

Change Notification for the UK Blood Transfusion Services

Date of Issue: 04 September 2023

Implementation: to be determined by each Service

No. 16 – 2023

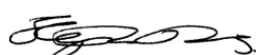
Red Book updates, Batch 2

Please note this document supersedes the version of CN 16-2023 which was circulated on 24/07/23.

Amendment: Proposed changes to Chapter 4.2 and 4.3 have been removed, pending further revision, and have been replaced with the existing published text. These sections are highlighted on [page 4](#).

This notification includes the following chapters:

	BM-DSG Bone Marrow & Peripheral Blood Stem Cell	CB-DSG Cord Blood	GDRI Geographical Disease Risk Index	TD-TSG Tissue – Deceased Donors	TL-DSG Tissue – Live Donors	WB-DSG Whole Blood & Components	Red Book Guidelines for the BTS in the UK
4 Premises and quality assurance at blood donor sessions (go to)	●	●	●	●	●	●	●
5 Collection of a blood or component donation (go to)	●	●	●	●	●	●	●
11 Reagent manufacture (go to)	●	●	●	●	●	●	●
12 Donation testing (red cell immunohaematology) (go to)	●	●	●	●	●	●	●
13 Patient testing (red cell immunohaematology) (go to)	●	●	●	●	●	●	●
14 Guidelines for the use of DNA/PCR techniques (go to)	●	●	●	●	●	●	●
15 Molecular typing for red cell antigens (go to)	●	●	●	●	●	●	●
16 HLA typing and HLA serology (go to)	●	●	●	●	●	●	●
17 Granulocyte immunology (go to)	●	●	●	●	●	●	●
18 Platelet immunology (go to)	●	●	●	●	●	●	●
22 Haemopoietic progenitor cells (go to)	●	●	●	●	●	●	●
A7 Requirements for the timing of testing for HPCs (go to)	●	●	●	●	●	●	●



Dr Stephen Thomas
Professional Director of JPAC

Changes are indicated using the following key

original text

«inserted text»

~~deleted text~~

Chapter 4 Premises and quality assurance at blood donor sessions

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This chapter applies to the collection of donations of whole blood and components at ~~«fixed» permanent~~ sites or by mobile blood collection teams.

4.1: Premises

Premises used for the ~~«collection of» preparation of components from~~ blood and ~~«components» plasma~~ have been subject to scrutiny by the Competent Authority, the Medicines and Healthcare products Regulatory Agency (MHRA), since 2005. Such facilities must comply with the principles embodied in the *Rules and Guidance for Pharmaceutical Manufacturers and Distributors 2007*.¹ ~~«to ensure the quality and safety of the collected blood and components, and to ensure the health and safety of donors and staff.»~~

~~Notwithstanding the fact that premises used for mobile donor sessions may often be accepted, from necessity, as the only local venue available, they~~ **«Premises»** must be of sufficient size, construction and location to allow ~~proper~~ operation, cleaning and maintenance in accordance with ~~«the blood service's procedures and national policies for infection prevention and control.» accepted rules of hygiene and in compliance with the WHO Expert Committee on Biological Standardization 43rd Report.~~²

«There must be provision for:

- the resuscitation equipment required by the blood service's policy
- a telephone so the emergency services can be called if required»

~~The designated person in charge of the blood collection team should in all cases be provided with a written plan of action appropriate to each venue. This can be used if conditions on arrival are not found to be acceptable. Care must be taken to avoid disturbances of any other activities within the venue if it is being shared.~~

4.1.1: Selecting a venue

«4.1.1.1: Collection process factors»

~~Whole blood and donor component procedures for the collection of plasma, platelets, red cells or combinations of these may be carried out at fixed or mobile collection sites.~~

~~Leucapheresis procedures to collect, for example, granulocytes, lymphocytes or peripheral blood progenitor cells, should only be performed at fixed component units.~~

~~In any apheresis unit, or at any blood donor session, a telephone must be immediately available so that the emergency services can be called at any time.~~

~~Resuscitation equipment, as required by local and national guidelines for blood donor sessions, must be available at all sessions undertaking routine component procedures.~~

Account must be taken of the **«space, services, equipment, and facilities required for the process of blood and component collection to ensure safety and minimise the risk of errors. The requirements include:»** ~~following activities/requirements when selecting a venue:~~

- registration of donors ~~and all other necessary data processing~~
- **«waiting area(s) with seats for donors»**
- **«privacy for confidential assessment of»** ~~appropriate facilities to assess~~ the fitness **«and suitability»** of individuals to donate
- **«sufficient space for chairs for beds for the donation and any equipment used during the donation (e.g. apheresis machine, blood mixer)»**
- ~~—withdrawal of blood from donors without risk of contamination or errors~~
- ~~social and medical~~ care of donors, including those who suffer reactions

- ~~sufficient seating should be provided for donors and staff, with allowance made for possible queues during busy periods~~
- storage of equipment, reagents and disposables
- storage ~~during the session~~ of blood and components ~~«prior to transportation», if they are not to be transferred immediately to the blood processing centre or to appropriate storage in the team vehicle~~
- ~~«a reliable» access to an adequate~~ electrical supply ~~«and adequate supply points for»~~ ~~to support all~~ electrical equipment ~~used for the session~~
- ~~«a reliable telecommunications network service»~~
- ~~the space required for these activities will depend on the anticipated workload~~
- ~~flooring should be non-slip.~~

«There must be an associated local contact whose details are available with the venue information for the designated person in charge of the blood collection team, for use if conditions or facilities are not suitable on arrival.»

«4.1.1.2:» ~~4.1.2:~~ Health and safety factors

«The blood service» ~~The requirements of the Health and Safety at Work Act 1974³ must be taken into account when selecting sessional venues. Each organisation~~ has the responsibility to ensure that venues comply with the Health and Safety at Work Act², and that staff are fully aware of their responsibilities under this ~~«and any other relevant»~~ legislation ~~«, and»~~. ~~It is the responsibility of all staff with supervisory or line management responsibility to ensure~~ that safe systems of work are in place ~~«and are upheld thereby minimising the risk of accidents and injuries.»~~ ~~at all times.~~ All venues should be formally assessed for suitability with an appropriate plan ~~«documented»~~ to manage risks. Premises should be safe, clean ~~«,»~~ and comfortable ~~«and convenient»~~ for donors and staff ~~«in regard to the following:»~~. ~~In particular, the following points should be borne in mind:~~

- ~~«Access:»~~ The venue should be ~~«located conveniently for the»~~ ~~as close as possible to the centre of~~ population being served. It should be possible for the sessional vehicle(s) to park in close proximity to the access doors, to facilitate off-loading if required. The ground to be covered by staff carrying equipment ~~«should»~~ ~~shall~~ be even and well lit. The space to be used should preferably not entail carriage of equipment on stairs. A similar safe approach should be ensured for donors, with as much provision as possible for car parking. Notices should be displayed, directing donors to the appropriate entry point of the building, and to the room being used.
- ~~«Flooring should be non-slip»~~
- Furniture and equipment ~~within the available space~~ should be arranged to ~~«allow a smooth and logical workflow without any obstruction to activities and movement within the session space, whilst also allowing»~~ ~~minimise crowding (with the increased risk of mistake or accident), enable~~ adequate supervision ~~and ensure a smooth and logical workflow.~~
- Fire exits must be unobstructed and operational. All sessional staff must be aware of the location of the fire extinguishers and exits.
- Lighting ~~«must»~~ ~~should~~ be adequate for all the required activities. Provision should be made for the use of emergency lighting in the event of interruption of the electricity supply.
- Environmental control may not be within the power of a mobile team, but every effort should be made to ensure that the space does not become too hot, cold or stuffy. ~~«Supplementary»~~ ~~Subsidiary~~ cooling fans and heating ~~«appliances»~~ should be carried on sessional vehicles, and used as necessary. This equipment should be subjected to a planned maintenance programme.
- Facilities for the provision of refreshments for donors and staff should be separated from the other activities of a donor session whenever possible. Every effort should be made to ensure that equipment used in this area poses the minimum threat of danger to all persons.
- Toilet facilities for ~~male and female~~ donors and staff should be provided
- Separate ~~«hand»~~ washing facilities are desirable for those staff involved in 'clean' procedures
- Adequate facilities must be available for the disposal of waste. On mobile sessions, all waste should be collected and contained in a suitable manner for subsequent disposal in accordance with relevant regulations.

