Irish Blood Transfusion Service
Seirbhís Fuilaistriúcháin na hÉireann

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Review

Review: IBTS DOC REVIEW AND APPROVAL

Level	Owner Role	Actor	Sign-off By
1	DOCUMENT CONTROLLER	GERMAN CARRARA CALMELS	GERMAN CARRARA CALMELS
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3	MOLECULAR BIOLOGY & GENETICS REVIEWER	OLUJIDE AJANI	
4	MOLECULAR BIOLOGY & GENETICS HEAD OF D	HELEN RYAN	
5	QUALITY ASSURANCE REVIEWER IBTS	REBECCA WALDEN	

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Change Orders

Changes as described on Change Order:	Change Order No.

Change Orders - Incorporated

Changes as described on Change Order:	Change Order No.
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Valid on Day of Printing, Verify Current Version
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TITLE: CUSTOMER MANUAL FOR THE BLOOD GROUP GENETICS LABORATORY

Change Description:

1. Expire IBTS/BGG/UG/0001 [3] and change to IBTS/MBG/CM
2. Update contact list
3. Number of current Blood Group Systems as defined by the ISBT updated.
4. Update method of reporting to include MediBridge
5. Appendix 1 updated to include additional sample referral and testing info.

Reason for Change:

1. New template and update manual
2. To reflect current staffing
3. The number of Blood Group Systems as defined by the ISBT has increased from 38 to 47.
4. To reflect current practice
5. Expand section to include questions that are commonly asked by referring hospitals, to improve the user understanding of results and significance

Change Order No.:

IBTS/CO/0510/24

Referenced Documents

N/A

SmartSolve Roles – *List roles as required for training in alphabetical order*

BGG MS IBTS
BGG SMS IBTS

Training Type

All Roles	Read and Understand

SmartSolve Document Category

Category	Mobile	Cryobiology	Website	GDP
Yes / No	*No*	*No*	*No*	*No*

TABLE OF CONTENTS

TITLE: CUSTOMER MANUAL FOR THE BLOOD GROUP GENETICS LABORATORY

1 CONTACT DETAILS

Location:	The Blood Group Genetics Laboratory is located within the Molecular Biology and Genetics Department of the Irish Blood Transfusion Service
Postal Address:	Blood Group Genetics Laboratory, Molecular Biology and Genetics Department National Blood Centre Irish Blood Transfusion Service James's Street Dublin D08 NH5R Ireland
Contactable hours:	09.00 to 17.00 – Monday to Friday (Excluding Public Holidays)

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ABOUT US

Molecular Blood Group typing is performed by the Blood Group Genetics Laboratory (BGGL) of the Molecular Biology and Genetics Department at the National Blood Centre, with the purpose of providing a molecular diagnostic service for blood group determination. This User Guide provides information on the services offered.

The Blood Group Genetics Laboratory operates under the Blood and Tissue Establishment Licence issued to the IBTS by the Health Products Regulatory Authority (HPRA). The IBTS operates to the relevant EU Blood Directives (2002/98/EC and 2004/23/EC) and Irish Statutory Instruments (SI 360/2005, SI 547/2006, SI 562/2006). These Directives and Statutory Instruments ensure the standard of quality and safety in relation to recruitment, donation, testing, processing, storage, and distribution of all blood and blood components and tissues including stem cells.

All activities are carried out within the framework of a documented Quality System. The department has a formal policy for internal quality control and participates in external quality assurance exercises for blood group genotyping. Information about patients and donors is held in compliance with the Data Protection and Freedom of Information acts.

2 ABOUT MOLECULAR BLOOD GROUP INVESTIGATION

DNA-based testing is increasingly being used to predict a blood group phenotype to improve practices in transfusion medicine. The red cell membrane contains many surface and trans-membrane proteins; many of which carry blood groups. Red blood cells (RBCs) carrying a particular antigen, if introduced into the circulation (through transfusion or pregnancy) of an individual who lacks that antigen, can elicit an immune response. The resultant production of antibodies can have significant effect on patient's morbidity and even mortality. It is for this reason that antigen-negative blood is often required for safe transfusion.

Most blood group antigens are glycoproteins and their specificity is mostly determined either by the oligosaccharide (e.g. ABO) or amino acid sequence (e.g. Kell, Duffy, Kidd). Human blood groups are classified and categorised according to criteria defined by the International Society of Blood Transfusion (ISBT). Each Blood Group System has fulfilled all criteria and represents either a single gene or a cluster of closely linked homologous genes.

Currently 47 Human Blood Group Systems have been identified, their genes cloned and the molecular basis associated with individual antigens determined; there are well over 300 individual blood groups recognised in the 47 Blood Group Systems.

The majority of blood groups result from single nucleotide polymorphisms (SNPs) encoding amino acid substitutions in either an extracellular domain of a red cell membrane protein or a glycosyltransferase. Antigen diversity also arises from other structural rearrangements in the genome (insertions, deletions, inversions) e.g. recombination events can result in novel functional hybrid genes (*RHD-RHCE*; *GYPA-GYPB*).

Molecular determination of blood groups offers a powerful method that overcomes many of the limitations of, and often offers higher resolution blood group typing than serological methods (e.g. *RHD* and *RHCE* variants, FY_{GATA} mutation).

