Fetal Genotyping

NHS Blood and Transplant

Optimising antenatal care

Chris Elliott
Pathology Assistant Director: Business Development and Transformation

Caring Expert Quality

Fetal genotyping tests

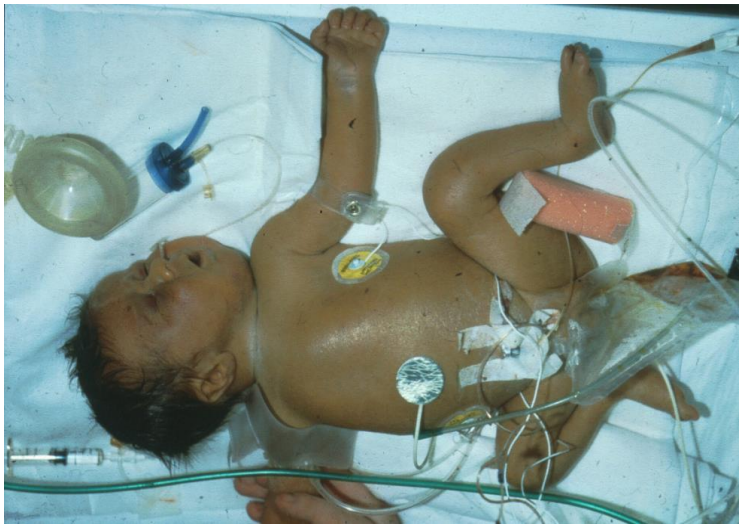
- Background info
- Why are we doing this test?
- Science and test design
- Ethics and benefits
- Test requirements
- Reasons for rejected samples
- Contact details

Caring Expert Quality

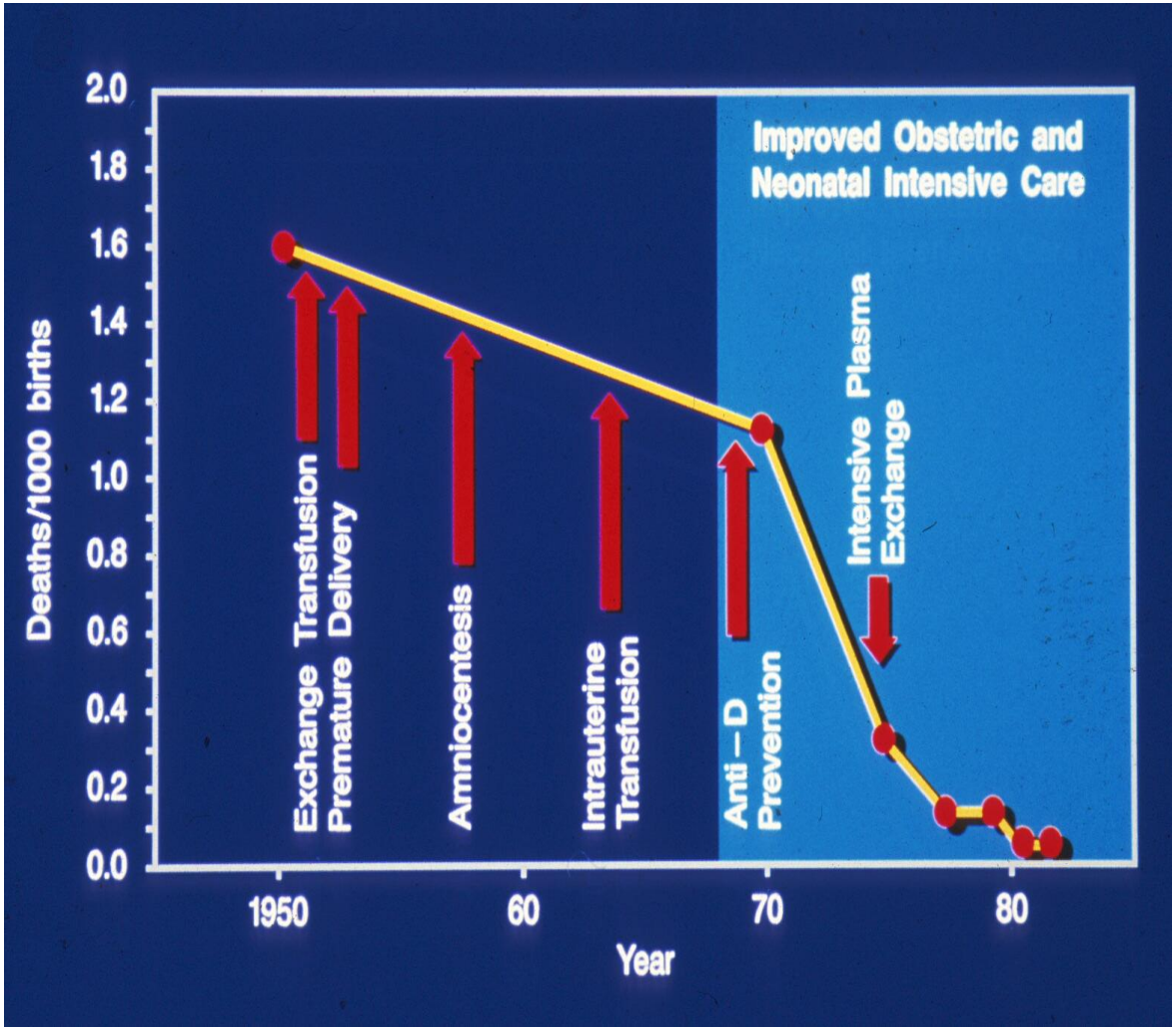
Haemolytic Disease of Foetus/Newborn (HDFN)