4.2: Staffing and training principles for donation sessions

A consultant with responsibility for the donors in consultation with nursing and operational managers should ensure that there are adequate staffing levels and that staff are properly trained. This consultant may delegate day-to-day clinical responsibility to appropriately trained clinical staff. When donors are undergoing leucapheresis procedures (e.g. granulocyte, lymphocyte and peripheral blood progenitor cell collections) a suitably trained doctor must be immediately available to attend to the donor.

At sessions where component collection is performed one or more suitably trained doctors or registered nurses must be responsible for supervising the performance of venepunctures and for the supervision of machine procedures.

The administration of drugs (e.g. local anaesthetic and citrate) must be supervised by a registered professional in accordance with *Standards for Medicines Management (2007)*.⁴ During donation, donors should never be left in a room without the presence of an appropriately trained doctor or registered nurse.

Training and certification of registered nurses undertaking donation procedures including training and monitoring of staff, performing venepunctures and obtaining informed consent, must be in accordance with the current *Nursing and Midwifery Council (NMC) Code of Professional Conduct*.⁵

The consultant, in consultation with the nurse manager, must ensure that there is an appropriate staffing level and skill mix to ensure donor safety and adequate monitoring of the equipment in use. They must ensure that, as a minimum requirement, all healthcare professionals involved with component procedures receive basic life-support training annually.

At sessions where component collection is performed planned staffing levels should ensure that normally there is at least one member of suitably trained staff present for every two machines in use. For leucapheresis procedures, higher staffing ratios are required. A programme should be established for initial and continued training to ensure an appropriate level of proficiency.

The consultant with responsibility for donors must ensure that a manual of standard operating procedures (SOPs) is compiled in accordance with local quality assurance systems for whole blood collection and each type of component collection procedure. These SOPs must be regularly reviewed and updated and must take into account the machine manufacturer's operating instructions. A current copy of the relevant manufacturer's manual for each type of machine in use must be available on-site.

4.3: Collection of the donation

The ultimate responsibility for ensuring that every unit of blood and blood components has been collected in accordance with the Blood Safety and Quality Regulations (2005) rests with the 'responsible person' for the Blood Establishment. The advocacy and guardianship of high-quality care for donors is the responsibility of the designated clinical lead in attendance, who must be a registered nurse or medical practitioner.

Guidance for whole blood and component donation procedures is given in Chapter 5.

Guidance for laboratory testing procedures is given in Chapters 9 and 12.

4.4: Donor identification

Donors must positively identify themselves at registration «and point of attendance» by volunteering their name, date of birth and permanent address. ~~Once-registered.~~ For subsequent identification «during the same attendance, provision of» their name and date of birth «during the process of blood collection» is sufficient. The identity of the donor must be recorded and linked to the donation record. «The donor record must be current and active to allow donation, and duplicate donor records identified.»

4.5: Labelling

Session staff must ensure that a set of labels with a unique number is assigned to each donation and that the same unique number appears on the donor session record, the ~~primary-and-secondary~~ collection «pack(s)» ~~packs~~ and ~~all~~ the sample tubes «from the same donor to allow full traceability.» ~~used.~~

~~Great caution is necessary to avoid crossover or duplication of numbers.~~ The working practice should be designed to minimise the risk of error ~~«including crossover»~~. ~~Arrangements should be such as to avoid the possibility of errors in the labelling of blood containers and blood samples.~~ The blood or component bags and corresponding samples must not be removed from the donor ~~«area» couch~~ until a satisfactory check on correct labelling has been carried out. It is recommended that each donor ~~«area» couch~~ has its own individual facilities for the handling of samples during donation and labelling.

Packs, sample tubes and the donor session record must never be relabelled. Unused sets of numbers must be accounted for. Labels which have been discarded must not be retrieved.

«Labels must comply with relevant regulations, ideally machine as well as eye-readable, and allow a system of complete traceability to all donor and donation information held by the blood service.»

4.6: Records

It is strongly recommended that all records pertaining to donor and donation identity be entered and maintained in an electronic format which can be accessed readily by approved and qualified personnel, and in a manner which preserves donor confidentiality in accordance with legal requirements. Machine-readable systems for identifying donors and donation derivatives are also recommended. Initial documentation – for example on session records – may be ~~«made» taken~~ manually and archived for the required period in law, with relevant portions transcribed electronically whenever convenient operationally.

4.6.1: Donor session records

~~A record of~~ The session ~~at~~ venue, the date, ~~the donation number~~ and the identity of all donors attending must be ~~«recorded.» maintained.~~

«The relevant medical history of all donors must be documented and» for any donors who are deferred ~~«or withdrawn», rejected or retired,~~ «and» the full details ~~«including the reasons»~~ must be recorded ~~and the reasons given for the action taken.~~

~~The records of blood donation sessions should allow identification of each important step associated with the donation.~~ All donations must be recorded including «type collected (blood or component(s)) and» the reason for any unsuccessful donations.

«The records should allow identification of each important step associated with the donation.»

All adverse reactions must ~~also~~ be recorded together with the action taken. Full details of any other incidents, including those only involving staff, must be recorded.

These records should be used for the regular compilation of statistics ~~«and review» which should be studied monthly~~ by those responsible for ~~activities concerned with~~ the organisation and management of blood collection sessions.

4.7: «Equipment and consumables» ~~Control of purchased material and services~~

«All equipment used for the collection of blood and components must be validated, calibrated, maintained and cleaned, and records kept. Use must be in accordance with the manufacturer's instructions. Faulty, defective, or damaged equipment or consumables must not be used and must be reported and managed through the blood service's quality system.»

4.7.1: Specification and inspection of blood bags

Blood ~~«must be collected» collection shall be~~ by aseptic techniques using a sterile closed system and a single venepuncture.

The integrity of the system must be checked prior to use ~~«looking for signs of damage or defects and/or contamination.» and~~ Measures must be taken to prevent non-sterile air entering the system.

~~Blood shall be collected into~~ «The» containers ~~«must be» that are~~ pyrogen-free and sterile, containing sufficient licensed anticoagulant for the quantity and purpose of blood to be collected.

~~The container label shall state the kind and amount of anticoagulant, the amount of blood that can be collected and the required storage temperature.~~

Manufacturers' directions regarding storage, use and expiry dates of ~~the~~ packs ~~whose outer containers have been opened and resealed~~ must be adhered to.

Batch numbers of the blood packs used ~~«must» shall~~ be recorded.

The donation number on the pack and «associated» sample tubes «and donor health questionnaire» should be checked at the end of the donation «that they are identical.» ~~to ensure that those for a given donation are identical; that is, the donation number on the donor health and lifestyle questionnaire, the primary and secondary collection packs and the sample tubes must all be identical.~~

Prior to release from the blood collection session the pack and its associated tubing should be reinspected for defects and its integrity should be checked by applying pressure to the pack to detect any leaks. Any defective pack should be marked for disposal and held separately from intact packs. Details of the defect(s) should be recorded «and reported» for ~~future~~ analysis and action (see section 5.11).

