Red Cell Immunohaematology

User Guide

Contents

This Guide

This guide outlines the Red Cell Immunohaematology (RCI) services provided by NHS Blood and Transplant (NHSBT). RCI is a UKAS accredited medical laboratory No. 8740.
This guide is of use to medical and scientific staff in hospital transfusion laboratories and others involved in, for example, antenatal care. The guide contains information about the organisation of the services and contact details for key staff.

NHS Blood and Transplant (NHSBT)

The National Blood Transfusion Service was founded in 1946 and was, until 1994, providing services regionally. In 1993 a Special Health Authority was created and in 2006 this Special Health Authority merged with UK Transplant, forming NHS Blood and Transplant (NHSBT). NHSBT is a national service and employs around 6,000 staff. The accountability for supplying blood services lies with NHSBT and the strategy for the service is formulated nationally, with local delivery. The core purpose is:

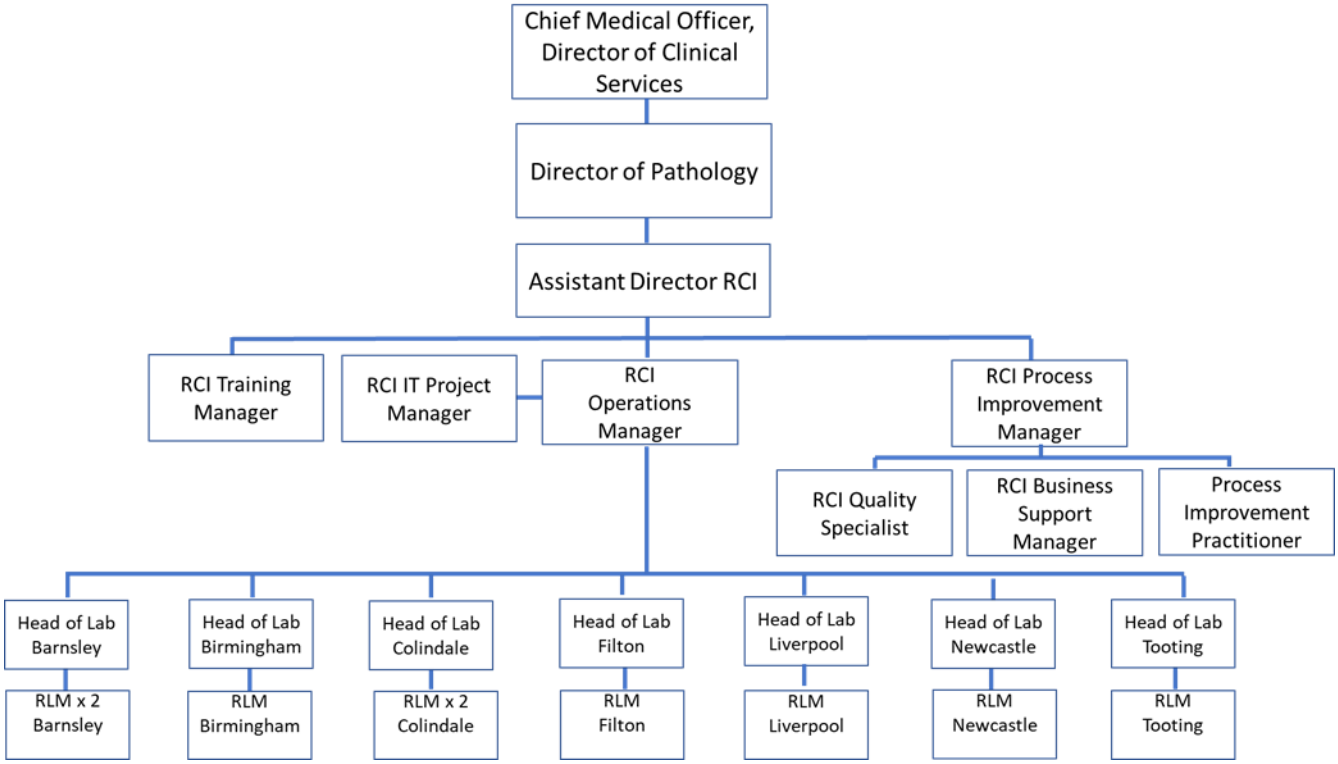
To save and improve patients' lives

Management of Red Cell Immunohaematology

RCI is managed nationally by the Assistant Director RCI, supported by National Operations and Process Improvement managers. Clinical support is provided by Consultant Haematologists and Consultant Clinical Scientists. The service is supported by the Quality function.

There are 7 laboratories providing RCI services at Barnsley, Colindale, Tooting, Filton, Birmingham, Liverpool, and Newcastle.
Each of the RCI laboratories is managed by a Head of Laboratory (HoL), and each laboratory is supported by a medical consultant. See Appendix 1 for contact details.
Samples may be sent to another site for testing and/or booking in.

Management diagram – RCI Reference



Quality statement

RCI is accredited to ISO15189 and RCI services, in common with other NHSBT services, are committed to a total quality philosophy. All work is carried out within the framework of a documented quality system, according to Good Laboratory Practice (GLP) and Good Manufacturing Practice (GMP) and in compliance with the Blood Safety and Quality regulations and Data Protection and Freedom of Information Acts. Techniques and procedures are validated, described in standard operating procedures (SOP) and conducted by staff whose proficiency is regularly monitored.

NHSBT Quality Managers carry out regular audits to establish and improve the level of BSQR and ISO15189 compliance. These complement external licensing and accreditation inspections by the Medicines and Healthcare Products Regulatory Agency (MHRA) and U.K. Accreditation Service (UKAS). RCI is committed to maintaining accreditation to ISO15189 as a managed network covering all its laboratories.

RCI is committed to standardising practice and strives for a consistent and high quality service for all RCI laboratories. Working together with the NHSBT medical directorate, clinical policies and procedures are implemented and developed in accordance with the principles of clinical governance.