Sensitive methods, such as quantitative polymerase chain reaction (qPCR), offer the ability to detect very low levels of DNA and are particularly applicable for the detection of fetal blood group genes in cell-free DNA extracted from maternal blood.

3 ABOUT OUR TESTS

The Blood Group Genetics Laboratory currently offers the following tests:

- Fetal *RHD* Screen
- Weak D Genotype investigation
- *RHD* variant investigation (includes normal *RHCE* determination)
- Full RBC Genotype investigation
- *RHCE* Variant investigation

See individual sections for detailed descriptions of tests.

4 NOMENCLATURE USED IN REPORTING

The Blood Group Genetics Laboratory will, where possible, use blood group allele nomenclature following the guidelines issued by the ISBT Working Party for Red Cell Immunogenetics and Blood Group Terminology (www.isbtweb.org/working-parties/red-cell-immunogenetics-and-blood-group-terminology/).

Reports are designed to be both user-friendly and follow ISBT recommendations. Examples include:

Common Term	Official ISBT	Alternative ISBT
Weak D Type 1	*RHD*01W.1*	*RHD*Weak D Type 1*
RhD variant DAR1	*RHD*09.01*	*RHD*DAR1*
C+e+	*RHCE*02*	*RHCE*Ce*
FY_{GATA}	*FY*01N.01*	-

Full RBC genotype results will be issued as a predicted phenotype.

5 FETAL RHD SCREEN

This investigation tests for the presence of fetal RHD in cell-free DNA (cfDNA) extracted from maternal blood.

Cell-free fetal DNA (cffDNA) is present in maternal circulation from as early as 6 weeks gestation. Therefore tests can be developed to detect various fetal genes, including *RHD*.

Fetal *RHD* genotyping of cffDNA relies upon the detection of fetal alleles which are absent from the maternal genome. In Caucasians, the RhD-negative phenotype is most commonly due to a homozygous deletion of the *RHD* gene. At the IBTS, qPCR (using hydrolysis probes) is used to amplify exons 7 and 10 of the *RHD* gene. This combination will detect RhD-positive, weak D and *RHD* variant genes, which results in a highly sensitive assay.

In African populations (or people of African descent), the majority of RhD-negative individuals are carriers of non-functional *RHDΨ* or *RHD-RHCE* hybrid genes. Although the RhD protein is not expressed in these individuals, *RHD* sequences present will be detected by our assay. Therefore these individuals will continue to receive RAADP. This exon combination was chosen to maximise the sensitivity of the assay, thereby minimising false negative results.

Indications for Testing

The Fetal *RHD* Screen is suitable for all pregnant RhD-negative women who ***DO NOT*** have alloanti-D.

Women with red cell alloantibodies should have their samples tested by IBGRL, Bristol for Diagnostic Fetal Genotyping (https://www.nhsbt.nhs.uk/ibgrl/services/molecular-diagnostics/fetal-genotyping-diagnostic/). These samples are not tested in the IBTS. Hospitals must refer the samples directly, taking note of the sample requirements by IBGRL.

The results obtained from the Fetal *RHD* Screen will allow clinicians and midwives to optimise the administration of anti-D.

The result of the Fetal *RHD* Screen can also be used to optimise the following:

- Bekte-Kleihauer Testing
- Feto-Maternal Haemorrhage Estimation
- Anti-D quantification (women following RAADP administration)
- Cord RhD phenotyping

Samples should be sent from 11 weeks gestation. Booking samples from all gestations will be accepted; however results will be issued as follows:

Fetal *RHD* Screen	Gestation	Summary of Result Issued
RHD-positive	All	*RHD*-positive fetus
RHD-negative	≥11	*RHD*-negative fetus
RHD-negative	<11	Inconclusive; Anti-D recommended; Repeat sample*
Inconclusive	All	Inconclusive; Anti-D recommended; Repeat sample
Maternal *RHD*	All	Inconclusive; Maternal *RHD*; Anti-D recommended

* see Limitations of Test

Limitations of the Test

We will issue results on samples sent to the laboratory at ≥11 weeks gestation. For samples referred with a gestation of <11 weeks and an '*RHD*-negative fetus' test result, an Inconclusive result will be issued with a request for a repeat sample at ≥11 weeks gestation.

The IBTS does confirm the presence of total cfDNA (through the detection of *GAPDH*); however we do not confirm the presence of ***fetal*** cfDNA in a maternal blood sample. As a result, failure to detect the presence of fetal *RHD* may be due to undetectable levels of cffDNA in the sample, rather than being suggestive of an *RHD*-negative fetus.

The test has been designed to minimise false negative results to <0.2%. Fewer than 2:1000 women predicted to be carrying a D negative fetus will therefore not receive anti-D during pregnancy when they may have benefited from it but should still receive post-natal prophylaxis if guidelines are followed. In up to 1% of tests the result may be incorrectly predicted to be RHD positive. This means that only 1% of RhD negative women receive antenatal prophylaxis unnecessarily, rather than 40% without using the NIPT. Incorrect prediction of a fetus to be *RHD*-positive can occur when a variant *RHD* gene is present in the fetus or the mother, or for inconclusive results in which case anti-D Ig administration is recommended. This is regarded as the safest option, as some individuals can possess an *RHD* gene but their blood group is RhD negative.
Testing performed on samples from RhD-negative women (determined by phenotyping) may sometimes detect the presence of maternal *RHD* gene sequences. The maternal *RHD* may not be expressed, or may express RhD variants (including weak D types and DEL variants), which are below the limit-of-detection of serological methods used by routine hospital RhD-phenotyping protocols. The presence of maternal *RHD* masks the presence of fetal *RHD*, therefore the *RHD* genotype of the fetus cannot be determined; prophylactic anti-D will be recommended in these cases.