4.7.2: Specification of apheresis sets

Blood components must be collected by apheresis using sterile, single-use, disposable items that are licensed and CE«/UKCA/UKNI» marked. The apheresis set for collection of components for direct clinical use must have a preconnected access needle to ensure a sterile pathway, and incorporate a bacterial filter in all «fluids that are not connected» ~~non-preconnected fluid lines~~ (e.g. «citrate anticoagulant»). ~~saline, SAGM, and the anticoagulant line [not required if the anticoagulant bag is preconnected]]. For dual needle procedures a preconnected needle is only essential for the access venepuncture.~~

A record must be kept of all lot and/or batch numbers of all the apheresis set components and injectable «/infused» materials used, in accordance with local quality systems.

4.7.3: Specifications for automated donor apheresis machines (see also section 8.5)

Machines must be correctly installed and commissioned according to each manufacturer's instructions. They must be CE«/UKCA/UKNI» marked.

The environment and operating area for each machine employed and the power supply available must conform to the manufacturer's recommendations for satisfactory machine performance.

Machines must comply with the relevant aspects of the Health and Safety at Work Act 1974² and the Good Automated Manufacturing Practice (GAMP) *Guide for Validation of Automated Systems in Pharmaceutical Manufacture*.³

Automated apheresis machines must have the following features:

- A manual override system so that the operator can stop the automatic cycle at any time during the procedure
- A blood flow monitor, to monitor blood flow during blood withdrawal and return. The purpose is to ensure that the selected donor flow rate does not cause collapse of the donor's vein and to monitor the venous pressure during the donor blood return cycle such that if any obstruction to flow occurs the blood pump will automatically reduce speed and/or stop. In either event a visual and audible alarm system should operate.
- An in-line air detector to protect the donor from air embolism. In the event of air entering the extra-corporeal circuit a visible and audible alarm must be activated, the return blood pump must automatically stop and the venous return line must automatically be occluded.
- A blood filter integral with the harness to prevent any aggregates formed during the procedure from being returned to the donor
- An anticoagulant flow indicator, providing a visible means of monitoring anticoagulant delivery throughout the procedure, and ideally an audible alarm if no anticoagulant is flowing
- A device for pre-setting the collection volume, monitoring the collection volume during the procedure and automatically ending the procedure. A system with a visual and audible alarm to notify the operator of the completion of the procedure may be provided.
- In the event of a power failure the machine must automatically enter a standby mode once power returns

Apheresis machines must be serviced in accordance with the manufacturer's instructions.

A planned maintenance scheme should be followed. Machine maintenance and servicing must be documented and be in accordance with the procedures outlined in the appropriate Medicines and Healthcare products Regulatory Agency publications: DB 9801, DB 9801 Supplement 1 and DB 2000(02). «6»⁷

Apheresis machines must be routinely cleaned with a suitable decontaminating agent on a daily basis. A standard procedure for dealing immediately with blood spillage must be in operation.

4.7.4: Anticoagulant

~~A licensed citrate anticoagulant must be used at a ratio which achieves a final plasma citrate concentration of 15–25 mmol/L in the collected component (see Chapter 3, Appendix II).~~

The anticoagulant must be in date, with no evidence of particles or leakage. Any suspect unit must not be used. The batch number must be recorded ~~on the session record~~ and any defect reported in accordance with local quality systems.

4.8: Protection and preservation of «donation» **product** quality

All ~~whole~~ blood and ~~apheresis~~ components must be transported, tested and stored in accordance with the specifications ~~for blood components~~ in Chapters 7 ~~and 8~~.

4.9: References

«

1. Medicines and Healthcare products Regulatory Agency (2017). *Rules and Guidance for Pharmaceutical Manufacturers and Distributors 2007 2017*. London: Pharmaceutical Press. Also available at www.mhra.gov.uk/publications/regulatoryguidance/medicines/othermedicinesregulatoryguidance/CON2030291
2. Health and Safety at Work Act 1974. Available at www.legislation.gov.uk
3. Good Automated Manufacturing Practice (GAMP) *Guide for Validation of Automated Systems in Pharmaceutical Manufacture*. Available at www.ispe.org
4. Standards for Medicines Management (2007). Available at www.nmc-uk.org/publications/standards
5. Nursing and Midwifery Council (NMC). Code of Professional Conduct. Available at www.nmc-uk.org
6. Medicines and Healthcare products Regulatory Agency publications, available at www.mhra.gov.uk
 - i. DB 9801, Medical Device and Equipment Management for Hospital and Community-based Organisations
 - ii. DB 9801 Supplement 1, Checks and Tests for Newly Delivered Medical Devices
 - iii. DB 2000(02), Medical Device and Equipment Management: Repair and Maintenance Provision.

»

- ~~1. Medicines and Healthcare products Regulatory Agency (2007). Rules and Guidance for Pharmaceutical Manufacturers and Distributors 2007. London: Pharmaceutical Press. Also available at www.mhra.gov.uk/publications/regulatoryguidance/medicines/othermedicinesregulatoryguidance/CON2030291.~~
- ~~2. World Health Organization (1994). WHO Expert Committee on Biological Standardization 43rd Report. Technical Report Series, 840, pp1–218.~~
- ~~3. Health and Safety at Work Act 1974. Available at www.legislation.gov.uk.~~
- ~~4. Standards for Medicines Management (2007). Available at www.nmc-uk.org/publications/standards.~~
- ~~5. Nursing and Midwifery Council (NMC). Code of Professional Conduct. Available at www.nmc-uk.org.~~
- ~~6. Good Automated Manufacturing Practice (GAMP) Guide for Validation of Automated Systems in Pharmaceutical Manufacture. Available at www.ispe.org.~~
- ~~7. Medicines and Healthcare products Regulatory Agency publications, available at www.mhra.gov.uk:~~
 - ~~• DB 9801, Medical Device and Equipment Management for Hospital and~~
 - ~~• Community-based Organisations~~
 - ~~• DB 9801 Supplement 1, Checks and Tests for Newly Delivered Medical Devices~~
 - ~~• DB 2000(02), Medical Device and Equipment Management: Repair and Maintenance Provision.~~

Chapter 5 Collection of a blood or component donation

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This chapter describes the steps involved in the collection of a blood or component donation from the information to be provided to a donor to the [«follow up of the donor post donation.»](#) ~~information required from the donor post donation.~~

Sections 5.1 and 5.2 are closely based on the Blood Safety and Quality Regulations 2005.¹

5.1: Information to be provided to prospective donors of blood or blood components

The following information must be provided to all donors:

- Accurate educational materials, which are written in terms which can be understood by members of the general public, about the essential nature of blood, the blood donation procedure, blood components and the important benefits to patients.
- For both allogeneic and autologous donations, the reasons for requiring a medical history, the testing of donations and the significance of informed consent.
- For allogeneic donations, the criteria for self-deferral, temporary and permanent deferral, and the reasons why individuals are not to donate blood or blood components if there could be a substantive risk for them or the recipient.
- For autologous donations, the possibility of deferral and the reasons why the donation procedure would not take place in the presence of a health risk to the individual whether as donor or recipient of the autologous blood or blood components.
- Information on the protection of personal data, including confirmation that there will be no disclosure of the identity of the donor, of information concerning the donor's health and of the results of the tests performed, other than in accordance with the requirements of these regulations.
- The reasons why individuals are not to make donations which may be detrimental to their health.
- Specific information on the nature of the procedures involved either in the allogeneic or autologous donation process and their respective associated risks. For autologous donations, the possibility that the autologous blood and blood components may not suffice for the intended transfusion requirements.
- Information on the option for donors to change their mind about donating prior to proceeding further, or the possibility of withdrawing or self-deferring at any time during or after the donation process, without any undue embarrassment or discomfort.
- The reasons why it is important that donors inform the Blood Establishment of any subsequent event that may render any prior donation unsuitable for transfusion.
- Information on the responsibility of the Blood Establishment to inform the donor, through an appropriate mechanism, if test results show any abnormality of significance to the donor's health.
- Information explaining why unused autologous blood and blood components will be discarded and not transfused to other patients.
- Information that test results detecting markers for viruses, such as HIV, HBV, HCV or other relevant blood transmissible microbiologic agents, will result in donor deferral and destruction of the collected unit.
- Information on the opportunity for donors to ask questions at any time.
- If the donated blood is to be used for purposes other than clinical transfusion or uses specified in the general consent materials, specific information must be provided.