All RCI laboratories participate in UK NEQAS (or EQA) exercises for all relevant disciplines. EQA results are promptly reviewed. All episodes of poor performance or trends that indicate as adverse impact on quality are reported on the QMS and managed through the root cause

and CAPA. RCI will feed back detail of the EQA performance, including and poor performance at the user group meetings.

NHSBT quality experts are always pleased to share their expertise with colleagues in the wider NHS where time and resources allow. If you need help, contact the Quality Department to discuss your requirements (Appendix 1).

Complaints / Compliments

NHSBT is committed to continuously improving the quality and range of services provided and welcomes any comments or suggestions from users. To ensure appropriate clinical governance, it is essential that failures of service and near misses which could have affected patient care are reported, and forms have been made available to hospital transfusion laboratories for this purpose. Please do not hesitate to discuss complaints with either Customer Services or the relevant medical consultant or Head of Laboratory. We always strive to provide a satisfactory response to any complainant. However, if you are unhappy with the handling of your complaint, please contact the Head of Customer Services (Appendix 1) Full details of the NHSBT complaints procedure can be found at:
Customer Service - Hospitals and Science - NHSBT (blood.co.uk)

Complaints must be clearly separated from communication about adverse reactions to transfusion, near misses or Patient Adverse Events which have or could have affected the quality of patient care. Such incidents and near misses often require immediate action and you are advised to discuss these with a NHSBT medical consultant, Consultant Clinical Scientist or a senior laboratory scientist at your local blood centre. Serious events must be reported to SABRE (appendix 2).

Services provided by RCI Laboratories

RCI's accredited services are listed at www.UKAS.com.
RCI provides the following services in support of hospital transfusion laboratories:

- Investigation of antibody problems, including crossmatching
- Investigation of haemolytic transfusion reactions
- ABO/D typing, including typing problems
- Investigation of positive direct antiglobulin tests, including autoimmune haemolytic anaemia (AIHA), crossmatching for these cases
- Determination of blood group by molecular methods when conventional serology is not applicable
- Investigation of IgA deficiency (provided nationally from Barnsley)
- Investigation of haemolytic disease of the fetus and newborn (HDFN)
- Antenatal reference services including quantification (testing is centralised and may be provided by another RCI laboratory) and titration of antibodies
- Determination of feto-maternal haemorrhage (FMH) (testing is centralised and may be provided by another RCI laboratory)
- Investigation of drug induced AIHA (provided nationally from Barnsley)
- Provision of suitable blood for IUT and at delivery
- ABO and D grouping of patient and donor transplant samples.
- Medical and scientific advice

RCI Guidance on Two Sample Policy

BSH guidelines require the ABO and D group of patients to be determined on two separate samples, the responsibility for ensuring this has been completed, and that the group is consistent with that determined by RCI in supplying crossmatched blood, remains with the hospital transfusion laboratory.

Type of investigations

Atypical antibodies, serological and crossmatching problems

Samples referred for the investigation of atypical antibodies will be tested both to confirm the specificity of the antibodies and to exclude the presence of additional alloantibodies. RCI reference laboratories have access to a large number of phenotyped red cells in addition to routine antibody identification panels to enable a full investigation to be completed. The patient's Rh and K phenotype will be performed on the first sample received from that patient, plus testing for any other implicated antigens. Clinical advice on further transfusion support is given as appropriate. Results are entered on the NHSBT national patient database and an antibody card will be issued for patients with irregular antibodies on first presentation. These results are also available on the Sp-ICE results viewer for registered hospitals

Serological and crossmatching problems can be discussed with the scientific staff and samples referred for investigation. Advice can be given as to the type of blood that is suitable for transfusion and the availability of such blood and in addition RCI will undertake crossmatching on request.

The turnaround time for these investigations is 4-6hours as per the Contract for the Supply of Blood Components, Reagents and Services Part 2 Section 3.5

Haemolytic Transfusion Reactions

See appendix 2 for guidance on reporting adverse reactions to transfusions.

ABO typing, Rh phenotyping, DAT and antibody screen / identification will be performed on both pre- and post-transfusion samples if available. If no antibodies are detected by standard methods a more sensitive method may be applied. Eluates will be prepared as necessary and tested against a panel of cells, including A_1 and B cells, by an IAT method. An ABO, Rh phenotype and DAT will be performed on any implicated red cell units and re-crossmatching will be carried out against the pre- and post-transfusion samples.
The tests above will be performed using plasma, however, if all tests for antibodies are negative and there is strong evidence of haemolysis, a serum sample may be requested in order to repeat the tests. It is possible that some weak, complement-dependent antibodies will be more readily detected in serum.
If all reactions are negative, further investigations will be considered for non-haemolytic transfusion reactions e.g. HLA antibodies, anti-IgA.

Problems with ABO/D grouping

Samples can be referred for investigation if anomalous results are obtained with routine ABO and/or D grouping, e.g. to distinguish between partial and weak D antigens. With ABO grouping problems saliva may be requested. It is important to include relevant clinical data on the request form. A blood group card may be issued if required.

Direct Antiglobulin Test (DAT)

In a high proportion of DAT positive cases with free autoantibodies referred to the RCI laboratories, alloantibodies are also present. Therefore it is recommended that samples from patients requiring a transfusion and with a positive DAT are referred so that clinically significant alloantibodies, which might be masked, can be detected and identified. In these cases absorptions may be performed and adequate volumes of samples are required.

In recently transfused patients who develop a positive DAT, this may be caused by alloantibodies bound to donor red cells (indicating a possible delayed haemolytic reaction); in such cases an eluate needs to be prepared for which at least 1mL of packed red cells are needed.

In patients with a positive DAT and a negative antibody screen who need transfusions, it is not necessary to refer samples prior to each transfusion episode. An eluate is indicated only if there is evidence of a delayed haemolytic transfusion reaction, a change in serology or if a higher frequency of transfusion than normal is required to maintain an adequate level of haemoglobin. The DAT may be positive in patients or in healthy individuals without overt haemolysis.

Autoimmune haemolytic anaemia

An ABO, Rh and K phenotype are performed on all referrals.
A DAT is performed with isotype and complement specific reagents (anti-IgG, -IgA, -IgM, -C3c, -C3d).