Maternal antibodies to red cells can cross placenta and affect the developing foetus

Most common antibody found causing problems is anti D



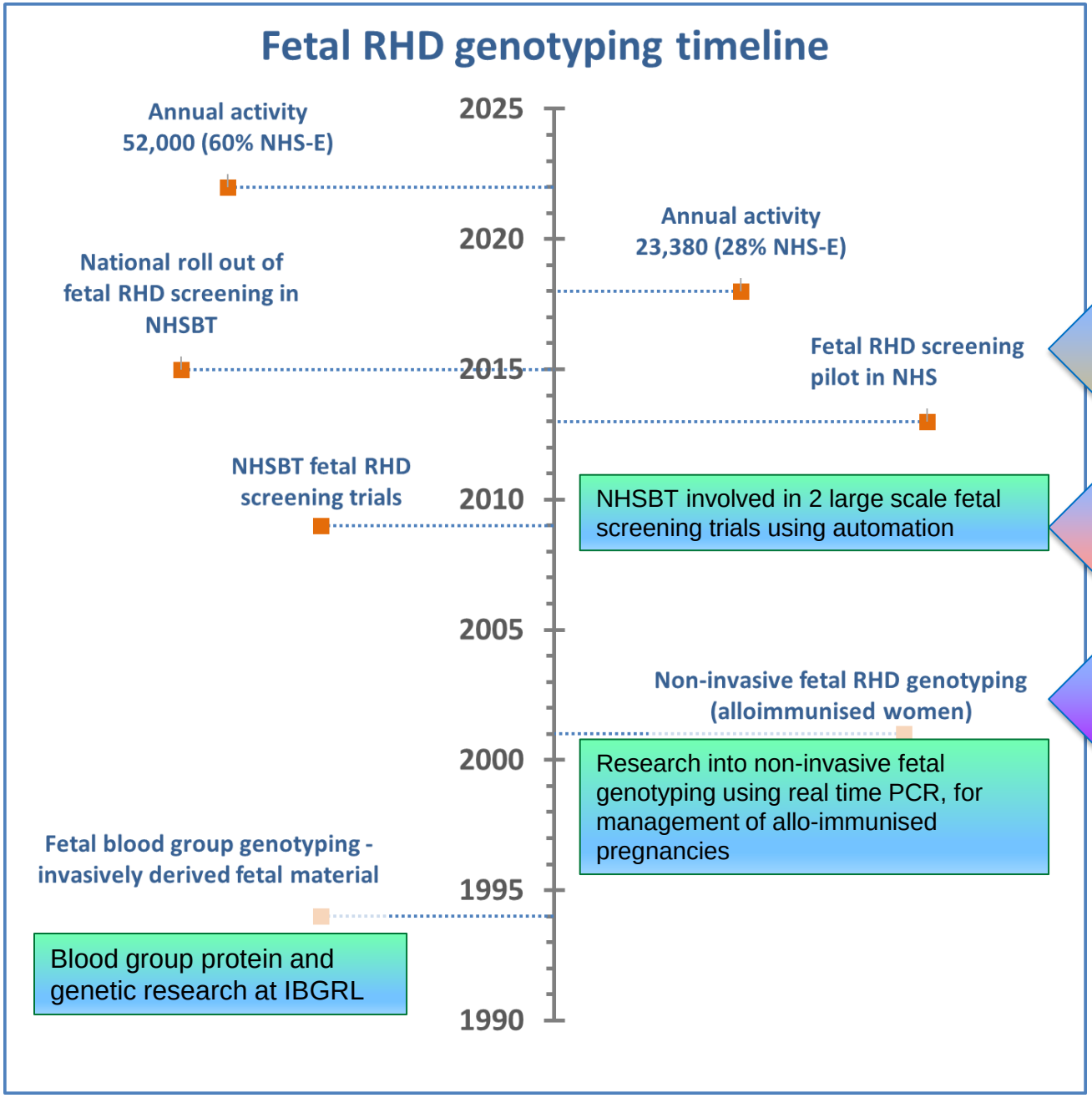
Introduction of Anti D prophylaxis



Routine inject of prophylactic anti D found to supress maternal sensitisation and prevent HDFN

Introduced at delivery in late 60s then extended to ante natal care in early 80s to all Rh D negative pregnancies

Historical timeline



NHS
Blood and Transplant

NICE endorse fetal *RHD* screening November 2016

NHS Blood and Transplant

Home › NICE Guidance › Conditions and diseases › Fertility, pregnancy and childbirth › Pregnancy

High-throughput non-invasive prenatal testing for fetal RHD genotype

Diagnostics guidance [DG25] Published: 09 November 2016

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, provided that the overall cost of testing is £24 or less. This will help reduce unnecessary use of a blood product in pregnant women, and conserve supplies by only using anti-D immunoglobulin for those who need it.

Cost savings associated with high-throughput NIPT for fetal *RHD* genotype are sensitive to the unit cost of the test, additional pathway costs and implementation costs. Trusts adopting NIPT should collect and monitor the costs and resource use associated with implementing testing to ensure that cost savings are achieved (see section 6.1).

NICE reviewed the evidence in April 2021 and found nothing new that affects the recommendations in this guidance.
www.nice.org.uk/guidance/dg25