5.2: Information to be obtained from donors by Blood Establishments at every donation

5.2.1: Donor identification

Donors must positively identify themselves by volunteering their name, date of birth and permanent address. The identity of the donor must be recorded and linked to the donation record.

5.2.2: Health and medical history of the donor

«The donor's» health and medical history, «obtained by» ~~provided on~~ a questionnaire and ~~through a~~ confidential personal interview ~~performed by a qualified health professional~~, must be assessed «by a suitably trained person». This will include relevant factors that may assist in identifying and screening out persons whose donation could present a health risk to others, such as the possibility of transmitting diseases, or health risks to themselves. Donors must be selected in accordance with the current JPAC *Donor Selection Guidelines*² which form a constituent part of Chapter 3.

5.2.3: Signature of the donor

The donor must sign the donor questionnaire. This must then be countersigned by the qualified health professional responsible for obtaining the health history confirming that the donor has:

- read and understood the educational materials provided
- had an opportunity to ask questions
- been provided with satisfactory responses to any questions asked
- given informed consent to proceed with the donation process (see Chapter 3)
- been informed, in the case of autologous donations, that the donated blood and blood components may not be sufficient for the intended transfusion requirements
- acknowledged that all the information provided by the donor is true to the best of their knowledge

«Where a suitable electronic system has been implemented, signatures from the donor and the qualified health professional can be accepted electronically. Any such system must meet quality and regulatory compliance standards, including verification of the identity of the individual signing the document.»

5.3: Haemoglobin screening

A validated haemoglobin screen should be applied to all donors prior to donation. The objective is to ensure that prior to each donation the donor has a minimum acceptable haemoglobin concentration (~~currently at least 125 g/L in females and at least 135 g/L in males~~, see section 3.15).

5.4: Preparation of the venepuncture site

Blood must be drawn from a suitable vein in the antecubital fossa in an area that is free of skin lesions. The veins can be made more prominent by using appropriate means of venous occlusion.

Although it is not possible to guarantee sterility of the skin surface for venepuncture, a strict standardised and validated procedure for the preparation of the venepuncture site should be in operation (see section 9.5).

The antiseptic solution used must be allowed to dry completely after application to the donor's skin, ~~or the skin must be wiped dry with sterile gauze before venepuncture~~. Thereafter, the prepared area must not be touched with fingers before the needle is inserted.

5.5: Preparation of the blood pack

5.5.1: Whole blood pack

The blood collection set must be in date and inspected for any defects. These are sometimes obscured by the label attached to the container, so careful inspection is required.

Moisture on the surface of a plastic pack after unpacking should arouse suspicion of a leak and if one or more packs in any packet is found to be abnormally damp, none of the packs in that container can be used. The solution in the set should be checked for clarity and must be clear before accepting the packs for use.

The blood pack is positioned below the level of the donor's arm and the blood collection tube must be clamped off.

The method used for monitoring the volume of blood removed shall be checked to be in working order and the pack placed in the correct position for the method to be effective.

5.5.2: Apheresis sets

The complete apheresis set and individual packaging must be thoroughly inspected for faults prior to use and during the setting up procedure. The set must be in date and a search must be made for set faults such as kinks, occlusions, points of weakness or leaks that may only become detectable during the setting up and priming procedure before the donor is attached to the set.

If an occlusive kink that cannot be remedied or a leak becomes apparent during a procedure then that procedure must be abandoned and any blood constituents remaining in the disposables must not be returned to the donor.

Any faults detected before or during a procedure must be recorded in accordance with local quality systems. Any defects must be reported (see section 5.11).

If there is any doubt about the integrity of any set, it must not be used but should be retained for inspection and returned to the manufacturer if deemed necessary.

5.5.3: Labels

Labelling: Whole blood and apheresis packs and donor sample tubes must be labelled in accordance with local standard operating procedures (SOPs).

All donors' records and labels should be checked for printing errors. Duplicate number sets «must» ~~shall~~ not be used «~~Both~~» ~~and~~ these and missing numbers «must» ~~shall~~ be reported via a designated senior «~~following documented local procedures.~~» ~~manager to the printer concerned and to the Chair of the National Working Party or equivalent on machine-readable labels.~~

5.6: Performance of the venepuncture

Venepuncture should only be undertaken by authorised and trained personnel. ~~If local anaesthetic is used, this should be a licensed medicinal product and injected in a manner which avoids any chance of donor-to-donor cross-infection (e.g. using individual disposable syringes and needles). A record of the batch number(s) should be made at each blood collection session and be capable of being related to individual donors.~~

~~Containers of local anaesthetic should be inspected for any leakage and if glass, inspected for cracks. Any suspect containers should be rejected.~~

~~Unused material must be discarded at the end of each donor session.~~

~~An aseptic technique must be used for drawing up the local anaesthetic into the syringe and the needle must be changed prior to the injection of the local anaesthetic.~~

Items used for venepuncture must be sterile, single-use and disposable. If the dry outer wrapping of sterile packs becomes wet the contents must not be used. Prior to use, session staff must ensure that the materials used for venepuncture are sterile, in date and suitable for the procedure to be undertaken. The sterile donor needle should not be uncovered and its tamper-proof cover should be checked for integrity immediately prior to the venepuncture.

As soon as the venepuncture has been performed, the clamp on the bleed line must be released.

It is important that a clean, skilful venepuncture is carried out to ensure the collection of a full, clot-free unit of blood suitable for the preparation of labile blood components.

The tubing attached to the needle should be taped to hold the needle in place during the donation.

5.6.1: Sample collection

At the start of the donation «an aliquot» ~~30 mL (up to 45 mL in some circumstances)~~ of blood should be diverted into a pouch. It is recommended that this pouch has a means of access opposite the entry line which allows blood to be sampled for ~~haematological and serological~~ testing without compromising the environmental integrity of the blood in the main pack. «Care should be taken that the volume of blood taken for samples does not lead to the total donated volume exceeding donation limits. For apheresis donors who give frequently, the total sample volume per year should also be considered.»

5.7: Whole blood donation

If necessary, the donor should be asked to open and close his/her hand slowly every 10–12 seconds to encourage a free flow of blood. The donor must never be left unattended during or immediately after donation and should be kept under observation throughout the phlebotomy.

5.7.1: Blood anticoagulation

The blood and anticoagulant should be mixed gently and periodically (at least every 60 seconds) during collection. Mixing should be achieved by manual inversion of the blood pack, or automatically by placing the blood pack on a mechanical agitator or by using a rocking device.

5.7.2: Blood flow

Blood flow should be constantly observed to ensure that the flow is uninterrupted.

The period of donation should not exceed 15 minutes.

5.7.3: Blood volume monitoring

The volume of blood withdrawn must be controlled to protect the donor from excessive loss of blood and to maintain the correct proportion of anticoagulant to blood.

The most efficient way of measuring the blood volume in plastic bags is by weight. The mean weight of 1 mL of blood is 1.06 g, and therefore, for example, a unit containing 470 mL of blood should weigh 470×1.06 g plus the weight of the pack(s) and the anticoagulant.

If it is not possible to adjust the weighing device in use for the tare weight of the container and anticoagulant solution it is advisable to record the minimum and maximum weight for the brand of pack in use as products from different manufacturers may vary considerably.

Several kinds of weighing equipment are available and such devices should be used according to the manufacturer's instructions for weighing blood into its plastic pack and periodically calibrated by appropriate techniques.

