

Guidelines for the Blood Transfusion Services

Chapter 13: Patient testing (red cell immunohaematology)

<http://www.transfusionguidelines.org/red-book/chapter-13-patient-testing-red-cell-immunohaematology>

Chapter 13:

Patient testing (red cell immunohaematology)

13.1: Scope

These specifications provide guidance on the tests required for investigations performed on patient samples in red cell immunohaematology (RCI) laboratories in UK Blood Transfusion Centres. These include pre-transfusion and compatibility testing, tests associated with supporting the prevention and treatment of Haemolytic Disease of the Fetus and Newborn (HDFN), assessment of fetomaternal haemorrhage, and titration studies supporting ABO mismatched transplant.

Extended testing of blood donors other than in the above contexts is covered in Chapter 12.

It is assumed that RCI laboratories comply with the following guidelines:

- *Guideline for Blood Grouping and Antibody Testing in Pregnancy* (British Committee for Standards in Haematology, BCSH)¹
- *The Specification and Use of Information Technology (IT) Systems in Blood Transfusion Practice* (BCSH)²
- *Guidelines for Pre-transfusion Compatibility Procedures in Blood Transfusion Laboratories* (BCSH)³
- *The Estimation of Fetomaternal Haemorrhage* (BCSH)⁴

And also comply with:

- UKAS Medical Laboratories accreditation (ISO15189)⁵
- The Blood Safety and Quality Regulations 2005⁶

Participation in relevant NEQAS BTLP schemes is recommended. Exercise results should be reviewed and any findings acted upon.

This chapter is intended to cover practice in areas not included in published UK guidelines at the time of writing. Where practice differs either from published guidance or this chapter, laboratory managers should formally document the reasons for doing so and assess the associated risk.

13.2: Sample acceptance and labelling

Visual inspection to determine the suitability for testing should consider the following in relation to the equipment, methods and samples used:

- the presence of haemolysis
- the presence of lipaemia
- the presence of an atypically large buffy coat layer
- the presence of clots in an anticoagulated sample
- a low sample volume
- an unusually high or low cell:plasma (serum) ratio.

If any of the above is identified, then this should be documented and appropriate action taken.

Any systematic variation from BCSH guidelines must be covered by a risk assessment. Tests performed on individual samples not complying with guidelines are documented on an authorised concession.

All samples are labelled with both barcoded and eye-readable numbers.

Samples that are separated prior to referral to the laboratory (e.g. samples separated at 37°C for Paroxysmal Cold Haemoglobinuria investigations) should be clearly labelled and signed to indicate the person separating the samples. Accompanying documents should clearly state the nature of the samples, the person separating the samples, and the time and date of sample separation.

13.3: Pre-transfusion testing

13.3.1: Resolution of anomalous grouping

ABO grouping is the most important pre-transfusion serological test performed. Fully automated ABO and D grouping procedures have significantly improved the accuracy and security of results, and should be used wherever possible.

When anomalous ABO groups are encountered laboratory protocols should support investigation of the following findings.

Missing agglutinins in reverse grouping:

- obtain the patient's history, and review for information which may explain missing agglutinin (e.g. age, immunodeficiency, previous haemopoietic stem cell transplant)
- repeat the reverse group, increasing the sensitivity of the test, consider the use of tube techniques, lower incubation temperature, increased plasma:cell ratio and enzyme-treated red cells.

Unexpected additional reactions in the reverse group:

- investigate the presence of allo- or autoantibodies active at temperatures below 37°C
- consider repeating the reverse group at 37°C
- consider repeating the reverse group using cells negative for any identified alloantibody.

Unexpected reactions in the forward or D grouping, including positive diluent control:

- check for immunoglobulin coating of the patient's cells by performing a direct antiglobulin test (DAT)
- consider repeating tests using unpotentiated reagents in tube techniques
- consider techniques to remove or reduce immunoglobulin coating (e.g. warm wash or use 0.2M DTT to remove IgM) and repeat tests with appropriate controls.

Unexpectedly weak or mixed field reactions in forward or D group:

- obtain the patient's history, and review for information which may explain results (e.g. recent non-ABO identical transfusion, haemopoietic cell transplant)
- consider additional investigations which may include adsorption/elution, and flow cytometry
- panels of monoclonal anti-D reagents may be commercially available for the investigation of partial and weak D phenotypes.

