

Guidelines for the Blood Transfusion and Tissue Transplantation Services in the UK

12. Donation testing (red cell immunohaematology)

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12.1. Scope

These specifications provide guidance on the tests required for blood donations in the UK.

12.2. General requirements

Secure and effective procedures must be in place to ensure that:

- Specific procedures are written in the form of standard operating procedures.
- Blood donations, components and their laboratory samples are correctly identified by barcoded and eye-readable numbers.
- Donations can be linked to their donor.
- A donor's record is reviewed every time they donate.

12.3. Samples

Samples may be ethylenediamine tetra-acetic acid (EDTA) or clotted.

Where equipment/reagent manufacturers have defined protocols for storage and preparation, then these must be followed.

In the absence of protocols or recommendations from manufacturers, then validated protocols for sample storage and preparation must be defined.

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

- haemolysis
- lipaemia
- clots
- volume
- cell:plasma (serum) ratio
- the buffy coat layer – please note, a large buffy coat layer in the sample may give rise to erroneous results

Labels should be examined for defective labelling.

Reconciliation of all samples to be tested should be completed prior to testing.

12.4. Reagents and test kits

Acceptance testing should be performed on each batch/delivery of reagents and test kits.

Reagents and test kits should be stored and used according to the manufacturer's instructions.

Reagents and test kits outwith these instructions must be validated.

Reagent antisera must comply with specificity and potency as per chapter 11.2.

A system of inventory control must be in place that records, as a minimum, the reagent or test kit:

- lot number
- expiry date
- supplier
- stock levels

Procedures should ensure the traceability of the batch number and manufacturer of reagents and kits and, if relevant, the serial number of equipment used to test every donation.

12.5. Equipment

Test equipment should be validated before being introduced into routine use and procedures must be in place to ensure that test systems and equipment are able to produce consistent and valid results.

Equipment must be used, cleaned, calibrated and maintained in accordance with the manufacturer's instructions and written procedures. It is recognised that during maintenance procedures equipment may be compromised and therefore a protocol for reinstatement of the equipment for routine use is required.

Any deviations from the manufacturer's instructions should be validated and documented.

An equipment log covering the following must be readily available for all equipment:

- service contract details
- downtime
- faults
- maintenance
- calibration
- software version

These logs must be retained.

12.6. Test procedure

Test procedures must:

- be validated before being introduced into routine use
- be written in the form of standard operating procedures
- be performed in compliance with the standard operating procedures
- be monitored and reviewed
- be performed by trained/competent staff and the training records must be maintained
- include the recording of test results

12.7. Reporting of results

The report must indicate the result of each and every test, by a system that provides positive sample identification.

Reporting a series of tests by an 'assumed negative' procedure is potentially dangerous and not acceptable.

The acceptance and release of test results will be the responsibility of designated personnel of proven proficiency.

Information must be archived.

12.8. Release of tested components

Standard procedures must ensure that blood and blood components cannot be released for issue until all the required laboratory tests (mandatory and additional) have been completed, documented and approved within a validated system of work. Compliance with this requirement may be achieved by the use of a computer program, or suite of programs, which requires the input of valid and acceptable test results for all the mandatory and additional laboratory tests before permitting, or withholding, the release of each individual unit.

Where a computer-based system has failed, compliance may be achieved by the use of a system, which requires documented approval for the release of each unit, by a designated person.

12.9. Laboratory test categories

Laboratory tests include the following categories:

Mandatory tests	Required as part of the criteria for release of all blood donations and components for clinical use. Currently these are ABO and D blood grouping and irregular red cell antibody screening.
Additional tests	Undertaken in special circumstances: • increase the safety of transfusion for susceptible patients or clinical effectiveness of specific transfusions, e.g. by providing HbS screened red cells • while not required for all blood donations or components, when such tests are performed to meet a specific need the results are an essential part of the criteria for release of that component.

12.10. Mandatory testing of blood donations

Blood groups shall be determined using reagents that comply with chapter 11.

All mandatory tests must be performed using an automated test system in the first instance (see chapter 12.13). Any persistent failures may be resolved using manual methods (see chapter 12.14).

12.10.1. ABO blood grouping

- The ABO blood group must be determined on each blood donation.
- For a donor whose ABO blood group is unknown to the test centre (e.g. a first-time donor), the ABO blood group must be determined by testing the plasma/serum with group A₁ and B red cells. The red cells of the donation must be tested twice with anti-A and anti-B as a minimum. The ABO group can only be accepted if the results are in agreement.
- If the security of sampling analysis and data transfer is assured, it is sufficient to test the red cells from previously tested donors with anti-A and anti-B once. There is no requirement to test the plasma. The ABO blood group shall be accepted only if the results are in agreement with those of previous tests.
- Where an anti-A which detects A_x is deployed in the testing of all donations, anti-A,B is not required.