Increases in cost since 2016

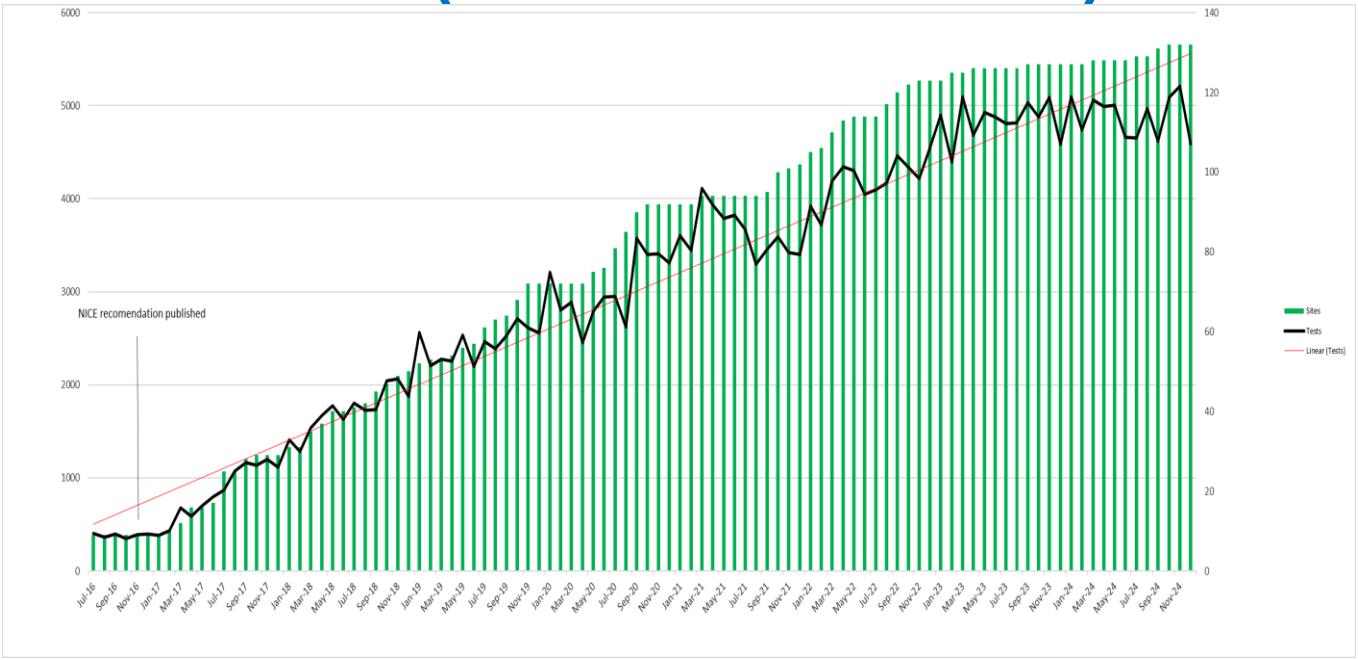
NHS Blood and Transplant

Year	Fetal *RHD* screening test Price	Anti-D Ig price
2015/16	£19.58	£38.00
2016/17	£19.58	£38.00
2017/18	£19.58	£39.00
2018/19	£21.50	£39.00
2019/20	£21.50	£40.63
2020/21	£22.50	£40.63
2021/22	£22.50	£42.89
2022/23	£26.60	£56.10
2023/24	£27.60	£63.49
2024/25	£28.68	£63.49

- Cost of Fetal *RHD* screening has increased by £9.10 (46.4%) over last 10 years
- Cost of prophylactic anti D has increased by £27.49 (72.3%) over the last 10 years

Progressive adoption of fetal RhD screening by English hospitals over last 6 years with c58,600 tests in 23-24 (out of 72.5k maximum)

NHS Blood and Transplant



Test Results- All hospitals	April 2017 - December 2022
Positive	55.6%
Negative	34.8%
Inconclusive	4.6%
Not tested	5.1%

So c35% of Rh negative women are receiving more personalised medical care as a result of testing

- The sample referrals have increased progressive to ~5,100 samples per month with only 19 English hospital sites not testing or referring work.
- **Lab using Continuous Improvement to help us scale up this new test over the last 5 years!**
- NHSBT currently test over 80% of English eligible pregnancies and are aiming for ~90% English Rh D negative pregnancies in the next 12 months.
- Both Scotland and Northern Ireland are asking NHSBT to take on their work, Wales are developing their own in-house testing

Fetal Genotyping: Why?

