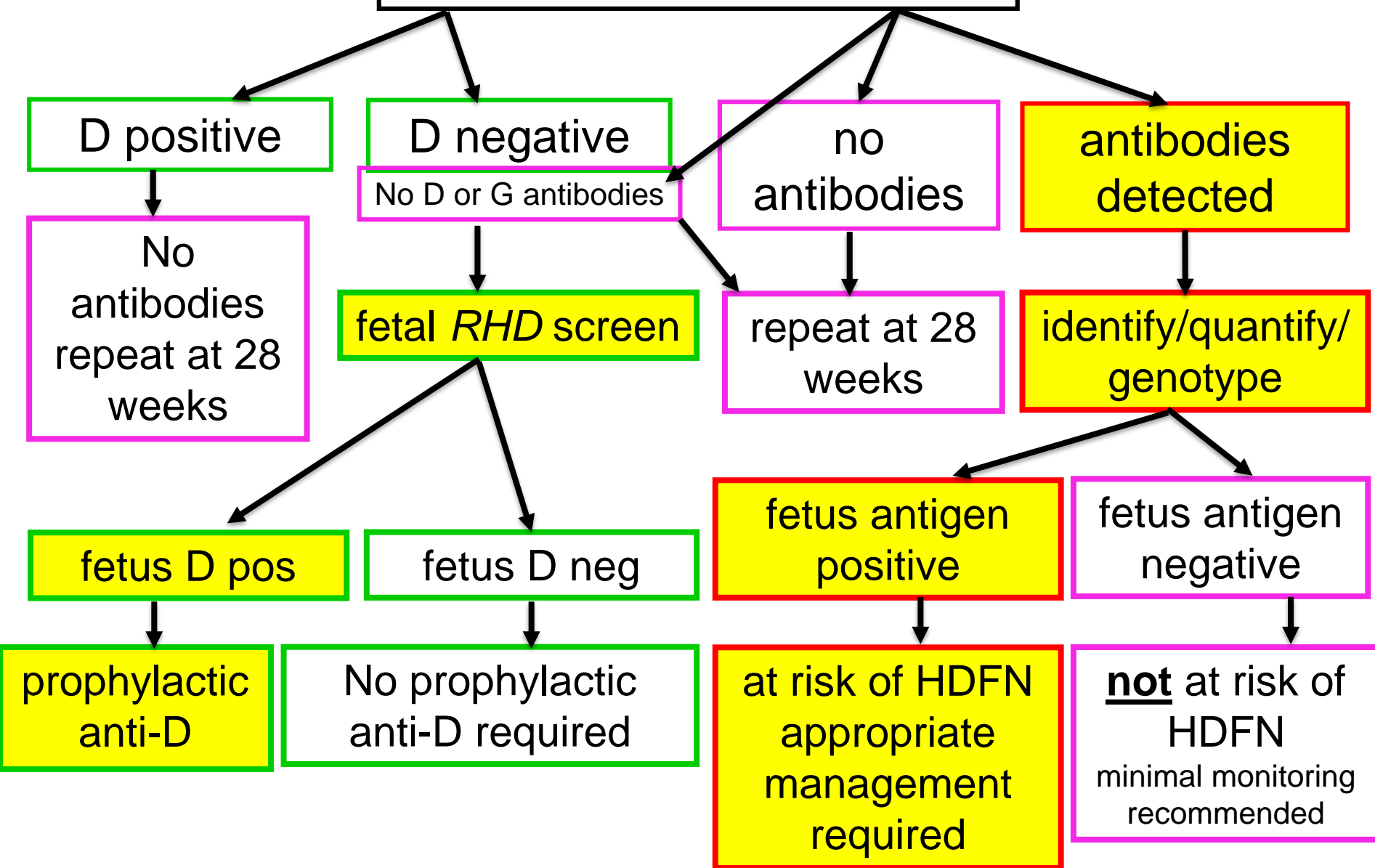
Antenatal

Newborn



Antenatal and Newborn Screening Timeline - optimum times for testing

Rh and Ab Screening



Cell free fetal DNA from maternal plasma

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

10–20 weeks:

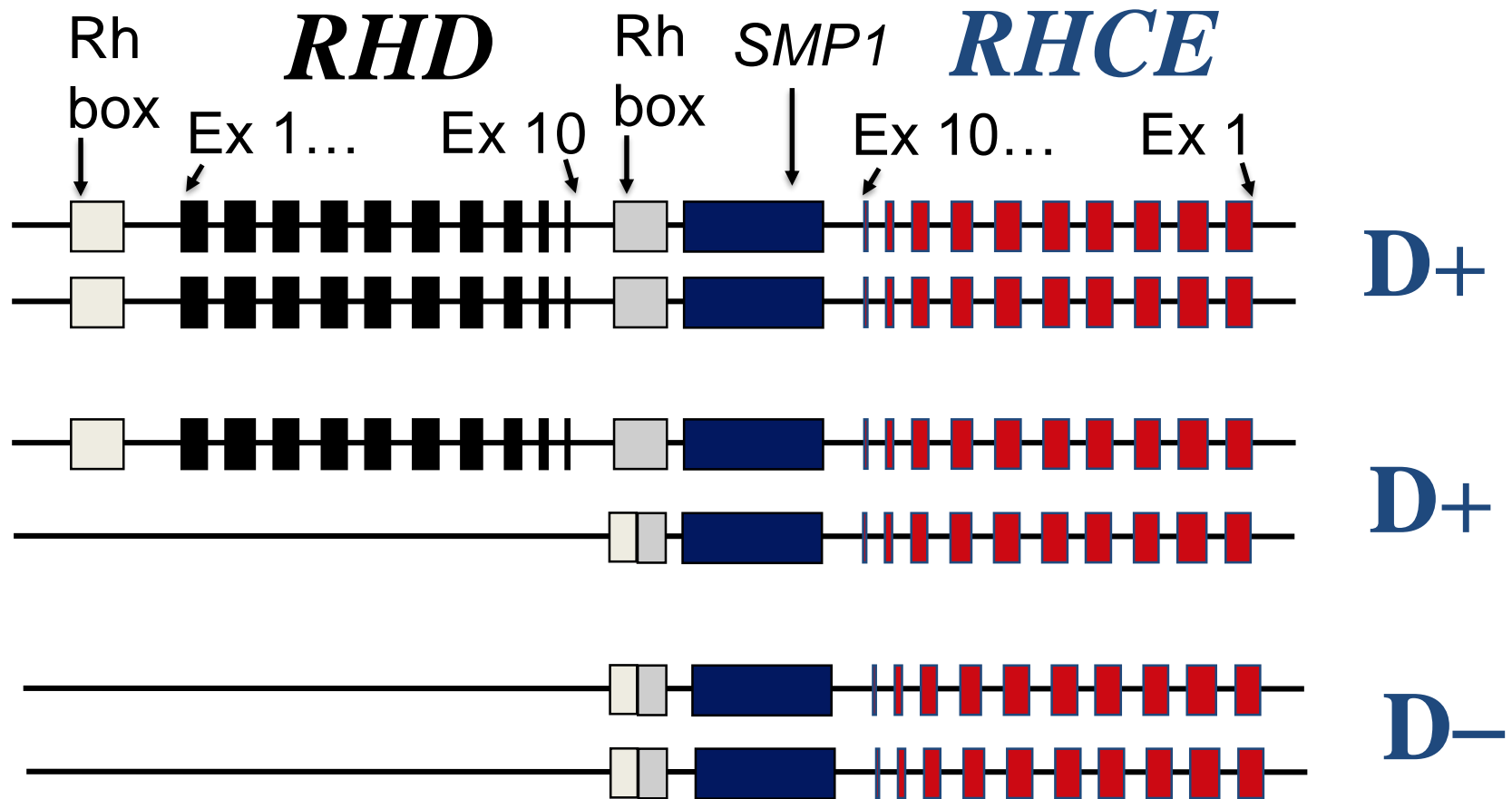
85-90% is maternal DNA
but antigen-negative for the antibody she has

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

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?