12.10.2. Quality control of ABO blood grouping

- Quality control (QC) procedures recommended by reagent and equipment manufacturers should be followed.
- The following minimum test monitors are required for each batch of ABO blood grouping tests:
 - anti-A, anti-B (and anti-A,B where used) must give appropriate reactions with A₁, B and O cells. A₂ and A₂B cells may also be used; however, where CE-marked reagents, validated as per chapter 11.2 are used, they are not mandatory
 - Reagent red cell samples must give appropriate reactions with anti-A, anti-B (and anti-A,B where used).

12.10.3. D grouping

- The D blood group must be determined on each donation of blood.
- In the testing of donors being grouped for the first time, 2 anti-D blood grouping reagents should be used capable of detecting between them D^I, D^V and D^{VI} antigens. If two monoclonal anti-D reagents are used, they should be from different clones.
- Donors whose blood gives an unequivocal positive reaction with both anti-D reagents should be regarded as D positive.

- Donors whose blood is unequivocally negative with both anti-D reagents should be regarded as D negative.
- If the results with the anti-D reagents are discordant or equivocal, the tests should be repeated. Where the D group is in doubt, it is safer to classify such donors as D positive.
- For known (repeat) donors, 1 anti-D reagent, or blended reagent, that detects weak D, D^{IV}, D^V and D^{VI} can be used.

12.10.4. Quality control of D grouping

- QC procedures recommended by reagent and equipment manufacturers should be followed.
- The following minimum test monitors are required for each batch of D grouping tests:
- Each series of D blood grouping tests must obtain appropriate reactions with R₁r red cells as a positive and with r'r or rr red cells as a negative
- Appropriate reactivity with red cell samples expressing weak D should also be assured as a minimum during validation as indicated in chapter 11.2.

12.10.5. Antibody screening

Blood and blood components with antibodies of probable clinical significance may be released, as shown in Table 12.1.

12.10.5.1. Routine antibody screen

- All donations must be tested for the presence of red cell antibodies. This is achieved by testing the donor's serum or plasma using a validated technique capable of detecting anti-D at 0.5 IU/mL or lower.
- Reagent red cells for routine antibody screening (see chapter 11.2.3.7) may be:
 - Provided from a minimum of 2 individual donations (not pooled), or
 - As a pool of red cells in equal proportions from no more than 2 donations, or
 - Red cells from a single donation.
- As a minimum, the following antigens should be expressed:
 - D, C, c, E, e and K
- Each batch of tests must include a test monitor of ≤0.5 IU/mL anti-D.
- Donations found to be reactive in the routine antibody screen should be further tested by an indirect antiglobulin test to determine the fate of the products as specified in Table 12.1.

12.10.5.2. Antibody screen for blood for neonates

- Blood for neonatal use must be screened and found negative for antibodies by an indirect antiglobulin test, performed using a two-cell panel expressing the following antigens as a minimum:
 - C, c, D, E, e, K, k, Fy^a, Fy^b, Jk^a, Jk^b, S, s and M

Table 12.1: Minimum release criteria for blood products with antibodies of probable clinical significance

Component	Release criteria
For neonatal use	Antibody screen for blood for neonates - negative
Red cells in SAGM	Donation plasma sample diluted 1 in 10 - negative
All other components	Donation plasma sample diluted 1 in 50 - negative

12.11. Additional testing

12.11.1. Antibody identification

Donations found to be reactive in the routine antibody screen may be further investigated for specificity.

12.11.2. Blood and blood components from group O donors with high titres of anti-A, anti-B and/or anti-A,B

Red cells, platelets and fresh frozen plasma from group O donors with high titres of anti-A, anti-B and/or anti-A,B can result in haemolytic transfusion reactions (HTRs) when given to non-group O patients. Such group O donors are generally termed 'high-titre group O donors'.

Reactions are more likely to occur when:

- The serological titre of the anti-A, anti-B and/or anti-A,B in the component is high.
- The plasma volume of the transfused product is high.
- The blood volume of the recipient is small.