As the Rh blood group system is complex with many variants, the genotype identified may not always reflect the phenotype determined by standard serological testing. All clinical decisions should be taken with this in mind.

Please refer to Appendix I – Fetal RHD Screen for additional information

Referring Patient Samples

Use the test-specific Test Request Form for Fetal *RHD* Screen (BT-638). ***Follow sample-labelling criteria at the back of BT-638. Labels and Request Forms generated from the Maternal & Newborn Clinical Management System (MN-CMS) will also be accepted***

- Samples will only be accepted from pregnant RhD-negative women with NO alloanti-D.
- Samples are accepted from all gestations. However samples with an earlier gestation (<11 weeks) may require repeat testing at ≥11 weeks' gestation, depending on the result.
- Blood samples must reach the laboratory no more than 5 days post-phlebotomy; this reduces the likelihood of fetal cfDNA degradation.
- ≥ 8ml peripheral blood in EDTA sample tubes is required (please contact laboratory if this is not possible)
- The blood sample must not be opened following blood collection or used for any testing prior to being sent to the laboratory; this avoids contamination of samples.
- Haemolysed samples will not be tested and will be rejected.
- Samples must be stored and transported at room temperature.

Test Summary

Fetal *RHD* Genotyping - Summary of essential referral information
Samples only accepted from RhD-negative women with no alloanti-D
Sample requirements: All gestations; 8ml peripheral blood in EDTA; ≤5 day old sample; unused samples only accepted; store and transport at room temperature

6 WEAK D GENOTYPING

This test identifies RHD alleles specific for Weak D Types 1, 2 and 3.

Many serological weak D phenotypes are associated with amino acid substitutions as a result of single nucleotide polymorphisms (SNPs) in the *RHD* gene. These amino acid changes in the RhD protein result in decreased antigen expression on the red cell surface. Sequence specific primer polymerase chain reaction (SSP-PCR) is used by the IBTS to identify the SNPs that determine the Weak D type 1, Weak D type 2 and Weak D type 3 genotypes, through amplification of target regions in genomic DNA. Non-weak D types 1-3 can also be determined by this method.

Benefits and Indications for Testing

It is generally accepted that Weak D type 1, 2 and 3 (WD1-3) individuals can be safely transfused RhD-positive red cells. However, other Weak D types have the potential to make alloanti-D, or the data is not available to make any conclusion regarding alloimmunisation risk, and should therefore be treated as RhD-negative.

Limitations of the Test

There are some rare situations where the genotype determination will not correlate with the serological phenotype. All clinical decisions should be taken with this in mind.

Of the 147 Weak D types published to date, this test is designed specifically to detect Weak D type 1, Weak D type 2 and Weak D type 3 genotypes and a limited amount of uncommon weak D genotypes.

Non Weak D 1-3 results exclude the presence of the most common weak D alleles but do not rule out the presence of a rare weak or partial *RHD* allele that is outside the scope of this test.

A non-weak D 1-3 result for obstetric patients may warrant further investigation by the IBTS to determine the most appropriate transfusion pathway.

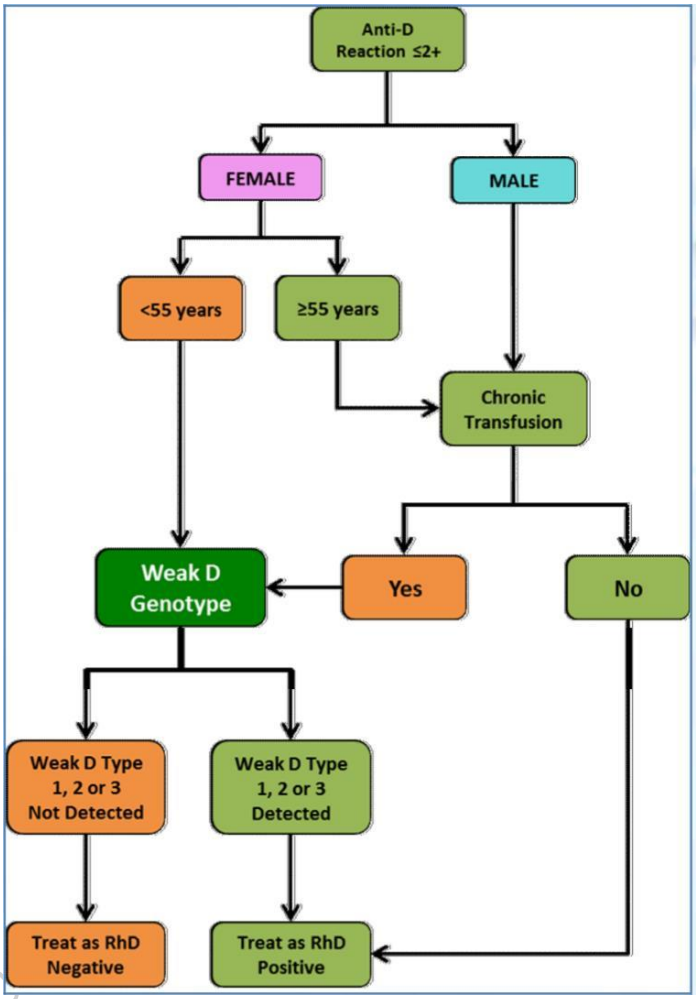
Genotype	Weak D Type	Recommended Transfusion Protocol
*RHD*01W.1*	Weak D Type 1	RhD Positive +
*RHD*01W.2*	Weak D Type 2	RhD Positive +
*RHD*01W.3*	Weak D Type 3	RhD Positive +
Non Weak D 1-3	Non Weak D 1-3	RhD Negative -

