NHS
Blood and Transplant

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

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. The guide is intended to be accessed via the website so that links to relevant documents or sections of the NHSBT hospital and science website https://hospital.blood.co.uk/ or Molecular Diagnostics website https://ibgrl.blood.co.uk/services/molecular-diagnostics/ are available to the reader.

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 immunoglobulin[1]. Tools to put this NICE guidance into practice are available https://www.nice.org.uk/guidance/dg25.

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General information

This user guide is written for obstetricians, midwives and scientific staff in hospital transfusion laboratories and others involved in antenatal care. The Molecular Diagnostics Laboratory (MD) is a UKAS accredited medical laboratory (number 9765) providing specialist diagnostics services in NHS Blood and Transplant (NHSBT). All work carried out within the framework of a documented quality system (please refer to the website licensing and accreditation section for details). The department regularly participates in external quality assurance exercises to predict fetal D blood group from maternal blood.

This guide is intended to assist referral of D-negative pregnant women **who have not made anti-D (or –G) antibodies** for fetal *RHD* screening to determine their need for antenatal anti-D prophylaxis. To refer samples from women who have made anti-D or -G antibodies please refer to our diagnostic service information and user guide INF1135. Please note there is a different sample requirement.

New customers:
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 for comprehensive information and assistance to establish this service within your hospital / clinic. All relevant contractual paperwork, including FRM5578, must be completed a minimum of three months prior to sending samples and is subject to agreement between NHSBT and the hospital / clinic of a start date to refer samples.

Ordering referral forms:
If you are an existing customer of the fetal *RHD* screening service and need to order request forms, please order them via the distribution hub on the Hospitals and Science website.

Clinical advice:
Please contact Molecular Diagnostics if you require clinical advice. Requests for clinical advice will be referred to the relevant NHSBT consultant haematologist or clinical scientist.

Laboratory contact details

Tel: 0117 921 7572 **Email**: molecular.diagnostics@nhsbt.nhs.uk
Website: https://ibgrl.blood.co.uk/services/molecular-diagnostics/fetal-RHD-screen/

Postal address for samples:

Molecular Diagnostics
NHS Blood and Transplant
500 North Bristol Park
Northway
Filton
BS34 7QH

Normal working hours: Monday to Friday (07:00 – 18:00) Saturday (07:00-16:00)

An answerphone system is in place to receive calls where required and out of normal working hours. Samples received out of working hours will be receipted by NHSBT 'Goods in' but will not be processed by MD until the next working day. MD is closed on all public holidays, Christmas Day and Boxing Day.

Customer complaints and suggestions

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 in the first instance regarding complaints and suggestions via email: molecular.diagnostics@nhsbt.nhs.uk or by telephone 0117 921 7572. 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.

Data protection / privacy assurance / consent for genetic testing

All information provided to NHS Blood and Transplant is used in accordance with the General Data Protection Regulation and all other relevant privacy and data protection laws. To find out more about your privacy rights please visit our website https://www.nhsbt.nhs.uk/privacy/. Staff access to the patient information is on a need-to-know basis for clinical care purposes only and patient confidentiality is respected at all times.

All genetic testing requires informed consent. It is the responsibility of the test requester to ensure that appropriate patient consent has been obtained. The laboratory assumes that, on receipt of a clinical sample and a completed referral form, consent for genetic testing has been obtained. Where appropriate, extracted DNA is stored for NHSBT quality assurance purposes or service development within NHSBT. Genetic material is not distributed outside of NHSBT unless specific informed consent from the individual is obtained.

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, 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 ($\leq$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 and the specificity of the test, 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 but the report will not differentiate between singleton and multiple pregnancies. 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 via the distribution hub on the Hospitals and Science website.

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.

Samples / labelling of samples/ completion of request forms

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

Sample requirements

One EDTA blood tube containing at least **6mL** maternal blood.

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

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. If you would like NHSBT to consider accepting samples from your hospital / Trust which have been labelled using a demand printed labelling system, please complete the application form.

Handwritten 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.

