International Blood Group Reference Laboratory (IBGRL)

IBGRL provides specialist diagnostic 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 participates in external quality assurance exercises for blood group genotyping. 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.

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User guide for referring samples to IBGRL Molecular Diagnostics

General information:

Terms and conditions
Please see our website for terms and conditions when referring samples to IBGRL Molecular Diagnostics. Referral of samples accompanied by a signed and completed Molecular Diagnostic request form (FRM4674 and FRM4739) and acceptance of this sample for testing by the laboratory constitutes an agreement between the Requester and NHS Blood and Transplant as outlined in the terms and conditions.

Please note that if your Trust or hospital has signed a service level agreement (or agreement under Schedule 8 of the Contract for the Supply of Blood, Blood Components and Services) with NHS Blood and Transplant, then that agreement will supersede the terms and conditions referenced above and a signature on the referral form is not required.

Laboratory contact details
For enquiries please contact the IBGRL Molecular Diagnostics laboratory. Requests for clinical advice may be referred to the relevant NHSBT consultant haematologist.

Tel: +44 (0)117 921 7572 or +44 (0)177 921 7574
Email: molecular.diagnostics@nhsbt.nhs.uk
Website https://ibgrl.blood.co.uk/

Laboratory address (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

Pricing and Request Forms
Please contact the laboratory for current pricing information and request forms molecular.diagnostics@nhsbt.nhs.uk

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 https://hospital.blood.co.uk/commercial-and-customer-service/complaints-compliments-and-feedback/
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Fetal RhD, Kell, Rhc, RhE, RhC and sex genotyping from maternal blood

Introduction
Cell-free fetal DNA is normally present in maternal blood plasma throughout pregnancy (Lo et al, 1998). This DNA can be analysed following maternal venepuncture, without risk to the fetus, and molecular techniques can be used to predict blood group status or gender of fetuses at risk of hemolytic disease of the fetus and newborn (HDFN) or X-linked genetic conditions respectively. The amount of fetal DNA in maternal blood increases throughout gestation. At early stages of pregnancy, the amount of fetal DNA may be too low to detect. The amount of fetal DNA in maternal blood generally increases throughout the pregnancy but is rapidly cleared from the mother’s blood following birth.

Technical aspects of the testing procedure
Analysis of free fetal DNA in maternal blood relies upon the detection of fetal alleles or genes which are absent in the maternal genome.

Fetal RHD Genotyping. There are several genetic causes for the RhD-negative phenotype. In the majority of Caucasian RhD-negative individuals the RHD gene is absent, however, the majority of RhD-negative black African individuals have an intact but non-functional RHD gene, termed the RHD pseudogene (Singleton et al, 2000). Quantitative real-time PCR is used to amplify exons 4, 5, 7 and 10 of the RHD gene in order to distinguish the expressed RHD gene from the most common non-expressed or variant RHD alleles. However, the molecular biology of the Rh system is incompletely understood and due to its complexity and ethnic diversity, there remains the possibility that on very rare occasions, genotyping results may not correspond to phenotype by conventional serology. Clinical decisions should not, therefore, rely solely upon genotyping results.

Genotyping for other blood groups
The molecular bases of most blood groups associated with HDFN are known and real-time PCR is used to target blood group alleles not present in the mother’s genome and thereby to predict the blood group status of her unborn baby.

Fetal Sex Typing This test uses real-time PCR to amplify DNA sequence (DYS14) located on the Y chromosome.