Each Blood Establishment should have a testing and issuing policy to avoid the use of high-titre anti-A and/or anti-B in instances where a significant adverse clinical reaction is likely. The policy should cover the following components:

- Whole blood and plasma reduced red cells (excluding red cells in additive solution).
- Fresh frozen plasma.
- Apheresis platelet donations.
- Pooled platelets containing plasma from a single 'high-titre' group O donor.
- Blood/components for neonatal use, and infants under 1 year.

Where high-titre anti-A/B testing is deemed necessary, a saline agglutination test (performed as detailed in chapter 11) should give a negative result, at a dilution of 1 in 128, or an equivalent dilution by other techniques.

There should be a procedure in place to collect and review testing and patient outcome data and to implement changes in policy in the light of continuing clinical experience with the plasma-containing blood products issued.

Components from group O donors with 'low titres' of anti-A, anti-B and/or anti-A,B can cause intravascular haemolysis in non-group O recipients if given in sufficiently large volumes.

It is important to recognise that although testing for high-titre ABO antibodies in blood donors may reduce the risk of HTR in 'out of group transfusion', it cannot be entirely eliminated through this route. Group O platelets can cause HTR even when tested and labelled negative for high-titre haemolysins. They should only be used for non-group O patients (particularly paediatric patients) as a last resort.

12.11.3. Additional phenotyping

Red cell components should only be labelled with confirmed extended phenotypes.

A confirmed phenotype is one where the typing has been carried out and results concur:

- In duplicate on the current donation, or
- Once on the current donation and the result is in agreement with historical data from previous donations, or
- On 2 previous donations from that donor.

For labelling to be carried out under the last of these conditions, the security of the donor data, testing methodology used on each occasion and that of the historical test result data must be assured through validation and risk assessment.

12.11.4. Quality control of additional phenotyping

Quality control of procedures recommended by reagent and equipment manufacturers should be followed.

The test monitors shown in Table 12.2 are required for each batch of tests.

Within some test procedures reagent cross-contamination may occur. Test monitors should be selected in order to maximise the detection of such contamination.

Table 12.2: Test monitor red cell samples for blood grouping reagents

Reagent	Positive samples	Negative samples
anti-C	R ₁ r	R ₂ r or rr
anti-E	R ₂ r or r''r	R ₁ r or rr
anti-c	R ₁ r or r'r	R ₁ R ₁
anti-e	R ₂ r or r''r	R ₂ R ₂
anti-K	K+k+	K-k+
Other specificities	Heterozygous positive	Antigen negative

12.11.5. HbS screening

Unless the Blood Centre recommends that screening of donations for HbS is unnecessary, each Blood Establishment should have a protocol in place which ensures:

- The use of donations which are HbS screen negative for the manufacture of whole blood and red cell components for intrauterine transfusion, neonatal exchange transfusion and for the transfusion of children and adults with haemoglobinopathy. This protocol may be extended to further red cell products as deemed necessary by the Blood Establishment.

- Confirmatory testing for donors who are found to be HbS screen test positive.

Please note, where the laboratory information management system (LIMS) in use allows recording of the donor's HbS status, historical information may be used for the purposes described above, provided that the security of the donor data, testing methodology and that of the historical test result data has been assured through validation and risk assessment.

12.12. Donations found to have a positive direct antiglobulin test

Direct antiglobulin test (DAT) positive donations may be identified incidentally by testing laboratories when:

- the autologous/reference control is positive in ABO/RhD blood grouping
- the antibody screen is positive
- anomalies are identified in extended phenotyping tests

Non-red cell components may be prepared and issued from DAT positive red cell donations. Red cell units may be prepared and issued from DAT positive red cell donations provided that:

- the ABO and RhD groups are confirmed
- red cell antibodies have been excluded as per the mandatory antibody screening (see Table 12.1 in chapter 12.10)

Donors who have been found incidentally to have a positive DAT at donation testing may remain as blood donors provided they continue to pass the health screening questionnaire and have a normal haemoglobin.

12.13. Automated testing

An automated system as a minimum must accomplish the following:

- positive sample identification, reading and interpretation of results
- matching of results to sample identification
- electronic transfer of results

There should be documented contingency plans for the breakdown or total failure of automated testing systems. Protocol settings for automated systems must be documented and version controlled. Where possible, current versions of software and settings for automated systems should be backed up and readily available.

12.14. Manual testing

A manual testing system is one in which the minimum automated testing criteria have not been met.

Manual testing can be used to resolve anomalous results.

Measures should be taken to minimise the testing batch size to avoid the potential for errors.

Manual tests must be performed and controlled according to the manufacturer's instructions.

Test results must be recorded.

There must be a secure and validated method of entering results onto the host computer. Post result entry verification should be performed.