Genotyping is useful in resolving grouping problems, particularly weak and partial D types (see section 15.2). Genotyping alone must not be used to determine the ABO group for use in selection of blood for transfusion. Where the patient ABO group cannot confidently be assigned by serology, group O (high-titre negative) blood must be selected.

13.3.2: Antibody identification

In all cases of the investigation of alloantibodies laboratories should focus on:

- secure identification of alloantibodies detected
- exclusion of additional specificities to those identified
- selection of blood for transfusion (Daniels *et al.* 2002)⁷

When antibodies which cannot be identified have been detected, laboratories should consider referral to the International Blood Group Reference Laboratory (IBGRL).

Antibody identification techniques and protocols are described in BCSH guidelines and should be adhered to. More complex problems encountered by RCI laboratories and not covered by BCSH are considered below.

13.3.2.1: Complex antibody mixtures

When investigating complex antibody mixtures RCI laboratories should consider:

- extended phenotyping of the patient, e.g. C, c, D, E, e, K, M, N, S, s, P, γ , Le^a, Le^b, Fy^a, Fy^b, Jk^a, Jk^b

- if this is not possible due to previous transfusion or IgG sensitisation, blood group genotyping should be considered
- extending the range of techniques and incubation temperature to identify component antibodies
- using cells matching the patient's phenotype/genotype to confirm the presence of multiple antibodies rather than an antibody to a high-frequency antigen
- careful use of alloadsorption/autoadsorption techniques to confirm the specificity of elements of the mixture

13.3.2.2: Antibodies known as high-titre low-avidity (HTLA)

Antibodies traditionally known as HTLA include anti-Ch, –Rg, –Kn^a, McC^a, –Yk^a, –Cs^a and –Si^a. Typically HTLA antibodies present as reacting with most panel cells by indirect antiglobulin test (IAT) with variable strength, with or without similar patterns using enzyme-treated cells. Experienced operators can recognise characteristic agglutination by microscopic examination of tube IAT, which have been described as 'loose', 'stringy', 'fluffy', 'delicate' or 'gritty'. In investigating samples suspected to contain HTLA antibodies RCI laboratories should consider:

- Neutralising anti-Ch or -Rg specificities by incubating the patient's plasma with pooled group AB donor plasma before IAT is undertaken. Reactivity of these antibodies is usually abolished. A dilution control in which the patient's plasma is incubated with phosphate-buffered saline should be prepared and tested in parallel with the neutralised plasma.
- The use of soluble recombinant CR1 proteins for inhibition studies
- The use of a panel of cells lacking HTLA antigens

13.3.2.3: Antibodies to high-frequency antigens (HFA)

Typically antibodies to HFA present with positive reactions of similar strength against all routine screen and identification panel cells. The most commonly encountered specificities include anti-k, –Lu^b, –Kp^b, –Vel, –Co^a, –Yt^a, –Fy3, –U and –In^b. In investigating samples suspected to contain antibodies to HFA, RCI laboratories should consider:

- the ethnicity of the patient
- extended phenotyping as in section 13.3.2.1
- testing against a panel of rare reagent red cells
- typing the patient's red cells with antibodies to HFA. Where possible, CE-marked reagents must be used, otherwise results must be considered in context of the reliability of the reagent in use, supported by adequate controls.

13.3.2.4: Antibodies to low-frequency antigens (LFA)

Typically antibodies to LFA present with a negative antibody screen and are detected in crossmatch or investigation of HDFN. The most commonly encountered specificities include anti-Kp^a, –Lu^a, –Wr^a and –Co^b. In investigating samples suspected to contain antibodies to LFA, RCI laboratories should consider:

- testing the patient's plasma with a panel of red cells expressing LFA

- phenotyping the incompatible unit(s)/cells for LFA

13.3.3: Autoantibodies

Autoantibodies are frequently encountered in pre-transfusion testing, and may be the cause of autoimmune red cell destruction, or may be clinically benign. In either case autoantibodies may interfere with pre-transfusion testing, either due to coating of patient's cells with immunoglobulin, or as pan-reactive antibody in patient's plasma. In providing safe transfusion in the presence of autoantibodies, RCI laboratories may adopt the following strategies.