Referral form requirements

Request form FRM5197 must accompany every sample (unless the hospital's electronically generated request form has been approved by NHSBT - applies to existing service users only, new customers will be required to use FRM5197). Instructions for completing FRM5197 are detailed in INF1340 available on the website. Verbal test requests are not accepted.

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 (NOT the Trust name)

ii. The hospital NHS code (5-digit number)

This information is very important as it will determine the destination for the report and invoice. Request forms that contain hospital name abbreviations or partial NHS codes so the referral location is not clear will not be tested. A 'Not-Tested' report will be generated once we are contacted by the referring hospital.

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. The name of the person taking the sample (on the sample and the request form)

viii. Estimated delivery date by dating scan (the gestational week is not acceptable)

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

Please do not place the hospital laboratory / pathology sample number on the blood tube as MD staff need to view the contents to assess suitability for testing and the sticker has to be removed before loading the tube onto the analyser. If Trust policy mandates use on the blood tube, avoid covering the clear sample window so that the blood inside the tube is still visible. Refer to the website for guidance on labelling sample tubes for fetal *RHD* screening.

- MD will include the hospital laboratory / pathology sample number on the report if it is in the labelled, designated area of the request form so that it is clear this is the hospital laboratory / pathology sample number. (For hospitals using a historically approved electronic request form, if the form does not have a labelled, designated area, 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).

- If your Trust policy mandates a hospital / pathology sample number on the tube it must match the request form or the sample will be rejected.

- If the hospital / pathology sample number is only on the tube, the number will not be recorded.

Packaging of samples

It is the responsibility of the requester to ensure that all samples are packaged in accordance with the current European agreement concerning Carriage of Dangerous Goods by Road Regulations, and IATA (packaging instruction 650), to prevent breakage or spillage in transit. For all transport purposes, pathogens are assigned according to categories A and B. Unless it is known or reasonably believed to contain infectious substance of category A (e.g. haemorrhagic fevers), all human or animal material is regarded as category B, UN 3373.

Category A

Category A includes higher risk infectious micro-organisms, defined as an infectious substance which is transported in a form that when exposure to it occurs is capable of causing permanent disability, life threatening or fatal disease in otherwise healthy humans or animals. If sending category A substances, please phone the testing laboratory and discuss the arrangements before sending.

Category B

Category B includes infectious substances that do not meet the criteria for inclusion in category A, and include human and animal material such as, but not limited to, excreta, secreta, blood and its components, tissue and tissue fluids, and body parts being transported for purposes such as research, diagnosis, investigational activities, disease treatment or prevention. All diagnostic or clinical samples are to be deemed and labelled Biological Substance Category B, UN 3373 and must meet Packaging Instruction 650:

- Primary inner receptacle and secondary receptacle, both must be sealed and leak proof
- Rigid outer packaging (the outer package must also have one side with the minimum dimensions of 100mm x 100mm)
- All packages must use a visible diamond-on-point label UN3373 (min require dimension of this diamond are 50mm x 50mm)
- Adjacent to the diamond must be the label 'Biological Substance, Category B' and all text MUST be 6mm in height
- Packaging Instruction 650 now permits up to 1 litre per primary receptacle with a total of 4 litres per package for liquids and 4kg for solids. Either primary or secondary receptacle must withstand pressure of 95kPa and a 1.2 meter drop test

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. Please post samples by 1st class post or courier if NHSBT transport is not available. Samples must be received at MD in time to be processed within 7 days of venepuncture.

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

NHSBT reserves the right to refuse to handle any samples which are inappropriately packaged or labelled; customers sending unsatisfactorily packaged samples will be contacted.

Turnaround time for results

MD aims to report 98% of samples within 10 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. https://hospital.blood.co.uk/diagnostic-services/sp-ice/

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 blood and DNA will be retained for seven months from date of receipt and may be used to assist with investigations in the rare circumstance that an incorrect D negative test result has been brought to the attention of NHSBT. Additional test requests cannot be requested for the archived cells or DNA.

Our requirements of the requester

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 by telephone 0117 921 7572. Please retain a sample of cord and maternal blood and forward them to Molecular Diagnostics for further investigation.

Please note that a final report of the findings of the investigation will be issued via your local NHSBT Customer Services manager and will not be available on Sp-ICE.

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.