5.7.4: Completion of the donation

«If used,» the pressure cuff must be deflated and the needle then removed from the arm. Immediate pressure must then be applied to the venepuncture site through a suitable clean dressing.

«Local procedures must give clear instruction on sealing the pack and removal of the needle for all pack types in use.» The needle must be discarded into a special container designed to minimise risk to personnel.

The pack must be inverted gently several times to ensure the contents are thoroughly mixed.

~~For pack systems designed for in-line leucodepletion in which the donor line becomes detached from the final red cell pack, and hence unavailable for compatibility testing, the line should be sealed close to the collection pack, according to clearly defined procedures. This sealing may be done without expressing the contents of the line into the main pack if the contents of the line are deemed to be of no further use.~~

The arm and general well-being of the donor should be checked before the donor leaves the session venue.

5.8: Component donation by apheresis

Guidance for collection procedures is identical to that for normal whole blood donations except for the points listed below.

Performance of the venepuncture: Once the venepuncture is performed subsequent procedures such as releasing clamps on the bleed line should follow the protocol for the particular type of apheresis procedure being undertaken.

Anticoagulation: This occurs automatically in apheresis, but instructions are needed to ensure apheresis machine operators monitor the flow of anticoagulant.

Consideration should be given to withdrawing donors who repeatedly show signs and/or symptoms of citrate toxicity from the apheresis panel. ~~The practice of~~ Prophylactic oral supplementation with calcium should be discouraged.

Blood flow and monitoring: Blood flow occurs automatically in apheresis unless a satisfactory flow rate cannot be maintained.

Instructions are needed for the apheresis operator in the event of a low-flow or no-flow situation. Particular care is needed when monitoring the return flow rate since most apheresis procedures operate with a pumped red cell return such that haematomas can rapidly form unless appropriate action is taken to prevent this from occurring.

Sample collection: ~~In apheresis sampling~~ should take place at the beginning of a «component» donation. ~~The methods employed shall ensure an aseptic technique with no risk of contamination and be clearly defined in the sessional procedures SOP manual.~~

Completion of the donation and quality control samples: «Local procedures must give clear instruction on removal of the apheresis harness, sealing the component bag(s) and removal of the needle for all harness types in use.» ~~A length of tubing should be left attached to the collection pack(s) as required for laboratory testing purposes.~~ All used disposable equipment must be discarded in such a way as to prevent any risk to personnel, according to Health and Safety regulations.

Final donation inspection: The collected apheresis components must be inspected routinely for the presence of haemolysis, unwanted red cell contamination, other abnormal appearance or evidence of clotting. Such changes may require a review of the apheresis procedure and/or equipment. Any suspected apheresis component abnormality must be recorded, and the donation must be identified and reported in accordance with local quality systems.

5.9: Information to be provided to the donor post-donation

The donor must be provided with information on care of the venepuncture site and requested to report any illness occurring within 14 days of donation. They will already have been made aware of the importance of informing the Blood Establishment of any event that may render their donation unsuitable for clinical transfusion.

5.10: Adverse reactions in donors

The care of all donors at blood collection venues should incorporate research-based therapeutic interventions to reduce the risk of adverse events of donation. ~~An example of the preventative measures that can be implemented are described in 'Points of care' used within one UK Blood Service (see Appendix I at the end of this chapter). This is a donation care pathway designed to minimise vasovagal events, bruising and re-bleeding from the venepuncture site.~~

All adverse reactions in donors should be documented and reported according to standard protocols. It is recommended that as a minimum data are collected and reviewed on all donor adverse events of donation using the International Haemovigilance Network (IHN) definitions of «complications related to blood donation.³ The blood services in the UK have also agreed definitions for Serious Adverse Events of Donation (SAEDs, see Appendix I).» ~~DAEDs (Appendix II) or similar and a standard data set for Serious Adverse Events of Donation (SAEDs) that is in line with the IHN definitions of SAEDs (Appendix III).~~ This will allow comparison over time and between services of event rates, and monitor «ing of» the effectiveness of any interventions to reduce event rates. SAEDs should ~~all~~ be ~~fully~~ investigated with ~~a~~ root cause analysis or similar tool «s» to ensure that proper preventative and corrective actions are implemented.

Serious adverse reactions occurring in donors during or post-donation must be reported to the Competent Authority according to the Blood Establishment protocol.

5.11: Adverse events

All adverse events must be documented and reported according to standard protocols.

All bag/harness defects (e.g. pinhole leaks) must be recorded and all defects should be reported to the Quality Assurance Manager. If the defect appears to be batch-related, all packs and blood collected in them must be set aside for further investigation.

Any safety-related defects in equipment, including single-use items, must be reported «and escalated as per local procedures,» ~~via the head of department to the Department of Health~~ in accordance with the requirements of the Competent Authority, currently the Medicines and Healthcare products Regulatory Agency (MHRA).

Serious adverse events must be reported to the Competent Authority according to the Blood Establishment protocol.

~~Online reporting to the MHRA is available at www.mhra.gov.uk.~~

5.12: Donor compensation

The Blood Transfusion Services should have established procedures to ensure that any claim by a donor for compensation for any injury or loss allegedly attributable to having donated blood or components will be dealt with in a timely manner and within a legal framework.

5.13: References

«

1. Statutory Instrument 2005 No. 50. The Blood Safety and Quality Regulations 2005. Available at www.legislation.gov.uk
2. Joint UKBTS Professional Advisory Committee's (JPAC) Whole Blood and Component *Donor Selection Guidelines*. Available at www.transfusionguidelines.org
3. Working Group on Donor Vigilance of the International Society of Blood Transfusion Working Party on Haemovigilance (2014). Standards for the Surveillance of Complications Related to Blood Donation.

»

- ~~1. Statutory Instrument 2005 No. 50. The Blood Safety and Quality Regulations 2005. Available at www.legislation.gov.uk~~
- ~~2. Joint UKBTS/HPA Professional Advisory Committee's (JPAC) Donor Selection Guidelines. Available at www.transfusionguidelines.org.uk.~~

«Appendix I – Serious Adverse Events of Donation – UK Blood Services Definitions

SAED categories	
01	Death within 7 days of donation
02	Hospital admission within 24 hours of donation
03	Injury resulting in a fracture within 24 hours of donation (including fractured teeth)
04	Road traffic collision within 24 hours of donations
05a	Problems relating to needle insertion persisting for more than one year (this mainly includes suspected or confirmed nerve and tendon injuries)
05b	Problems relating to needle insertion requiring hospitalisation/intervention (this mainly includes vascular complications)
06	Acute coronary syndrome diagnosed within 24 hours of donation
07	Anaphylaxis (component donation)
08	Haemolysis (component donation)
09	Air embolism (component donation)
10	Other event related to donation resulting in: <ul style="list-style-type: none"> • Hospital admission, • Intervention, or • Disability or incapacity lasting more than one year and not included above»

Appendix I – Points of care

AI.1 Welcomer

- ~~A principle role of the Welcomer is to reduce potential anxiety in the donor, observe for donors in a 'hyper vigilant' state and refer where appropriate.~~
- ~~Professionalism, including appearance, is crucial in order to assure the donor of a safe and positive experience.~~
- ~~The Welcomer should greet the donor with a warm welcome, thank them for attending the session and giving up their time to donate blood.~~
- ~~The Welcomer needs to promote drinks to the donors. Offer the donor 500mls of fluid to stretch the stomach (gastric dilation) and raise blood pressure reducing the risk of vaso vagal (VV) episodes. This offer or promotion of drinks must be emphasised quite strongly in order for the donor to understand the importance of taking the fluid. Ideally the fluid should be consumed over 5 minutes rather than sipped, and should be taken no longer than 20 minutes prior to donation for best effect. The nurse or supervisor may wish to change the position of the water area on session in line with donor waiting times. An information leaflet for donors is available.~~
- ~~If possible, donors who are queuing to give their details must be offered fluids along with an explanation of why there is a delay. Back pod support/ nurses should help the Welcomer give drinks if the start of the session is busy.~~
- ~~Donors who are waiting to be screened must not be seated facing the front of the donation chairs. The eyes of all waiting donors ideally need to be focused away from clinical activity.~~
- ~~The Welcomer should ensure all donors are given the Welcome folder to read prior to screening.~~

