Fetal *RHD* Screening User Guide

NHS Blood and Transplant

Cell-free fetal DNA screening for D blood group to determine need for anti-D prophylaxis during pregnancy

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This guide outlines the cell-free fetal DNA screening service for determination of fetal D blood group in D negative pregnant women to guide the requirement for antenatal anti-D prophylaxis. High- throughput non-invasive prenatal testing for fetal *RHD* genotype is recommended by the National Institute for Health and Care Excellence (NICE) as a cost-effective option to guide antenatal prophylaxis with anti-D immunoglobulin1. Tools to put this NICE guidance into practice are available https://www.nice.org.uk/guidance/dg25.

This user guide is written for obstetricians, midwives and scientific staff in hospital transfusion laboratories and others involved in antenatal care. The International Blood Group Reference Laboratory (IBGRL) provides specialist diagnostics services to NHS Blood and Transplant (NHSBT). The Molecular Diagnostics department is an accredited laboratory and all work is carried out within the framework of a documented quality system. The department regularly participates in external quality assurance exercises to predict fetal RhD blood group from maternal blood. Information about patients and donors is held in compliance with the EU General Data Protection Regulation. Staff access to the patient information is on a need-to-know basis for clinical care purpose only and patient confidentiality is respected at all times.

This guide concerns fetal D blood group testing for pregnant women **who have not made anti-D (or –G)** and who require the test to determine their need for antenatal anti-D prophylaxis. To refer samples from women who have made anti-D antibodies, please consult INF1135 'User Guide for referring samples to IBGRL Molecular Diagnostics' (there is a different referral form and sample requirement). See the IBGRL website http://ibgrl.blood.co.uk/services/user-guides/

If you are a potential new customer for the NHSBT Fetal *RHD* screening service, please contact Erika Rutherford, Business Development Manager: erika.rutherford@nhsbt.nhs.uk. If you are an existing customer of the fetal *RHD* screening service and wish to order request forms or require clinical advice, please contact the Molecular Diagnostics laboratory. Requests for clinical advice may be referred on to the relevant NHSBT consultant haematologist.

Laboratory contact details

Tel: 0117 921 7572
Email: molecular.diagnostics@nhsbt.nhs.uk
Website http://hospital.blood.co.uk/diagnostic-services/red-cell-immunohaematology/antenatal-screening-services/#fetal

Postal address for samples:

Molecular Diagnostics
International Blood Group Reference Laboratory
NHS Blood and Transplant
500 North Bristol Park
Northway
Filton
BS34 7QH

Normal working hours: Monday to Friday 09:00 – 17:00

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Customer complaints and suggestions

IBGRL Molecular Diagnostics is committed to continuously improving the quality and range of services provided and welcomes any comments or suggestions from users. Please contact the Laboratory Manager or Head of Department in the first instance regarding complaints and suggestions. Complaints are managed via our Quality Management system or Customer Services as appropriate. We always strive to provide a satisfactory response to any complaint. In the unlikely event that your complaint is not resolved to your satisfaction please refer to the NHSBT complaints procedure http://hospital.blood.co.uk/customer-services/complaints-compliments-and-feedback/

Technical aspects, limitations of the test and factors affecting test performance

During pregnancy a small amount of cell-free fetal DNA is present in maternal blood. This DNA can be analysed for *RHD* exons 5 and 7 using real-time polymerase chain reaction to predict the baby's D blood group to see if it differs from that of the mother. The test is highly accurate and can be performed from 11^{+2} weeks' gestation (crown rump length $\geq$ 45mm). However, owing to the sensitivity of the test, there is a small chance (0.1%) that a fetus predicted to be D negative will be D positive at birth. Please inform the Molecular Diagnostics department as soon as possible if this occurs, and send a sample of cord and mother's blood if available. Owing to the presence of rare variant *RHD* genes, up to 2% of fetuses predicted to be D positive will in fact be D negative at birth.

Women who have been confirmed to be weak D or D variant are unlikely to benefit from the fetal *RHD* screening test because the maternal *RHD* gene will prevent prediction of fetal D phenotype and an inconclusive test result will be issued. Women who are confirmed weak D should be treated as D positive and prophylactic anti-D is not required. Women who are confirmed D variant should be given anti-D prophylaxis in line with local policy.

The amount of fetal DNA in maternal blood increases throughout gestation, therefore it is essential that maternal blood is not taken before 11^{+2} weeks' gestation. Samples taken too early in gestation will not be tested.

Blood samples which have significantly haemolysed may contain a very high background of maternal DNA which could interfere with detection of fetal DNA, therefore haemolysed samples will not be tested.

Samples from women carrying multiple babies can be tested. A D positive test result means that at least one fetus is predicted to be D positive. A D negative test result means that all the fetuses are predicted to be D negative.

Measurement uncertainty for the assays used in the laboratory has been established and is available upon request.

Request forms, samples, labelling requirements

Request forms

Request form FRM5197 can be ordered free of charge from IBGRL. Trusts wishing to use their own electronically generated referral form must have the form approved by NHSBT before sending samples.

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Patient information leaflets

Patient information leaflets (INF1263: D negative mother's blood test to check her unborn baby's blood group) can be ordered free of charge via the distribution hub on the Hospitals and Science website https://hospital.nhsbtleaflets.co.uk/Login.html

Samples / labelling of samples/ completion of request forms

Samples will only be accepted for testing if the following conditions are met:

Sample requirements

It is the responsibility of the test requester to ensure that patient consent has been obtained.

A minimum of 6mL maternal EDTA blood (pink or purple blood tubes).

