Guidelines for pre-transfusion compatibility procedures in blood transfusion laboratories*

British Committee for Standards in Haematology

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INTRODUCTION

The effective development and maintenance of satisfactory standards in pre-transfusion testing requires a structured approach in the adoption of a quality management system. Technical errors, clerical errors, the use of non-validated techniques or equipment and non-compliance with established procedures may result in missed incompatibilities and immediate or delayed haemolytic transfusion reactions (SHOT, 1996–2010; Stainsby et al., 2006). The purpose of these guidelines, which replace those published in 2004 (Chapman et al., 2004), is to define the laboratory processes and procedures that should be adopted to undertake pre-transfusion testing.

The guideline group was selected to be representative of UK-based medical, scientific and technical experts. The writing group produced the draft guideline which was subsequently revised by consensus by members of the Transfusion Task Force of the British Committee for Standards in Haematology. The guideline was then reviewed by a sounding board of approximately 50 UK haematologists, the BCSH (British Committee for Standards in Haematology) and the Transfusion Laboratory Managers Working Group of the National Blood Transfusion Committee and its equivalent in the other three countries, and comments incorporated where appropriate.

These guidelines are formulated from expert opinion and based on the requirements of the Blood Safety and Quality Regulations (BSQR, 2005), and the recommendations of Clinical Pathology Accreditation (CPA, 2010), Guidelines for Blood Transfusion Services in the UK (UK Blood Transfusion Services, 2012), the UK Transfusion Laboratory Collaborative (Chaffe *et al.*, 2009), and data from UK NEQAS (BTLP) (Knowles *et al.*, 2002; UK NEQAS ANNUAL REPORTS) and the Serious Hazards of Transfusion (SHOT) haemovigilance scheme annual reports (SHOT, 1996–2010). Where evidence exists to support new and potentially contentious recommendations, this is referenced in the text.

SUMMARY OF KEY RECOMMENDATIONS

1. The laboratory must identify all critical control points in pre-transfusion testing and build in security at these points. See Appendix 1 for examples.
2. Laboratories must have contingency plans for action to be taken when normal systems are not available.
3. The laboratory should have a policy with respect to the manual editing and authorisation of test results.
4. Serological studies should be performed using blood collected no more than 3 days in advance of the actual transfusion when the patient has been transfused or pregnant within the preceding 3 months.
5. A pre-transfusion sample should be retained for at least 3 days post-transfusion, to ensure that repeat ABO grouping of the pre-transfusion sample can be performed in the event of an acute transfusion reaction.
6. ABO grouping is the single most important serological test performed on pre-transfusion samples and the sensitivity and security of testing systems must not be compromised.
7. Fully automated systems should be used where possible to reduce the risks of interpretation and transcription errors.
8. Any abbreviation of the ABO group must be fully risk assessed.
9. The patient demographics on the sample should be checked against the computer record prior to validation of results (preferably prior to testing) to ensure that they match and that no errors have been made during data entry onto the Laboratory Information Management System (LIMS).
10. If the patient is known to have formed a red cell alloantibody, each new sample should be fully tested to exclude the presence of further alloantibodies.
11. When one antibody specificity has been identified, it is essential that the presence or absence of additional clinically significant antibodies is established.
12. Unless secure electronic patient identification systems are in place, a second sample should be requested for confirmation of the ABO group of a first time patient prior to transfusion, where this does not impede the delivery of urgent red cells or other components.
13. The indirect antiglobulin test (IAT) crossmatch is the default technique which should be used in the absence

of functioning, validated IT or when electronic issue is contra-indicated.
14. An IAT crossmatch must be used if the patient's plasma contains or has been known to contain, red cell alloantibodies of likely clinical significance.
15. The overall process for determining eligibility for electronic issue (EI) must be controlled by the LIMS and not rely on manual intervention or decision making.
16. Laboratories should have written protocols in place which define the responsibilities of all staff in dealing with urgent requests.
17. For genuinely unknown patients, the minimum identifiers are gender and a unique number.
18. Following an emergency rapid group, a second test to detect ABO incompatibility should be undertaken prior to release of group specific red cells.
19. If the direct antiglobulin test (DAT) is positive in a patient transfused within the previous month, an eluate made from the patient's red cells should be prepared and tested for the presence of specific alloantibodies.

1. ORGANISATION OF THE GUIDELINES

The quality section includes all of the quality recommendations from the whole guideline, so those using this guideline should refer back to the quality section for advice relating to individual sections.

All aspects of testing relating to emergency situations have been put into a separate section – Section 8. Other sections now relate solely to routine testing.

Efforts have been made to avoid duplication and overlap with other guidelines. This guidance is complementary to the BCSH guidelines that cover transfusion of paediatric patients, antenatal serology, information technology (IT) systems, administration of blood components and validation in the transfusion laboratory (BCSH, 2004, 2006a,2006b, 2009, 2010a), and these should be available for reference. The referenced versions of these guidelines were current at the time of publication of this document but it is recognised that they may be updated during the lifetime of this guideline, and reference should always be made to the current version.

Where expansion on the decision making on the recommendations is required, this is covered in a series of appendices.

Recommendations are based on overriding principles, but it is recognised that a safe outcome may be achieved using a different approach, whilst still complying with minimum standards. In these circumstances, a fully documented risk assessment is required.

Exceptions to policy relating to individual patients are now covered by a statement relating to concessionary release and an example is given in Appendix 9.

There is an additional section relating to what happens after components have been issued, and the serological investigation of a suspected transfusion reaction.

There are new flow charts for anomalous D typing and selection of blood in this circumstance, and for anomalous ABO typing (Appendix 3).

There are worked examples of antibody identification in Appendix 4.

2. QUALITY MANAGEMENT IN PRE-TRANSFUSION TESTING

2.1. Quality management system

2.1.1. In keeping with all other clinical laboratories, the transfusion laboratory must have an operational and documented Quality Management System, clearly defining the organisational structure, procedures, processes and resources necessary to meet the requirements of its users, to accepted standards of good practice.

2.1.2. From November 2005, all Hospital Blood Banks and Blood Establishments have been subject to the Blood Safety and Quality Regulations (BSQR, 2005). Article 2 of European Commission directive 2005/62/EC gives details of the Quality System standards and specifications required.

2.1.3. Transfusion laboratories must use equipment, information systems and test systems that have been validated against the documented requirements of the laboratory.

2.1.4. The systems must enable a full audit trail of laboratory steps, including the original results, cross-referenced to associated internal controls, interpretations, amendments, authorisations, and the staff responsible for conducting each critical step.

2.1.5. The laboratory must identify all critical control points in pre-transfusion testing, and build in security at these points. See Appendix 1 for examples.

> KEY RECOMMENDATION: The laboratory must identify all critical control points in pre-transfusion testing and build in security at these points.

2.1.6. A programme of regular independent internal audits must be instituted to assess compliance with laboratory processes.

2.1.7. The laboratory management must conduct regular reviews of quality incidents including: untoward laboratory incidents [including those reported to the Medicines and Healthcare products Regulatory Agency (MHRA) and SHOT via the SABRE system], complaints, external quality assessment reports, internal audits of the laboratory procedures, concessionary release, recalls and process deviations.

2.1.8. The laboratory should participate in relevant accredited External Quality Assessment (EQA) Schemes. Approved EQA schemes are those that have been accredited to standards based on ILAC G13:2000 Guidelines for the Requirements for the Competence of Providers of Proficiency Testing Schemes, or to ISO 17043:2010: General Requirements for Proficiency Testing.

2.1.9. Laboratories must have contingency plans for actions to be taken when routine systems are not available. These plans should include manual systems to deal with loss of automation and LIMSs. Examples include: suspending testing that is not absolutely necessary; recording information for uploading later to ensure the audit trail; consideration given to sending routine samples to another site with same LIMS; suspending the use of electronic issue.

> KEY RECOMMENDATION: Laboratories must have contingency plans for actions to be taken when normal systems are not available.

2.2. Staff training and competency

2.2.1. There must be a documented programme for training laboratory staff, including on-call staff not routinely working in the laboratory, which covers all tasks and testing performed appropriate to the grade of staff and which fulfils the documented requirements of the laboratory (Chaffe *et al.*, 2009). It must also include handling major incidents and emergency situations including contingency plans for major system failures.

2.2.2. Staff must receive regular update training on the principles of Good Manufacturing Practice (GMP).

2.2.3. Laboratory tasks must only be undertaken by appropriately trained staff.

2.2.4. There must be a documented programme for assessing staff competency in all laboratory tasks.

2.2.5. Where decisions are required about interpretation of results, component selection and/or specialist requirements, the staff involved must have the required knowledge (supported by relevant qualification) to do this safely.

2.2.6. Specialist clinical and technical advice should be available at all times from staff who have demonstrated sufficient knowledge, training and competency to do so (Chaffe *et al.*, 2009). This could be from within a network or Blood Service reference laboratory if not available from within a single centre.

2.3. Reagents and test systems

2.3.1. Reagents used for ABO and D grouping must be CE marked, and be stored and used in accordance with the manufacturers' instructions.

2.3.2. Reagents used for all other tests should be, where available, CE marked, and be stored and used in accordance with the manufacturers' instructions.

2.3.3. All critical processes, equipment, facilities or systems in the transfusion laboratory must be validated in accordance with the BCSH Guidelines for Validation & Qualification, including Change Control, for Hospital Transfusion Laboratories (BCSH, 2010a).

2.3.4. All changes to critical process, equipment, facilities or systems in the transfusion laboratory must be validated in accordance with the BCSH Guidelines for Validation & Qualification, including Change Control, for Hospital Transfusion Laboratories (BCSH, 2010a).

2.3.5. Laboratories should ensure that they have a way of identifying and documenting changes made by manufacturers to the raw material used for reagents or test systems; if this occurs, the performance of the system should be reviewed and revalidated.

2.3.6. There should be a record of all batch numbers and expiry dates of all reagents used in the laboratory. The record should either include the time periods during which they are in use or should link directly to the test result.

2.3.7. All laboratory equipment must be regularly maintained in accordance with the manufacturer's instructions. Where appropriate, calibration of equipment must include details of the reference standard used.

2.3.8. There should be a record of instrument failure, subsequent corrective action and 'downtime'. There should be a process for trending instrument failures and for returning equipment to use after corrective action.

2.3.9. Appropriate quality control (QC) must be used for all parts of the test system. The type and frequency of any controls used should be based upon appropriate risk assessments.

2.3.10. There must be a documented procedure for dealing with QC failure with all actions documented. The process should include action to be taken with regard to results already released since the previous QC testing. Result acceptance should only continue once the QC failure has been satisfactorily resolved.

2.3.11. There should be a documented programme to ensure the efficacy of the IAT, including efficacy of the cell washers, where used (Voak *et al.*, 1988).

2.4. Information systems

2.4.1. The LIMS should comply with the requirements described in the BCSH guidelines for the specification and use of information technology systems in blood transfusion practice (BCSH, 2006b).

2.4.2. The system must be validated in accordance with the BCSH Guidelines for Validation & Qualification, including Change Control, for Hospital Transfusion

Laboratories (BCSH, 2010a), Guidelines for validation of automated systems in blood establishments (ISBT, 2010), GAMP®5: A Risk-based Approach to Compliant GxP Computerised Systems (ISPE, 2008), and Eudralex Vol 4 GMP guidelines (EUDRALEX, 2011).

2.4.3. The system must be revalidated following the installation of any upgrades or changes in interface. The level of revalidation required will depend on the magnitude of the changes in the upgrade or interface alteration.

2.5. Automated blood grouping and antibody screening systems

2.5.1. Prior to introduction and use, the system must be validated in accordance with the BCSH Guidelines for Validation & Qualification, including Change Control, for Hospital Transfusion Laboratories (BCSH, 2010a).

2.5.2. Planned preventative maintenance/emergency repair will require a documented 'return to service' procedure to be undertaken.

2.5.3. The laboratory should have a policy with respect to the manual editing and authorisation of test results; this should include the designation of staff allowed to edit results, with password controlled access where possible (SHOT, 1996–2010).

> KEY RECOMMENDATION: The laboratory should have a policy with respect to the manual editing and authorisation of test results.

2.5.4. Automated grouping and antibody screening systems should have, wherever possible, safeguards built into the systems to detect possible failures; these could include but are not limited to:
   i. notification of failure to dispense and/or aspirate samples, reagents or wash solutions;
   ii. presence of a level check on final test mixture.

3. SAMPLES AND DOCUMENTATION

3.1. Introduction

Errors in patient identification and sample labelling may lead to ABO-incompatible transfusions. Evidence for this is well documented in the annual reports of the SHOT steering group (SHOT, 1996–2010) and by others (Stainsby *et al.*, 2006; Sazama, 1990).

3.2. Written/electronic requests

3.2.1. There should be written policies for generating blood transfusion requests and for the collection of blood samples for pre-transfusion compatibility testing. This

should specify grades of staff authorised to request blood and to take samples for pre-transfusion testing. Reference should be made to the guidelines on the administration of blood components (BCSH, 2009).

3.2.2. It is essential that the request form and sample conform to the requirements as described in the guidelines on the administration of blood components (BCSH, 2009).

3.2.3. Electronic ward requesting should comply with all the same minimum standards, although in the absence of a facility for signing the request form, the system must/should capture the identity of the requester. The system must comply with the recommendations described in the IT guidelines (BCSH, 2006b).

3.3. Telephone requests

3.3.1. There should be a procedure for documenting telephone requests. This procedure should identify what core patient identifiers need to be provided at the time of request/enquiry by the clinician and also what patient identifiers and information are recorded by the laboratory.

3.4. Retention of request documentation

3.4.1. It is important that all request documentation for transfusion testing be kept available for appropriate lengths of time. Each department should have a clear policy on document retention that complies with the guidelines on retention and storage of pathological specimens and records (RCPATH, 2009).

3.5. Duplicate records

3.5.1. Duplicate patient records must be avoided, to prevent essential transfusion or antibody history being overlooked. There should be a policy to identify and link separate records that exist for each patient at the time of the request.

3.5.2. The user must be alerted at the time of a request entry into the LIMS that there are existing records for a patient or patients with the same name and date of birth (BCSH, 2006b).

3.6. Sample requirements

3.6.1. EDTA samples (plasma) are most appropriate for use in automated systems, whilst clotted samples (serum) remain suitable for use in manual systems. For the purposes of this guideline, the use of the term 'plasma' will be used to cover all requirements irrespective of specimen type, unless specifically stated. It should be remembered that:

    i. Weak, complement-binding antibodies are more likely to be missed when using plasma (refer to 9.3.2).

    ii. When using serum, haemolysis can indicate a positive reaction in the reverse group (refer to 4.3.1 vi) or the IAT.

3.6.2. Laboratories should have a sample acceptance policy, which covers labelling and condition of samples, and should comply with the guidelines on the administration of blood components (BCSH, 2009).