Alloantibodies in addition to autoantibodies: samples are tested to ensure clinically significant alloantibodies that may be masked by autoantibodies are detected and identified.
Eluates are prepared and tested only if the patient has been recently transfused, has received a haemopoietic stem cell or bone marrow transplant or has signs of haemolysis. If other tests have proved inconclusive an eluate might also be of value.

Red Cell Genotyping

Red cell genotyping is undertaken in RCI laboratories to support and enhance timely decision-making to improve the safety of transfusion and improve patient outcomes.

In particular, the ability to determine blood type in previously transfused patients, and those with immunoglobulin coated cells will be of significant benefit. The service is available for referrals from all users but is provided from Tooting, Birmingham, Barnsley, Filton, and Newcastle sites.

Low throughput, rapid turnround genotyping is performed using the Innotrain Fluogene system. The FluoGene detection system is based on polymerase chain reaction (PCR) sequence specific priming (SSP) using the Amplification Refractory Mutation System (ARMS) principle
The test enables predicted phenotypes for commonly found antigens with the Rh (C,c,E,e,Cw), Kell, Kidd, Duffy, MNS and Dombrock blood group systems to be given.

The use of this test will be determined by RCI and clinical staff as part of the investigation.

Drug associated Auto-Immune Haemolytic Anaemia

Haemolysis suspected to be associated with the use of a certain drug can be investigated, but advice should be sought from an RCI medical consultant before sending samples.

Cold Agglutinins / haemolysins and cold haemagglutinin disease

A DAT is performed and the plasma is investigated for the presence of clinically significant red cell alloantibodies at 20°C. Subsequent tests may need to be performed at 4°C and 30°C where indicated to establish clinical significance and thermal amplitude. It is not necessary to warm separate samples unless titration studies are required or specifically requested to do so by the RCI laboratory. Cold agglutinin titrations at 4°C can be performed on request in Cold Haemagglutinin Disease patients.

Transfusion advice: SAGM-suspended cells that are ABO compatible, K negative and of a Rh phenotype matched with that of the patient are selected for transfusion. If the patient has clinically significant red cell antibodies units must also be negative for the relevant antigen(s). The blood is crossmatched by a standard antiglobulin technique strictly at 37°C. Advice regarding the use of blood warmers for patients with cold agglutinins will not be included in RCI reports.

Biphasic haemolysins and paroxysmal cold haemoglobinuria (PCH)

Biphasic haemolysins as a cause of AIHA are extremely rare and mainly seen as a post-viral event in children. Routine investigations for AIHA do not include the test for biphasic haemolysins but where indicated, or on request, the Donath-Landsteiner test can be performed if paroxysmal cold haemoglobinuria (PCH) is suspected. If positive, the specificity of the antibody can be determined to confirm the diagnosis.
The Donath-Landsteiner test requires a separated serum sample which has been separated from a whole blood sample that has been allowed to clot at 37ºC. Please contact your local RCI laboratory for advice regarding sample referral.
Transfusion advice: The haemolysis in PCH is generally self-limiting. Transfusion requirements can be discussed with a NHSBT medical consultant.

Paroxysmal nocturnal haemoglobinuria (PNH)

If you require investigation of a suspected case of PNH the RCI laboratories will refer you to a specialist centre such as

Haematology Malignancy Diagnosis Service at Leeds Teaching Hospitals. Tel 0113 392 6285. Hospitals may refer samples there directly for testing.

Birmingham Heartlands: 0121 424 0706

Haematology dept. King's College Hospital, London 0203 299 3520

Transfusion advice: Previously washed red cells were recommended for PNH patients. However, there is no evidence that the survival of washed red cells is better than that of those suspended in SAG-M.

Patients receiving monoclonal antibody therapy.

Reactivity typically presents as either an autoantibody or an antibody to a high frequency antigen, with a pan-agglutinin present in IAT tests. There may be a positive or negative auto control, and a positive DAT. Different therapies may affect serological testing methods in a variety of ways. MoAb induced reactivity may persist for several months after the last treatment infusion.

Before commencing MoAb based therapies, all patients should have an ABO group, Rh & K typing, an antibody investigation, direct antiglobulin test (DAT) and a full red cell phenotype.

If the patient has already received cellular transfusion, is DAT positive and has a positive autologous control, or has already commenced MoAb based therapies then a red cell genotype is performed in place of the phenotype. Where a genotype is performed, this will be performed on the original referred sample but will be reported under a different RCI sample number to the serology results.

Provision of crossmatched units in difficult cases

Where the provision of crossmatched units is problematic, the RCI laboratory can undertake the crossmatch. Please make the request for crossmatched units clear on the request form. All crossmatch requests required during routine hours must be telephoned to the local RCI laboratory. Out of hours, only crossmatch requests that are required to be tested within the out of hours period must be telephoned. If the request is sent overnight for investigation and provision of crossmatched units later the following day, please telephone the RCI lab as soon as possible during routine hours the following day to confirm. In the event of difficulty in contacting RCI, please contact Hospital Services department.

Crossmatched units may sometimes be labelled by RCI as "suitable", rather than "compatible" for a patient. If this is because either a) serology testing is not complete within RCI or b) serology cannot be resolved and the sample needs to be sent to IBGRL for further investigation, but transfusion is required urgently before allo-antibodies have been identified / excluded – then hospital laboratory staff should inform their Consultant Haematologist, who may wish to discuss with an NHSBT Consultant, whether steroid or IVIg cover for transfusion may be appropriate. Transfusion observations should be reviewed and investigation for any signs of delayed haemolytic transfusion reaction (low Hb, raised LDH, bilirubin, reticulocytes) and if these are present renal function should be monitored.

If units are labelled as “suitable” because either a) an auto-antibody / pan-reactive antibody is present, but no alloantibodies are detected, b) the patient is on Daratumamab treatment or c) other reasons such as tests were performed with modified plasma e.g. neutralised for Chido/Rodgers, or allo-adsorbed, or d) cross-matching for a baby was performed using the mother’s plasma – then the additional clinical measures above are not required.