Limitations of Non-Invasive Genotyping
Please note that we cannot confirm the presence of fetal DNA in a maternal blood sample. There is a possibility that failure to detect the fetal gene of interest may be due to undetectable levels of fetal DNA in the sample and may not indicate that the fetus is negative for that blood group or lacks the Y chromosome. There is a theoretical possibility that in a very small number of pregnancies we may detect fetal DNA from a fetus that has subsequently been lost as a result of the ‘vanishing twin’ phenomenon (Landy & Keith, 1998). In addition, due to the complexity of some blood group systems, there remains the possibility that on very rare occasions, genotyping results may not correspond to phenotype by conventional serology. Measurement uncertainty for the assays used in the laboratory has been established and is available upon request.
Referral of maternal blood samples

Gestational age

RhD, Rhc, RhC and RhE samples are accepted from 16 weeks gestation. In some circumstances we will test samples before the recommended gestation (please contact the laboratory to discuss). In such cases, where a negative blood group is predicted, we recommend that another sample is sent after 16 weeks gestation, and this test will be chargeable.

Kell samples are accepted from 20 weeks gestation. Unless the fetus is found to be Kell positive, the report will carry a recommendation to send a repeat sample after 28 weeks gestation. A Kell negative prediction will only be reported when a confirmatory sample has been tested after 28 weeks gestation and found negative. It is recommended that pregnancies with anti K antibodies should continue to be monitored by Doppler ultrasound until the results of the second confirmatory sample have been obtained.

Fetal sex prediction samples can be sent from 7 weeks onwards.

Sample and request form requirements

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

16ml maternal blood in EDTA tubes per test requested.

3ml paternal blood sample should be sent, if available, when requesting fetal RhD status. A paternal sample is not essential for most fetal RhD status requests but can help to establish the presence of a variant RHD gene in the fetus.

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

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

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

A request form must accompany every sample and must be signed by the requester (please see Terms and Conditions section for signature requirement).

Please complete the appropriate referral forms

- **FRM4674** for fetal blood group genotyping from maternal blood
- **FRM4739** for fetal sex typing from maternal blood

Instructions for completing **FRM4674** and **FRM4739** are detailed in **INF1343** and **INF1342** retrospectively, available on the website [http://ibgrl.blood.co.uk](http://ibgrl.blood.co.uk)

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

**Minimum patient identification**
- Surname/family name and first name(s) in full (surname and first name are one identifier)
- NHS number, hospital number or unique identification number (the same number must be on both the tube and the form)
- Date of birth.

The following information must also be provided:
- Date of venepuncture
- Estimated delivery date (by scan) or gestational age in weeks.

**Samples which do not meet the above specification will be rejected at receipt**

**Preparation of frozen samples**
Samples referred from outside the UK can be sent as frozen plasma aliquots prepared to a protocol provided by IBGRL – see INF1291. Please contact the laboratory prior to sending frozen plasma. Frozen plasma aliquots that do not meet the sample labelling requirements listed in INF1291 will not be tested.

**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 and DAT2132. For advice on posting samples see Royal Mail website. The outside of the box or package containing the samples must be clearly addressed to the appropriate department.

**Transport of samples**
Samples must reach the laboratory in time to be processed during laboratory working hours within set time limits after venepuncture. Sample reception is open around the clock, but samples must arrive during working hours Monday to Friday 09:00 to 17:00, so that they can be processed within the time limits below:
- Referrals for Kell: must be processed within 2 days of venepuncture
- Referrals for RhD, Rhc, RHE and RhC: must be processed within 3 days of venepuncture
- Referrals for Fetal sex: must be processed within 7 days of venepuncture

Time-dependent samples (especially samples for fetal K status determination) should not be sent on NHSBT transport but should be sent by first class post or courier. Timing starts on day of venepuncture.

Timing is a guide to suitability of samples for testing, but other factors may affect sample condition (e.g. exposure to fluctuation in temperature, heat, incompletely filled EDTA tubes). Samples which have lysed due to prolonged time or adverse conditions in transit or which are not in the requested anticoagulant will be rejected and reported as such.

Blood samples should be shipped at room temperature.

Aliquots of maternal plasma, processed following the protocol listed in INF1291, should be shipped frozen on dry ice. Samples must be received within 5 days and must be frozen on receipt.
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Turnaround time & reports (including sample rejection notification)
Our target is to issue reports within:
- 5 working days from date of sample receipt for fetal sex typing from maternal blood
- 7 working days from date of sample receipt fetal blood group genotyping from maternal blood.