3.7. Timing of sample collection in relation to previous transfusions

3.7.1. Transfusion or pregnancy may stimulate the production of unexpected antibodies against red cell antigens through either a primary or secondary immune response. The timing of samples selected for cross-matching or antibody screening should take account of this, as it is not possible to predict when or whether such antibodies will appear. It is also important to note that all cellular blood components contain residual red cells and may elicit an immune response.

3.7.2. To ensure that the specimen used for compatibility testing is representative of a patient's current immune status, serological studies should be performed using blood collected no more than 3 days in advance of the actual transfusion when the patient has been transfused or pregnant within the preceding 3 months, or when such information is uncertain or unavailable. The 3 days includes the dereservation period, e.g. if the sample was 1-day old, the blood would have to be transfused within 2 days. Where there has been no transfusion or pregnancy within the preceding 3 months, the sample is valid for up to 3 months. See Table 1 for summary of sample validity and Appendix 2 for further discussion.

> KEY RECOMMENDATION: Serological studies should be performed using blood collected no more than 3 days in advance of the actual transfusion when the patient has been transfused or pregnant within the preceding 3 months.

3.7.3. A formal deviation from the 3-day rule may be considered for chronically transfused patients with no alloantibodies, following multiple repeated transfusion episodes, allowing samples to remain acceptable for up to 7 days. However, alloimmune response to red cells is unpredictable and may be first detected after many transfusions. Data from patients with sickle cell disease suggest that the proportion of patients developing antibodies increases with the number of transfusions, possibly plateauing at approximately 100 transfusions (Reisner, 1987). Great care should therefore be

Table 1. Working limits for use of stored whole blood and plasma for pre-transfusion testing

	Sample type		
Patient type	Whole blood at room temperature	Whole blood at 2–8 °C	Plasma at −30 °C
Patient transfused or pregnant in last 3 months	Up to 48 h	Up to 3 days[1]	N/A
Patient not transfused and not pregnant in last 3 months	Up to 48 h	Up to 7 days	3 months

[1]This is the time between the sample being taken and the subsequent transfusion.

taken, and there should be a formal assessment of risk and benefit for each patient undertaken by a haematologist as part of their management plan, and this should be recorded on the LIMS and documented in the patient's record. Each individual assessment should be reviewed on an annual basis, or immediately in the event of a change in serological status.

3.7.4. This principle may be extended to pregnant women with no clinically significant alloantibodies who, for example, require blood standing by for potential obstetric emergencies, e.g. placenta praevia. Fetomaternal haemorrhage (FMH) constitutes a smaller stimulus than transfusion, because the number of foreign antigens is limited, and in many pregnancies the volume of red cells transferred from foetus to mother is too small to stimulate a primary response (Klein & Anstee, 2005a).

3.8. Storage of samples

3.8.1. Whole-blood samples will deteriorate over a period of time. Problems associated with storage include red cell lysis, bacterial contamination, decrease in potency of red cell antibodies, particularly immunoglobulin M (IgM) antibodies and the loss of complement activity in serum samples.

Table 1 gives suggestions for working limits (if times are extended this must be supported by local risk assessment prior to implementation).

3.8.2. While antibodies are probably stable for up 6 months in frozen-stored samples, the risk of intervening transfusion or pregnancy and the risk associated with the sample identification of separated plasma samples should be assessed before considering the use of stored samples for crossmatching, and it is recommended that samples be considered suitable for crossmatching (including electronic issue) for no more than 3 months.

3.8.3. Manual separation of plasma for storage is a critical point and, if performed, maintaining correct patient identification and sample identification on the secondary tube is essential, and the process should allow for a fully auditable trail (including who separated the sample). Secondary, physical separators (see Appendix 2) may be a safer alternative, allowing samples to be frozen within their original bottles while ensuring red cell lysis does not 'contaminate' the plasma, so preserving plasma for serological testing.

3.9. Retention of pre-transfusion samples

3.9.1. In the event of a haemolytic transfusion reaction it is good practice to retest a pre-transfusion sample, allowing determination of whether there have been any systemic or individual failures in grouping or antibody detection, or whether the reaction was truly unpredictable prior to transfusion.

3.9.2. It is recommended that a pre-transfusion sample be retained for at least 3 days post-transfusion, to ensure that repeat ABO grouping of the pre-transfusion sample can be performed in the event of an acute transfusion reaction (RCPATH, 2009). Laboratories should consider how best to achieve this, whilst reducing the risks of an out-of-date sample being selected for crossmatching.

> KEY RECOMMENDATION: A pre-transfusion sample should be retained for at least 3 days post-transfusion, to ensure that repeat ABO grouping of the pre-transfusion sample can be performed in the event of an acute transfusion reaction.

3.9.3. It is useful to keep plasma available for 7–14 days post-transfusion for investigation of delayed transfusion reactions (SHOT, 1996–2010). There should be a process to prevent these samples from being used inappropriately for further crossmatching.

For more discussion on the recommendations regarding timing of sample collection, storage of samples and use of physical separators, see Appendix 2.

4. ABO AND D GROUPING

4.1. Introduction

4.1.1. ABO grouping is the single most important serological test performed on pre-transfusion samples and the sensitivity and security of testing systems must not be compromised.

> KEY RECOMMENDATION: ABO grouping is the single most important serological test performed on

pre-transfusion samples and the sensitivity and security of testing systems must not be compromised.

4.1.2. Fully automated systems should be used where possible to reduce the risks of interpretation and transcription error. SHOT data (SHOT, 1996–2010) has demonstrated that the vast majority of ABO grouping errors occur in manual systems, and the UK Transfusion Laboratory Collaborative recommends the use of full automation for all but the smallest laboratories (Chaffe *et al.*, 2009).

KEY RECOMMENDATION: Fully automated systems should be used where possible to reduce the risks of interpretation and transcription errors.

4.1.3. Although full grouping, including a reverse group, is the default position, there has been a gradual move towards abbreviating ABO grouping in the UK, in certain circumstances. It should be remembered that the reverse group acts as a valuable in-built check of the forward group and plays an important role in highlighting anomalies following transfusion and stem cell transplantation, as well as those due to pathological conditions, such as cold agglutinins.

4.1.4. Interpretation of D grouping has become more complex, with the increase in variety of monoclonal reagents, and molecular testing. The historical distinction between weak and partial D, based on whether the individual is able to make anti-D, has become blurred and a new algorithm is included in Fig. 1.

4.2. Reagent selection

4.2.1. Those responsible for choosing test systems and blood grouping reagents should take into account the specificity and sensitivity of the reagents available, and the requirements for diluent controls.

4.2.2. It should also be noted that some column agglutination technology (CAT) card/cassette profiles will give the wrong group if read in the wrong orientation; an example is shown in Fig. 2.

4.2.3. Some commercial reagents contain potentiators such as polyethylene glycol (PEG), to enhance reactions in forward (ABO and D) or reverse grouping. High levels of these potentiators can cause false positive reactions in the presence of *in vivo* immunoglobulin coating of the patient's red cells. Where they are used, there should be a process in place to reduce the risks of misinterpretation of false positives; a diluent control should be included in accordance with

manufacturers' requirements and instructions, and alternative reagents should be available to investigate anomalies.

4.2.4. The anti-B reagent should not react with acquired-B antigen.

4.3. Test selection

4.3.1. ABO grouping.

i. A full ABO group comprises a forward group and a reverse group; the forward group should be performed using monoclonal anti-A and anti-B blood grouping reagents, and the reverse group using A_1 and B reagent red cells.

ii. A full group must be performed on all samples from first time patients, with the exception of neonates, where the reverse group is unlikely to be helpful, as any ABO antibodies are likely to be maternal in origin.

iii. Consideration can be given to omitting the reverse group on subsequent samples, where secure, fully interfaced automation is used and a risk assessment has been undertaken to ensure that the forward group is not compromised. The risk assessment should include the possibility that the first sample may have been taken from the wrong patient, an event estimated to occur at a rate of 1:2000 samples (Dzik *et al.*, 2003; Murphy *et al.*, 2004).

KEY RECOMMENDATION: Any abbreviation of the ABO group must be fully risk assessed.

iv. The following should apply before consideration is given to omitting the reverse group:

- There should be no manual intervention or manual editing of results;
- The current cell group must be identical with the historical record;
- There must be at least one valid historical record where testing included a reverse group. The historical group should have been performed in a fully automated system, in control of the LIMS or analyser, with no manual edits; however, further aspects of validity should be locally defined, with consideration given to where and when the group was performed and recorded.

v. The risks involved with omitting the reverse group decrease with the number of matching historical records. Where there is only one historical record, the first sample could have been taken from the wrong patient, and a grouping anomaly in the subsequent sample could be overlooked without a reverse group, e.g. mixed field reactions (potentially indicating an ABO incompatible transfusion) are sometimes not detected or are misinterpreted.

vi. Where serum is used, it is recommended that diluent containing EDTA is used for re-suspension of reverse
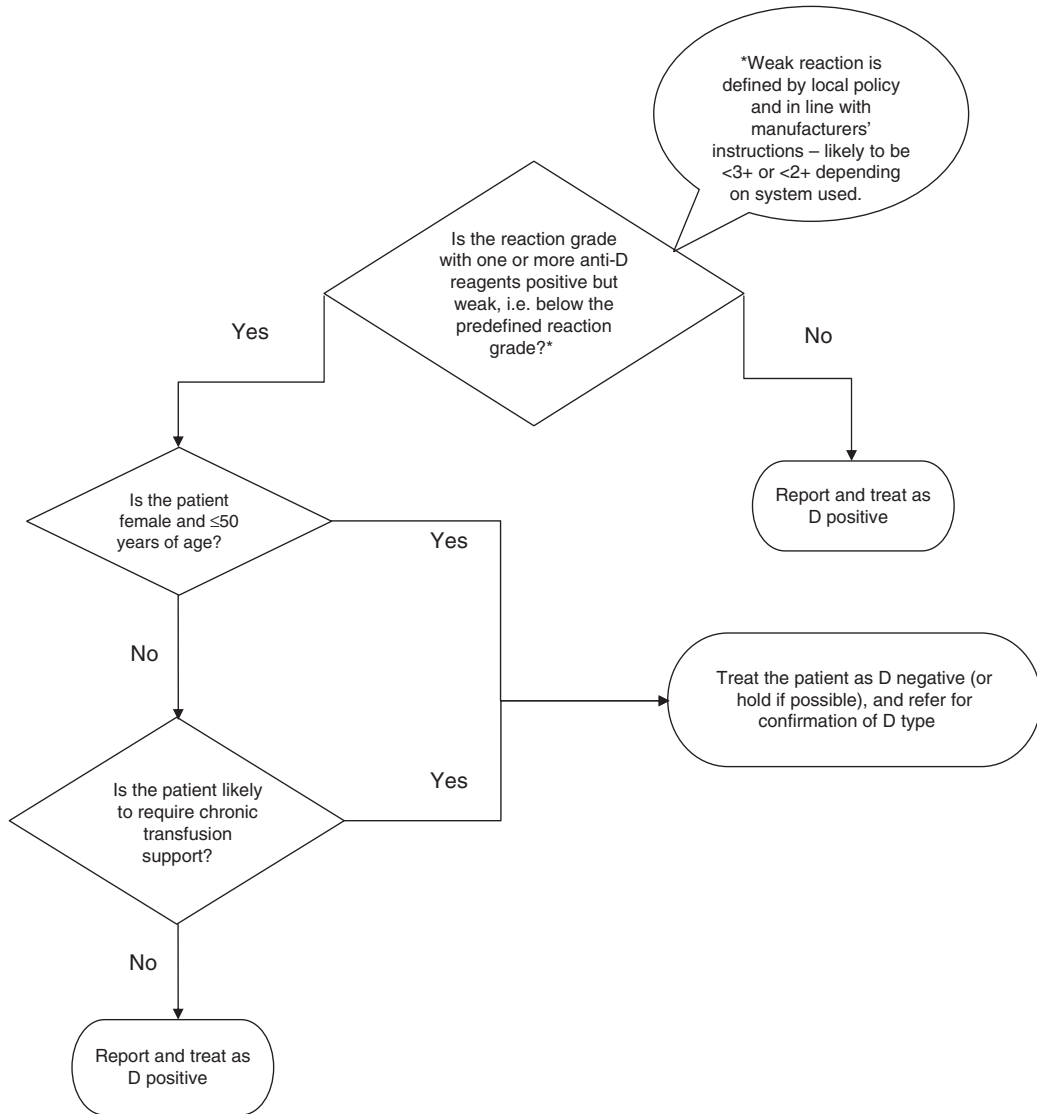
Fig. 1. Reporting of D typing anomalies and selection of red cells.

Example: an O D negative sample in a card read from correct side					
Anti-A	Anti-B	Anti-D	Ctrl	A₁ Cells	B cells
-	-	-	-	+	+
Same card read from the reverse side appears to be AB D negative					
B cells	A₁ Cells	Ctrl	Anti-D	Anti-B	Anti-A
+	+	-	-	-	-

Fig. 2. Example of a card/cassette read in the wrong orientation.

grouping cells to prevent misinterpretation of results due to haemolysis.

4.3.2. D typing

i. Where secure automation is used, D typing may be undertaken using a single IgM monoclonal anti-D reagent, which should not detect DVI. In the absence of secure automation, each sample should be tested in duplicate,

either with the same reagent or with two different IgM monoclonal anti-D reagents; this is to reduce the risk of reagent cross-contamination and the potential for procedural error where manual testing is undertaken.

ii. Potent monoclonal IgM anti-D reagents will detect all but the weakest examples of D and confirmatory tests on apparently D negative samples are not routinely required for the purposes of transfusion. Use of such tests, e.g. an antiglobulin test, creates an unnecessary risk that a

DAT positive (D negative), or a DVI sample will be misinterpreted as D positive.

iii. Anti-CDE reagents are of no value for routine typing of patients' red cells and have led to misinterpretation of r′ and r″ red cells as D positive in UK NEQAS exercises. It is therefore recommended that anti-CDE reagents are not used for routine pre-transfusion testing.

iv. There is minimal evidence that foetal red cells expressing the DVI antigen can cause maternal sensitisation. Therefore, the use of different reagents (DVI positive) for typing cord samples is not recommended, as the risks of using the wrong reagent for routine testing, outweighs the risk of missing a DVI cord sample.

v. It is important to note that monoclonal anti-D reagents vary widely in their ability to detect both partial and weak D. If using two different reagents it may be helpful to use those of similar affinity to reduce the number of discrepancies due to the detection of weak D.

4.4 Controls

4.4.1. Positive and negative controls should be used on a regular basis; the exact frequency will depend on work patterns.

4.4.2. Controls should always be included when changing reagent lot numbers and when starting up an analyser.

4.4.3. When using automated systems, control samples should be loaded onto the analyser in the same way as patient samples.

4.4.4. Controls should be included at least once every 12 h when the analyser is in use. Timings should take into account the length of time that reagents have been out of temperature control on the analysers.

4.4.5. When working manually in batches (one or more samples at a time), using a tube or microplate technique, controls should be included in every batch.