NHS Blood and Transplant

Optimising appropriate antenatal care

Introduction of the **fetal *RHD* screening test** for mothers without D or G antibodies

- Giving prophylactic anti-D Ig only to those women who need it

The **Fetal genotype diagnostic test** for mothers with antibodies

- 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)
- Anti-D, c, C, E, K are the main antibodies who cause HDFN (others rare)

Accuracy

NHS Blood and Transplant

Fetal *RHD* screen

≤0.1% for false negative predictions (currently 0.046%) or less than 1 in every 2,000 Rh D neg births

Fetal D genotype

sensitivity of 99.8% and specificity of 99.2%
2,514 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

Difference between the two tests

NHS Blood and Transplant

RHD screening to determine requirement for antenatal anti-D

Automated test

RHD exons 5 & 7

Low resolution test – 3 possible results: positive, negative or inconclusive (treat as D positive)

Designed to minimise false negative results

Allo-immunised women to determine obstetric care during pregnancy

Manual test

RHD exons 4, 5, 7, 10

Higher resolution test, may detect some D variants

Designed to minimise both false negative and false positive results

Rh typing 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 week 16-22

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

Caring Expert Quality

Ethics and benefits for mothers with antibodies

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 or titration

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 – appropriate treatment
Samples will be taken at the time when women are being seen 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
Reduce referrals for antibody quantification
Reduce Fetal maternal Haemorrhage (FMH) measurement for mothers having repeated PV bleeds
Reduce need for cord bloods or bleeding neonates

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

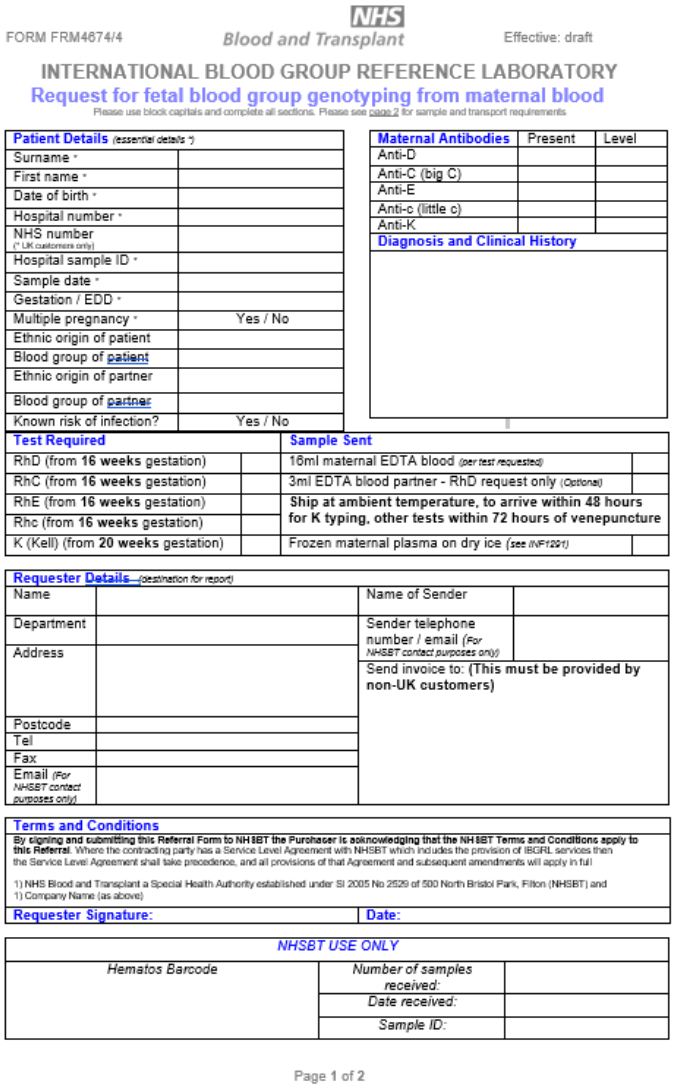
Sample volume:
6mL EDTA

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**

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

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

ROUTINE

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

Diagnostic Specimen **STORE at room temperature**

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

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

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
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:

(Template Version 01/11/13)

Author(s): Laura Johnson **Page 1 of 1**

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 abbrieviate.