Urgent samples can be tested more quickly by prior arrangement with the laboratory.

The requester will be notified by email or telephone if a significant delay in reporting is anticipated.

A single report will be posted to the address indicated on the referral form and reports are also available on the Sp-ICE reporting system.

Additional requests
Maternal plasma samples will be archived for one year from date of receipt. Additional requests for testing may be made within six months of the first request, subject to there being sufficient plasma to complete the additional test request. If there is insufficient plasma, a new sample must be sent. Additional test charges will apply in either case.

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. We appreciate receiving information on the infant’s blood group or sex after delivery. If there is a discrepancy between the baby’s phenotype at birth and the predicted phenotype or baby’s sex, please inform the IBGRL laboratory as soon as possible.

If samples are referred for fetal RhD, C, c or E genotyping or sexing before the recommended gestation, and an antigen negative or female gender result is predicted, the requester should send a repeat sample after the recommended gestational age. This will reduce the small chance of a false negative genotyping result being undetected during the pregnancy. Requests for fetal Kell genotyping before 20 weeks gestation will be rejected.

References


User guide for referring samples to IBGRL Molecular Diagnostics

Blood group genotyping from blood sample or other tissue (e.g. amniotic fluid or chorionic villus)

Introduction
In multi-transfused patients or DAT positive patients, the presence of transfused red cells or auto-antibodies can prevent determination of blood group phenotype by serological techniques, however analysis of genotype can be used to predict blood group phenotype. The blood group phenotype of a fetus can be determined by analysis of DNA derived from fetal cells in amniotic fluid or chorionic villus (CV). IBGRL Molecular diagnostics uses various techniques to determine blood group genotype: allele-specific PCR, real-time PCR (allelic discrimination using Taqman probes) and HEA Beadchip. The technique used will depend on the test requested and the current workload in the laboratory.

Available genotyping tests (please request the current price list for costs)

STANDARD BLOOD GROUP GENOTYPE
RhD, c, C, E, e, K/k, Jk^a /Jk^b, Fy^a /Fy^b, MN, S/s, U- /Uvar

EXTENDED BLOOD GROUP GENOTYPE
RhD, c, C, E, e, V, VS, K/k, Jk^a /Jk^b, Fy^a /Fy^b, Fy^e, MN, S/s, U- /Uvar, Do^a /Do^b, Js^a /Js^b, Kp^a /Kp^b, Lu^a /Lu^b, Co^a /Co^b, Di^a /Di^b, Sc^1 /Sc^2, LW^a /LW^b

EXTENDED GENOTYPE: HAEMOGLOBINOPATHY PATIENT ARRAY (a genotyping test array particularly suited for haemoglobinopathy patients). RhD, C, c, E, e, (including common RhD, C and e variants), V, VS, hr^d, hr^s, K/k, Kp^a/b, Js^a/b, Do^a/b, Fy^a/b, Jk^a/b, M/N, S/s, U-, U^var

ABO GENOTYPE – please note that clinical decisions relating to transfusion / transplantation must not be made on the basis of the ABO group predicted from genotyping results.

RHD ZYGOSITY
This test can be used to determine the RHD zygosity of partners of D- women. The tests predict whether the test subject carries one or two copies of the RHD gene.

Limitations and caveats
The molecular biology of blood groups, and particularly of the Rh system, is complex and genetic differences may be found in ethnic groups. It remains a possibility that on very rare occasions, genotyping results may not correspond to serological phenotype. Clinical decisions should not, therefore, rest solely on genotyping results.

People who have received a transplant following which incomplete engraftment has taken place may exhibit chimerism in their blood cell populations and therefore in their DNA; this may give incorrect genotyping results or prevent a conclusive result being issued. Clinical history of transplantation should be recorded on the request form.