4.4.6. Where a manual CAT technique, incorporating pre-dispensed reagents, is used, it is not necessary to include controls with each batch. However, laboratory policy should ensure that all other relevant quality measures are in place, such as validation of the reagents prior to use, and monitoring of the storage conditions. In this case, controls should be included every 12 h of working.

4.4.7. Controls for manual and automated procedures are shown in Table 2.

4.4.8. Where controls do not give the expected reactions, investigations must be undertaken to determine the validity of all test results subsequent to the most recent valid control results. Refer to quality Section 2.

4.4.9. Where recommended by the manufacturer, a diluent control reagent should always be tested against the patient's red cells, as part of the ABO and/or D

Table 2. Use of positive and negative controls for ABO/D grouping

Reagent	Positive control cells	Negative control cells
Anti-A	A	B
Anti-B	B	A
Anti-D	D positive	D negative

grouping procedure. If positive (even weakly), the test result is invalidated.

4.4.10. A diluent control(s) must always be used as part of the ABO/D grouping procedure in cases where there is evidence that a strong cold auto-antibody is present, or when auto-agglutination in the patient's sample has been detected. In such cases, washing the cells with warm saline prior to testing may also be helpful (Fig. A1).

4.5. Interpretation of results

4.5.1. Manual intervention may be required in automated systems, but this should be auditable: reaction patterns and any associated edits should be stored and accessible on the LIMS in accordance with Royal College of Pathologists (RCPath) guidelines relating to quality records (RCPATH, 2009). Although reactions may be stored pictorially on the analyser, reliance should not be placed on these as they are likely to be lost when the analyser is replaced.

4.5.2. There should be a documented policy for dealing with edited results in relation to the sample on which the result was edited, and any subsequent samples on the same patient. The policy may differ with respect to editing of forward and reverse grouping reactions and interpretations. The risk of an incorrect edit combined with a subsequent sample from the wrong patient (or vice-versa) should be assessed.

4.5.3. Reactions brought forward for review should be visually inspected before decisions are made – inspecting the computer image alone might be insufficient to detect weak mixed field reactions.

4.5.4. Where manual systems are used, the risk of error can be minimised by separating the procedure into distinct tasks and, wherever possible, using different members of staff to perform each task. Suggested options for achieving this are:
   i. Separating the documentation of reaction patterns from the final interpretation.
   ii. Separating the interpretation and documentation of the forward and reverse groups.

4.5.5. The chosen procedure should be suitable for use 24/7.

4.5.6. Where manual CAT testing is used, there should be a process in place to manage the risk of misinterpretation

due to the card/cassette being read in the wrong orientation. See reagent selection 4.2.2.

4.6. Verification of results

4.6.1. Barcode labelling of the sample is a manual task and a critical point in the process. It is strongly recommended that the patient demographics on the sample are checked against the computer record prior to validation of results (preferably prior to testing) to ensure that they match and no errors have been made during data entry onto the LIMS.

KEY RECOMMENDATION AND CRITICAL POINT: The patient demographics on the sample should be checked against the computer record prior to validation of results (preferably prior to testing), to ensure that they match and no errors have been made during data entry onto the LIMS.

4.6.2. The ABO and D group must be verified against any previous results for the patient. When verification checks against historical results reveal a discrepancy, a further sample must be obtained and tested immediately.

4.6.3. If transfusion is required in the meantime, group O should be given.

4.6.4. Any manual editing of results should be performed in accordance with Section 2.1.4.

4.7. Grouping anomalies

4.7.1. The following are some examples of blood group anomalies:

i. *Mixed-field reactions*. Any samples showing mixed-field reactions must be repeated and/or investigated prior to group authorisation or issue of red cells. These reactions may represent an ABO/D mismatched or even incompatible transfusion, mismatched haemopoietic stem cell transplant, an A3 or B3, a large FMH, or (rarely) a chimera, following a twin-to-twin transfusion.

ii. *Intrauterine transfusions*. For a period of several months post-delivery, infants who have received intrauterine transfusions may appear to be the same ABO and D group as that of the transfused red cells, due to bone marrow suppression.

iii. *Presence of cold-active alloantibodies*. An unexpected reaction with the reverse grouping cells may be obtained if these cells express an antigen for which a cold-active alloantibody is present in the patient's plasma other than anti-A or anti-B. Where possible, the reverse group should be repeated at a higher temperature,

or using reverse grouping cells that lack the implicated antigen.

iv. *A/B Variants*. Variants of A and B may give weak or negative reactions with monoclonal reagents, and referral to a reference centre may be required to confirm the group.

v. *Other reverse grouping anomalies*: Potentiators in the reverse grouping reagents may cause IgG antibodies such as anti-c to be detected in the reverse group.

vi. *Partial and weak D*: Historically, it has been accepted that patients with weak D cannot make anti-D and can therefore be regarded as D positive, whereas those with partial D can make anti-D to the epitopes they lack and should therefore be treated as D negative. Evidence from molecular testing and from testing with an increasing number of monoclonal antibodies suggests that this is not necessarily the case, and some individuals classed as weak D have made anti-D. The distinction between weak and partial D is no longer considered to be straightforward (Daniels *et al.*, 2007) and therefore a new patient-based algorithm is recommended (Fig. 1).

4.8. Resolution of grouping anomalies

4.8.1. Anomalies should be resolved prior to provision of red cells or other cellular components unless this seriously compromises the clinical care of the patient. See Fig. A1 for guidance.

4.8.2. If transfusion is required before the anomaly is resolved, group O red cells should be given – see 7.7.2.

4.8.3. If it is not possible to obtain a reliable reverse grouping result due to the age of the patient or to insufficient sample, and there is no historical group against which to validate, the cell group must be repeated.

4.8.4. Where there is a discrepancy in reaction strength between different anti-D reagents, or where the reagent fails to give a clear-cut strong positive reaction,[1] a decision to investigate further needs to be made based on whether the development of anti-D is likely to cause clinical problems.

i. Females of childbearing potential or patients who are likely to require long-term transfusion should be treated as D negative until a confirmed group is assigned and appropriate advice given by a reference laboratory; all others can be treated as D positive without confirmation (Fig. 1).

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[1]Clear cut reaction is defined by local laboratory policy and in line with manufacturers' instructions (likely to be >2+ or >1+), depending on the system used.

ii. Patients with a known partial D status should be regarded as D negative, but the findings explained clearly to the patient in order to prevent misunderstandings.

5. ANTIBODY SCREENING

5.1. Introduction

5.1.1. The aim of antibody screening is to determine the presence of atypical red cell antibodies of likely clinical significance. When the antibody screen is positive, further testing is required to identify the responsible antibody(ies). This process ultimately enables the laboratory to select suitable units should transfusion be required. Reporting of a positive antibody screen also serves to alert the clinician to possible delay in the supply of compatible blood.

5.1.2. Antibody screening should always be performed as part of pre-transfusion testing as it provides the laboratory with a more reliable and sensitive method of detecting a red cell antibody than serological crossmatching, due to the following:

    i. Homozygous antigen expression on screening cells compared with variable antigen expression of donor cells (refer to Section 5.3.3): for example, the homozygous genotype $Jk^a Jk^a$ often results in a higher expression of the Jk^a antigen than the heterozygous genotype $Jk^a Jk^b$, and the former can result in a stronger reaction with a weak example of anti-Jk^a.

    ii. Antigen preservation of screening cells (5.3.4).

    iii. Although crossmatching can be automated, it is possible to transpose donor cell suspensions during manual preparation, and there is less standardisation in preparation of cell suspensions in crossmatching compared with antibody screening.

5.2. Choice of IAT technology

5.2.1. Automated and manual techniques for antibody screening vary in sensitivity and specificity, and should be evaluated in consideration of local requirements.

5.2.2. A low ionic strength solution (LISS) IAT is considered to be the most suitable for the detection of clinically significant antibodies because of its speed, sensitivity and specificity. Different technologies (e.g. column agglutination, solid-phase) have different strengths and weaknesses and should be subject to local validation before their introduction into routine use.

5.3. Reagent red cells for use in antibody screening

5.3.1. As a minimum, the following antigens should be expressed within the screening cell set, which should comprise a minimum of two donors:

    i. One reagent red cell should be R_2R_2; the other R_1R_1 (or $R_1{}^wR_1$).

    ii. The following antigens should additionally be present in the screening cell set: K, k, Fy^a, Fy^b, Jk^a, Jk^b, S, s, M, N, P_1, Le^a and Le^b.

5.3.2. The screening cells should not be pooled.

5.3.3. The screening cell set should include at least one cell with homozygous expression of the Fy^a, Fy^b, Jk^a, Jk^b, S and s antigens. These recommendations are, in part, based on UK data regarding the incidence of delayed transfusion reactions, and the need for a high sensitivity in the detection of Kidd antibodies (SHOT, 1996−2010; Knowles *et al.*, 2002).

5.3.4. Red cells for antibody screening should be preserved in a temperature-controlled environment in a diluent shown to minimise loss of blood group antigens during the recommended storage period.

5.3.5. The stability of screening cells should be validated locally for routine use in the laboratory whether located on the analyser or bench or in a refrigerator.

5.3.6. This validation should be used to determine a time limit for each bottle of cells once opened, and should be repeated when / if storage conditions or usage patterns change.

5.3.7. Reagents must not be used past the manufacturer's expiry date.

5.4. Controls

5.4.1. The principles for use of controls within automated and manual testing are the same as those described in the grouping Section 4.4.

5.4.2. An autologous control or DAT need not form a part of antibody screening.

5.4.3. A weak anti-D control (containing anti-D at a level of less than 0.1 IU mL^{-1}) should be used on a regular basis to assure the efficacy of the whole test procedure (see 5.4.6). The exact frequency will depend on work patterns.

5.4.4. The use of further controls, containing weak examples of antibodies with specificities known to be clinically significant when present in patients' plasma (e.g. anti-Fy^a) is also recommended to assure the sensitivity of the test procedure and the integrity of antigen expression of reagent red cells during storage; this is especially important for the more labile antigens, e.g. Fy^a.

5.4.5. Laboratories should assure themselves that denaturation of the S antigen does not occur as a result of any hypochlorite decontamination process. Weak anti-S can be used as a control.

5.4.6. Controls for antibody screening should be selected so that each screening cell is expected to give both a positive and negative reaction as per the example in Table 3.

Table 3. Example of controls for antibody screening

QC reagent	Screening cells		
	Cell 1	Cell 2	Cell 3
Weak anti-D	+	+	−
Weak anti-c	−	+	+
Weak anti-Fy[a]	+	−	+

5.4.7. The specificity of controls should be reviewed against the antigen profile of any new lot of screening cells.

5.4.8. The antibody screening control pass or fail criteria should ensure that each cell gives the expected reaction (positive or negative) with the chosen antisera. Reaction strength should be checked to detect any adverse changes, e.g. weakened due to deterioration of antigen expression. Limits for acceptable reaction grades can often be set within the automation, e.g. pass if the reaction strength is > 1+.

5.4.9. Weak IgG-coated red cells should be used to control the washing phase of liquid-phase tube or microplate IAT techniques. For all negative IAT results, agglutination should occur when IgG-coated red cells are added. A positive result indicates the presence of free anti-IgG, thus validating the washing procedure and the test result. If the result is negative, the test is invalid and should be repeated in its entirety.

6. ANTIBODY IDENTIFICATION

6.1. Introduction

6.1.1. When an alloantibody is detected in the screening procedure, its specificity should be determined and its likely clinical significance assessed (Daniels *et al.*, 2002).

6.1.2. If the patient is known to have formed a red cell alloantibody, each new sample should be fully tested to exclude the presence of further alloantibodies (as described in 6.2.5), within the sample timing limits described in Section 3.7.

> KEY RECOMMENDATION: If the patient is known to have formed a red cell alloantibody, each new sample should be fully tested to identify or exclude the presence of further alloantibodies.

6.1.3. If there is any doubt concerning the identity of any antibodies present, or the ability to exclude clinically significant antibodies, a blood sample should be sent to a red cell reference laboratory.

6.1.4. Laboratories that are not registered for antibody identification in an accredited external quality assessment scheme should refer samples from all patients that have given positive results in the antibody screen to a laboratory that is registered for antibody identification.

6.1.5. Examples of antibody identification and exclusion are included in Appendix 4.

6.2. Principles of antibody identification

6.2.1. The patient's plasma should be tested against an identification panel of reagent red cells and should always include an IAT.

6.2.2. Antibody screening results can contribute to assigning the antibody specificity and the exclusion of additional specificities. A check should be made to ensure that the panel results do not conflict with the antibody screening results, which may reflect manual tests being performed on the wrong sample, or the correct sample containing an antibody directed against a low frequency antigen expressed on screening cells but not the identification panel.

> CRITICAL POINT: A check should be made to ensure that the panel results do not conflict with the antibody screening results which may reflect manual tests being performed on the wrong sample.

6.2.3. Inclusion of the patient's own red cells as an auto control may be helpful, for example, in recognition of an antibody directed against a high frequency antigen. However, the presence of a positive auto does not exclude the presence of an alloantibody.

6.2.4. Antibody specificity should only be assigned when the plasma is reactive with at least two examples of reagent red cells expressing the antigen and non-reactive with at least two examples of reagent red cells lacking the antigen.

6.2.5. When one antibody specificity has been identified, it is essential that the presence or absence of additional clinically significant antibodies is established. This can only be demonstrated by choosing cells that are antigen negative for the recognised specificity, but positive for other antigens to which clinically significant antibodies may arise, e.g. by using a panel of R_1R_1 (CCDee) cells when testing plasma known to contain anti-c.

6.2.6. Failure to recognise all of the antibody specificities within a sample may lead to a haemolytic transfusion reaction (SHOT, 1996–2010). In particular, the presence of anti-Jk[a], anti-Jk[b], anti-S, anti-s, anti-Fy[a] and anti-Fy[b] should be excluded using red cells having homozygous expression of the relevant antigen. A single example only of each phenotype is sufficient for exclusion.

KEY RECOMMENDATION: When one antibody specificity has been identified, it is essential that the presence or absence of additional clinically significant antibodies is established.

6.2.7. It is acceptable to exclude Rh antibodies using validated techniques incorporating enzyme treated cells (see Appendix 4 for examples).

6.2.8. The patient's red cells should be phenotyped using a reagent of the same specificity as the antibody assigned, with suitable positive and negative controls. The incorporation of a reagent control or AB serum control used by the same technique as the phenotyping reagent should be included where recommended by the manufacturer. A positive result in this control test invalidates the phenotyping test results.

6.3. Reagent red cells in use in antibody identification

6.3.1. An identification panel should consist of red cells from eight or more group O donors. For each of the more commonly encountered clinically significant red cell antibodies, there should be at least two examples of phenotypes lacking and at least two examples of phenotypes expressing the corresponding antigen. In addition, the panel should support resolution of as many common antibody mixtures as possible.