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.**

Turnaround time – 10 working days

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

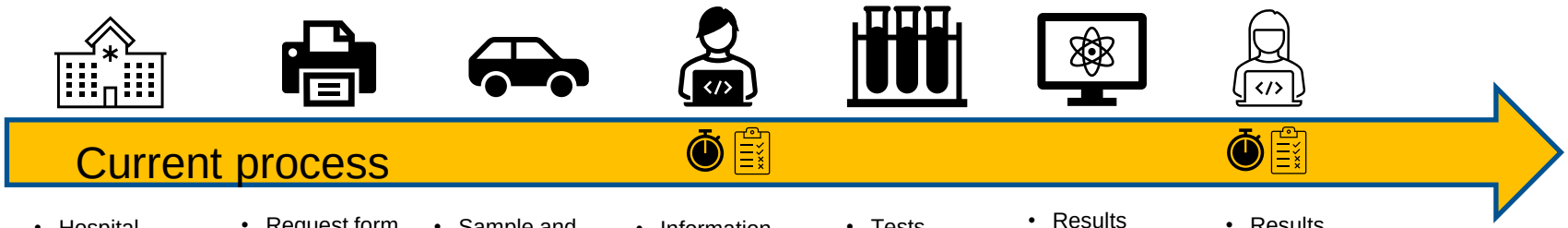
RHD screening sample criteria and service

NHS Blood and Transplant

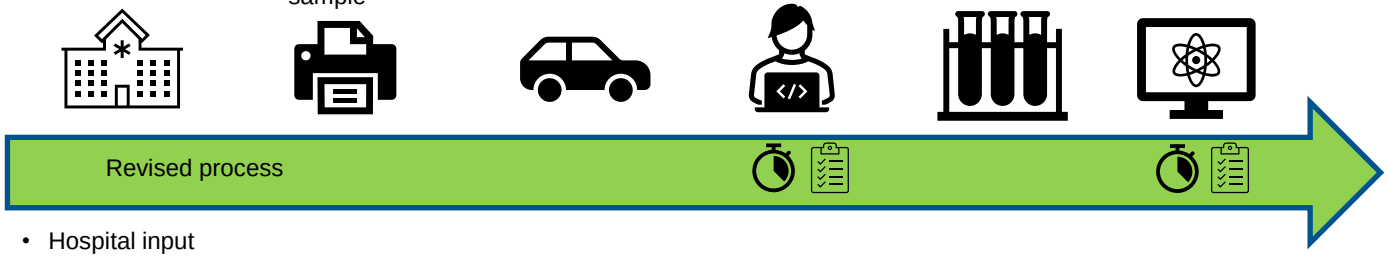
- Sample acceptance criteria
  - ≥11+2 weeks gestation
  - **Must provide EDD** from dating scan (key identifier of the fetus)
  - Sample tested within 7 days of venepuncture
  - Mother **must not have anti-D or –G** or historical record of these antibodies. (A different "diagnostic" test is available).
- Service features
  - Sample taken at routine antenatal visit
  - Sample storage and transport at ambient temperature (via NHSBT transport network)
  - Inconclusive results not re-tested (4-5% inconclusive)
  - Electronic reporting via Sp-ICE (consider giving Drs and midwives access)
  - High accuracy: false negative rate <0.05%
  - Only investigate false negative results (not false positives)
  - **New role out for electronic requesting and reporting – now live for Clinisys Winpath Enterprise LIMS users and also for EPIC EPR users. Currently 23 sites live for electronic requesting/reporting (as of Jan 25)**

Electronic requesting and reporting of fetal RHD screening test

NHS Blood and Transplant



- Hospital input information into LIMS
- Request form completed/ printed
- Included in bag with sample
- Sample and paper request form transported to Bristol
- Information cross-checked and manually typed in
- Tests carried out
- Results reported back to hospitals via SPICE
- Results transferred from SPICE to hospitals LIMS