Single nucleotide polymorphisms (SNPs) in the relevant genes outside of the targeted regions will not be detected by SSP-PCR. In addition, novel mutations leading to altered or partial antigen expression and null phenotypes may not be detected.

Results from bone marrow or stem cell transplant patients may not match the genotype obtained from other tissues.
Patients who have received a transplant from which incomplete engraftment has taken place, may exhibit chimerism in their DNA which may prevent a conclusive result being issued.

Referring Patient Samples

- Please select **Weak D Genotyping** on the Test Request Form for Red Cell Genotyping (BT-637).
- Include all details of RhD phenotyping already performed.
- The IBTS recommends confirmation of Weak D status by hospital transfusion laboratories for women of childbearing age potential and those who are transfusion dependent to confirm the result (see Figure 1 for IBTS Suggested Algorithm for Referral and Results). The IBTS will however, test any sample that is referred provided the reason for testing is to determine the correct transfusion or obstetric management of the patient (see next point).
- ***This test is not appropriate for neonate samples to determine if prophylactic anti-D is required for the mother after birth. Serological investigation is recommended as a first tier test in this instance; especially where administration of anti-D is required within 72 hours.***
- At least 3ml peripheral blood in EDTA sample tubes is required. If only a small volume is available please contact the laboratory.
- The blood sample must not be opened following blood collection or used for any testing prior to being sent to the laboratory.



Test Summary

Weak D Genotyping - Summary of essential referral information

Please refer to above chart for patient referral criteria; unused samples only; store and transport at room temperature; ≥3ml peripheral blood in EDTA

7 RHD/RHCE GENOTYPING

This test identifies RHD-positive, RHD-negative, RHD variant alleles, and RHCE alleles (C, c, E, e, C^W).

In addition to the RhD-positive and RhD-negative phenotypes, variable expression of the RhD antigen can occur in individuals with *RHD* variant alleles, who consequently can develop alloanti-D. *RHD* variant alleles occur in all populations, however most commonly occur in individuals of African origin.
RhD variants are most commonly expressed as the result of single nucleotide polymorphisms or hybrid *RHD-RHCE* alleles; produced by gene conversion events between the highly homologous *RHD* and *RHCE* genes on chromosome 1p34-p36.
A wild type *RHD* gene can be distinguished from the most common *RHD* variant alleles by molecular detection of the different exons and mutations of the *RHD* gene, using Sequence-Specific Primer Polymerase Chain Reaction (SSP-PCR). This test also allows the detection of normal *RHD*, the most common and important *RHD* variant alleles; it also allows detection of the C, c, E, e and C^W antigens and the allelic variants *RHD*08N.01* [*RHDΨ]* and *RHCE*01.20* [r'^S].

Benefits and Indications for Testing

Patients who may benefit from *RHD*/*RHCE* genotyping:

- Transfused patients where determination of RhD and RhCcEe group by phenotyping is not reliable
- RhD-positive individuals with certain variant *RHD/RHCE* genes who may produce alloanti-D
- Patients where it is unclear if they have auto or alloanti-D
- Patients of African origin in which *RHD* variant genes are more common

If an *RHD* variant allele is detected in a patient of African origin, then an *RHCE* Variant investigation is usually also indicated. The *RHCE* Variant Investigation is an additional test and would incur an additional charge.

Limitations of the Test

There are some rare situations where the genotype determination will not correlate with the serological phenotype. All clinical decisions should be taken with this in mind.

Single nucleotide polymorphisms (SNPs) in the relevant genes outside of the targeted regions will not be detected by SSP-PCR. In addition, novel mutations leading to altered or partial antigen expression and null phenotypes may not be detected.

Results from bone marrow or stem cell transplant patients may not match the genotype obtained from other tissues. Patients who have received a transplant from which incomplete engraftment has taken place, may exhibit chimerism in their DNA which may prevent a conclusive result being issued.

Referring Patient Samples

Please select ***RHD/RHCE* Genotyping** on the Test Request Form for Red Cell Genotyping (BT-637).

- At least 3ml peripheral blood in EDTA sample tubes is required. If only a small volume is available please contact the laboratory.
- The blood sample must not be opened following blood collection or used for any testing prior to being sent to the laboratory.
- Include any details of RhD phenotyping already performed.

Test Summary

***RHD*/*RHCE* Genotyping - Summary of essential referral information**

≥3ml peripheral blood in EDTA; store and transport at room temperature; patient referral – Patients with autoantibodies (standard *RHD* genotype), multi-transfused patients, *RHD*-variants in African populations.

8 EXTENDED RBC GENOTYPING

This test identifies common clinically significant blood groups.

The MBG laboratory offers a test profile that predicts the blood group phenotype for the most clinically significant blood groups.