In cases where the delay in waiting for crossmatched units to arrive from RCI may adversely impact on the patient’s condition (e.g. Hb <60g/L or the patient is bleeding or actively haemolysing), then it is advised that hospital laboratory staff escalate the case to their local Consultant Haematologist to ensure there is a plan in place to mitigate the risk of any delay in blood provision. Advice is available from NHSBT Consultant Haematologists and RCI if required.

Investigation of IgA deficiency

In cases of anaphylactic transfusion reaction or other indications, samples can be referred to test for IgA deficiency and the presence of antibodies to IgA. A card is issued to the requesting clinician together with the RCI report for IgA deficient patients in whom IgA deficiency is diagnosed as part of a transfusion reaction, with or without anti-IgA. who have experienced transfusion reactions.
The turnaround time for issuing IgA deficiency results is 5 working days.
Please contact Barnsley RCI for advice of how to send samples for IgA investigation before sending the referral.

Haemolytic disease of the fetus and newborn (HDFN)

Suspected cases of HDFN can be investigated by RCI reference laboratories to detect and identify the causative red cell antibody, including immune ABO antibodies. Samples are required from mother and newborn, carefully labelled to distinguish between them.

Antenatal reference service

RCI offers an antenatal reference service for women whose plasma contains irregular antibodies. The specificity of the antibody is determined or confirmed and the concentration is measured. All investigations related to the prevention of HDFN are in accordance with the Guideline for the Investigation and Management of Red Cell Antibodies in Pregnancy.
https://b-s-h.org.uk/guidelines/guidelines/

Pregnant women with a positive red cell antibody screen

If the antibody is confirmed and is of clinical significance to the fetus, the antibody will be quantified or titrated and follow-up tests performed as recommended by BSH guidelines above. Antibody cards and explanatory leaflets are issued to all women who have clinically significant antibodies.

The follow-up investigations are:

- Monitoring maternal red cell alloantibody levels
- Identification of possible additional clinically significant antibodies

In addition the following may be recommended:
- Red cell phenotyping of the father
- Fetal genotyping (at IBGRL) using peripheral maternal blood samples

Measurement of maternal antibody levels during pregnancy (quantification and titration)

Measurement of antibody concentration depends on the antibody specificity:
- anti-D and anti-c are quantified in comparison to a national standard (see below)
- other antibodies, including anti-K, are titrated by testing a series of doubling dilutions of the antibody. Where available, the previous archive sample will also be titrated. The result is expressed as a titre, i.e. the greatest dilution of plasma which gives a serological reaction. A titre of 32 or above is generally considered to have the potential to cause HDFN.
- antibodies such as anti-P_1, anti-Le^a and anti-Le^b, which do not cause HDFN, do not require measurement.

Anti-D and anti-c are the antibodies most likely to cause significant fetal disease. Therefore, pregnant women with these antibodies should be followed-up at monthly intervals until 28 weeks gestation and at two-weekly intervals thereafter to term. Anti-D and anti-c are quantified against a national standard and the strength of the antibody is reported in international units (IU/mL).

The measurement of antibody in international units is one of the tools available for assessing the likelihood of HDFN. An increase by 50% or greater over the previous level indicates a significant rate of increase, irrespective of the period of gestation.

Generally the significance of anti-D and anti-c levels during pregnancy is as follows:

Anti-D less than 4 IU/mL	HDFN unlikely
Anti-D 4-15 IU/mL	Moderate risk of HDFN, requiring referral to a specialist obstetric unit
Anti-D greater than 15 IU/ mL	High risk of HDFN, requirement as above

NB. It should be noted that HDFN has been reported at levels less than 4 IU/mL.

Anti-c less than 7.5 IU/ mL	HDFN unlikely, continue to monitor
Anti-c 7.5 – 20 IU/mL	Moderate risk of HDFN, requiring referral to a specialist obstetric unit
Anti-c greater than 20 IU/mL	High risk of HDFN, requirement as above

RCI antenatal reports provide clinical advice regarding risk of HDFN and follow-up required.

It is essential that pregnant women with levels of anti-D of ≥ 4 IU/mL or anti-c at ≥ 7.5 IU/mL are referred to an obstetric unit with experience in non-invasive monitoring for

fetal anaemia at the earliest opportunity. RCI provides this advice in the antenatal reports.

Measurement of uncertainty data for quantification, titration and FMH estimation is available on request.

Due to the adoption of the NICE guidance recommending routine antenatal anti-D prophylaxis for D negative women, there is a marked increase in the incidence of low level anti-D in antenatal samples taken after anti-D injection. BSH guidelines recommend that all antenatal samples where anti-D has been detected are referred for quantification by a reference laboratory (unless an in house fully validated technique is used). RCI will provide repeat sampling recommendations based on the BSH guidelines and information provided on the request form.

Anti-K is a significant cause of HDFN. More than 80% of women who have anti-K, have K negative partners. Guidelines recommend a sample from the father of the pregnancy should be K typed. RCI will not routinely request paternal samples, but will recommend on the maternal report that the results of paternal testing could provide useful information to support the management of the pregnancy. If the paternal sample is typed as K negative either by RCI, or the hospital transfusion laboratory provides written confirmation that they have typed a paternal sample as K negative, samples from the mother will be requested for re-testing at 28 weeks gestation. The report will state the current paternal details and it will be the responsibility of the healthcare professional monitoring the pregnancy to confirm these are correct. If the paternal sample is K positive or if there is any doubt, samples will be requested at monthly intervals to 28 weeks and fortnightly thereafter to term.

The concentration of **anti-K and other clinically significant antibodies** is assessed by testing dilutions of the patient's plasma and the results are reported as a titre.

Pregnant women with clinically significant antibodies other than anti-D, anti-c and anti-K should normally be re-tested at 28 weeks gestation. The results at this time will determine the frequency of follow-up testing. These antibodies are titrated as for anti-K.