- Hospital input information into LIMS/EPR
- Information received by Labgnostic (formerly known as NPeX)
- **Shipping manifest completed/ printed**
- Sample and **shipping manifest** transported to Bristol
- Information cross-checked
- **Barcode scanned in from shipping manifest and sample as part of booking in**
- Tests carried out
- **Results reported back directly into NHSBT LIMS and into hospital LIMS/EPR via Labgnostic**

Benefits

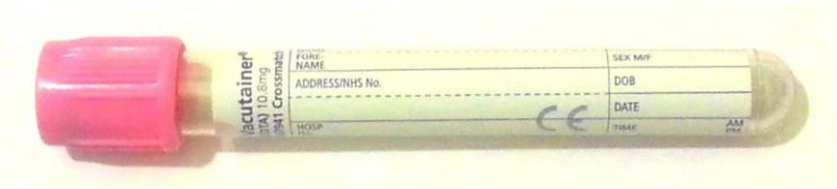
- Reduction in transcription errors
- Savings in lab processing time

Requirements:

- Estimated date of delivery needs to be included in HL7 message
- Date format YYYY/MM/DD
- Labgnostic middleware available in transfusion lab

NHS
Blood and Transplant

Fetal *RHD* screen: One 6ml EDTA sample should be collected and sent

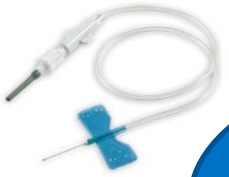


Documentation of the test result should be made in the maternity record

Please Note: women with antibodies must have 16mls blood sample sending to the lab

NHS
Blood and Transplant

Practice Points



Butterfly needles can be used if it is a 'closed' system

EDD on request form must be taken from the dating scan

Send blood to local lab and they will forward to NHSBT for processing

Blood sample have to go directly into closed containers (vacutainers) not drawn from a needle and syringe