6.3.2. There should be at least one example of each of the phenotypes R_1R_1 (CCDee) and $R_1^wR_1$ (C^{w}CDee). Between them, these two cells should express the antigens K, k, Fy^a, Fy^b, Jk^a, Jk^b, S, s. There should be at least one example of each of the phenotypes R_2R_2 (ccDEE), r′r (Ccddee) and r″r (ccddEe), and at least three examples of the phenotype rr (ccddee), including at least one K+, and collectively, homozygous expression of k, Jk^b, S, s, Fy^a, and Fy^b.

6.3.3. It is important to recognise the limitations of the panel in use. A single panel may not permit identification of some common combinations of antibodies. A selection of two different panels increases the probability of being able to identify a mixture of antibodies, whilst excluding additional antibodies of likely clinical significance, and is strongly recommended for laboratories undertaking antibody identification. Laboratories should assure themselves that the antigenicity of panel cells is not compromised during the working life of the panel.

6.4. Resolving antibody identification problems

6.4.1. Additional techniques may be helpful in antibody identification, and some are listed in Appendix 5. Workers should consider the value and limitations of such techniques when selecting them and interpreting the results obtained. However, it is recommended that a panel of enzyme treated cells is available as it has been shown to improve the chances of correctly identifying an antibody mixture where at least one of the antibodies is directed against an antigen affected by enzymes (Knowles *et al.*, 2002).

6.4.2. The patient's red cells should be phenotyped as described in 6.2.8. If this test is positive or mixed field:

   i. the patient may recently have been transfused with antigen-positive blood;

   ii. the antibody may be an autoantibody (in which case the patient's cells will normally but not always be DAT positive); and/or

   iii. the patient's cells may be coated with immunoglobulin or complement components (if an antiglobulin or potentiated test method has been used), in which case the DAT will be positive; or

   iv. the assignment of the antibody specificity may be incorrect, or

   v. the wrong sample may have been tested.

6.4.3. If the patient is known to have been transfused in the previous three months, phenotyping may be misleading due to the presence of transfused cells; In this case genotyping can be used as an alternative and is available from specialist laboratories.

6.4.4. When an Rh antibody is suspected, the C, c, E, e types should be determined to aid in the selection of appropriately phenotyped red cells. See Section 7.9 and Table 4.

Table 4. Selection of Rh phenotyped red cells for patients with Rh antibodies

Rh antibody	Probable genotype/ phenotype of patient	Donor red rell (Rh) selection
Anti-D	rr (ccddee)	D- C- E- (rr)
	r′r (Ccddee)	D- C- E- (rr)
	r″r (ccddEe)	D- C- E- (rr)
Anti-E	R_1R_1 (CCDee)	c- E- (R_1R_1)
	R_1r (CcDee)	E-
	R_0r or R_0R_0 (ccDee)	C- E- (R_0 or rr)
	rr (ccddee)	D- C- E- (rr)
	r′r (Ccddee)	D- E- (rr)
Anti-C	R_2R_2 (ccDEE)	C- e- (R_2R_2)
	R_2r (ccDEe)	C-
	R_0r or R_0R_0 (ccDee)	C- E- (R_0 or rr)
	rr (ccddee)	D- C- E- (rr)
	r″r (ccddEe)	D- C- (rr)
Anti-c	R_1R_1 (CCDee)	c- E- (R_1R_1)
	r′r′ (CCddee)	D- c- E (r′r′)
Anti-e	R_2R_2 (ccDEE)	C- e- (R_2R_2)
	r″r″ (ccddEE)	D- C- e- (r″r″)

6.4.5. Where antibody exclusion is problematic, more extensive phenotyping may be informative to identify antigens to which the patient may be alloimmunised.

6.4.6. When red cells taken from a blood donation are found to be positive in an IAT crossmatch against patient's plasma, but no activity is detected in the plasma against red cells in an identification panel, it is likely that either:

  i. the plasma contains an antibody to a low frequency antigen expressed on the donor's cells; or
  ii. the red cells in the donation are DAT positive; or
  iii. blood of the wrong ABO group has been selected for crossmatching.

6.4.7. A positive DAT may be encountered as part of an investigation into haemolytic anaemia or transfusion reaction. When the DAT is positive in patients transfused within the previous month, an eluate should be prepared and tested for the presence of specific alloantibodies. The results should be used in selection of blood for transfusion.

6.4.8. In cases where all panel cells are positive but the DAT or auto are repeatedly negative, an antibody to a high frequency antigen should be suspected. This will require full investigation by a reference laboratory. However, anti-HI is a commonly found antibody in non-group O patients and its presence should first be excluded – this can be achieved by testing the plasma with cells of the same group as the patient (e.g. A or B reverse grouping cells) which would give negative reactions with anti-HI. The presence of underlying alloantibodies should be excluded before transfusion.

6.4.9. Software packages are available as an aid to interpretation of reaction patterns in antibody identification. These should be validated before use. Antibody identification software should be seen as an aid to trained and experienced staff rather than a replacement.

6.5. Autoantibodies

6.5.1. Many autoantibodies cause no clinical problems. In patients with autoimmune haemolytic anaemia (AIHA), autoantibodies directed against red cell antigens are responsible for shortening red cell survival that may lead to severe anaemia. Clinically benign autoantibodies often cause laboratory problems making determination of ABO and Rh groups problematic and preventing effective antibody screening due to cross-reacting antibody (Maley *et al.*, 2005).

6.5.2. Serological investigations in AIHA should focus on determination of the correct ABO, CcDEe and K status of the patient, and determination of the possible presence of an underlying alloantibody. It may be necessary to refer cases of AIHA to a red cell reference laboratory dependent on of the complexity of the investigation required.

6.5.3. Selection of blood for transfusion may be influenced by the presence of an autoantibody of simple or mimicking specificity (e.g. auto anti-e). However, specialist advice should be sought before selecting antigen-negative blood in these circumstances, as prevention of the development of alloantibodies is usually of more importance.

6.5.4. Cold-type AIHA or cold haemagglutinin disease: typically the patient's red cells have a strongly positive DAT due to coating with complement components (most commonly C3d). Where bound IgM causes a positive DAT or control, washing red cells at 37 °C can be useful to remove autoantibody. The presence of alloantibodies can usually be excluded using screening cells and patient's plasma separately prewarmed to 37 °C before use in IAT. The use of anti-IgG in place of polyspecific antiglobulin reagent may also be helpful.

6.5.5. Warm-type AIHA: red cells from the patient typically have a positive DAT due to coating with IgG and, sometimes, complement components. When a panreacting autoantibody prevents detection of underlying alloantibody using unmodified patient's plasma, adsorption should be performed.

  i. Autoadsorption using the patient's red cells can remove autoantibody from the patient's plasma. The efficiency of autoadsorption can be improved using the ZZAP method (Branch & Petz, 1982).
  ii. In many cases, autoadsorption may be difficult or undesirable (e.g. when there is a limited volume of patient's red cells or within 3 months of transfusion). Allogeneic adsorptions using selected red cells can be performed as an alternative, to facilitate the exclusion of alloantibodies.
  iii. After adsorption, plasma may be used in standard screening and/or antibody identification and crossmatch techniques, but it should be noted that the adsorption might result in a slight reduction in the serological activity of alloantibodies.

6.5.6. In regularly transfused patients, advice should be sought from the local reference laboratory in regards to the frequency of repeat testing and documented in an individual management plan.

6.6. Antibodies showing no obvious specificity at 37 °C

6.6.1. Where antibodies are detected at 37 °C but no specificity is identified despite thorough investigation, every effort to exclude the presence of clinically significant antibodies should be made (see 6.4). Such cases might be technology specific and consideration should be given to using different technologies.

6.6.2. Antibodies reacting preferentially at temperatures below 37 °C are sometimes detected in antibody screening. In many cases, specificity is determined using standard IAT methods, but sometimes it is not possible to determine an unequivocal specificity. If it is suspected that a cold-active antibody is present in a patient's sample, it is unnecessary to identify the specificity if the antibody screen using cells and plasma prewarmed to 37 °C is negative. Further guidance on the selection of blood for patients with cold-active antibodies is given in Appendix 6.

7. SELECTION AND ISSUE OF RED CELLS

7.1. Introduction

7.1.1. Selection and issue of red cells are inherently high risk procedures and all care should be taken to reduce the risk (NPSA, 2006). The transfusion of ABO-incompatible blood is now classed as a 'never event' (DH, 2011).

7.1.2. This guideline defines a crossmatch as the final part of the process which determines the compatibility of donor red cells with the patient. This may be serological or electronic.

7.2. General principles

7.2.1. Safety of transfusion begins with collection of the sample. It has been estimated that 1 in 2000 samples is from the wrong patient, commonly known as 'wrong blood in tube' (Dzik *et al.*, 2003; Murphy *et al.*, 2004). SHOT near-miss data confirm that this continues to be a serious problem (SHOT). The use of secure bedside electronic patient identification systems reduces this risk; however, in the absence of such systems, it is highly recommended that a second sample is requested for confirmation of the ABO group of a first time patient, where this does not impede the delivery of urgent red cells or other components. See Appendix 7 for further discussion.

KEY RECOMMENDATION: Unless secure electronic patient identification systems are in place, a second sample should be requested for confirmation of the ABO group of a first time patient prior to transfusion, where this does not impede the delivery of urgent red cells or other components.

7.2.2. As the sensitivity of the antibody screen has improved and with the increased security afforded by the use of automated systems interfaced with laboratory information systems, electronic issue (EI) has increasingly replaced the IAT crossmatch. It is recommended that electronic issue is the method of

choice when a laboratory wishes to replace the IAT crossmatch with an alternative method. The system requirements identified in the current IT guidelines (BCSH, 2006b) should be met.

7.2.3. Whatever crossmatching procedure or technique is used, it should be capable of detecting an ABO incompatibility.

7.2.4. Ideally, one person should carry out the crossmatching procedure from beginning to end, one crossmatch at a time. Where this is not possible, there should be an audit trail of any individuals involved in any stage of the procedure.

7.2.5. MHRA guidance (MHRA, 2010) requires that EI is controlled by the LIMS; however, there are supplementary checks which should be manually performed regardless of the crossmatch method employed. These include:
  i. Review of the clinical details on the request form.
  ii. Checking for potential duplicate patient registrations and associated special transfusion requirements.

7.3. IAT crossmatch

7.3.1. The indirect antiglobulin test (IAT) is used to detect ABO and non-ABO red cell antibody incompatibility between donor cells and patient plasma, by testing each donor unit against the plasma of the intended recipient.

7.3.2. The (IAT) crossmatch is the default technique which should be used in the absence of functioning, validated IT or when electronic issue is contra-indicated (refer to Section 7.5). Methods are not described here as there are many variations depending on the technology in use.

KEY RECOMMENDATION: The indirect antiglobulin test (IAT) crossmatch is the default technique which should be used in the absence of functioning, validated IT or when electronic issue is contra-indicated.

7.3.3. An IAT crossmatch must be used:
  i. If the patient's plasma contains or has been known to contain, red cell alloantibodies of likely clinical significance. This recommendation is based on the need to:
  - provide assurance that the phenotype of the donor red cells correct;
  - detect additional specificities which may have been masked or undetected in antibody identification;
  - provide assurance that the assigned antibody specificity is correct.
  ii. If the antibody screen is positive. See Appendix 8 for further discussion.

iii. For neonates or foetuses when a maternal IgG antibody is present, see 7.16.7.

iv. If the patient has had an incompatible haemopoietic stem cell transplant (HSCT).

v. If the patient has had an incompatible solid organ transplant in the previous three months. This is necessary to detect IgG anti-A or anti-B produced by passenger lymphocytes in the transplanted organ (Sokol *et al*., 2002).

> KEY RECOMMENDATION: An IAT crossmatch must be used if the patient's plasma contains, or has been known to contain, red cell alloantibodies of likely clinical significance.

7.3.4. A variation of this technique is the strict 37 °C IAT crossmatch, which is useful when there are cold-reacting antibodies present.

7.3.5. An appropriate positive control should be set up with every crossmatch, e.g. a group O, R_1r or R_1R_1 cell tested against a weak anti-D. This provides assurance that the overall process has been performed correctly.

7.4. Saline spin crossmatch

7.4.1. Commonly known as the 'immediate spin' crossmatch, the saline spin crossmatch is now rarely used as a routine compatibility test. It is used mainly as a rapid means to detect ABO incompatibility.

7.4.2. It cannot be relied on to detect ABO incompatibility in patients with weak anti-A or anti-B.

7.4.3. It is not a suitable substitute for an IAT crossmatch because it does not detect incompatibility due to IgG antibodies.

7.4.4. The saline spin technique is a manual tube method with a number of variables that are difficult to standardise (O'Hagan *et al*., 1999). The broad principles to ensure optimum sensitivity require a 2:1 ratio of patient plasma to saline suspended donor red cells (2–3%), a tip and roll reading technique and a short incubation time of 2–5 min before centrifugation. The appropriate centrifugation speed and time should be determined via local validation.

7.4.5. If using serum samples, the donor red cells should be suspended in EDTA saline in order to prevent prozone (by overcoming steric hindrance of agglutination by C1) (Judd *et al*., 1988).

7.4.6. There are no agreed standard controls for a saline spin crossmatch.

7.5. Electronic issue

7.5.1. Electronic issue (EI) is the selection and issue of red cell units where compatibility is determined by the LIMS without serological testing of donor cells against patient plasma.

7.5.2. The BCSH guidelines for the specification and use of information technology systems in blood transfusion practice previously included detailed guidance on the testing aspects of EI, which has now moved to this guideline. Reference should still be made to the IT guidelines (BCSH, 2006b) for the IT requirements relating to EI.

7.5.3. Laboratories that routinely perform electronic issue should have a documented contingency plan, including validated manual processes, in case of IT failure.

7.5.4. The ability to perform EI depends on three main factors:

i. Having the recommended quality management system and laboratory processes in use.

ii. Having LIMS control of the issue of blood components as recommended in the BCSH IT guidelines.

iii. The specific patient's transfusion and antibody history and serological status of the current sample.

7.5.5. If ALL of the following process criteria are met, then EI is acceptable:

i. Testing and result entry of the group and antibody screen are fully automated.

ii. Reagents, cells and technology used for grouping and antibody screening meet the criteria as outlined in Sections 4 and 5.

iii. Samples and reagents are registered and identified within the analyser via a unique barcode or equivalent.

iv. Results are transmitted electronically from the analyser to the LIMS.

v. The LIMS controls the suitability of patients and their samples for EI.

vi. The LIMS enables permanent exclusion of patients from EI in the presence of antibodies of likely clinical significance.

vii. The LIMS enables temporary exclusion of patients from EI, e.g. limited period exclusion for 3 months following transplantation of solid organs (Sokol *et al*., 2002).

viii. Stock entry of unique donation number, blood group, component code and expiry date from the unit(s) is by barcode reader or other electronic means.