The sample tube must not be opened following blood collection or used for any testing prior being sent to IBGRL.

The sample tube should be stored at room temperature prior to reaching the laboratory within 7 days following venepuncture and must not be lysed on receipt.

Samples **MUST** be labelled, dated and signed by the person taking the blood.

Labels pre-printed prior to phlebotomy e.g. addressograph labels, are not acceptable on samples. They are, however, acceptable on request forms providing they do not obscure other vital details

Samples must have handwritten labels unless demand printed labels are produced at the time of phlebotomy. NHSBT must be informed in writing if demand printed labels are in use. Hand written alterations on either the sample or request form may make the sample invalid for testing. Any minor alterations must be initialled by the person taking the sample to be acceptable for testing.

Request form FRM5197 must accompany every sample unless the hospital trust's electronically generated request form has been approved by NHSBT. Instructions for completing FRM5197 are detailed in INF1340 available at http://hospital.blood.co.uk/diagnostic-services/red-cell-immunohaematology/antenatal-screening-services/#fetal

Request forms are the basis of the correct identification of the patient and the requesting hospital. The points of identification provided on the request form must match the information provided on the sample. NHSBT will not test samples unless three or more identical points of identification for the patient are used on both forms and tubes.

Minimum hospital identification (request form)

i. The correct hospital name written out in full (no abbreviations)

ii. The hospital NHS code (5 digit number)

This will determine the destination for the report and the invoice.

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Minimum patient identification (request form and sample tube/s)

iii. Surname/family name and first name/s in full (surname and first name are one identifier).

iv. NHS number or hospital number (the same number must be on both the tube and the form)

v. Date of birth

The following information must also be provided

vi. Date of venepuncture (on sample tube/s)

vii. Estimated delivery date by dating scan (on request form)

Requests which do not meet the above minimum specification for hospital and patient identifiers as well as EDD and date of venepuncture will be rejected at receipt.

Use of the hospital laboratory / pathology sample number

IBGRL will only include the hospital laboratory / pathology sample number on the report if the following conditions are met:

i. The hospital laboratory / pathology sample number must be on both the sample tube and the request form. The number must be in a labelled, designated area of the request form so that it is clear this is the hospital laboratory / pathology sample number.

- **If the hospital / pathology sample number on tube and request form differ, the sample will be rejected.**
- If the hospital / pathology sample number is only on the tube OR the request form, the number will not be recorded.
- If the hospital / pathology sample number is not in the designated area of the request form, the number will not be recorded.

ii. For hospitals that use their own approved electronically generated request form and the form does not have a labelled, designated area for the hospital / pathology sample number, a hand-written label “Hospital / pathology sample number” can be written next to the number, otherwise the number will not be included on the report.

Packaging of samples

It is the responsibility of the sender to ensure that all samples are packaged in accordance with the current Transport of Dangerous Goods: United Nations Model Regulations to prevent breakage or spillage in transit. For advice on posting samples see www.royalmail.com. The outside of the box or package containing the samples must be clearly addressed to the appropriate department. Address labels are available on our website http://hospital.blood.co.uk/diagnostic-services/red-cell-immunohaematology/antenatal-screening-services/#fetal

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Transport of samples

Samples and referral forms should be sent to the hospital pathology (or Send Away) laboratory for forwarding to the NHSBT postal address above using NHSBT transport where available. Samples must be received at IBGRL within 7 days of venepuncture.

Turnaround time for results

The Molecular Diagnostics department aims to report 98% of samples within 10 business days of receiving the sample. Customers will be informed by email or phone in the unlikely event that a delay is anticipated.

Reports

Reports (including sample rejection notifications) are issued to the hospital blood transfusion laboratory (or other referring laboratory) via the Sp-ICE reporting system.

The referring hospital, primary care trust or clinic is responsible for:

i) ensuring that a risk assessment has been performed to address and mitigate against the occasions when Sp-ICE may not be available and to accommodate the possibility of NHSBT having to revert to issuing hard copy reports, which will not incur a cost in these circumstances.

ii) there are systems in place within the Trust and all linked Trusts to ensure that all clinical and scientific staff who require access to reports will receive them within a time-scale commensurate with their clinical urgency.

On exceptional occasions, if paper reports are required, these may be provided for an additional administration charge by prior approval and arrangement with NHSBT.

Customers should contact their local hospital transfusion laboratory for fetal *RHD* screening results in the first instance.

Additional requests

Residual maternal plasma samples will be archived for nine months from date of receipt and will be re-tested in the rare circumstance that an incorrect D negative test result is brought to the attention of NHSBT. Additional test requests cannot be requested for the archived plasma.

Our requirements of the requester

In order to ensure the standards of our service are maintained and to aid improvement, we try to monitor the accuracy of our testing procedures. **If the fetal *RHD* screening test has predicted a fetal D negative phenotype, and serological testing of cord blood indicates a D positive baby, please inform us as soon as possible by email: molecular.diagnostics@nhsbt.nhs.uk or telephone. Please retain a sample of cord and maternal blood and forward them to IBGRL Molecular Diagnostics for further investigation.**

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References:

1.High-throughput non-invasive prenatal testing for fetal RHD genotype. https://www.nice.org.uk/guidance/dg25

2.Diagnostic accuracy of routine antenatal determination of fetal *RHD* status across gestation: population based cohort study. Chitty, L et al, *BMJ*: (2014): 349

3.Use of cffDNA to avoid administration of anti-D to pregnant women when the fetus is RhD-negative: implementation in the NHS. Soothill, P et al. *BJOG*, 2015 Nov;122(12):1682-6.