AI.2 Screening

- ~~The Screener should enquire as to whether the donor has had any previous problems when donating blood and try to relieve any anxiety.~~
- ~~They should ask the donor about their preparation for giving blood e.g. have they had their usual meals today? If they have undertaken any strenuous activity or exercise, not usual for them, prior to attending are they fully recovered and rehydrated?~~
- ~~If a previous adverse event is identified or the donor has an increased risk of an adverse event, a nurse should be asked to speak to the donor. The nurse will also instruct the donor on how to do applied muscle tension (AMT) exercises to raise the blood pressure if appropriate.~~
- ~~The Screener should ask the donor if they have consumed the recommended volumes of fluid prior to the screening. If they have not, they should explain to the donor why drinking fluids is important and offer again. If the donor agrees to drink, give the fluids whilst talking.~~
- ~~The Screener should ensure new donors and those with a previous history or higher risk of VV episode/s are asked for their permission to discretely identify them throughout their visit so they can receive extra attention. Ensure the vulnerable donor identification card is then included in the pack box.~~
- ~~Once screening is complete, the Screener should show the donor to the waiting area, which must not have chairs facing the clinical area. Reading material should be available to provide a distraction for waiting donors. It is important to reduce tension and anxiety that will be experienced by many first time donors and those who may have had a problem donating or an adverse event in the past. Additional fluids could be offered too but not as an alternative to moving the main water station at busy times.~~

AI.3 Donation

- ~~To preserve donor dignity and keep the viewing of clinical activity to a minimum donation chairs should not be facing each other.~~
- ~~Staff should prioritise donors and provide appropriate therapeutic attention. Talking to donors will allow you to recognise their coping strategies and how best to put them at their ease.~~
- ~~If required, in order to raise the donor's blood pressure once they are on the chair, ask the donor to commence AMT exercises. This keeps their mind occupied as they are counting and their focus is away from the venepuncture (VP).~~
- ~~The donation chair should be placed steadily into the R position to donate.~~
- ~~If a vaso vagal episode occurs, call for help, reassure the donor and encourage them to commence AMT to prevent the vasovagal episode from worsening. The chair can be taken steadily into U position.~~
- ~~A nurse should decide if it is clinically necessary to screen off the donor to ensure privacy for the person involved and to avoid raising anxiety levels in those who are waiting. Screens should be placed around the donor, but initially if necessary, place your body between the donor and the waiting donors to block their view until screens arrive. Donors should never be left unattended behind a screen.~~
- ~~Non donating family/friends are welcome, but must sit at the other side of the chair from the agitator.~~
- ~~Once the donation is complete, remove the needle and cover the VP site with gauze, asking the donor to apply firm pressure with 3 fingers to the dressing.~~

- ~~Start the graduated recovery stage bringing the donor back up to O position in stages over a timed 2 minute period.~~
- ~~Those who are identified as vulnerable may require more, smaller recovery stages.~~
- ~~Encourage all donors to do AMT exercises during this stage to assist in the prevention of hypotension.~~
- ~~A staff member should stay with the donor until they leave the chair. Use the time to complete any observations, give advice to the donor, assess pallor and ensure the donor is applying the correct pressure to their arm and the arm remains straight.~~
- ~~If the VP site is observed then do so by lifting the gauze without removing it, to protect the donor from any blood splash. This also shields the donor from seeing the VP site, if there is no new bleeding, apply the dressing.~~
- ~~Support the donation arm as the donor leaves the chair.~~
- ~~Give vulnerable donors their identification card and ask them to place it in front of them as a place mat on the refreshment table.~~

AI.4 Appointments and teas

- ~~Set the refreshment table up with chairs to three sides to enable clear observation of all recovering donors.~~
- ~~Staff should ensure the computer does not obscure direct vision of the donors.~~
- ~~Ensure there is adequate space around the tea table and chairs to reduce the risk of injury should any falls occur.~~
- ~~Move any cages out of the refreshment area and ensure the safe placement of hot water boilers /cables.~~
- ~~Emphasise to donors who refuse a drink, the importance of having a post donation drink to replace fluid depletion.~~
- ~~Encourage those identified as vulnerable to have plenty of cold drinks and snacks.~~
- ~~Encourage those identified as vulnerable to stay in the refreshment area for a minimum of 15 minutes post donation and to continue with AMT exercises if necessary.~~
- ~~It may be necessary for donors who want to use the toilet immediately after donating to be escorted by a member of staff.~~
- ~~Deal with rebleeds promptly. Try to ensure nearby donors see as little as possible.~~
- ~~If a donor becomes unwell, stay with them and call for assistance.~~
- ~~Use the mat and wedge in order to get the donors legs elevated as soon as possible.~~
- ~~Encourage AMT exercises to prevent the vasovagal episode worsening.~~
- ~~When the donor is ready to transfer to the donation recovery chair assist them onto the chair and place in the U position. Slowly bring them through from U position to O giving the blood pressure time to adjust to each position.~~
- ~~Give post donation advice when the donor is ready, ensuring this advice is given in a confidential manner with regards to the dignity and well being of both the individual and other donors.~~

Appendix II—International Haemovigilance Network categories for donor adverse events

All.1 Complications mainly with local symptoms

All.1.1 Complications mainly characterised by the occurrence of blood outside the vessels

Haematoma

A haematoma is an accumulation of blood in the tissues outside the vessels.

Arterial puncture

Arterial puncture is a puncture of the brachial artery or of one of its branches by the needle used for bleeding of the donor.

Delayed bleeding

Delayed bleeding is spontaneous recommencement of bleeding from the venepuncture site, which occurs after the donor has left the donation site.

All.1.2 Complications mainly characterised by pain

Nerve irritation

Irritation of a nerve by pressure from a haematoma.

Nerve injury

Injury of a nerve by the needle at insertion or withdrawal.

Tendon injury

Injury of a tendon by the needle.

Painful arm

Cases characterised mainly by severe local and radiating pain in the arm used for the donation and arising during or within hours following donation, but without further details to permit classification in one of the already more specific categories mentioned above.

All.1.3 Other kinds of categories with local symptoms

~~Thrombophlebitis~~

~~Inflammation in a vein associated with a thrombus.~~

~~Allergy (local)~~

~~Allergic type skin reaction at the venepuncture site caused by allergens in solutions used for disinfection of the arm or allergens from the needle.~~

All.2 Complications mainly with generalised symptoms

All.2.1 Vasovagal reaction

~~A vasovagal reaction is a general feeling of discomfort and weakness with anxiety, dizziness and nausea, which may progress to loss of consciousness (faint).~~

All.2.2 Immediate vasovagal reaction

~~Symptoms occurred before donor has left the donation site.~~

All.2.3 Immediate vasovagal reaction with injury

~~Injury caused by falls or accidents in donors with a vasovagal reaction and unconsciousness before donor has left the donation site.~~

All.2.4 Delayed vasovagal reaction

~~Symptoms occurred after donor has left the donation site.~~

All.2.5 Delayed vasovagal reaction with injury

~~Injury caused by falls or accidents in donors with a vasovagal reaction and unconsciousness after donor has left the donation site.~~

All.3 Complications related to apheresis

All.3.1 Citrate reaction

All.3.2 Haemolysis

All.3.3 Generalised allergic reaction

All.3.4 Air embolism

All.4 Other complications related to blood donation

Appendix III – International Haemovigilance Network definition of severe donor adverse events