7.5.6. If ALL of the following patient and sample criteria are met, then electronic issue is acceptable for that patient sample;

i. Blood group interpretation on the current sample is identical to the historical record.

ii. No manual amendments have been made to automated results.

iii. The current antibody screen is negative. See Appendix 8 for further discussion.

iv. The patient's group and antibody screen results are complete and fully authorised in the LIMS.

v. The patient does not have a previously known antibody of likely clinical significance.

vi. Patient is not excluded on clinical grounds – see 7.3.3.

vii. The current sample meets the sample timing and storage requirements detailed in 3.7 and 3.8.

KEY RECOMMENDATION: The overall process for determining eligibility for EI must be controlled by the LIMS and not rely on manual intervention or decision making.

7.6. Selection of blood

7.6.1. The correct selection of red cells is paramount for safe transfusion. Ideally this should be controlled as far as possible by the LIMS and supported by clearly written and unambiguous policies, with continuing training of laboratory staff.

7.6.2. The LIMS must not allow selection of ABO incompatible red cells.

7.6.3. The LIMS should allow selection of ABO or D non-identical but compatible units and should issue an alert.

7.6.4. The LIMS should control selection and issue of red cells and other blood components, e.g. antigen negative, irradiated, etc., where applicable to an individual patient. This should also include special requirements based on patient demographics, e.g. age and gender.

7.6.5. The LIMS should prevent issue of red cells against a sample which is not valid for red cell issue, e.g. time expired.

7.6.6. Robust procedures must be put in place to ensure that the wrong blood cannot be selected and issued in error during LIMS downtime.

7.6.7. In non-computerised laboratories, robust procedures must be put in place to ensure that the wrong blood cannot be selected and issued in error.

7.7. ABO selection

7.7.1. Red cell components of the same ABO group as the patient should be selected whenever possible.

7.7.2. If ABO identical blood is not available for group A or B patients, group O blood should be used, and provided it is in additive solution, it does not need to be tested for high titre haemagglutinins as the volume of residual plasma is too small to cause haemolysis (AABB, 2011).

7.7.3. Group AB should be used for AB patients, but if unavailable, group A or B red cells should be selected rather than group O.

7.7.4. Group O red cells should be used in the following situations where transfusion cannot await full investigation and resolution because transfusion is deemed clinically urgent:

i. Where the ABO group has not yet been determined (see Section 8).

ii. Where there is a discrepancy between the ABO group on the current blood grouping sample and a historical blood grouping result.

iii. When there are mixed field ABO reactions that have not been confirmed to be related to compatible non-ABO identical transfusion.

iv. For any ABO grouping anomaly that cannot be explained and is pending investigation or repeat samples.

7.8. D selection

7.8.1. Selection of D matched blood is the recommended best practice, and D positive blood should be selected for D positive patients according to the definition in the flow chart (Fig. 1, Section 4). However, in order to preserve supplies of D negative red cells for D negative women of child bearing potential, D positive red cells may be selected for D negative patients in the following situations:

i. Female patients > 50 years.

ii. Adult males who are D negative or whose D status is unknown.

iii. Patients undergoing a large volume transfusion (> 8 units), excluding children, females of childbearing potential and patients with immune anti-D.

7.8.2. The policy for use of D positive red cells to a D negative recipient should be documented and controlled by validated rules in the LIMS where applicable.

7.8.3. D negative red cells should always be selected for:

i. D negative women of childbearing potential (<51 years).

ii. D negative patients <18 years old.

iii. Patients who have formed immune anti-D, even if not currently detectable.

iv. Transfusion-dependant D negative adults.

7.9. Females of child-bearing potential

7.9.1. Females of child-bearing potential should receive K negative red cells unless they are unavailable in an emergency (Lee & de Silva, 2004; BCSH, 2006a).

7.10. Patients with red cell alloantibodies of likely clinical significance

7.10.1. Red cells should be selected which have been phenotyped and found negative for the relevant antigen. It is good practice to give K negative red cells to these patients because it is sometimes difficult to exclude anti-K in the presence of other antibodies and easy to select K negative units.

7.10.2. Antigen negative red cells should also be selected when a clinically significant antibody has previously been identified, but cannot be detected or identified in the current sample.

7.10.3. Patients with anti-D who are rr (ccddee) should receive rr (D- C- E-), K negative blood.

7.10.4. Patients with other Rh antibodies should be additionally matched for C, c, E and e in order to prevent further Rh alloimmunisation, provided this does not impede delivery of effective transfusion support. See Table 4 for details.

7.11. Patients with known alloantibodies of likely clinical significance to low-frequency antigens, e.g. anti-Wr[a]

7.11.1. IAT crossmatch-compatible red cells may be issued rather than selecting antigen-negative units. Advice should be sought from a blood service reference centre if necessary.

7.12. Patients with alloantibodies considered unlikely to be of clinical significance

7.12.1. IAT crossmatch-compatible red cells may be issued; it may be necessary to perform a strict $37\,^{\circ}$C IAT crossmatch when the antibody has a low thermal range. See Appendix 6.

7.13. Patients with autoimmune haemolytic anaemia.

7.13.1. While transfused cells are present in the patient's circulation, alloantibodies can be made which may be masked by a strong autoantibody. It may be necessary to refer samples to a reference laboratory to exclude underlying alloantibodies. See Section 6.

7.13.2. In cases where a clinically significant alloantibody has been detected, an IAT crossmatch using absorbed plasma should be performed, which is likely to be in a reference laboratory. See 6.5.5.

7.13.3. Whilst adsorption techniques offer a rapid and effective approach to reducing the risks of transfusing red cells that are antigen positive for masked alloantibodies, they may result in slight reduction in the serological activity of alloantibodies. Test results using absorbed plasma (see 6.5.5) should be reported in a manner which reflects this (e.g. blood should be labelled 'suitable' rather than 'compatible').

7.13.4. Where alloantibodies have been excluded and IAT crossmatching using unmodified patient's plasma cannot be expected to add value to the compatibility testing process (i.e. it is strongly pan-reactive) it may be omitted. An immediate spin crossmatch using unmodified plasma can be used to exclude ABO incompatibility (Lee *et al.*, 2005).

7.13.5. Risk of transfusion reaction due to underlying alloantibodies can further be reduced by matching blood with the patient's own type. Determination of the phenotype in multiply transfused and/or DAT+ patients may be problematic and determination of genotype offers useful information in managing these complex cases; this particularly applies to regularly transfused cases and those with autoantibodies resistant to removal by adsorption. By agreement of a consultant haematologist, cases managed in this way may be subject to reduced frequency of testing or have the serological crossmatch omitted. Such a decision should be made on a case-by-case basis in otherwise stable patients and be subject to review if the status of the patient changes. The potentials risks and benefits to the patient should be clearly documented in the clinical notes.

7.14. Panagglutination

7.14.1. Panagglutination may be present in circumstances other than AIHA, including where the patient has an antibody to a high frequency antigen or anti-HI (see 6.4.8).

7.14.2. Selection of blood depends on the exclusion of underlying alloantibodies and should be considered on a case-by-case basis.

7.14.3. Panagglutination can sometimes be caused by technique-specific problems, e.g. a LISS-only non-specific antibody. In such cases use of a different technique should be considered, e.g. normal ionic strength saline (NISS) IAT.

7.15. Massive blood transfusion

7.15.1. Massive blood loss can be defined as the loss of one blood volume within a 24-h period or, in the acute situation, a 50% blood volume loss within 3 h or a rate of loss of 150 mL min^{-1} in an adult.

7.15.2. If group O red cells have been given initially, it is recommended to switch to the patient's own ABO group as soon as it has been determined to the standards set out in 8.4, which includes testing a second sample as soon as possible. Reference should be made to Appendix 7.

7.15.3. Once the volume of blood transfused in any 24 h period is equivalent to the patient's own blood volume

(adults 8–10 units, children 80–100 mL kg^{-1}), ABO and D compatible blood can be issued without the need for a serological crossmatch.

7.15.4. For patients with clinically significant red cell antibodies, antigen negative blood can be given in the same way as in 7.14.3; where demand outstrips supply, untyped units might be required, but decisions will need to be made on a case-by-case basis and should be subject to the concessionary release process. Specialist advice may be required in these circumstances.

7.16. Foetal transfusions

7.16.1. Red cells for foetal transfusion should be selected to comply with current 'Red Book' guidelines (Guidelines for the Blood Transfusion Services in the UK 2012) and BCSH Transfusion Guidelines for Neonates and Older Children (BCSH, 2004).

7.16.2. D negative, K negative (and further antigen negative where appropriate) units should be crossmatched against the maternal plasma by IAT if the maternal plasma contains red cell antibodies of likely clinical significance.

7.17. Transfusions for neonates and infants up to 4 months post-delivery

7.17.1. Red cells for transfusions to neonates and infants up to 4-months old should comply with the 'Red Book' (Guidelines for the Blood Transfusion Services in the United Kingdom 2010) and BCSH transfusion guidelines for neonates and older children (BCSH, 2004). For the purposes of the rest of this document, the term 'neonate' includes infant up to the age of 4 months.

7.17.2. Unless local policy is to issue only group O to neonates, red cells must be of an ABO group which is compatible with both mother and neonate.

7.17.3. If the maternal group is unknown or uncertain, group O red cells should be selected.

7.17.4. Where there are no maternal IgG alloantibodies and the neonatal DAT is negative, group O, D compatible red cells suitable for neonatal transfusion can be issued without a serological crossmatch.

7.17.5. Where there are known maternal IgG alloantibodies, ABO and D compatible, antigen negative red cells should be selected. These should be IAT crossmatch-compatible with maternal plasma or, if unavailable, neonatal plasma.

7.17.6. Once the antibody screen and DAT on the neonatal sample are negative it is not necessary to continue crossmatching against maternal plasma.

7.17.7. If a neonate has a positive DAT due only to maternal anti-A, anti-B or anti-A,B (as demonstrated by testing

an eluate), group O red cells, suitable for neonatal use, may be issued to the neonate without a serological crossmatch.

7.18. Patients with sickle cell disease

7.18.1. There is a high incidence of red cell alloantibodies in patients with sickle cell disease, and severe haemolytic transfusion reactions are not uncommon.

7.18.2. The patient's red cells should be phenotyped as fully as possible prior to transfusion. Where patients have already been transfused, the genotype can be determined.
   i. An extended phenotype (or genotype) should include C, c, E, e, K, k, Jk^a, Jk^b, Fy^a, Fy^b, S, s.
   ii. If S- s-, then U typing should be performed.

7.18.3. As a minimum, red cells should be matched for Rh and K antigens.

7.18.4. R$_0$ blood should be selected for patients who are R$_0$ if available, otherwise rr.

7.18.5. Red cells should be HbS negative where possible.

7.18.6. Where possible, red cell survival post-transfusion should be maximised by selection of 'fresh' red cells. The Sickle Cell Society (SCS, 2008) recommends red cells less than 10-days old for top-up transfusions and less than 7-days old for exchange transfusion, but this may not be possible where the patient has multiple red cell alloantibodies. In such situations freshest available suitable units may be transfused.

7.19. Other transfusion-dependent patients (excluding those with sickle cell disease)

Transfusion-dependent patients are those who require frequent and long-term transfusion support to sustain life. This includes thalassaemia syndromes, aplastic anaemia and paroxysmal nocturnal haemoglobinuria (PNH), myelodysplastic syndromes (MDS) and other congenital or acquired chronic anaemias.

7.19.1. Approximately 70% of these patients do not produce alloantibodies despite repeated transfusion of red cells matched only for ABO and D (Spanos *et al.*, 1990; Ameen *et al.*, 2003). It should be a local decision whether to provide red cells that have been additionally matched for Rh (CcEe) and K to minimise the risk of red cell alloimmunisation.

7.19.2. An exception to this is transfusion support of thalassaemia syndromes where Rh and K matching is recommended (UK Thalassaemia Society 2008).

7.19.3. Where possible, red cell survival post-transfusion should be maximised by selection of 'fresh' red cells. NHS Blood and Transplant recommends using blood less than 14 days from date bled (NHSBT, 2011).

7.20. Recipients of allogeneic haemopoietic stem cell grafts

7.20.1. Recipients of allogeneic haemopoietic stem cell transplants present blood grouping complexities with associated red cell selection problems. The transplant may introduce a new ABO antigen (major mismatch) or a new ABO antibody (minor mismatch) or both.

7.20.2. The transfusion laboratory should obtain relevant details of all haemopoietic stem cell allograft donors.

7.20.3. It is recommended that group O red cells (in additive solution or high-titre negative) be selected when there is a recipient/donor ABO mismatch.

7.20.4. It has been highlighted by SHOT (SHOT, 1996−2010) that there have been issues with correct ABO group selection for transfusion support when there is a recipient/donor ABO incompatibility. Laboratories should recognise the limitations of their LIMS to control red cell group selection for ABO or D mismatched allograft cases when the patient's own group is not the appropriate group to transfuse.

7.20.5. Post engraftment, when ABO antibodies to the donor ABO type are undetectable and the DAT is negative, the donor group may be selected. However, it should be noted that it is increasingly common for multiple cord donations to be used and that each donor cord may be of a different ABO and/or D group. Post-engraftment transfusion management should be decided on a case-by-case basis and will depend on which cord engrafts.

7.20.6. When either the recipient or donor is D negative, D negative red cells should be selected.

7.20.7. If graft rejection occurs, selection of red cells should remain compatible with both the patient and donor until complete reversion to the original recipient ABO and D type.

7.20.8. All red cells should be irradiated to prevent transfusion-associated graft-versus-host disease (BCSH, 2010b).

7.21. The compatibility tag

7.21.1. The labelling of blood components is a critical step, and red cell units must be securely identified with a compatibility tag before issue.

7.21.2. The compatibility tag should contain the patient's and component details:
    i. Last name;
    ii. First name;
    iii. Date of birth;
    iv. Unique patient identification number;
    v. First line of address (Wales only);
    vi. Ward or location;
    vii. Patient ABO and D group;
    viii. Donation number;
    ix. Component type;
    x. Donor ABO and D group.

7.21.3. If the blood group of the unit and the patient are not identical, a comment should be printed on the compatibility tag highlighting the difference but stating that the red cells are suitable for transfusion.

7.21.4. If routine pre-transfusion testing has not been carried out, this should be stated on the compatibility tag.

7.21.5. Previous compatibility tags should be removed.

7.21.6. It is vital that the correct compatibility tag is attached to the correct unit. Final verification of the label and unit after attaching tags is recommended. Ideally, this should be an electronic check but could be a visual confirmation.

7.21.7. Blood component labels should only be printed and attached for one patient at a time to avoid the risk of transposition of labels between units for different patients.

7.21.8. If duplicate labels are produced, the laboratory should have a robust procedure (line-clearance) to prevent incorrect labels being available to be inadvertently attached to components for a subsequent patient.

7.21.9. Many hospital blood transfusion laboratories no longer issue an additional compatibility report, based upon National Patient Safety Agency (NPSA) advice (NPSA, 2006) that this should not be used in the final bedside check.