Many red cell antibodies have the potential to cause HDFN. Therefore, it is essential for ALL pregnant women found to have clinically significant red cell antibodies to be referred to a hospital obstetric unit for management of their pregnancy. For women with clinically significant antibodies, including anti-K, with a titre of ≥ 32 it is also recommended that referral to a specialist obstetric unit be considered (as for women with significant levels of anti-D or anti-c - see above).

Clinical advice is given on reports of all pregnant women with antibodies that could cause fetal disease.

Exclusion of further antibodies

All samples referred for antibody monitoring are tested to exclude further clinically significant red cell antibodies that might have developed.

Paternal phenotype

When an antibody with a high chance of causing HDFN is detected (i.e. anti-D, anti-c, anti-K or other significant antibody with a high titre) the maternal report will recommend that a sample from the partner of the fetus is phenotyped, because paternal testing can be useful to guide clinical management.

Fetal genotype

If there is a high risk of severe HDFN, caused by anti-D, anti-c or anti-K, then prediction of the relevant genotype of the fetus should be considered on the advice of a Consultant in fetal medicine. Fetal D, C, c, E and K genotyping can be performed on a sample of maternal blood. The molecular typing service is provided by IBGRL. www.blood.co.uk/ibgrl

Feto-maternal haemorrhage estimation

In any case where the acid elution test indicates a FMH > 2mL of fetal red cells, or where the test result is equivocal, a sample (the same as used for the acid elution test) can be referred to RCI for confirmation by flow cytometry. Advice on how much additional anti-D prophylaxis is recommended for large bleeds will be provided by an RCI Consultant Haematologist or a Consultant Clinical Scientist.

To ensure timely testing, please telephone your local RCI FMH testing laboratory if you intend to send samples for flow cytometry. In the event of difficulty in contacting RCI, please contact Hospital Services department.

Please note that for large bleeds >40mL an intravenous (IV) anti-D preparation is recommended in preference to intramuscular injection

Provision of blood for Intra-Uterine Transfusion (IUT)

RCI will crossmatch units for IUT on request. It is essential that RCI is notified in advance of any planned IUT in order that suitable units can be identified in a timely manner. RCI will only perform crossmatching for IUT out of hours if the case is clinically urgent.

ABO and D grouping of donor/patient transplant samples

This service is available for transplant samples including patients and donors. The type of sample must be clearly indicated on the request form and it is essential that samples are collected in an **EDTA individual tube** and must conform to BSH labelling guidelines (see section on labelling requirements for samples and request forms). The results are available by Sp-ICE, reports will not be issued from RCI via email or hard copy.

Medical and scientific advice

Advice on all aspects of RCI investigations can be obtained from Consultant Haematologists and senior scientists. Contact details are given in Appendix 1. This advice is available 24 hours per day and details of how to contact us outside our normal working hours are given in 'On Call' below.

Reports include advice on selection of units for transfusion and, in the case of antenatal samples, advice on the significance of antibodies in pregnancy and the requirements for re-testing.

Out of hours/On Call

All blood centres can be contacted 24 hours per day, every day of the year (Appendix 1).
Out of hours hospitals should contact RCI via Hospital Services rather than directly on all sites except Filton where a Smart Divert number: 01179693927 is in use for hospitals to contact RCI on call BMS directly. Please contact the Hospital Services Department at the site where the referred samples will be processed.

Urgent cases

RCI aim to report results within five working days. Any referral which requires a result before this time, or any crossmatch request should be treated as an urgent request. Interim results might be incomplete and subject to change/addition before issue of the final report and may require review before they can be given. Please notify the RCI laboratory by telephone of any urgent requests. All crossmatch requests required during routine hours must be telephoned to the local RCI laboratory. Out of hours, only crossmatch requests that are required to be tested within the out of hours must be telephoned.

During normal working hours contact the local RCI laboratory or Consultant Haematologist. Out of normal RCI laboratory working hours, for clinical and scientific advice and discussion about urgent cases and requests for urgent investigations, phone the Hospital Services department. They can put you in contact with a RCI laboratory scientist or a medical consultant.

In the event of difficulty in contacting RCI, please contact Hospital Services department.

Both during and outside RCI laboratory working hours your contact will ask you for information about urgent referrals in order to understand the immediate requirement and urgency of the case and to assist in the appropriate investigation. In addition to the patient demographics you will be asked for: the patient's condition, current haemoglobin level, transfusion and/or obstetric history, drug history, if relevant, and transfusion requirement (special requirements etc.). We will also ask for a summary of your serological findings.

Collection of data on the outcome of pregnancies with clinically significant antibodies.

In order to collect and collate data on the effect of red cell antibodies on the fetus and newborn, RCI will issue a questionnaire for selected women who have red cell antibodies. The questionnaire requests information about the outcome of the pregnancy and condition of the baby. The aim is to build up a body of evidence about the effect of antibodies of known specificity and strength which will be valuable in determining future policies and practices for the testing and treatment of pregnant women with antibodies. Expectant mothers are informed via the antenatal information leaflet "Blood Groups and Red Cell Antibodies in Pregnancy" and on the information sheet issued with cards sent to women with significant red cell antibodies. Data Protection requirements are satisfied by the statements on the questionnaires together with the Service Level Agreement between the NHSBT and your Trust. The help and co-operation of users in completing these questionnaires is an essential contribution to this initiative.

Patient information cards

A patient information card will be sent for patients with clinically relevant irregular red blood cell antibodies or IgA deficiency with a history of transfusion reaction.

Request forms, samples: labelling requirements

Our investigations may require testing of the patient's DNA, and storage for possible testing in the future. RCI have reviewed the relevant legislature and guidance, which confirms that there is no legal or professional requirement to obtain specific informed patient consent for red cell genotyping. Genotyping data may be processed within General Data Protection Regulation (GDPR) legislation as they are considered to be in the "vital interests" of the patient.

Red cell genotyping is purely for diagnostic purposes and only provides information about the patient's predicted red cell phenotype. There are no implications for the patient's family, it cannot predict future disease risk and RCI cannot pick up incidental findings of potential significance. Under guidance from the Joint Committee on Genomics in Medicine, RCI genotyping samples can be treated as standard pathology samples, without the need for separate patient consent.