Conditions which define a case as severe are:

- ~~Hospitalisation: If it was attributable to the complication.~~
- ~~Intervention:~~
 - ~~to preclude permanent damage or impairment of a body function~~
 - ~~to prevent death (life-threatening).~~
- ~~Symptoms: Causing significant disability or incapacity following a complication of blood donation and persisted for more than a year after the donation (long-term morbidity).~~
- ~~Death: If it follows a complication of blood donation and the death was possibly, probably or definitely related to the donation.~~

For the purpose of consistent reporting of SAEDs the UK Blood Transfusion Services have adopted these categories:

- ~~death within 7 days of donation~~
- ~~hospital admission within 24 hours of donation~~
- ~~injury resulting in a fracture within 24 hours~~
- ~~road traffic collision (RTC) within 24 hours of donation~~
- ~~acute coronary syndrome (ACS) diagnosed within 24 hours of donation~~
- ~~problems relating to needle insertion persisting for more than a year~~
- ~~anaphylaxis, haemolysis or air embolism (component donors).~~

Chapter 11 Reagent manufacture

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11.1 Guidelines for reagent manufacture

11.1.1: Introduction

All reagents used to determine the group of human red cells and to detect red cell antibodies must comply with «[Medical Devices Regulations 2002 \(SI 2002 No 618, as amended\) \(UK MDR 2002\)](#)»¹ ~~Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on in vitro diagnostic medical devices~~¹ and all associated standards.

General guidelines for reagent manufacture are presented in this section. In other sections additional guidelines are given for particular reagents.

~~This document uses Fisher's notation to describe the presumed Rh genotype of red cell samples to be used. Where R_{zr} or r^r red cells samples are to be used, the probable genotype should be confirmed, for example by extended Rh phenotyping.~~

Where specific reference to British Standard European Standard (BS EN) documents is given this is the most recent version. It is intended that these guidelines refer to the current requirements contained in the applicable documents so the phrase 'and subsequent revisions' should be assumed whenever a specific reference is given.

11.1.2: Reference preparations

«[Reference preparations for use with these guidelines](#)» ~~The following reference preparations are available for use with these guidelines. Further details of these preparations~~ can be found on the National Institute for Biological Standards and Control (NIBSC) website at «www.nibsc.org» ~~www.nibsc.ac.uk~~:

- ~~WHO International Standard for Minimum Potency of anti-A Blood Grouping Reagents~~
- ~~WHO International Standard for Minimum Potency of anti-B Blood Grouping Reagents~~
- ~~WHO International Standard for Minimum Potency of anti-D Blood Grouping Reagents~~
- ~~ICSH/ISBT anti-Human Globulin Standard~~
- ~~ICSH/ISBT Papain Reference Preparation~~
- ~~ICSH/ISBT anti-D (for use with Papain Reference Preparation)~~

See section 11.3 for further information.

11.1.3: Definitions

Antibody identification is a test or combination of tests designed to determine the specificity of «[atypical](#)» ~~irregular~~ antibodies.

Antibody screening is a test or combination of tests designed to detect «[atypical](#)» ~~irregular~~ antibodies.

A **batch of reagent** is a defined quantity of material or of bulk, intermediate or finished product that is intended or purported to be uniform in character and quality, and which has been produced during a defined cycle of manufacture. A batch may be divided into sub-batches. A batch is sometimes described as a 'lot'.

A **batch of tests** is defined as a number of tests set up at the same time, under the same conditions and processed in a similar manner.

A **blood grouping kit** comprises a set of blood grouping components (reagents or materials) ~~and 'instructions for use'~~, packaged together, intended by the manufacturer to be used together for determining one or more blood groups.

A **blood grouping reagent** is a reagent, used alone or in combination with other materials, intended by the manufacturer for the determination of a blood group of an individual.

- A blood grouping reagent recommended by the manufacturer for the detection of A (i.e. subgroups A_1 and A_2), A_x and B should be named **anti-A,B blood grouping reagent**.
- ~~A reagent recommended by the manufacturer for the detection of A (i.e. subgroups A_1 and A_2) and B but not of A_x , should be named anti-A+B blood grouping reagent.~~

A **blood grouping system** is an *in vitro* diagnostic medical device intended by the manufacturer to be used for determining one or more blood groups.

Clinically important or clinically significant antibody is a red cell antibody which will produce significantly accelerated red cell destruction when combined *in vivo* with its corresponding antigen.

Expiry date is the date beyond which performance of the reagent cannot be assured and is based upon the stability of the reagent.

Fresh serum for complement activity stored in the liquid state should be used within eight hours of donation. When used after storage at –70°C or below, the 8-hour liquid storage period refers to the time both before and after frozen storage. Unless validated, the maximum period of frozen storage shall be six months at this temperature.

An **immediate container** is a medium adequate to protect the content(s) from contamination and/or physical damage. For example, a sealed vial, ampoule or bottle, a foiled pouch or a sealed plastic bag. ~~The European Standard BS-EN-375 requires a label on the immediate container and the outer container that is the material used in the packaging of the immediate container(s) of a product. It is a valid interpretation of that Standard that a microplate presented within a sealed pouch or foiled pouch does not require any label. It is considered by the Standing Advisory Committee for Immunohaematology that this interpretation will contribute to errors in identifying microplates in use within the laboratory. Therefore, in addition, the body of microplates presented in sealed bags or foiled pouches should be marked with a unique identifier to enable identification and traceability. Vials, ampoules, bottles and micro-well plates used as containers for a reagent for blood group serology should be transparent to permit visual inspection of the contents and consist of a material which does not cause deterioration of the reagent over the period recommended for use by the manufacturer.~~

«**Atypical**» ~~Irregular~~ **blood group antibodies** are those of «non-ABO» specificity ~~other than anti-A or anti-B.~~

The **manufacturer** is «a natural or legal person who manufactures or fully refurbishes a device or has a device designed, manufactured or fully refurbished, and markets that device under its name or trademark.» ~~is the natural or legal person with the responsibility for placing the device on the market under his or her own name, regardless of whether he or she has designed, manufactured, packaged, or labelled the device.~~

The name for a blood grouping reagent derived from monoclonal materials should include the word **monoclonal**.

A **monospecific blood grouping reagent** is one containing an antibody or blend of antibodies specific for one antigen, e.g. anti-A, anti-IgG.

A **polyspecific blood grouping reagent** is one containing a blend of antibodies specific for more than one antigen.

Polyspecific anti-human globulin reagent should be the name for a reagent which contains anti-human IgG and anti-human complement (C3d) activity, and is recommended by the manufacturer for use in both the direct and indirect anti-human globulin techniques, i.e. for the detection of red cell bound human IgG, and C3 complement in the form E1C3b and EC3d irrespective of the presence of other human immunoglobulin or human complement specificities.

Potency titre is a term used to describe the highest dilution of a reagent that effects a grade 2 endpoint reaction «in tube or a grade 1 endpoint in column agglutination technologies».

Prozone is the term used to denote the absence or weakening of agglutination with excess of antibody.

A **reagent control** is a reagent made to the same formulation as a blood grouping reagent but without the specific blood group antibody reactivity. If the reagent control contains serum or plasma, the reagent control should be shown to be free from specific blood group antibody reactivity.

A **reference preparation** is prepared nationally or locally and contains a known or agreed concentration of the activity being measured. It should be assayed to establish the sensitivity or calibration of a test procedure or reagent.

Sensitivity in relation to these guidelines is a term defining the limit of detectable specific reactions using reagents or test systems. These guidelines specify levels of sensitivity that should be achieved.

Shelf life is the period until expiry date.

Specificity in relation to these guidelines is a term defining the ability of a reagent or test system to react selectively. In particular terms, it represents the absence of unwanted or false-positive reactions.