Benefits and Indications for Testing

A full RBC genotype can detect various human red cell blood group alleles to enable identification of an extended predicted phenotype. This test will predict the patient's phenotype for the following blood groups:

- RhD, C/c, E/e, C^{W}, K/k, Fya/Fyb, Jka/Jkb, M/N/S/s, Doa/Dob, Vel

The rare *RHD*08N.01* (*RHD*Ψ*) allele and some *RHD* variant alleles will be detected (see *RHD*/*RHCE* Genotyping).

The *FY*01N.01* (FyGATA; -67T>G) and *FY*02W.01/.02* (Fyx; 265C>T) alleles can be detected. The current testing is not able to detect mutations that define the JK_{null} phenotype or the U-/$U+^{Var}$ phenotype.

Patients that would benefit from this test include, but are not limited to:

- Those with unresolved blood group determination, due to multi-transfusion of red cell units
- Patients generating allo- or auto-antibodies
- Patients who have a positive direct antiglobulin test (DAT)
- Patients with autoimmune haemolytic diseases (e.g AIHA, CAD)
- Patients undergoing daratumumab treatment or other anti-CD38 mono-clonal antibodies.
- Patients on trials for anti-CD47 (Camellia), e.g. AML or MDS patients.
- Haemoglobinopathy patients

Limitations of the Test

There are some rare situations where the genotype determination will not correlate with the serological phenotype. All clinical decisions should be taken with this in mind.

Single nucleotide polymorphisms (SNPs) in the relevant genes outside of the targeted regions will not be detected by SSP-PCR. In addition, novel mutations leading to altered or partial antigen expression and null phenotypes may not be detected.

Results from bone marrow or stem cell transplant patients may not match the genotype obtained from other tissues. Patients who have received a transplant from which incomplete engraftment has taken place, may exhibit chimerism in their DNA which may prevent a conclusive result being issued.

Referring Patient Samples

Please select ***Full RBC Genotyping*** on the Test Request Form for Red Cell Genotyping (BT-637)

- At least 3ml peripheral blood in EDTA sample tubes is required. If only a small volume is available please contact the laboratory.
- The blood sample must not be opened following blood collection or used for any testing prior to being sent to the laboratory.
- Include any details of blood group phenotyping already performed.

Test Summary

Full RBC Genotyping - Summary of essential referral information

≥3ml peripheral blood in EDTA; store and transport at room temperature; patient referral - haemoglobinopathy patients (standard genotype), multi-transfused patients, FY_{GATA} in African populations

9 RHCE VARIANT INVESTIGATION

This test identifies the most common RHCE variant alleles.

In addition to *RHD* variant alleles, *RHCE* variant alleles are often seen in ethnically diverse populations; although they are more commonly found in individuals of African origin. Individuals with *RHCE* variant alleles will normally exhibit variable expression of either or both Rhe and RhC antigens. Certain *RHCE* variant alleles will also encode RhCE proteins that lack various High Frequency Antigens (e.g. hr^S-, hr^B-, RH18-, RH34-).

Individuals with variable expression of the RhCE antigen have been shown to make alloantibodies with anti-e-like and/or anti-C-like specificity (e.g. anti-hr^S, anti-hr^B, anti-RH18 and anti-RH34), which are difficult to identify serologically. These clinically significant antibodies have been shown to cause transfusion reactions. Different combinations of *RHCE* variants can often be inherited with an *RHD* variant allele; these individuals may therefore make antibodies to RhCE antigens in addition to anti-D.

Benefits and Indications for Testing

Patients that would benefit from this test include:

- Haemoglobinopathy patients – to identify *RHCE* variants before transfusion
- Haemoglobinopathy patients – to identify *RHCE* variants in patients with anti-e-like or anti-C-like alloantibodies
- Obstetric patients of African origin – to identify *RHCE* variants in patients with anti-e-like or anti-C-like alloantibodies
- Other patients with variant expression of RhC or Rhe antigens.

Limitations of the Test

There are some rare situations where the genotype determination will not correlate with the serological phenotype. All clinical decisions should be taken with this in mind.

Single nucleotide polymorphisms (SNPs) in the relevant genes outside of the targeted regions will not be detected by SSP-PCR. In addition, novel mutations leading to altered or partial antigen expression and null phenotypes may not be detected.

Results from bone marrow or stem cell transplant patients may not match the genotype obtained from other tissues. Patients who have received a transplant from which incomplete engraftment has taken place, may exhibit chimerism in their DNA which may prevent a conclusive result being issued.

Referring Patient Samples

Please tick ***RHCE*** **Variant Genotyping** on the Test Request Form for Red Cell Genotyping (BT-637)

- At least 3ml peripheral blood in EDTA sample tubes is required. If only a small volume is available please contact the laboratory.
- The blood sample must not be opened following blood collection or used for any testing prior to being sent to the laboratory.

- Include any details of blood group phenotyping already performed.

Test Summary

***RHCE* Variant Investigation - Summary of essential referral information**

≥3ml peripheral blood in EDTA; store and transport at room temperature; patient referral - haemoglobinopathy patients, African obstetric patients, Rhe-positive patients with an anti-e-like alloantibody, RhC-positive patients with an anti-C-like alloantibody.

10 MOLECULAR INVESTIGATION FOR OTHER BLOOD GROUPS

This test identifies blood group alleles not identified by tests already described in this User Guide. These include ABO and rare/novel blood group alleles that are better identified by gene sequencing.