Request forms

Request forms can be ordered free of charge from any NHSBT RCI laboratory.

Sample requirements

Full details of sample requirements are listed on the reverse of the request forms.

Haemolysed and Lipaemic samples

Sending haemolysed and/or lipaemic samples should be avoided where possible as free haemoglobin and/or fatty plasma can produce test result errors especially when using

automated equipment. Such samples may have to be rejected. However, it is recognised that there are situations when haemolysis, in particular, is a result of the patient's condition.

Labelling of samples / completion of request forms

All samples must be accompanied by a request form specifying the tests or investigations required. Please ensure that relevant details are included on the request form to help determine suitability of samples for investigation.

Request forms are the basis of the correct identification of the patient. The SHOT scheme has shown that serious hazards of transfusion are often caused by clerical errors. The points of identification provided on the request form must match the information provided on the sample. RCI will not accept referrals with an inadequate request form or sample labelling (see Guidelines for Pre-Transfusion Compatibility Procedures in Blood Transfusion Laboratories) https://b-s-h.org.uk/guidelines/guidelines/

In the event that the sample and request form do not match, but it is confirmed that the details on the samples are correct, RCI will request a replacement request form with the correct information. In these cases results/crossmatched units/reports will not be released until the replacement request form is received.

In case of a clinical emergency RCI may agree with the requesting Consultant Haematologist or laboratory scientist to proceed with the requested investigations under concession. In such cases reports will carry an explicit warning that the three points of identification were not used for the samples and / or request form and advice that caution should be taken when accepting the results.

The requester is advised to check the identifiers and to obtain reassurance about the identifiers used for the linking between patient and sample.

Samples labelled with *Addressograph* labels, i.e. pre-printed labels, are not accepted by RCI. Demand printed labels which have been generated and attached at the bedside at the time of phlebotomy from scanning bar-coded wristbands on an automated system are acceptable for samples. Since it is not possible to distinguish reliably between demand printed and *Addressograph* labels they can be accepted only from referring organisations which have informed RCI, in writing, that their sample labels are generated in an audited system and are demand printed at the time of phlebotomy. Please contact your local Customer Services Manager if you wish to discuss this.

RCI will not normally test samples unless three or more identical points of identification are used on both request forms and samples.

Minimum patient identification. Surname and first name are one identifier:

- Surname / family name
- First name(s) in full (correctly spelt)
- NHS number or hospital number
- Date of birth

While RCI will accept hospital number as a third identifier, we would remind users that the hospital number does not specifically identify your patient. Since some hospitals use the same numbering systems and RCI stores patient data on a national database, the use of hospital number without other points of identification may lead to errors. Please use the NHS number where available. RCI are working towards a zero-tolerance policy for inadequately labelled samples.

The sample tube must be signed by the person taking the sample and must be dated to ensure that it is fresh enough to give accurate results within the parameters of the test(s) requested. For pre-transfusion samples, the time the sample was taken must also be on the sample tube. If this is not provided RCI will use a default time of 00:00 (midnight)

The following additional information is required on the request form:

- Requesting hospital (Please use your NHS code)
- Name of consultant or clinician
- Ethnic origin of patient
- Type of investigations requested, including requests for crossmatched units
- Clinical condition
- Current haemoglobin level (for cross match requests)
- Recent transfusion history (for hospital reference cases)
- Dates and doses of recent anti-D prophylaxis

RCI should be informed if samples are from private patients. The terms and conditions of service provision for the NHS by the NHSBT are agreed with the National Commissioning Group. Service provision for private patients may be charged differently.

Clinical information is essential for providing the most appropriate laboratory tests. The quality of clinical advice will also depend on provision of adequate clinical information. Absence of clinical information may lead to a delay in processing of the sample if the requester needs to be contacted to agree on the type of investigations. In particular notification of the administration of therapeutic monoclonal antibodies such as anti-CD47 or anti-CD38 is of critical importance

The sample must be dated as the outcome of several tests may be influenced by the age of the sample. It also allows RCI to monitor turnaround times.

The type of investigation requested and reason for the request must be clearly identified on the request form.

Due to the size of neonate sample tubes, these may be accepted with an attached label, that has been completed at the time of phlebotomy, which meets the sample acceptance criteria.

The labelling requirements for samples and request forms is summarised in the following table:

	Sample **Note** all RCI samples must be hand written /demand printed labels	Request Form
NHS number	**Essential (if available)**	**Essential (if available)**
Name First and last name spelt correctly Unless patient/donor identity is confidential	**Essential**	**Essential**
Date of Birth	**Essential**	**Essential**
Hospital Number or temporary unique identification number.	Essential - if NHS number is not available	Essential - if NHS number is not available
Address	Will only be accepted for paternal samples or samples from private patients	Will only be accepted for paternal samples or samples from private patients
Date sample taken	**Essential**	**Essential**
Time sample taken	**Essential for pre-transfusion samples**	**Essential for pre-transfusion samples**
Signature of person taking sample	**Essential**	Essential
Requesting institution name and location code	Not required	**Essential**
Requesting Clinician	Not required	**Essential**
Signature of requester	Not required	**Essential**
Clinical information: clinical condition, current Hb, recent transfusion history, dates and doses of anti-D	Not required	**Essential**
Tests required	Not required	**Essential**

Following guidance from the UKTLC, RCI will consider samples bled more than 10 minutes apart as separate phlebotomy events. The samples will be booked into the RCI LIMs under separate sample numbers, however the second sample will only be processed if RCI are unable to complete the investigation on the first sample. If the second sample is not processed, the referring hospital will not be charged for the second sample.

Tubes

Glass tubes are not acceptable.
It is the responsibility of the referrer to ensure samples tubes are in date. NHSBT will reject blood samples that are taken into expired sample tubes as it is against manufacturer's recommendations. In circumstances where there is a clinical need to test such samples a concession will be required.