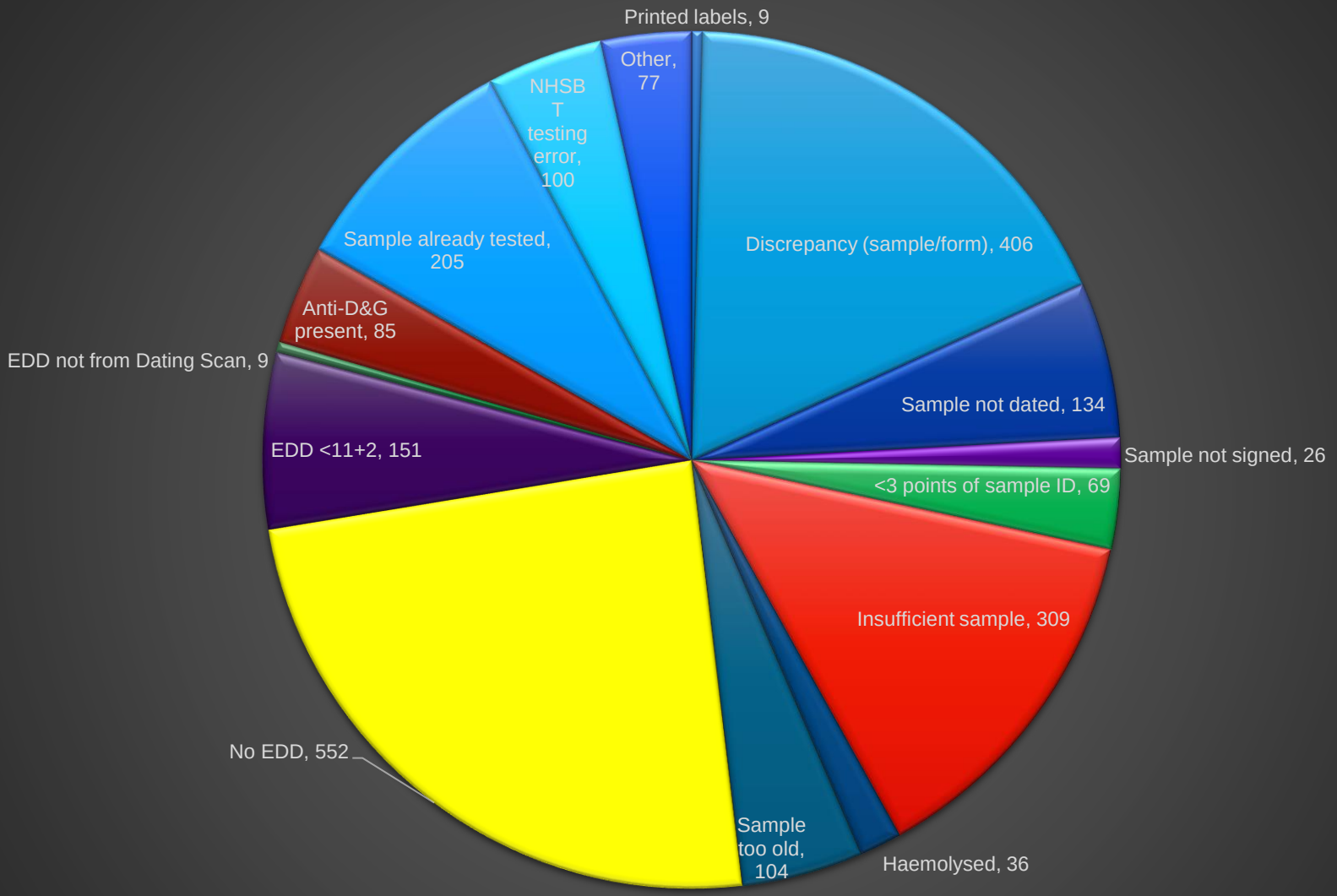
Samples must be room temperature and not put in the fridge

Each rejected sample costs the Trust £13.94. Samples are mainly rejected because:

- EDD has not been put on the form
- Incorrect demographics entered on the blood form

Routine anti-D prophylaxis is offered at 28 weeks to those women who have a predicted fetal D positive or inconclusive result

Not tested by category Oct 2020-Dec 2021



Why should fetal screening samples arrive as early as possible?

NHS Blood and Transplant

Although samples have to reach Filton within 7 days of venepuncture, Molecular Diagnostics would appreciate if they could receive the samples as fresh as possible.

Molecular Diagnostics streamlines their processes in order to keep the test price as low as possible.

A “fast track” process for day 7 samples increases test complexity and drives up cost.

Possible reasons for delay:

1. Samples from the community
2. NHSBT logistics
    - Transport route (via several centres)
    - Number of deliveries to Trusts

Please send samples every day!
We work on Saturdays!

Caring Expert Quality

NHS
Blood and Transplant

Just a few final notes:

1. The EDD is the **only** identifier for this fetus and this pregnancy! Please ensure that you check the EDD when looking up results
2. There is a question and answer document on our website. The questions are linked to the answers, please use this as an online document.
3. When the baby is born you will now know the D group of the baby already. You can give anti-D accordingly even before the laboratory confirms the D group
4. If baby was predicted RhD neg but is phenotyped post delivery as RhD positive then a false negative investigation will need to be performed.

Contact details

NHS Blood and Transplant

- Erika Rutherford NHSBT Business Development Manager Erika.Rutherford@nhsbt.nhs.uk – until end of May 2025
- Filton Molecular Diagnostics Laboratory Molecular.Diagnostics@nhsbt.nhs.uk
- Website: www.nhsbt.nhs.uk/ibgrl/services/molecular-diagnostics/fetal-rhd-screen

NHS
Blood and Transplant

Any questions