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 *RHD* Ψ

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

Alloimmunised women

RHD exons 4, 5, 7, 10

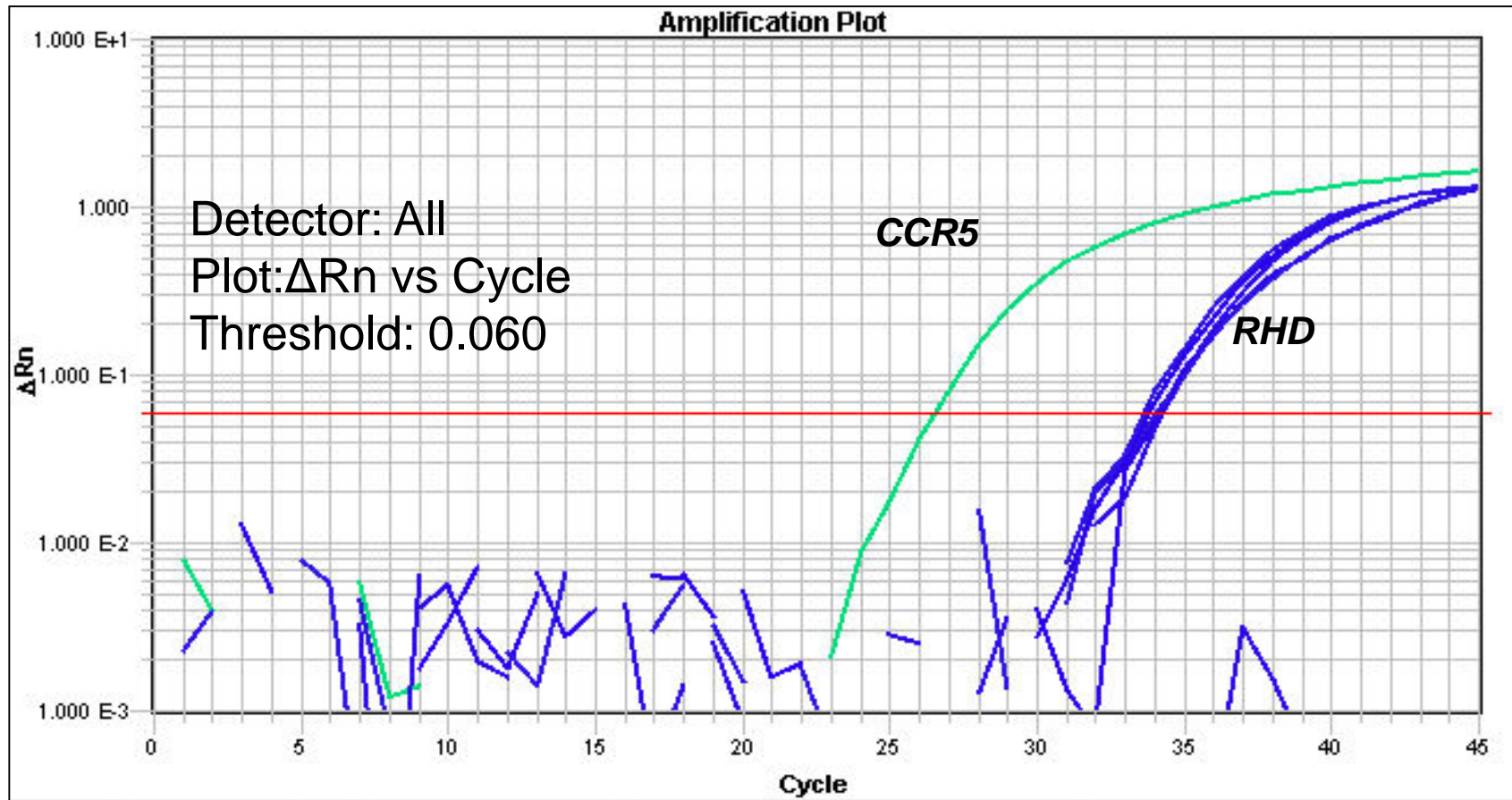
Manual extraction, Real-time Quantitative PCR

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

Fetal *RHD* screen

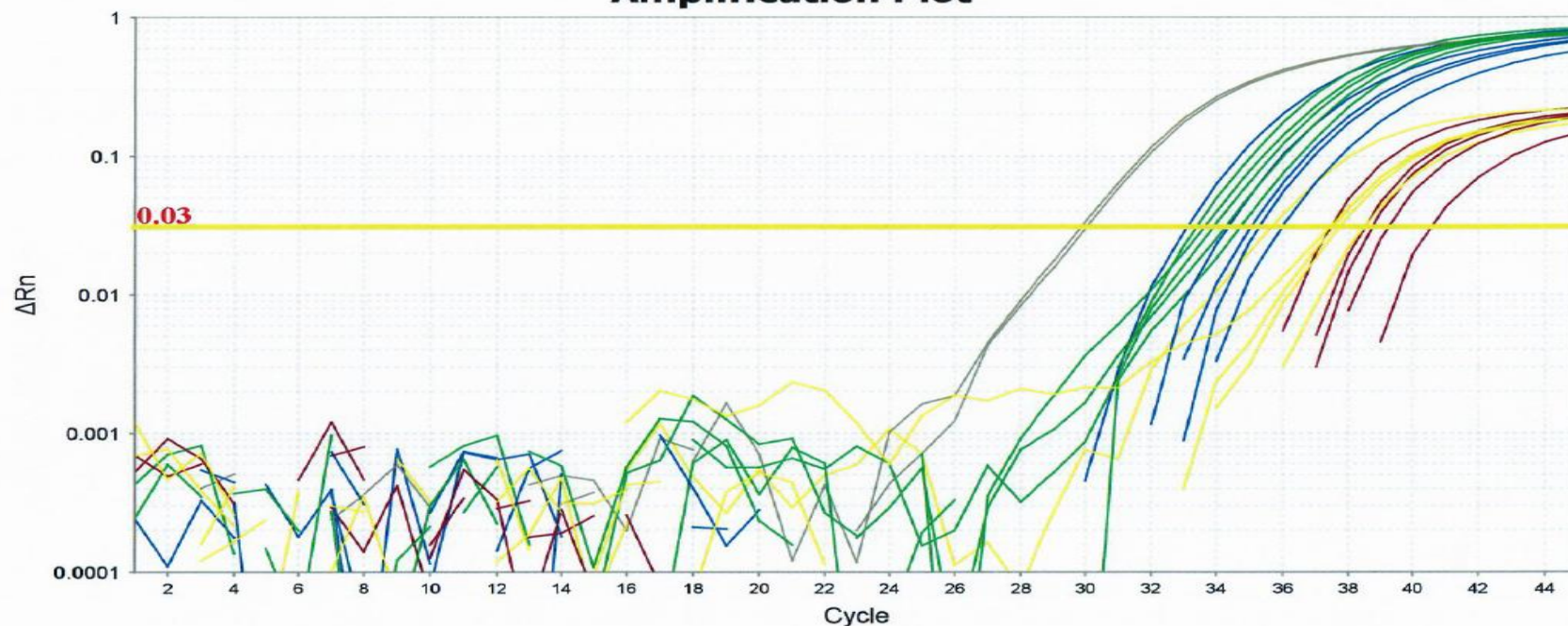
DNA is extracted robotically and amplified by real-time PCR.