Packaging of samples

It is the responsibility of the sender to ensure that all samples are packaged in accordance with the current recommendations on the Transport of Dangerous Goods: United Nations Model Regulations to prevent breakage or spillage in transit. The outside of the box or package containing the samples must be clearly addressed to the appropriate Red Cell Immunohaematology Department with storage instructions for labile material. NHSBT reserves the right to refuse to handle any samples which are inappropriately packaged or labelled.
For advice on posting samples see www.royalmail.com

Transport of samples

The storage and transport of samples from referring laboratories, prior to receipt at RCI, may impact upon the condition of the specimen and its suitability for testing. RCI request that hospitals follow the recommendations of BSH guidelines for pre-transfusion compatibility procedures in blood transfusion laboratories, which state that whole blood samples are suitable for testing if they have been stored at room temperature for up to 48 hours and beyond 48 hours (and up to 7 days, dependent on the history of the patient), whole blood should then should be stored at 2–8 °C.
Referring hospitals should ensure they have local policies or guidelines in place which detail how blood samples should be stored and transported to RCI in a timely manner, including emergency and urgent samples and at Bank Holidays. RCI sample reception procedure includes a check of condition and age of sample to ensure samples are tested within 7 days of date taken. Any sample which cannot be tested within 7 days of being bled will be rejected.

All packages must be clearly labelled to ensure samples do not go astray. **Please do not put samples for RCI in the same box as samples for other NHSBT laboratories as this could result in a delay in the samples being received by RCI.** Printable address label templates are available on the Hospitals and Science website : https://hospital.blood.co.uk/diagnostic-services/red-cell-immunohaematology/address-labels-for-sample-boxes. Using these labels will help NHSBT staff to identify where the boxes need to be delivered to after they arrive on site, and reduce the risk of samples being incorrectly stored or forwarded to the wrong department.
The labels can be printed onto labels measuring 99.1 x 38.1 mm
Blood centre location maps can be provided on request.

Routine samples

Samples for non-urgent testing by RCI can be given to the NHSBT blood delivery driver.
Please contact Barnsley RCI department for advice of how to send samples for IgA investigation before sending the referral.

Urgent samples

For urgent samples during or after normal working hours, please phone the RCI laboratory and discuss the arrangements for sending the samples. In the event of difficulty in contacting RCI, please contact Hospital Services department.
Urgent samples should be transported directly from the hospital transfusion laboratory or requesting clinician to the blood centre. **NHSBT transport is not suitable for samples requiring urgent investigation.**

Reports

A medical consultant, consultant clinical scientist or a senior scientist sees all non-routine reports before they are issued and relevant clinical comments are made. The basic principle for reporting is to send the report to the requester. RCI reports on referrals received through hospital transfusion laboratories will be addressed to the requesting laboratory, however the final responsibility lies with the hospital Consultant Haematologist who may or may not delegate that responsibility to the laboratory manager.

Report turnaround time

RCI will produce final authorised reports on all referred samples. Normally these will be available on more than 95% of referrals within 5 working days of receipt.
Reporting performance is monitored on a monthly base and if the target is consistently not met or not met for an extended period then users will be informed.

Sp-ICE

Registered users may access RCI results on the NHSBT web browser at any time. Results are placed on the web browser within one hour of the final report being printed. For further details about access to the web browser contact the NHSBT Information Governance Manager (Tel. 0191 202 4581).
Where the referring hospital has not entered into a Sp-ICE sharing agreement, results will not be given to a third-party hospital without consent from the referring hospital. RCI will advise that the third-party hospital contacts the referring hospital directly for the results.

Patient records

The RCI laboratories are supported by a national computer system, Hematos, on which patient data are stored. Information about patients is held in compliance with the EU General Data Protection Regulations. Staff access to patient information is on a need-to-know basis for clinical care purposes only and patient confidentiality is respected at all times.

Appendix 1

All blood centres can be contacted 24 hours per day, every day of the year (Appendix 1). Out of hours hospitals should contact RCI via Hospital Services rather than directly on all sites except Filton where a Smart Divert number: 01179693927 is in use for hospitals to contact RCI on call BMS directly. Please contact the Hospital Services Department at the site where the referred sample will be processed.

CONTACT DETAILS

		Mobile
Assistant Director RCI	Wisdom Musabaike	0738 553 0092
National Operations Manager – RCI	Sarah Thompson	0782 335 1741
Process Improvement Manager – RCI	Tess Winfield	0759 035 2173
Associate Medical Director for RCI and Genomics	Dr. Cath Booth	0738 503 7538
Quality Project Specialist Clinical Services	Donna Blair	0788 930 4259
Head of Hospital Customer Service	Chris Philips	0788 930 4517

RCI:

BIRMINGHAM - Routine opening hours: 08:00-23:00 Mon to Fri
Address: Vincent Drive, Edgbaston, Birmingham, B15 2SG

		Office	**Mobile**
Centre switchboard		0121 278 4000	
Hospital Services Department		0121 278 4037	
RCI Consultant Haematologist	Dr Suzy Morton		0747 114 7753
Head of Laboratory	Ian Skidmore	0121 278 4125	0780 890 6443

NHS email – rcibirmingham@nhsbt.nhs.uk

FILTON - Routine opening hours: 08:00-22:00 Mon to Fri, 08:00-16:00 Sat
Address: North Bristol Park, Northway, Filton, Bristol, BS34 7QH

		Office	**Mobile**
Centre switchboard		0117 921 7200	
Hospital Services Department		0117 921 5724	
RCI Consultant Haematologist	Dr Tom Latham	0117 921 7474	0751 576 1072
Head of Laboratory	Abi McNeill	0117 921 7511	

NHS email – rcifiltonmailbox@nhsbt@nhs.uk

COLINDALE Routine opening hours: 08:00-20:00 Mon to Fri, 09:00-17:00 Sat
Address: Colindale Avenue, London, NW9 5BG

		Office	Mobile
Centre switchboard		0208 957 2700	
Hospital Services Department		0208 957 2800	
RCI Consultant Haematologist	Dr. Fiona Regan	0208 957 2834	0771 144 7235
Head of Laboratory	Julia Mahmood	0208 957 2743	0771 144 7196