7.22. Visual inspection of the red cell unit

7.22.1. Each red cell unit should be visually inspected at the point of selection and at the point of labelling. If there is any evidence of the examples below, the red cells should not be used and the supplying blood centre should be informed as soon as possible:
    i. Leaks at the ports and seams.
    ii. Discolouration of red cells.
    iii. Haemolysis in the plasma or at the red cell: plasma interface.
    iv. Presence of clots.

7.23. Remote issue

7.23.1. Remote issue (RI) is the selection and electronic issue of compatible red cell units from an electronically-controlled blood refrigerator in a location outside of the testing laboratory. The fridge is either connected to or contains an integrated computer kiosk from which only trained and authorised users can dispense blood on demand for a patient.

7.23.2. A compatibility label should automatically be printed at the time of dispensing, to be affixed to the red cell unit and then verified by scanning.

7.23.3. The blood fridge system should be interfaced with the LIMS of the testing laboratory to ensure real-time integrity of patient and component data.

7.23.4. The movement of blood in and out of the remote issue fridges should be logged in real time with a full audit trail of actions.

7.23.5. The rules for remote release of red cells should be identical to those used in the laboratory for electronic issue. This means that patients who are not eligible for electronic issue or do not have a current valid group and screen sample should not be eligible for remote issue of red cells.

7.23.6. The system must be fully validated to ensure that it is compliant with the criteria for electronic issue and to ensure that it not possible to release units which are ABO D incompatible.

8. TESTING AND RED CELL ISSUE IN NON-ROUTINE SITUATIONS

8.1. Introduction

8.1.1. The previous sections have dealt with procedures and testing associated with routine situations. In an emergency, where blood is required before full routine compatibility testing can be completed, procedures may need to be adapted, changed or omitted, to supply red cells and blood components in a clinically relevant timeframe. This inevitably increases the risk of an incompatible transfusion due to ABO and non-ABO antibodies.

8.1.2. The risks of an incompatible transfusion, or conversely, failure to supply blood to meet clinical demand, are increased if staff are inadequately informed, appropriate urgent procedures are not in place, or because staff are not clear about their own responsibilities or the responsibilities of other staff groups in the transfusion chain (NPSA, 2010).

8.1.3. Where full patient identification is not available, transfusion of group O blood may be a safer option, but supply of group O is limited and its use should be restricted, with a safe blood group being established as soon as possible.

8.1.4. Robust procedures must in place for component recall and reporting if the antibody screen is found to be positive post release of red cells. Donor cells should be retained until antibody screen results are obtained in case retrospective serological crossmatching is required.

8.2. Responsibilities and communications

8.2.1. Laboratories should have written protocols in place which define the responsibilities of all staff in dealing with urgent requests. These should include:
    i. Who is permitted (e.g. which grades of staff / clinicians) to authorise different types of exceptions.

    ii. The communication pathways between relevant personnel, e.g. the clinician responsible for the patient, the medical staff responsible for transfusion and laboratory BMS.

    iii. The contact details and triggers for referring a case to blood service medical staff.

KEY RECOMMENDATION: Laboratories should have written protocols in place which define the responsibilities of all staff in dealing with urgent requests.

8.3. Sample acceptance

8.3.1. There should be a sample acceptance policy defining the minimum patient identification required to release group identical (rather than group O) red cells. For known patients, this might be the same as for routine samples, but for unknown patients, i.e. where no name or DOB is available, this may be gender and a unique number, but could include an indication of age, which would be helpful in rules-based component selection. The patient should continue to be transfused on this identification until the record is updated and a new, fully labelled sample is tested.

8.3.2. Where the sample label does not meet the sample acceptance policy, group O blood should be issued until an acceptable sample has been tested.

KEY RECOMMENDATION: For genuinely unknown patients, the minimum identifiers are gender and a unique number.

8.4. ABO and D grouping

8.4.1. When red cells are required urgently, there may be insufficient time for routine ABO and D grouping prior to selection of blood components; however, accurate determination of the ABO group is the main priority. See Appendix 7 for further discussion about the risks of WBIT in these circumstances.

8.4.2. A different (e.g. manual) or abbreviated system is often used in an emergency for blood grouping. The risks associated with the issue of group-specific red cells against a manual, rapid group must be considered and mitigated as far as possible. This includes, but is not limited to validation and use of controls.

8.4.3. Emergency groups performed in these circumstances must include a test against anti-A, anti-B and anti-D, with appropriate controls or a reverse group. Particular care should be taken when results indicate that the patient is group AB D positive, as anomalies such as cold agglutinins may not be detected without adequate controls.

8.4.4. The result must be documented and confirmed as soon as possible by routine methods if these differ from emergency procedures.

8.4.5. A second sample should be sought and tested as soon as possible.

8.4.6. There must be an effective system for recalling units issued in the event of a discrepancy in the confirmatory group.

8.5. Antibody screening

8.5.1. In emergency situations blood may be issued without an antibody screen. Retrospective antibody screening should be performed where blood has been issued in an emergency. It is not acceptable to perform a crossmatch in place of an antibody screen.

8.6. Selection and issue of red cells

8.6.1. Following a rapid group, at least one the following should be performed before issuing group specific red cells:

  i. a reverse group, using a new aliquot from the patient's sample;

  ii. a repeat forward group using a new aliquot from the patient's sample;

  iii. a saline spin crossmatch (see 7.4).

KEY RECOMMENDATION: Following an emergency rapid group, a second test to detect ABO incompatibility should be undertaken prior to release of group specific red cells.

8.6.2. Group O red cells should be used in emergency situations where the ABO group has not yet been established.

8.6.3. For large volume blood replacement (e.g. more than 8 units of red cells), D positive red cells should be issued to females over the age of 50 and adult males in whom no anti-D is detectable, thus preserving stocks of O D negative red cells for women of child bearing potential. (NBTC, 2009).

8.6.4. The following should be covered by a concessionary release procedure (an example is shown in Appendix 9):

  i. Use of D positive blood for a D negative patient who would normally be excluded from receiving D positive units.

  ii. Use of antigen positive or un-typed red cells in patients with atypical red cell antibodies.

  iii. Issue of red cells to patients with AIHA without the necessary exclusion of underlying antibodies. This is the only circumstance where 'least incompatible' red cells might be the best option.

  iv. Issue of components that do not meet known special requirements, e.g. CMV negative or irradiated.

9. POST ISSUE OF BLOOD COMPONENTS

9.1. Transfusion history

9.1.1. Positive evidence of the transfusion of each component must be fully documented in accordance with local policies and procedures using electronic or manual systems. This allows access to an accurate transfusion history which may be required for investigation of blood group anomalies or delayed transfusion reactions.

9.2. Return or dereservation of unused components

9.2.1. There should be clear locally defined times for how long units will be reserved for an individual patient.

9.2.2. The interval between issue and return to stock of untransfused units (the dereservation time) should be as short as possible for two reasons: firstly, it provides information about the transfusion status of the patient and hence the validity of samples for pre-transfusion testing; secondly it increases the non-allocated blood stocks and reduces wastage.

9.2.3. Compatibility labels should be removed, ensuring that no previous patient data is visibly remaining.

9.3. Serological investigation of a suspected haemolytic transfusion reaction (HTR)

9.3.1. Serological investigation of HTRs should concentrate on looking for possible blood group mismatches and/or atypical antibodies. The exact testing requirements will vary depending on whether the reaction is acute (immediate) or delayed. Full serological investigation is only warranted where there is evidence of haemolysis, either clinical, e.g. post-transfusion fever and jaundice, or laboratory based, e.g. falling Hb, raised bilirubin or LDH.

9.3.2. The use of serum samples is recommended wherever possible for the post-transfusion antibody investigation in order to identify weak antibodies (e.g. anti-Jk^a), which might only be detectable by the complement they bind to red cells. The post-transfusion antibody screen should involve a polyspecific (IgG and C3) antiglobulin reagent if serum is used.

9.3.3. Investigation of an acute haemolytic reaction should begin with the following:

  i. Rechecking of the pre-transfusion sample label and the crossmatch labels, which should match the post-transfusion samples.

  ii. Visual inspection of the transfused packs to look for signs of deterioration (haemolysis or discolouration); if this is found it may indicate

bacterial contamination and should be referred to the supplying blood centre immediately.

9.3.4. Minimum tests to be performed on both post-transfusion sample and retrospectively on the pre-transfusion sample, where this is still available:

  i. Visual inspection of the plasma pre and post centrifugation for signs of haemolysis.

  ii. ABO and D group.

  iii. IAT antibody screen.

  iv. IAT crossmatch (of suspected units if still available).

  v. Direct antiglobulin test (DAT) – this needs to be performed on a well mixed sample as the transfused cells are older and denser than the patient's own cells and may sit towards the bottom of a centrifuged sample.

9.3.5. If the patient had a known antibody pre-transfusion, the following should be performed:

  i. Visual inspection of the plasma pre and post centrifugation for signs of haemolysis.

  ii. More extensive antibody identification using additional cells and panels if necessary. See Section 6.

  iii. Phenotype check of the units.

9.3.6. Further testing will be required depending on the results of the above testing, and it should be noted that it is not uncommon for haemolysed samples to be the result of poor phlebotomy technique, and where this is suspected, a repeat sample should be sought:

  i. Any new antibodies must be identified and the corresponding phenotype of the implicated unit or units determined; if a causative antibody is found, the results should be referred to a clinical haematologist.

  ii. If the DAT is positive, an eluate made from the patient's red cells should be tested for the presence of antibodies. It is not unusual for the causative antibody to be present in an eluate but absent in the plasma (SHOT, 1996–2010).

  iii. If the DAT is negative, but there is clear evidence of haemolysis, an eluate should still be tested, as the DAT may be falsely negative.

  iv. If the crossmatch is positive and antibody screen negative, this may indicate an antibody to low frequency antigen, and is likely to require referral to a reference laboratory.

KEY RECOMMENDATION: If the DAT is positive, an eluate made from the patient's red cells should be prepared and tested for the presence of specific alloantibodies. It is not unusual for the causative antibody to be present in an eluate but absent in the plasma.

9.3.7. If a patient shows signs of active haemolysis following transfusion but no antibodies are detectable and no other possible cause is known (e.g. sickle cell crisis or mechanical haemolysis due to heart valve failure), this can be due to very rare examples of antibodies which are not detectable by normal serological techniques. In this case:

  i. Serological investigation should be undertaken using more sensitive techniques such as Polyethylene Glycol or enzyme IAT, which may require referral to a reference centre.

  ii. It may be appropriate to fully red cell genotype the patient and select donor blood matched as closely as possible.

  iii. Advice should be sought from a clinical haematologist or from a reference laboratory at the local Blood Centre.

APPENDIX 1 EXAMPLES OF CRITICAL CONTROL POINTS

Table A1 shows examples of critical control points in the compatibility process and risk reduction strategies. The list is not exhaustive but gives examples of some critical control points. Mapping the full compatibility process in each laboratory will aid in identifying these points.

APPENDIX 2 TIMING OF SAMPLE COLLECTION IN RELATION TO PREVIOUS TRANSFUSIONS AND STORAGE OF SAMPLES POST-TRANSFUSION

Timing of sample collection

There is a dearth of published data regarding when red cell alloantibodies form and are first detectable following a stimulating event (be it a primary or secondary response).

Of the papers available for review it is clear that only a very small percentage of antibodies which are below detectable level pre-transfusion become detectable in the first 72 h, estimated at 2.3% (Schonewille *et al.*, 2006), and supported by SHOT data (SHOT, 1996–2010). Mollison reports that red cell destruction does not begin before the 4th day post-transfusion (Klein & Anstee, 2005b). Following this time, most developing antibodies will manifest themselves within the next 30 days (there are occasional stragglers), and by 3 months post-transfusion very few antibodies will develop. SHOT data shows that the majority of delayed haemolytic transfusion reactions are noted 3–14 days post-transfusion. It was on this basis that the previous guidelines recommended a 24-h lifespan for a sample when the patient had been transfused within the previous 3–14 days.

A survey of UK laboratory practice, undertaken by the writing group (through UK NEQAS), revealed that a minority of laboratories comply with this guideline. However, when taking into account the combination of the age of the sample and the length of time that the blood sits in the issue fridge, approximately 80% transfuse within 72 h of a new sample

Table A1. Examples of critical control points in the compatibility process and risk reduction strategies

Critical control point	Examples of risk	Examples of risk reduction measures
Barcode labelling of samples and request forms	• Mixing up labels between different samples and request forms	• Labelling samples from a single patient only • Checking sample barcode against LIMS system after booking in
Testing samples and entering results	• Manual testing – possible transcription errors • Automated testing – possible interface / testing errors	• Use automated testing in both routine and emergency situations • Validation of testing system and interface
Reservation of red cells	• ABO mismatching • Special requirements missed • Labelling wrong donations – mix up between patients	• Validating LIMS to show wrong ABO cannot be reserved • Warning in LIMS system if wrong component is selected • Highlighting requirements on request form • Performing only one crossmatch / electronic issue labelling at a time

being taken (Milkins *et al.*, 2010). The vast majority of all UK laboratories report through SHOT, and there do not appear to be significant numbers of additional delayed haemolytic transfusion reactions being reported as a result of this. It would seem that empirical evidence would point to the previous 24 h recommendation being unnecessarily tight. The writing group also noted recommendations from other countries which have longer times, e.g. the Joint Commission on Accreditation of Healthcare Organizations (JCAHO) in the USA require an antibody test within 3 days prior to red cell transfusion, while the Canadian Society for Transfusion Medicine recommends that a specimen be collected within 96 h prior to transfusion.

With a significant number of laboratories unable to achieve the existing guideline combined with the empirical evidence above, the writing group felt that a change that represented a balance of safety with achievability was required. With this in mind, the group took the decision to change the model to the length of time of red cell units issued against a particular sample are available. With regard to the published data on alloantibody formation, transfusion reaction reporting, and the survey, it was felt that a blanket 3-day period up to 3 months post-transfusion,

offered the best balance of safety and achievability. A laboratory could interpret this as a 24-h sample life + 48-h reservation period, or as a 48-h sample life with 24-h dereservation period, or some other combination as they felt best met local conditions. It is recognised by the group that the scientific evidence for such a decision is limited and that this should be considered as a baseline only. Those laboratories wishing to have stricter time frames more in line with the existing guidance could do so; those wishing to use more lenient time frames would have to support their decision through a local risk assessment.

It may be that some feel able to accept these new time frames for patients with no previous antibody history but may be more reluctant for patients with existing antibody histories (there is some evidence that the presence of an antibody is likely to predict further formation of antibodies as it indicates 'good responders') or those in high risk groups, e.g. those with sickle cell disease. One size fits all does have the benefit of being understandable by all staff (clinical and laboratory).

Storage of samples

There is almost no published data on storage times, storage temperature or length of time a sample remains suitable for testing, so the writing group has decided not to significantly alter the existing recommendations until new data become available.