13.3.3.1: ABO and Rh grouping in the presence of autoantibodies

Most modern test systems support routine, accurate grouping of the majority of patients whose cells are coated with immunoglobulin and who give a positive DAT. Cases which are problematic may present with reaction patterns that cannot be assigned to an ABO group, weak additional reactions and positive reagent controls. Such cases should be investigated as in section 13.3.1.

Laboratories should make a clear documented assessment, based on the recommendations of reagent and test system suppliers, how to manage cases with anomalous ABO and D groups. This is particularly important when potentiated reagents are included in test systems.

13.3.3.2: Alloantibody detection and identification in the presence of autoantibodies

In dealing with cross-reacting autoantibodies, which complicate the detection and identification of underlying alloantibodies, RCI laboratories should consider:

- The characteristics of available, validated IAT in testing patient plasma-containing pan-reacting autoantibodies. Tube IAT may be less prone to interference by autoantibodies than column technologies.
- The use of the patient's own cells to adsorb autoantibody from the plasma, permitting detection and identification of alloantibodies.
- The use of cells from two or more selected donors to adsorb autoantibody. Typically these cells are enzyme treated to optimise removal of autoantibody. Alloantibodies to high frequency antigens are also likely to be removed by this technique.

13.3.4: Management of patients with autoantibodies

Consideration should be given to close matching of recipient and donor red cell types. This is to safeguard against the presence of alloantibodies undetected by tests on modified plasma, and to prevent further alloimmunisation. In patients who cannot be grouped by conventional serology, due to sensitisation of red cells or previous transfusion, genotyping offers a solution.

In patients with autoantibodies requiring regular transfusion, close matching of transfused red cells with the patient's own phenotype, to manage risk of transfusion reactions, may be used as a basis by scientists and clinicians to assess, and potentially reduce, the required frequency of testing. Such assessments should be fully documented and subject to planned review.

13.3.5: Therapeutic monoclonal antibodies

A number of conditions are treated using therapeutic monoclonal antibodies (TMABs). These therapies have the potential to adversely interfere with serological investigations and compatibility testing in the blood bank, potentially causing unnecessary delays in providing blood components for transfusion. This may

delay treatment of these patients, many of whom are transfusion dependent. Monoclonal antibody therapies may affect serological testing methods in a variety of ways, with the monoclonal antibody induced reactivity persisting for up to 6 months after the last treatment infusion.

The following testing protocols should be undertaken:

13.3.5.1 Management of patients on therapeutic monoclonal antibodies

Before TMAb therapy has started:

- Baseline ABO and D group (follow local policy for requirement of confirmatory sample rule for ABO and D group)
- Antibody screen, and antibody identification, if required
- Direct Antiglobulin Test (DAT)
- Extended phenotyping/genotyping for C, c, E, e, K, (k if K+), MNSs, Jk^a, Jk^b, Fy^a and Fy^b (genotyping to be used if the patient has been recently transfused, <3 months ago)

Once TMAb therapy has been commenced:

- ABO and D typing as per normal method (If the ABO group cannot be concluded, group O red cells may be required for transfusion)
- Antibody screening, and antibody identification if required, using a strategy to avoid the effect of the TMAb, e.g. reagent cells treated with 0.2M Dithiothreitol (DTT) used in patients on anti-CD38 TMABs
- Red cells should be matched for Rh (CcDEe) and K as well as for any alloantibodies
- In patients who have a strong panagglutinin, alloadsorption studies may allow satisfactory antibody detection / identification, alternatively use of a different serological technique, use of a soluble recombinant protein or use of an anti-human globulin reagent which lacks anti-IgG4 or use of rare phenotype red cells which lack the CD marker of interest may help with the resolution of the investigation.

13.4: Antibody quantification and titration

Antibody quantification and titration is performed in RCI laboratories on patients' samples, to support the prediction and management of HDFN and ABO mismatched organ transplant.