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

External Quality Assurance
The laboratory participates in a NEQAS scheme for blood group genotyping and participates in the HEA Beadchip EQA scheme provided by the supplier of this platform, Immucor.
Referral of blood samples or other tissues (not maternal blood for fetal genotyping)

Sample and request form requirements

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

**Blood.** minimum 0.5mL EDTA blood stored at room temperature.

**Ammniotic fluid.** 5ml sample of amniotic fluid should be received within 7 days of sampling. To avoid the possibility of contamination, it is preferable to dispatch the amniotic fluid without transferring it to a second container. If amniotic fluid is transferred from one container to another, then precautions should be taken to avoid contamination with material containing exogenous DNA.

**Chorionic Villus.** Pre-extracted DNA must be referred. The laboratory performing the DNA extraction should ensure that procedures are in place to prevent contamination of the DNA sample with extraneous DNA or other substances. DNA must be at a minimum concentration of 10ng / µL and a minimum volume of 60 µL.

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.

A request form must accompany every sample and must be signed by the requester (please see Terms and Conditions section for signature requirement).

Please complete referral form

- **FRM4738** for standard red cell genotype or extended blood group genotype *(including amniotic fluid or chorionic villus samples)
  - Instructions for completing **FRM4738** are detailed in **INF1341** available at [http://ibgrl.blood.co.uk](http://ibgrl.blood.co.uk)
- **FRM1597** Extended Genotype (haemoglobinopathy patient array - particularly suited for haemoglobinopathy patients)
  - Instructions for completing **FRM1597** are detailed in **INF417** available at [https://hospital.blood.co.uk/diagnostic-services/red-cell-immunohaematology/rci-test-request-form/](https://hospital.blood.co.uk/diagnostic-services/red-cell-immunohaematology/rci-test-request-form/)

Request forms are the basis of the correct identification of the patient.

The points of identification provided on the request form must match the information provided on the sample. IBGRL will not normally test samples unless three or more identical points of identification are used on both forms and tubes.

**Minimum patient identification**

- Surname/family name and first name(s) in full (surname and first name are one identifier)
- NHS number, hospital number or unique identification number (the same number must be on both the tube and the form)
- Date of birth.
  - The following information must also be provided.
  - Date of venepuncture / sampling

Samples not meeting the above specification will be rejected at receipt.
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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 and DAT2132. For advice on posting samples see Royal Mail website. The outside of the box or package containing the samples must be clearly addressed to the appropriate department.

Transport of samples
Blood samples should be sent at room temperature and received within 14 days of venepuncture. Amniotic fluid samples should be sent at room temperature and received within 7 days of sampling. CVS DNA samples may be sent frozen or at room temperature and should be received within 7 days.

Turnaround Time & reports (including sample rejection notification)
Our target is to issue reports within:

- 10 working days of sample receipt for standard red cell genotype and extended genotype
- 12 weeks of receipt for extended Genotype (haemoglobinopathy patient array - particularly suited for haemoglobinopathy patients).

Samples referred for Standard Genotype can be tested more quickly (and within 48 hours if required) by prior arrangement with the laboratory. However, an urgency premium charge (see current price list) will be applied for any sample which is referred with requested turnaround time less than 10 working days from sample receipt.

The requester will be notified by email or telephone if a significant delay in reporting is anticipated.

A single report will be posted to the address indicated on the referral form and reports are also available on the Sp-ICE reporting system. Extended Genotype (haemoglobinopathy patient array) reports are only distributed via Sp-ICE.

Additional requests
DNA samples will be archived for one year from date of receipt. Additional requests for testing may be made within one year of the first request, subject to there being sufficient DNA to complete the additional test request. If there is insufficient DNA, a new sample must be sent. Additional test charges will apply in either case.

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. It is known that genotype does not always reflect phenotype, however, if an unexpected discrepancy between genotype and phenotype is discovered we will be happy to investigate further. Please contact the laboratory to discuss.