Benefits and Indications for Testing

Patients that would benefit from this test include:

- Patient's with anomalous ABO phenotyping
- Patient's with variant RhD, RhC or Rhe expression, not resolved by *RHD*/*RHCE* Genotype or *RHCE* Variant Genotype.
- Patient's with alloantibodies, the specificity of which is not yet covered by an IBTS test (e.g. Js^b type)

Limitations of the Test

There are some rare situations where the genotype determination will not correlate with the serological phenotype. All clinical decisions should be taken with this in mind.

Single nucleotide polymorphisms (SNPs) in the relevant genes outside of the targeted regions will not be detected by SSP-PCR. In addition, novel mutations leading to altered or partial antigen expression and null phenotypes may not be detected.

Blood Group gene sequencing is suited to detecting both known and novel mutations in blood group genes. However, there may be occasions where blood group expression is governed by an accessory molecule; the mutation responsible for lack of/altered expression may be in the gene of the accessory molecule (e.g. mutations in the *XK* gene affecting the expression of the Kx protein; which is required for the expression of KEL proteins).

Results from bone marrow or stem cell transplant patients may not match the genotype obtained from other tissues. Patients who have received a transplant from which incomplete engraftment has taken place, may exhibit chimerism in their DNA which may prevent a conclusive result being issued.

Referring Patient Samples

Please tick ***Other Blood Group Genotype Investigation*** on the Test Request Form for Red Cell Genotyping (BT-637).

- At least 3ml peripheral blood in EDTA sample tubes is required. Although more difficult to extract DNA from clotted samples, we will accept these samples with prior agreement. If a smaller volume is all that is available please contact the BGG Laboratory.
- The blood sample must not be opened following blood collection or used for any testing prior to being sent to the BGG Laboratory.
- Include any details of blood group phenotyping already performed.

Test Summary

Other Blood Group Genotype Investigation - Summary of essential referral information
≥3ml peripheral blood in EDTA; store and transport at room temperature; patient referral is usually by request from Blood Group Genetics Laboratory, IBTS.

11 SENDING SAMPLES

Sample Labelling Requirements

There are specific sample requirements depending on the test requested; see individual sections in this Customer Manual or the back of the relevant Test Request Form.

The IBTS will use the Referring Laboratory's Sample Reference Number as the main identifier for charging. Please ensure you include this, ideally on both Test Request Form and sample.

No sample criteria can be waived for testing that is performed on cell-free DNA extracted from peripheral blood plasma. Samples must either have handwritten labels, or demand-printed labels produced at the time of phlebotomy (including those generated by the MN-CMS).

Test Request Forms

The laboratory has two Test Request Forms. Both are available on the Blood Group Genetics Homepage. Test Request Forms will not be supplied to referring laboratories, instead they are to be downloaded and printed from our webpage (www.giveblood.ie/Clinical-Services/Blood-Group-Genetics/Customer-Resources/Sending-Samples/).

Test Request Form	IBTS Code	Colour	Use for these tests	Comments
Red Cell Genotyping	BT-637	Dark Red	Weak D Genotype	
			RHD/*RHCE* Genotype	
			Full RBC Genotype	
			RHCE Variant Genotype	
			Other Blood Groups	
Fetal *RHD* Screen	BT-638	Yellow	Fetal *RHD* Screen	NO alloanti-D present

The laboratory will also accept Test Request Forms generated by the MN-CMS.

Additional Information Required

Our Test Request Forms have been designed to capture the required information for testing and result interpretation. These include:

Additional Requested Information	Reason
Ethnic Origin	Ethnic origin is useful in the interpretation of results. Many blood groups are found exclusively in certain populations.
Clinical information including transfusion and transplant history	Clinical and transfusion history can be useful for result interpretation. The presence of DNA from transplanted material can affect the result interpretation.

Sample Packaging

Diagnostic samples must be packaged for transport to meet the requirements of IATA Packaging Instructions 650. The outside of the box must be clearly labelled and addressed to the appropriate laboratory. See website for address labels.

Packaging should be of good quality and strong enough to withstand the shocks and loading normally encountered during transport. Packaging must be constructed and closed so as to prevent any loss of contents that might be caused under normal conditions of transport.

The packaging consists of

1. **Primary receptacle** must be leak-proof and sealed, containing the specimen, not exceeding 1000ml, individually wrapped with enough absorbent material to absorb all fluid in the event of a leakage or breakage.
2. **Secondary packaging**, durable and leak-proof container to protect the primary receptacle. Multiple individually wrapped primary receptacles may be placed in one secondary packaging with sufficient absorbent material to absorb the entire contents.
3. **Outer packaging**, to protect the secondary packaging and contents from outside influences such as physical damage.

Sample Type

Test	Tested By	Sample Type	Required Volume
Fetal *RHD* Screen	IBTS	EDTA	≥8ml
Weak D Genotype	IBTS	EDTA	≥3ml*
***RHD*/*RHCE* Genotype**	IBTS	EDTA	≥3ml*
Full Genotype	IBTS	EDTA	≥3ml*
***RHCE* Variant Genotype**	IBTS	EDTA	≥3ml*
Full Genotype**	IBGRL	See IBGRL sample acceptance criteria	
ABO Genotype	IBGRL		

***RHD* Sequencing**	IBGRL	
***RHCE* Sequencing**	IBGRL	
Other blood groups	IBGRL	

* If 3ml EDTA whole blood is not possible please contact the BGGL (office or laboratory)
** Full genotype for Haemoglobinopathy offered by IBGRL

Due to the low concentration of cell-free fetal DNA present in maternal whole blood samples, pre-used samples will NOT be accepted for the Fetal RHD Screen. Fresh samples only (≤5 days post-phlebotomy) MUST be sent for Fetal RHD Screening.