Because of a number of unpublished communications within and outside the writing group where patients not seen for some time appear for treatment and have, unknown to laboratory, been transfused elsewhere, it was felt that a period of no more than 3 months should be recommended as a maximum for samples to remain suitable for issue of red cells. This should allow laboratories to cater for electronic issue of blood on preoperative assessed patients' samples while limiting the possibility of unexpected transfusion at an alternative site.

The availability of pre-transfusion samples to investigate transfusion reactions varies considerably from site to site. The group felt that having such a sample to test as part of a transfusion reaction investigation represented best practice, allowing determination of whether the antibody was previously undetectable or had been missed as a result of system frailty. It is usual in Good Manufacturing Practice (GMP) industries to ensure sample availability for testing in the event of subsequent product problems.

The group particularly felt that having a pre-transfusion sample to test for ABO status in the event of an acute transfusion reaction was highly desirable and so recommended that systems are put in place to ensure that a sample for testing was available for a minimum of 3 days post-transfusion.

The writing group suggests that being able to retain a plasma/serum sample for up to 14 days post-transfusion would be desirable for delayed transfusion reaction testing for similar reasons to those of an acute transfusion reaction. This would require separation and freezing of the plasma from the red cells. Laboratories are, for good reasons, not comfortable with separation and the risk associated with the labelling, into separate

plasma pots. However, if physical separators (these are devices that are commercially available that can be introduced post routine testing into the primary sample, inserting a physical barrier between the red cells and plasma) are used this then negates the need for plasma separation while retaining plasma for testing and the original sample tube for inspection of patient ID as necessary.

The writing group noted that other countries also required retention of samples post-transfusion (e.g. JCAHO in USA requires samples to be retained for at least 7 days following a transfusion and 10 days following a crossmatch).

The writing group recognises that implementation of these recommendations and suggestions may entail changes in laboratory procedures and investment in new equipment. However, it feels that the benefits of ensuring a full audit trail of transfusion reaction events to patient, individual laboratories and transfusion medicine as a whole (by allowing collection of data in an area of non-existent data) are substantial enough to support their inclusion.

APPENDIX 3 RESOLUTION OF GROUPING ANOMALIES

Figure A1 shows a flowchart for the resolution of ABO grouping anomalies.

APPENDIX 4 WORKED EXAMPLES OF ANTIBODY IDENTIFICATION

In the following examples, the shaded cells show specificities which can be excluded with one or more examples of homozygous expression (or in the case of Kell, Kk) on ID panel or screening panel.

In addition, antibodies to antigens of unlikely clinical significance or low incidence are also shaded where appropriate, to demonstrate their exclusion. However, it is not necessary to routinely exclude such specificities, unless there are positive reactions unaccounted for once all antibodies of likely clinical significance have been identified.

Where reference is made to exclusion based on negative results using an enzyme panel, this assumes that a validated two-stage test has been used, i.e. using enzyme pre-treated cells.

Example 1

Figure A2 shows the results of the identification panels for Example 1.

Interpretation: Shaded cells show that all antibodies of likely clinical significance can be excluded on the identification panel except anti-s, anti-c and anti-E.

Anti-s can be excluded by considering the negative result with the c-, ss screen cell 1.

Specificities which still cannot be excluded are: anti-c, -E.

Positive IAT with all c+ cells (>2 available) and negative with all c- negative cells (>2 available including screen cell 1).

Identification: anti-c

Unable to exclude anti-E, however, if CCDee cells are selected for transfusion it is unnecessary to do so. Use of CCDEe cells may be required for antenatal samples.

Additional work: Rh phenotype

Example 2

Figure A3 shows the results of the identification panels for Example 2.

Interpretation: Rh specificities can be excluded using negative enzyme panel results. Shaded cells show that all other specificities can be excluded except anti-M.

Positive IAT with all M+ cells (>2 available) and negative with all M- negative cells

Identification: anti-M

Additional work: M phenotype

Example 3

Figure A4 shows the results of the identification panels for Example 3.

Interpretation: Rh specificities other than anti-D and -C can be excluded using negative enzyme panel results.

Anti-M, -S, are excluded using screening cell 3.

Shaded cells show that all other specificities of likely clinical significance can be excluded except anti-D, -C, -Jk^b

Anti-D identified using enzyme panel results: all D+ cells (> 2 available) positive and all D- cells (>2 available) negative except r′r suggesting additional anti-C.

Positive with all Jk(b+) cells (>2 available) and negative with all Jk(b-) negative cells (>2 available, which are also D- and C-).

Anti-Le^a cannot be excluded but the reactions do not interfere with the identification criteria for the above antibodies.

Identification: anti-D+ Jk^b+ probable anti-C

Additional work: Jk^b phenotype; test plasma vs additional D-, C+ cell to confirm anti-C.

Example 4

Figure A5 shows the results of the identification panels for Example 4.

Interpretation: IAT positive with all cells

Enzyme positive with all cells

Identification: Unable to identify

Exclusion: Unable to exclude

Additional work: Auto IAT result negative, indicates one or more alloantibody, rather than pan-reacting/autoantibody

Patient phenotype: C+c-D+E-e+, M-N+, S-s+, P$_1$+, K-, Le(a-b+), Fy(a+b-), Jk(a+b-).

Patient can therefore produce alloanti-c, -E, -M, -S, -K, -Fy^b, -Jk^b

Figure A6 shows the results of the additional panel cells used to Identify/exclude additional specificities:

Discrepancy between forward and reverse group – Causes to consider:
Forward group:
- Mixed field reactions (see section 4.7)
- Weak A or B subgroup
- Technical problems with procedure or reagents
- Weakening or loss of antigen due to disease
- Positive DAT
- Polyagglutination

Reverse group:
- Missing agglutinin in reverse group
- Cold reacting allo or auto antibodies
- Technical problems with procedure or reagents
- Haemolysis or lipaemia

Depending on nature of discrepancy as listed below, some or all of the following might be undertaken:
- a patient history
- a repeat test
- a repeat sample
- referral to a reference centre
- any or all of the additional tests in the boxes below

Additional reactions in forward group and/or control

Apparent missing agglutinins in reverse group (might actually be due to antigen loss)

Additional reactions in reverse group

- Patient history – neonate/old age or immunodeficiency
- Enhance reactions by:
  o lowering temperature of test
  o using enzyme treated cells
  o increasing plasma/cell ratio

- Perform a DAT
- Repeat using unpotentiated reagents in a tube
- Warm wash and repeat

- Identify cold antibodies
- Perform a DAT if auto positive
- Pre-warm plasma and cells
- Select reverse cells negative for relevant antigen

If unable to obtain reactions in reverse group, report group based on two forward groups

If anomaly is resolved, report group

If anomaly is unresolved, refer to reference laboratory

The usual checks must be in place to mitigate the risks of manual testing and recording of results, e.g. the results should be recorded twice, independently.

Fig. A1. Resolution of ABO grouping anomalies.

Alloantibodies excluded either on patient phenotype, or cells 15 (anti-M, -S) and 16 (anti-Fy^b), except anti-c, -E, -K, -Jk^b.

Interpretation:
positive with 3 c+, K-, Jk(b-) demonstrating presence of anti-c positive with 3 c- K+, Jk(b-), demonstrating presence of anti-K

positive with 4 c-, K-, Jk(b+), demonstrating presence of anti-Jk^b

negative with 2 c-, Jk(b-), K- confirming presence of all 3

Identification Anti-c+(+/− anti-E), -K, +Jk^b

Antibody screen

	Rh	C	c	D	E	e	Cw	M	N	S	s	P1	K	Lea	Leb	Fya	Fyb	Jka	Jkb	IAT
1	R1R1	+	-	+	-	+	-	-	+	-	+	+	-	+	-	+	-	-	+	-
2	R2R2	-	+	+	+	-	-	-	+	-	+	-	+	-	+	+	-	+	-	+
3	rr	-	+	-	-	+	-	+	-	+	-	+	-	-	+	-	+	+	-	+

Antibody identification panel

	Rh	C	c	D	E	e	Cw	M	N	S	s	P1	K	Lea	Leb	Fya	Fyb	Jka	Jkb	IAT	Enz
1	R1wR1	+	-	+	-	+	+	+	-	+	-	+	-	+	-	-	+	-	+	-	-
2	R1R1	+	-	+	-	+	-	+	+	+	+	-	+	-	+	+	-	+	-	-	-
3	R2R2	-	+	+	+	-	-	+	-	+	-	+	-	+	-	+	-	+	-	+	+
4	r'r	+	+	-	-	+	-	-	+	-	+	+	-	-	+	-	+	-	+	+	+
5	r''r	-	+	-	+	+	-	+	+	+	+	+	-	-	+	+	-	+	-	+	+
6	rr	-	+	-	-	+	-	-	+	-	+	-	-	-	+	-	+	-	+	+	+
7	rr	-	+	-	-	+	-	-	+	-	+	+	+	+	-	-	+	+	-	+	+
8	rr	-	+	-	-	+	-	+	-	+	-	+	-	-	+	+	-	-	+	+	+
9	rr	-	+	-	-	+	-	+	-	-	+	-	-	-	-	+	-	-	+	+	+
10	rr	-	+	-	-	+	-	-	+	+	-	+	+	-	+	+	-	-	+	+	+

Fig. A2. Antibody identification panels for Example 1.

Antibody screen

	Rh	C	c	D	E	e	Cw	M	N	S	s	P1	K	Lea	Leb	Fya	Fyb	Jka	Jkb	IAT
1	R1R1	+	-	+	-	+	-	-	+	-	+	+	-	+	-	+	-	-	+	-
2	R2R2	-	+	+	+	-	-	-	+	-	+	-	+	-	+	+	-	+	-	-
3	rr	-	+	-	-	+	-	+	-	+	-	+	-	-	+	-	+	+	-	+

Antibody identification panel

	Rh	C	c	D	E	e	Cw	M	N	S	s	P1	K	Lea	Leb	Fya	Fyb	Jka	Jkb	IAT	Enz
1	R1wR1	+	-	+	-	+	+	+	-	+	-	+	-	+	-	-	+	-	+	+	-
2	R1R1	+	-	+	-	+	-	+	+	+	+	-	+	-	+	+	-	+	-	+	-
3	R2R2	-	+	+	+	-	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-
4	r'r	+	+	-	-	+	-	-	+	-	+	+	-	-	+	-	+	-	+	-	-
5	r''r	-	+	-	+	+	-	+	+	+	+	+	-	-	+	+	-	+	-	+	-
6	rr	-	+	-	-	+	-	-	+	-	+	-	-	-	+	-	+	-	+	-	-
7	rr	-	+	-	-	+	-	-	+	-	+	+	+	+	-	-	+	+	-	-	-
8	rr	-	+	-	-	+	-	+	-	+	-	+	-	-	+	+	-	-	+	+	+
9	rr	-	+	-	-	+	-	+	-	-	+	-	-	-	-	+	-	-	+	+	+
10	rr	-	+	-	-	+	-	-	+	+	-	+	+	-	+	+	-	-	+	-	-

Fig. A3. Antibody identification panels for Example 2.

APPENDIX 5 ADDITIONAL TECHNIQUES FOR ANTIBODY IDENTIFICATION

Table A2 gives examples of additional techniques than can be useful for antibody identification.

APPENDIX 6 CLINICAL SIGNIFICANCE OF RED CELL ANTIBODIES

- Clinically significant antibodies are those that are capable of causing patient morbidity due to the accelerated destruction of a significant proportion of transfused red cells.
- Anti-A, anti-B and anti-A,B must always be regarded as being of clinical significance.
- With few exceptions, red cell antibodies which are likely to be of clinical significance are only those which are reactive in the indirect antiglobulin test (IAT), performed strictly at 37 °C.

- Recommendations for the selection of red cells for transfusion to patients with alloantibodies are given in Table A3.

Table A3 shows the likely clinical significance of red cell alloantibodies, and recommendations for the selection of blood for patients with their presence.

APPENDIX 7 REQUIREMENT FOR TWO SAMPLES FOR ABO/D GROUPING PRIOR TO ISSUE OF RED CELLS

This recommendation is based on the evidence from the BEST studies as referenced in 7.2, and on data from the IBCT and the Near Miss chapters in recent SHOT reports (SHOT, 1996–2010) – 386 cases of 'wrong blood in tube' (WBIT) were reported as near misses in 2010.

Whenever possible a second sample should be obtained. The urgency of the situation should always be considered, as delays in provision of blood could compromise patient outcome.

Antibody screen																			
	Rh	C	c	D	E	e	Cw	M	N	S	s	P1	K	Lea	Leb	Fya	Fyb	Jka	Jkb	IAT
1	R1R1	+	-	+	-	+	-	-	+	-	+	+	-	+	-	+	-	-	+	+
2	R2R2	-	+	+	+	-	-	-	+	-	+	-	+	-	+	+	-	+	-	+
3	rr	-	+	-	-	+	-	+	-	+	-	+	-	-	+	-	+	+	-	-

Antibody identification panel																				
	Rh	C	c	D	E	e	Cw	M	N	S	s	P1	K	Lea	Leb	Fya	Fyb	Jka	Jkb	IAT	Enz
1	R1wR1	+	-	+	-	+	+	+	-	+	-	+	-	+	-	-	+	-	+	+	+
2	R1R1	+	-	+	-	+	-	+	+	+	+	-	+	-	+	+	-	+	-	+	+
3	R2R2	-	+	+	+	-	-	+	-	+	-	+	-	+	-	+	-	+	-	+	+
4	r'r	+	+	-	-	+	-	-	+	-	+	+	-	-	+	-	+	-	+	+	+
5	r''r	-	+	-	+	+	-	+	+	+	+	+	-	-	+	+	-	+	-	-	-
6	rr	-	+	-	-	+	-	-	+	-	+	-	-	+	-	-	+	-	+	+	-
7	rr	-	+	-	-	+	-	-	+	-	+	+	+	-	+	-	+	+	-	-	-
8	rr	-	+	-	-	+	-	+	-	+	-	+	-	-	+	+	-	-	+	+	-
9	rr	-	+	-	-	+	-	+	-	-	+	-	-	-	+	-	-	+	+	-
10	rr	-	+	-	-	+	-	-	+	+	-	+	+	-	+	+	-	-	+	+	-

Fig. A4. Antibody identification panels for Example 3.