NHS email – COLSMA73@nhsbt@nhs.uk

LIVERPOOL Routine opening hours: 08:00-19:00 Mon-Fri, 09:00-17:00 Sat
Address: 14 Estuary Banks, Speke, Liverpool, L24 8RB

		Office	Mobile
Centre switchboard		0151 268 7000	
Hospital Services Department		0151 268 7170	
RCI Consultant Haematologist	Dr Therese Callaghan	0151 268 7012	0771 1447383
Head of Laboratory	Daniel Palmer	0151 268 7144	0752 529 9023

NHS email – RCI.Liverpool@nhsbt@nhs.uk

NEWCASTLE Routine Opening hours: 08:00-21:00 Mon to Fri
Address: Holland Drive, Newcastle upon Tyne, NE2 4NQ

		Office	Mobile
Centre switchboard		0191 202 4400	
Hospital Services Department		0191 202 4500	
RCI Consultant Haematologist	Dr Andrew Charlton	0191 202 4548	0747 114 8121
Head of Laboratory	David Bruce	0191 202 4416	

NHS email – RCINewcastle@nhsbt@nhs.uk

Barnsley Routine opening hours: 08:00-22:00 Mon to Fri 09:00-17:00 Sat
Address: Capitol Park, Dodworth, Barnsley S75 3FG

		Office	Mobile
Centre switchboard		0122 686 8000	
Hospital Services Department		0122 686 8061	
RCI Consultant Haematologist	Dr Therese Callaghan	0151 268 7012	0771 1447383
Head of Laboratory	Tracey Watson	0122 686 8012	0787 263 6762

NHS email – NHSBT.rcibarnsley@nhs.net

TOOTING Routine opening hours: 08:00-21:00 Mon to Fri.
Address: 75 Cranmer Terrace, Tooting, London, SW17 0RB

		Office	Mobile
Centre switchboard		0203 123 8300	
Hospital Services Department		0203 123 8352	
RCI Consultant Haematologist			
Head of Laboratory	Doris Lam	0203 123 8346	0772 027 5322

NHS email – RCI.Tooting@nhsbt.nhs.uk

Local Customer Service Managers - please see Contact page on the Hospital & Science web site for contact details
Customer Service support team email NHSBTCustomerService@nhsbt.nhs.uk
Customer Services Response Desk
Mon-Fri 09:00-17:00 except Bank Holidays: 0208 201 3107

Appendix 2

Reporting adverse reactions to transfusions

There is a regulatory requirement in the UK under the terms of the Blood Safety and Quality Regulations 2005 to report adverse reactions related to transfusion. The Medicines and Healthcare Products Regulatory Agency (MHRA) has been appointed the Competent Authority on behalf of the Secretary of State to administer the regulations, and has developed a web-based haemovigilance reporting system called SABRE (Serious Adverse Blood Reactions and Events) to facilitate reporting.

All Trusts in the UK should be registered with the MHRA and must submit a 'notification' report to them as soon as possible following a reaction. At the time of reporting, there is the opportunity to tick a box allowing SHOT (the Serious Hazards of Transfusion confidential enquiry) access to the report details. Failure to tick this box will result in a call from the MHRA Adverse Incident Centre to advise the reporter of the need to report also to SHOT.

The SHOT Office will review the incident details and, if appropriate, will assign a questionnaire for the reporter to complete on-line. An automated e-mail is generated informing the reporter which questionnaire has been allocated and containing a link to access it. This SHOT questionnaire should be completed and returned via SABRE as soon as possible. Automated reminders will be sent at regular intervals until the questionnaire is completed.

Following investigation of the incident by the reporting hospital, and where appropriate by the blood services, the reporter is required to submit a 'confirmation' report to MHRA via SABRE which effectively closes the case, provides an assessment of the likelihood of the reaction being due to the blood component and details, where appropriate, any corrective and preventative actions put in place to reduce the likelihood of the event recurring.

Current SHOT reporting categories may be found at;
https://www.shotuk.org/wp-content/uploads/myimages/SHOT-Definitions-update-FINAL-April-2018.pdf

Cases in which the wrong blood component was transfused (IBCT), cases of acute transfusion reactions (ATR) including anaphylaxis and delayed haemolytic transfusion reactions (HTR) may be referred to RCI for investigation.

Appendix 3 Information for ISO15189 4.5 Examination by referral laboratories

Test Name	TAT	Confirmation that method has been validated / verified	Ref Range, include UoM if relevant (if N/A please comment)	Name of EQA scheme –include scheme ref (if N/A please comment)
Antibody identification	**95% reported within 5 working days**	**Yes, accredited to ISO15189 as per schedule 8740**	UoM for antibody screening has been determined acceptable range = >95% of results within +/- 1 reaction grade of target	**NEQAS BTLP**
Blood grouping and phenotyping (including anomalous types)	**95% reported within 5 working days**	**Yes, accredited to ISO15189 as per schedule 8740**	UoM has been determined for automated and manual grouping acceptable range = >95% of results within +/- 1 reaction grade of target	**NEQAS BTLP NEQAS phenotyping pilot**
Antenatal antibody titration/quantification	**95% reported within 5 working days**	**Yes, accredited to ISO15189 as per schedule 8740**	**Titration = >95% results are +/- 1 dilution** **Quant < 25%**	**NEQAS antibody titration pilot AAQS anti-D and anti-c quantitation**
FMH quantitation	**95% reported within 5 working days**	**Yes, accredited to ISO15189 as per schedule 8740**	**<15%**	**NEQAS FMH quantitation**
Investigation of autoantibodies / transfusion reactions and other complex red cell serology investigation	**95% reported within 5 working days**	**Yes, accredited to ISO15189 as per schedule 8740**	**See UoM for titration which is manual IAT**	**NEQAS BTLP NEQAS genotyping pilot**
Anti-A and anti-B titres	**95% reported within 5 working days**	**Yes, accredited to ISO15189 as per schedule 8740**	**See UoM for titration which is manual IAT**	**NEQAS ABOI titration pilot**