If DNA is the only test material available, please contact the laboratory before referral. ***We will NOT accept DNA for the Fetal RHD Screen under any circumstances.***

12 EXTERNAL REFERRALS BY BLOOD GROUP GENETICS

On occasion samples may need to be sent to the International Blood Group Reference Laboratory in Bristol for testing of blood groups we currently do not perform. Following analysis by the IBGRL, results are provided in the form of a written interpretive report based on best practice guidelines for pre-transfusion compatibility procedures in blood transfusion laboratories, and will be sent from the Blood Group Genetics Laboratory to the referring clinician or referring laboratory.

13 REPORTING OF RESULTS

Molecular Blood Group Typing uses eTraceline for registration of samples, and test result management. Reports will be issued in one of three formats, depending on the test requested. Results are currently issued as paper reports or via MediBridge.
Please contact IBTS to discuss setting up electronic transmission of results.

14 TURN AROUND TIMES

The BGG Laboratory endeavours to deliver results in a timely manner, however to maintain cost-effectiveness most genotyping tests are batched. IBTS turn-around-times will be monitored and adjusted as appropriate.

Test	By	Target TATs*
Fetal *RHD* Screen	MBG	14 days
Weak D Genotype	MBG	14 days
***RHD*/*RHCE* Genotype**	MBG	14 days
Full RBC Genotype	MBG	14 days
***RHCE* Variant Genotype**	MBG	21 days

* calendar days (including weekends and public holidays)

Urgent Requests

With the exception of the **Fetal *RHD* Screen**, all blood group typing tests performed by the laboratory can be performed on an emergency basis. These samples will be processed individually and will therefore incur an 'emergency' charge. Results will be

available within 48 hours of receipt (Monday-Friday; excluding weekends and public holidays).

Please ensure all urgent samples arrive to the lab before midday 12:00 if a result is required on the same day.

Turn around times for routine samples are calculated from the time the sample is received by the IBTS to the time the report leaves the IBTS (either by post or electronically).

15 CHARGING

The BGG Laboratory endeavours to provide a quality yet cost-effective service. Current charges have been approved by the Department of Health. These current charges have been distributed to hospitals:

Test	Code	Reason
Fetal *RHD* Screen	FRS1	Fetal *RHD* Screen result issued
Fetal *RHD* Screen	FRS2	Inconclusive Fetal *RHD* Screen
Weak D Genotype	WDG	Weak D Genotype result issued
RHD/RHCE Genotype	RHD	*RHD*/*RHCE* Genotype result issued
Full RBC Genotype	FGS	RBC Genotype result issued
RHCE Variant Genotype	RHV	*RHCE* Variant Genotype result issued
SCD Combination	SCD	SCD patient requiring RHD, RHV and FGS (determined by BGGL)
Sample Rejected (Not Tested)	REJ1	Sample rejected: registered but not tested
Sample Rejected (In Progress)	REJ2	Hospital cancelled request, but after testing had started
Process Failure	PFA	Failure of process [BGGL]
Emergency Charge	EMM3	Request for test result to be issued ≤48 hours [BGGL]
Emergency Charge (NHSBT)	EMM4	Fee charged by IBGRL for urgent investigation
Courier Charge	BGGC	To cover cost of DHL incurred by IBTS when sending to IBGRL
Miscellaneous Charge	MISC	For any cost incurred not covered by above

The laboratory will use the Referring Laboratory's sample reference number as the main means of identification for charging. It is therefore vital that this be included on at least one of either the Test Request Form or sample.

16 DATA PROTECTION

Patient's Personal Data

The Irish Blood Transfusion Service is committed to ensuring that the personal data it collects and processes in the course of performing its functions, is managed appropriately and securely at all times. To this end the IBTS complies with the Data Protection Acts 1988 – 2018 and the General Data Protection Regulation 679/2016 (GDPR).

The IBTS acts as Data Processor for patient data. The IBTS keeps patient and donor data on both a computer system and on a paper filing system. The data held can include some or all of the following:

Requested/Stored Information	Reason
Name	Critical identification information
Date of Birth	Critical identification information
Gender	Critical identification information
Address	Useful identification information
Medical Record Number	Critical identification information
Ethnic Origin	Useful information that can be vital for result interpretation
Laboratory Referral Number	Essential information. The IBTS will use this for charging.
Referring laboratory test method and result	Useful information that can guide which test is to be used by BGGL. Can also be vital for final result interpretation.
Transfusion history	This not essential, however can be useful in result interpretation.
Transplant history	The presence of DNA from transplanted material can affect the result interpretation.
Obstetric history	This not essential, however can be useful in result interpretation.
Clinical information pertinent to the specific test	This not essential, however can be useful in result interpretation.
Dates specimens collected, received, tested and reported	Requirement for ISO:15189 compliance
Test results and interpretations	Requirement for ISO:15189 compliance
Test methods used	Requirement for ISO:15189 compliance

Blood/plasma/DNA samples for Fetal *RHD* Screen and red cell genotyping are kept for a period of at least one month after the date on which the initial test results are reported. The specimen tubes are discarded periodically after this date (as workload allows).