CCR5 used to confirm successful extraction



Fetal genotype diagnostic

Amplification Plot



Legend

EXON7 CCR5 EXON4 EXON5 EXON10

NHS
Blood and Transplant



Sensitivity & Specificity

| Result | <i>RHD</i> Screening Test (High sensitivity) | <i>RHD</i> 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

Fetal *RHD* screen

$\leq 0.1\%$ for false negative predictions

Fetal D genotype

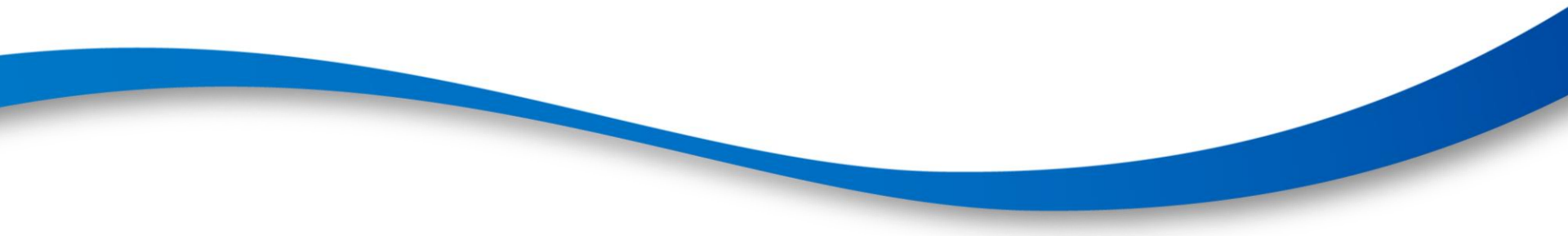
sensitivity of 99.8% and specificity of 99.2%
2514 tests – 5 false pos / 2 false neg

Fetal C, c, E genotype

we have not been informed of any false results

Fetal K genotype

$\leq 0.5\%$ for false negative predictions

- 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
- 
- A thick blue wavy line that curves across the bottom of the slide, starting from the left edge and ending at the right edge.

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



Blood and Transplant

Fetal genotyping for alloimmunised women

Send by 1st class post

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 <small>(* UK customers only)</small> | |
| 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 IVF1221) |

| 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 is 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: |

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

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

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

Fetal *RHD* screen

INFORMATION DOCUMENT INF1340/1

Effective: 01/02/17

Guidance for completion of Molecular Diagnostics Request Form FRM5197

Send via NHSBT routine transport

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

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
NHS Blood and Transplant - Filton

500 North Bristol Park, Northway
Filton, Bristol
BS34 7QH

ROUTINE

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

Diagnostic Specimen STORE at room temperature

FRM5197/1

Request for cell free fetal DNA (cffDNA) Screen **Blood and Transplant**
RhD Fetal Genotyping Service

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

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 _____

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

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

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

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.

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.

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

Please provide 6ml EDTA blood sample from the mother

Date of sample taken _____ Name of person taking sample _____

Hospital and Requester Details:

Full Hospital Trust Name _____ Hospital NHS Code* _____

*ODS code (Formerly NACs code)

Midwife code _____ Practice code _____

Sender's name and address _____ For Hospital Laboratory use

Telephone: _____ Date received: _____

Email: _____ For NHSBT 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.

Turnaround time – 10 working days

<https://nhsbtdeb.blob.core.windows.net/umbraco-assets-corp/15885/ibgri-molecular-diagnostics-turnaround-times.pdf>





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Any questions