13.4.1: Antibody quantification of anti-D, and anti-c for management of HDFN

In UK laboratories it is standard practice to quantify anti-D and anti-c by continuous flow analyser against standard anti-D and anti-c preparations. In doing so, laboratories must:

- procure and maintain fully validated and supported quantification equipment
- procure and maintain fully validated dilution equipment

- prepare calibration curves from standard antibodies
- ensure operation consistency by running archive samples in parallel with all alloimmune anti-D and all anti-c samples where available (the repeat archive test result value should be within 10% of its original reported value)
- participate in the NHSBT's Antibody Quantification Quality Assurance Scheme, regularly review the results and act on the findings

13.4.2: Antibody titration of antibodies capable of causing HDFN

RCI laboratories undertake IAT titration to assess all antibodies capable of causing HDFN other than anti-D and anti-c. Protocols for these tests should focus on achieving reproducible results by:

- specifying the phenotype of red cells for use with each antibody specificity
- describing the dilution medium and method
- using calibrated pipettes for dilution and dispense of reagents
- using IAT for titration, typically column technology
- establishing means of consistently identifying the endpoint for titration
- using parallel titration of previous archive samples from the patient where available
- managing cases where there is a difference between the current and archive sample endpoints
- participate in NEQAS BTLP antibody titration scheme, regularly review results and act on the findings

13.4.3: Antibody titration in ABO mismatched transplant

RCI laboratories undertake titration of ABO antibodies to allow clinical assessment of the feasibility of ABO mismatched transplant, and monitoring of treatment to reduce antibody titre in preparation for ABO mismatched transplant. Protocols for this procedure should consider all the previously listed elements of titration, and in addition:

- the use of the organ donor's cells for titration
- inactivation of the IgM component of ABO antibodies (e.g. dithiothreitol (DTT) treatment)
- participate in NEQAS BTLP ABO titration scheme, regularly review results and act on the findings

13.5: Fetomaternal haemorrhage estimation by flow cytometry

13.5.1 Indications for testing

For any D negative pregnant mother where the screening test indicates a fetomaternal haemorrhage which is >2mL of suspected D positive fetal red cells, a sample can be referred to RCI for confirmation by flow cytometry. Results are used to support the management of prophylactic anti-D dose recommendation to mitigate against maternal sensitisation.

13.5.2 Procedures for testing in the RCI laboratory

As per BSH guideline recommendations, referred samples must not have been used for determination of an ABO blood group due to the risk of foetal cells being removed at the RBC/plasma interface and therefore possible underestimation of FMH.

Flow Cytometry procedure

- RCI laboratories should procure and maintain fully validated and supported flow cytometers
- Direct staining is recommended using a commercially available fluorochrome conjugated IgG monoclonal anti-D known to have high avidity for the D antigen
- Testing must include a suitable range of control cells and negative control antibodies in line with BSH guidelines. Laboratories must ensure operational consistency by defining acceptance ranges for control cell populations and ensuring that they are met with each patient investigation.
- White cell populations have been demonstrated to cause interference in the testing of delivery samples for fetomaternal haemorrhage volume by flowcytometry. A fluorochrome conjugated antibody, raised against a white cell specific marker, can be used in combination with the anti-D and negative antibody control reagents to remove interference from testing e.g. PE conjugated anti-CD66b or PE conjugated anti-CD45.
- The Mollison formula should be used to calculate the packed fetomaternal haemorrhage cell volume, which considers the patient to be 75Kg in weight, have a red cell volume of 1800mL and that fetal cells are 22% larger than adult cells
- The uncertainty of measurement (UoM) associated with the test should be calculated and applied to ensure the correct dose of prophylactic anti-D is used to mitigate against sensitisation. Laboratory teams should undertake an annual exercise to determine the UoM for the test.
- Laboratories should participate in an external quality assessment scheme, regularly review the results and act on the findings

13.6: Post-examination

All patient records and test results should be maintained according to the requirements of the Caldicott Report (1997)⁸ and Data Protection Act (1998).⁹

Authorising and reporting of routine test results should be the responsibility of designated laboratory personnel. Consultant grade staff should authorise non-routine and discrepant results, or designate other senior staff to do so.

Results reported by email should be to a secure NHS email address. The sender should confirm the designated email address. The sender should indicate when the report will be sent and, following email

transmission of the report, confirmation that the email has been received should be obtained from the intended recipient.

Where electronic data interchange is in place either direct to surgeries/hospitals or onto a web browser the system should be based on the principles of the Caldicott Report.⁸ The system should be validated and password controlled with clearly defined access levels. Data should be encoded with an electronic signature to ensure that the information cannot be altered and can only be viewed by designated individuals.

13.7: References

1. British Committee for Standards in Haematology (2016). Blood Grouping and Antibody Testing in Pregnancy. Available at b-s-h.org.uk/guidelines/guidelines
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