Hard copies of reports and request forms are stored for 30 years.

Retention of DNA with blood group alleles of interest

The Laboratory is committed to working towards obtaining accreditation to the *ISO:15189 Standard for Medical Laboratories*, and accreditation to the AABB's *Standards for Molecular Testing for Red Cells, Platelet, and Neutrophil Antigens*.

A requirement for accreditation to the AABB's *Standards for Molecular Testing for Red Cells, Platelet, and Neutrophil Antigens* is the development of a repertoire of examples of DNA with various blood group SNPs. Occasionally samples will therefore be retained for this purpose.

If your laboratory prefers this not to happen, please let us know so we can discard such samples.

17 TERMS AND CONDITIONS

Referral of samples accompanied by a signed and completed Test Request Form (BT-637 Red Cell Genotyping, BT-368 Fetal *RHD* Screen) and acceptance of this sample for testing by the laboratory constitutes an agreement between the Requesting Laboratory and the MBG Laboratory.

Please note that if your hospital has signed a Service Level Agreement with the IBTS, this agreement overrides standard terms and conditions.

18 COMPLAINTS AND FEEDBACK

Your feedback is important to us. Any complaints, compliments or feedback may be directed to the Quality Assurance Department. Please follow directions on how to do this on the IBTS website (https://www.giveblood.ie/Clinical-Services/Quality/).

Non-Quality related feedback can be directed to the Laboratory (See Contact Details at the beginning of this document).

19 APPENDIX 1 – FETAL RHD SCREEN ADDITIONAL INFORMATION

Definition of 'false negative result'

In fetal blood group genotyping, a false negative result means that the test has predicted an antigen negative fetus when the baby is found to be antigen positive at birth.

Definition of 'false positive result'

In fetal blood group genotyping, a false positive result means that the test has predicted an antigen positive fetus when the baby is found to be antigen negative at birth.

How is accuracy calculated?

The IBTS performed an extensive assay development and validation project to confirm the accuracy of the assay ($LOD_{0.95}$ = 8 copies *RHD* per ml plasma; sensitivity = 100% (95% CI = 99.24 - 100)).

The accuracy of our test results are monitored and all discrepant results notified to us are investigated.

The importance of feedback on discrepancy between genotype and phenotype – procedure for the identification of a discrepancy.

User feedback from test results is essential for the maintenance of accurate data on how our tests perform. If a discrepant result is identified, please contact the laboratory as soon as possible.

What will the IBTS do if they are notified of an incorrect result?

Please note that we will not investigate false positive results for the fetal RHD screening test. The IBTS will re-test the archive plasma sample (if available) to confirm genotype. Errors (other than false positive cases) will be investigated and if an error in procedure is identified, corrective and preventative actions will be put into place. The user will be notified of the findings and any recommended further action.

Reasons for incorrect results:

Fetal DNA in maternal plasma represents a very small fraction of the total DNA in plasma. The amount of fetal DNA increases during pregnancy. However, it can vary between individuals and in some cases the amount of fetal DNA may be too low to detect, especially in early pregnancy (<11 weeks gestation). This can cause a false negative result.

- **Other possible causes of false negative results:**
  Error (human or mechanical) in testing
  Wrong blood in tube
  False positive results may, on rare occasions, be caused by presence of genes which are not expressed on red cell surface (i.e. the phenotype does not reflect genotype). Some blood group genes are inactivated by mutations distinct from the blood group gene itself.

- **Other possible causes of false positive results:**

Extraneous contamination of the blood sample
Extraneous contamination of testing reagents (all normal precautions are taken to ensure this does not occur)
Error (human or mechanical) in testing
Wrong blood in tube
Vanishing twin

Is the test suitable for patients who have weak D or RHD variant allele?
Women who have been confirmed to be weak D or carriers of an RHD variant are unlikely to benefit from the fetal RHD screen because the maternal RHD gene will prevent prediction of fetal RHD phenotype, and an inconclusive test result will be issued.

Can the test be used for patients who expect multiple births?
Yes the test is suitable for pregnancies with twins, but the report will predict the (single) fetus as being positive or negative. A positive result in this case means at least one of the babies is predicted RHD-positive. A negative result would mean that all the babies are predicted RHD-negative. The report does not distinguish between single and multiple pregnancies.

Common reasons a repeat sample may requested for testing:
- Insufficient patient identifiers on tube and/or referral form
- Insufficient sample <8ml
- Sample > 5 days old at time of receipt to the laboratory
- Sample damage e.g broken tube
- Sample haemolysed
- Sample tube opened or previously used for another test
- Inadequate sample storage temp
- GA <11 weeks and the fetal RHD result is predicted RHD-negative fetus
- Where a failure occurred during processing or testing so that a result cannot be issued

The importance of storing samples for fetal RHD screen at room temperature:
The white blood cells in the sample deteriorate when they are exposed to temperature changes. This results in high levels of maternal cell free DNA levels which can interfere with the test result.

Can samples be tested when pregnant women have already received Anti-D prophylaxis?
Yes, samples can be tested for fetal *RHD*. Anti-D prophylaxis does not interfere with this test.