Antibody screen																			
	Rh	C	c	D	E	e	Cw	M	N	S	s	P1	K	Lea	Leb	Fya	Fyb	Jka	Jkb	IAT
1	R1R1	+	-	+	-	+	-	-	+	-	+	+	-	+	-	+	-	-	+	+
2	R2R2	-	+	+	+	-	-	-	+	-	+	-	+	-	+	+	-	+	-	+
3	rr	-	+	-	-	+	-	+	-	+	-	+	-	-	+	-	+	+	-	+

Antibody identification panel																				
	Rh	C	c	D	E	e	Cw	M	N	S	s	P1	K	Lea	Leb	Fya	Fyb	Jka	Jkb	IAT	Enz
1	R1wR1	+	-	+	-	+	+	+	-	+	-	+	-	+	-	-	+	-	+	+	+
2	R1R1	+	-	+	-	+	-	+	+	+	+	-	+	-	+	+	-	+	-	+	+
3	R2R2	-	+	+	+	-	-	+	-	+	-	+	-	+	-	+	-	+	-	+	+
4	r'r	+	+	-	-	+	-	-	+	-	+	+	-	-	+	-	+	-	+	+	+
5	r''r	-	+	-	+	+	-	+	+	+	+	+	-	-	+	+	-	+	-	+	+
6	rr	-	+	-	-	+	-	-	+	-	+	-	-	-	+	-	+	-	+	+	+
7	rr	-	+	-	-	+	-	-	+	-	+	+	+	+	-	-	+	+	-	+	+
8	rr	-	+	-	-	+	-	+	-	+	-	+	-	-	+	+	-	-	+	+	+
9	rr	-	+	-	-	+	-	+	-	-	+	-	-	-	+	-	+	-	+	+	+
10	rr	-	+	-	-	+	-	-	+	+	-	+	+	-	+	+	-	-	+	+	+

Fig. A5. Antibody identification panels for Example 4.

When, in an urgent situation only, it is not possible to obtain a second sample, group-specific red cells should not be issued without a second ABO check for ABO compatibility. The options for this are a second group on the same sample, preferably undertaken using a different method/reagents from a fresh sampling and/or a serological crossmatch. In these circumstances a local risk assessment, including identification of clinical areas where WBIT errors have previously originated, systems currently in place for training of clinical and laboratory staff, and electronic systems for patient identification and sample collection, should be undertaken.

Depending on the outcome of the local risk assessment consideration should be given to whether it is safe to issue group-specific red cells or whether group O units should be transfused in an emergency until a second sample has been processed. It should be noted that this could make it difficult to obtain a clear ABO/D group from subsequent samples as mixed field reactions may be obtained.

Where the patient groups as O on the first sample, there is an argument for not requiring a second sample prior to transfusion, as the patient will safely receive group O red cells. There are two issues to consider and risk assess before implementing such a strategy: the first is whether this decision can be controlled by the LIMS (a requirement for electronic issue); the second is the potential for transfusion of large volumes of incompatible, potentially high-titre, group O plasma, and consideration should be given to selection of group AB fresh frozen plasma (FFP) and group A platelets in these circumstances, until the ABO group has been confirmed on a second sample.

Concerns have been expressed that the two samples may be taken at the same time, but one 'saved' to send to the transfusion laboratory at a later time. It is important to have a policy and process in place to assure that the two samples have been taken independently of one another, and those taking samples for transfusion, need to understand the reasons for requesting a second sample and the risk of WBIT.

	Rh	C	c	D	E	e	Cw	M	N	S	s	P1	K	Lea	Leb	Fya	Fyb	Jka	Jkb	IAT
11	R1wR1	+	-	+	-	+	+	+	-	+	-	+	-	+	-	-	+	-	+	+
12	R1R1	+	-	+	-	+	-	+	+	+	+	-	-	-	+	+	-	-	+	+
13	R1R1	+	-	+	-	+	-	+	-	+	-	+	+	+	-	+	-	+	-	+
14	R1R1	+	-	+	-	+	-	-	+	-	+	+	+	-	+	-	+	+	-	+
15	R1R1	+	-	+	-	+	-	+	-	+	-	+	-	-	+	+	-	+	-	-
16	R1wR1	+	-	+	-	+	+	-	+	-	+	-	-	+	-	-	+	+	-	-

Fig. A6. Additional panel cells used for Example 4.

Table A2. Additional techniques for antibody identification

Technique	Detail	Application
Strict 37 °C	Cells and plasma warmed before mixing	Should only be used to eliminate cross-reactions from cold autoantibodies which have previously been shown to interfere with routine antibody identification tests on the patient's plasma
Manual tube	IAT and direct agglutination tests at room temperature (DRT) in tube	Allows characterisation of agglutination especially helpful where weak non-specific reactions are noted
Enzyme IAT	Panel cells pre-treated with enzyme, used in IAT	Weak Rh and weak Kidd system antibodies; useful in resolving mixtures of antibodies
Low temperature	Direct agglutination at 4−20 °C, typically in tubes	Weak and inconclusive IAT panels due to cold reacting antibodies, e.g. anti-Le, -M, -P1
Neutralisation	Patient plasma incubated with a pool of plasma from 2 to 3 ABO matched, antibody screen negative patients before IAT phase	May neutralise antibodies to soluble antigens (e.g. anti-Ch, -Rg) permitting exclusion of additional antibodies. Dilution controls should be included and compatibility status of blood issued after tests with modified plasma considered. Immediate spin crossmatching may also be considered when dealing with clinically benign alloantibodies for which antigen negative blood is unavailable (e.g. anti-Ch, -Kn[a])

APPENDIX 8 THE NEED FOR AN IAT CROSSMATCH RATHER THAN ELECTRONIC ISSUE (EI) WHERE THE ANTIBODY SCREEN IS POSITIVE

As discussed in Section 7, the LIMS needs to be in full control of the algorithms associated with selection of patients for electronic issue, and one of the main exclusions are patients with clinically significant red cell antibodies, whether in the current sample or historically. This makes it difficult to safely issue blood by EI if the antibody screen is positive. MHRA recommends that there are no manual workarounds for selection of patients (MHRA, 2010).

It is recognised that the scenario of a positive screen due to prophylactic anti-D is likely to be contentious:

As described above, the main difficulty is managing the algorithm for patient acceptance for EI. Some laboratories use a set of rr screening cells in these circumstances, which would result in a negative antibody screen, allowing the sample to meet the criteria for EI. However, this in itself requires a human decision process which is not controlled by the LIMS, and is reliant on the correct clinical information being provided. Patients with immune anti-D could be missed. It should also be remembered that there is no reliable way of distinguishing serologically between immune and prophylactic anti-D, and mistakes have been made both ways as described in various SHOT reports (SHOT, 1996−2010). If electronic issue is undertaken in these circumstances there should a full risk assessment.

It should be recognised that these patients are a small minority of those requiring transfusion support and for these few patients it should not be onerous to perform a serological crossmatch. However, in a major obstetric haemorrhage, concessionary release could be used to rapidly issue D negative red cells without a serological crossmatch.

APPENDIX 9 EXAMPLE OF A CONCESSIONARY RELEASE FORM

Concessionary release of blood components or blood products, or acting contrary to an SOP, is sometimes the necessary and appropriate course of action in the best interest of patients. To act contrary to an SOP requires prior authorisation, or justifiable authorisation as soon after as is practicable, preferably by a haematologist or other suitably competent person who should discuss the clinical consequences with the clinicians in charge of the patient. An example is shown in Fig. A7.

APPENDIX 10 GLOSSARY

Accreditation procedure by which an authoritative body gives formal recognition that an organisation is competent to carry out specific tasks against defined standards.

Blood component a therapeutic constituent of human blood, as defined by BSQR, i.e. red cells, platelets, fresh frozen plasma, cryoprecipitate and granulocytes.

Table A3. Likely clinical significance of red cell alloantibodies, and recommendations for the selection of blood for patients with their presence

System	Specificity	Likely clinical significance in transfusion	Recommendation for selection of red cells for transfusion[1]
ABO	Anti-A$_1$	No	IAT crossmatch compatible at 37 °C
Rh	Anti-D, -C, -c, -E, -e	Yes	Antigen negative
Rh	Anti-C^w	No	IAT crossmatch compatible[2]
Kell	Anti-K, -k	Yes	Antigen negative
Kell	Anti-Kp^a	No	IAT crossmatch compatible[2]
Kidd	Anti-Jk^a, -Jk^b	Yes	Antigen negative
MNS	Anti-M (active 37 °C)	Yes	Antigen negative
MNS	Anti-M (not active 37 °C)	No	IAT crossmatch compatible at 37 °C
MNS	Anti-N	No	IAT crossmatch compatible at 37 °C
MNS	Anti-S, -s, -U	Yes	Antigen negative
Duffy	Anti-Fy^a, -Fy^b	Yes	Antigen negative
P	Anti-P$_1$	No	IAT crossmatch compatible at 37 °C
Lewis	Anti-Le^a, -Le^b, -Le^{a+b}	No	IAT crossmatch compatible at 37 °C
Lu	Anti-Lu^a	No	IAT crossmatch compatible at 37 °C
Diego	Anti-Wr^a (anti-Di3)	Yes	IAT crossmatch compatible[2]
H	Anti-HI (in A$_1$ and A$_1$B patients)	No	IAT crossmatch compatible at 37 °C
All	Others active by IAT at 37 °C	Yes	Seek advice from Blood Centre

[1]Where antigen negative red cells are recommended these should also be compatible in an IAT crossmatch.

[2]These recommendations apply when the antibody is present as a sole specificity. If present in combination, antigen negative blood may be provided by the blood centre, to prevent wastage of phenotyped units. This guidance is also suitable for patients undergoing hypothermia during surgery (Klein and Anstee, 2005b).

Blood product any therapeutic substance derived from human plasma e.g. human albumin solution, clotting factor concentrates, anti-D immunoglobulin and therapeutic immunoglobulins.

CE marking is a declaration by the manufacturer that a product meets all the appropriate provisions of the relevant legislation implementing certain European Directives. The letters CE stand for 'Conformité Européenne' which means 'European Conformity'.

Change control is a formal system to ensure that changes are introduced in a controlled and coordinated manner. Where proposed or actual change might affect the validated status of a system, equipment or process change control should ensure a continued validated state.

Childbearing potential females less than 50 years of age where sensitisation to an antigen could put a baby at risk of haemolytic disease of the foetus and newborn.

Clinically significant antibodies red cell antibodies that have the potential to cause a haemolytic transfusion reaction (HTR) and/or haemolytic disease of the foetus and newborn (HDFN). The word 'likely' or 'potentially' is used because although these specificities are known to frequently cause HDFN or HTRs, they may not do so in a particular patient.

Compatibility tags/forms tags/forms that are generated in the transfusion laboratory and attached to the blood component bags or accompanying the blood components bag. They contain the core patient identifiers and blood component details as well as additional information to support the safe administration of blood.

Competency assessment demonstration that an individual is capable of and proficient at performing a particular task.

Concessionary release blood components that do not conform to specified requirements may be issued for therapeutic use when any benefits of giving the component outweigh the risks as assessed by a medical practitioner on behalf of the patient.

Dereservation period the time between issue of a blood component and return of a non-transfused component to stock or discard.

Emergency a clinical situation where blood is required before full routine compatibility testing can be completed so procedures may need to be adapted, changed or omitted, to supply red cells and blood components in a clinically relevant timeframe. Many transfusion requests are urgent but this does not warrant omitting important steps in testing.

Infant a child under 1 year of age.

Must refers to a recommendation or action which is required to comply with the BSQR, or where the evidence for the recommendation is unequivocal.

Neonate for the purposes of pre-transfusion testing a child less than 3 months of age. The usual definition would be a child under 1 month of age.

Never events are serious, largely preventable patient safety incidents that should not occur if the available preventative measures have been implemented as defined by the DH and NPSA. For blood transfusion this includes giving an ABO incompatible transfusion.

Section A - Patient details and concession information		
First name	Last name	NHS number/Hospital number
Date of birth	Ward/location	Consultant
Brief description of reason for concession including justification:		
Completed by: Name: Signature:		Date / time:
Section B – Blood component or blood product details		
Description of component/product for concessionary issue	Donation number or batch number(s)	
Section C – Is the concession justifiable in the best interests of the patient?		
Haematologist authorisation: Name: Signature: Designation: Date: Time:		
Section D – Informing patient's clinical team		
Name of the doctor on the clinical team who has agreed to accept this concession for this patient: Name: Designation:		
Section E – Confirmation of concessionary issue		
Issuing BMS Name: Signature: Date: Time:		
Section F – Review of documentation of the event		
Signature and designation of person reviewing this concession (usually TLM or QM)		

Fig. A7. Example of a concessionary release form.

Patient core identifiers all patients should be identified by (minimum requirements) last name, first name, date of birth and a unique patient identification number, e.g. NHS number or equivalent.

Physical separators introduced post-testing to allow samples to be frozen within their original bottles while ensuring red cell lysis does not 'contaminate' the plasma, so preserving plasma for retrospective serological testing.

Plasma/Serum the term 'plasma' will be used to cover all requirements irrespective of specimen type, unless specifically stated. Plasma samples anticoagulated in EDTA are most appropriate for use in automated systems. Clotted (serum) samples remain suitable for use in manual systems.

Potentiators such as polyethylene glycol (PEG) are added to some reagents to enhance blood grouping reactions and high levels of these potentiators can cause false positive reactions in the presence of *in vivo* immunoglobulin coating of the patient's red cells.

Risk assessment is a systematic process for the assessment control, communication and review of risks to the quality of a system, equipment or process.

Should refers to a recommendation or action that is based on expert opinion and endorsed by the BCSH Transfusion Taskforce. Individual organisations may consider converting 'should' to 'must' within their own local policies/guidelines.

Unique (patient) identification number all patients should be issued with a unique patient identification number at their initial contact with the healthcare organisation. Wherever possible the national unique identification number should

be used (NHS number in England and Wales, HSC number in Northern Ireland and CHI number in Scotland.

APPENDIX 11 ACRONYMS AND ABBREVIATIONS

BSQR = Blood Safety and Quality Regulations
CAT = Column agglutination technology
CPA = Clinical Pathology Accreditation (UK) Ltd
DH = Department of health
DAT = Direct antiglobulin test
EQA = External quality assessment
EI = Electronic issue
EDTA = Ethylenediaminetetraacetic acid
FMH = Fetomaternal haemorrhage
GAMP = Good automated manufacturing practice
GMP = Good manufacturing practice
HB = Haemoglobin
HSCT = Haemopoietic stem cell transplant
HTR = Haemolytic transfusion reaction
IAT = Indirect antiglobulin test
ILAC = International Laboratory Accreditation Cooperation
ISO = International Organization for Standardisation
IT = Information technology
LDH = Lactate dehydrogenase
LIMS = Laboratory Information Management System

LISS = Low ionic strength solution
MHRA = Medicines and Healthcare products Regulatory Agency
NPSA = National Patient Safety Agency
QA = Quality assurance
QC = Quality control
QMS = Quality management system
RA = Risk assessment
RCPath = Royal College of Pathologists
SABRE = Serious adverse blood reactions and events
SHOT = Serious hazards of transfusion
SOP = Standard operating procedure
WBIT = Wrong blood in tube

CONFLICT OF INTEREST

None of the authors have declared a conflict of interest.

DISCLAIMER

While the advice and information in these guidelines is believed to be true and accurate at the time of going to press, neither the authors, the British Society for Haematology, nor the publishers accept any legal responsibility for the content of these guidelines.

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