Test monitors are a series of samples included as part of each batch of tests, which provide part of the release algorithm for a batch of tests.

Validation is the confirmation, through the provision of objective evidence, that the requirements for a specific or intended use have been fulfilled. Validation of a manufacturing method is to ensure that the product will be of the quality required for its intended use and that tests used in monitoring will accurately reflect the quality of the product.

Verification is the confirmation, through the provision of objective evidence, that specific requirements have been fulfilled.

Undiluted in these guidelines means the reagent as intended for use by the manufacturer. This term includes a diluted reagent if the reagent is supplied in a form requiring dilution by the user prior to use, as specified in the manufacturer's 'instructions for use'.

An **unequivocal** reaction in a test system is a reaction that is unambiguous. In the manual tube test, this is defined as a reaction of grade 3 or greater. In column tests this is defined as a «1+ or greater» ~~2+~~ reaction.

11.1.4: General manufacturing considerations

11.1.4.1: Good manufacturing practice

Reagents for blood group serology must be manufactured in accordance with «UK MDR 2002¹» ~~Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on in vitro diagnostic medical devices~~ and all associated documents.

Guidance on the principles of good manufacturing practice can be obtained from *Rules and Guidance for Pharmaceutical Manufacturers and Distributors* 2007.²

- The method of manufacture should result in a product within an immediate container that is homogeneous and free of properties which adversely affect its intended use throughout its recommended shelf life. The reagent should have no precipitate, particles or fibrin gel.
- Each batch or sub-batch should be specifically identified by a distinctive combination of numbers and/or letters (batch reference) which permits its history to be traced.
- Reagents should be produced by a validated process that is shown to be suitable for the intended purpose, including any methods for preserving red cells prior to their preparation as reagent red cells.
- The manufacturer should monitor the batch-to-batch performance of the blood grouping reagent (e.g. by the reaction against some internal reference material) in order to provide consistency of performance. This is particularly important when the blood grouping reagent is provided as a test system, kit or kit component, when the performance may be dependent on the characteristics of other system variables or kit components.

11.1.4.2: Risk management

Risk management should be performed in accordance with:

- BS EN ISO 14971:«2019»~~2012~~ Medical Devices – Application of Risk Management to Medical Devices
- BS EN 13641:2002 Elimination or Reduction of Risk of Infection Related to *in vitro* Diagnostic Reagents

11.1.4.3: Performance evaluation

Performance evaluation should be undertaken in accordance with:

- BS EN «ISO 23640 *In vitro* diagnostic medical devices. Evaluation of stability of *in vitro* diagnostic reagents» ~~113612:2002 Performance Evaluation of in vitro Diagnostic Medical Devices~~
- Reagents ~~listed in Annex II, List A, of the EU In Vitro Diagnostic Medical Devices Directive~~ must also comply with the Common Technical Specifications for *In Vitro* Diagnostic Medical Devices «(where they exist)» ~~(2009/408/EC)~~

11.1.4.4: Stability data

Stability testing should be performed in accordance with:

- BS EN «ISO 23640 *In vitro* diagnostic medical devices. Evaluation of stability of *in vitro* diagnostic reagents» ~~13640:2002 Stability Testing of in vitro Diagnostic Reagents~~

11.1.4.5: Date of manufacture

- For blood grouping reagents the date of manufacture is the date of commencement of the last potency test on the batch or sub-batch that indicates attainment of the required specification.

- For reagent red cells the date of manufacture is the date of collection from the donor. Where reagent red cells are prepared from more than one donor, the date of collection of the oldest donation should be recorded as the date of manufacture.
- Where a freezing process is used to preserve red cells before their preparation for issue as reagent red cells, the date of manufacture is the date of recovery from the frozen state.

11.1.4.6: Colour coding of reagents

No colouring agent should be added to reagents for blood group serology except that:

- Polyspecific anti-human globulin reagents may be coloured green, anti-A may be coloured blue, anti-B may be coloured yellow.
- The colorant should not interfere with the observation of the test result.
- 'Bespoke' antisera for use on automation may be coloured providing the information contained in the barcode on each bottle contains sufficient identifiers (specificity and lot number) to provide assurance that the intended test has been performed. The colours used for other specificities should not be coloured blue or yellow to avoid confusion with those for anti-A and anti-B reagents.

11.1.4.7: Freedom from microbial contaminants

- Reagents should be prepared using validated processes to produce a final product free from microbial contaminants that adversely affect the unopened product during storage at the recommended temperature. The manufacturer should routinely monitor the efficacy of the process used in the manufacture of the reagent.
- A preservative may be included in the reagent to minimise the effects of contamination during use if the preservative has been shown not to adversely affect the product during storage or use.
- Other than reagent red cells, all reagents for blood group serology recommended by the manufacturer for storage in the liquid state, should be filtered through a sterile filter of pore size not exceeding 0.22 µm. All reagents should be dispensed into the immediate container under aseptic conditions.
- Tests for contamination do not give absolute assurance of freedom from microbial contaminants. Bactericidal agents in common use for blood grouping reagents do not guarantee the absence of microbial agents after opening of the container.

11.1.4.8: Retained samples

- A minimum of 1% or three immediate containers, whichever is less, of each batch of reagents other than reagent red cells should be retained and stored as recommended by the manufacturer to enable analysis of reported defects. Such samples should be retained for at least six months beyond the expiry date.
- A minimum of two final containers of each batch of reagent red cells should be retained and stored as recommended by the manufacturer to enable analysis of reported defects. Such samples should be retained for at least ten days beyond the expiry date.

11.1.4.9: Tests required

The manufacturer should test, as described in these guidelines, each lot of a reagent obtained from the immediate container to be supplied for use (see section 11.2.1).

11.1.4.10: Human source material

Existing procedures in the UK Blood Transfusion Services for consent to donate are sufficient to allow cellular and plasma materials collected as part of the donation process to be used as reagents without further explicit consent.

Samples/donations that are obtained specifically for reagent purposes will require additional consenting of the donor, and must have appropriate ethical approval. Donor materials that are obtained and retained for genomic or nucleic acid testing must comply with the regulations laid down by The Human Tissue Act 2004 (except Scotland).³

Residual samples retained from patient testing laboratories may be used without further explicit consent, if anonymised.⁴ Additional samples taken from patients specifically for reagent use will require ethical approval and explicit consent. All patient samples acquired and retained must comply with the regulations laid down by the Human Tissue Act (2004).

Each individual donation or sample of human material in a reagent for blood group serology shall be tested and found negative for mandatory microbiological tests required by the UK Blood Transfusion Services for blood donations (see Chapter 9). A statement is required in the 'instructions for use' to this effect.

«In case there is a need to perform retrospective microbiological testing on material used to prepare *in vitro* diagnostic devices, an archive sample collected at the same time and from the same donor(s) used to prepare the device should be taken and stored for up to six months» ~~To ensure retrospective microbiological testing, an appropriate sample, collected at the same time as the donation used in the formulation of a particular reagent, should be archived until at least 6 months after the expiry date of the last batch of the reagent made from that material.~~

11.1.4.11: Label requirements

The label must conform to the requirements of BS EN 18113:2011 Information Supplied by the Manufacturer with *in vitro* Diagnostic Reagents for Professional Use.

~~In addition, the instructions for use should meet the following criteria:~~

- The label fixed to the immediate container of a reagent should leave uncovered sufficient area of the full length or circumference of the container to allow ready visual inspection of the contents.
- The specificity of the reagent for blood group serology should be of a print size which is clearly legible. The print size of other information on the label should not exceed that used for the specificity of the reagent.
- The typeface used should clearly differentiate between antigens and related antibody specificities represented by upper and lower-case characters, e.g. C/c, S/s and K/k.
- For products needing to be prepared in the final form by the user following the instructions of the manufacturer and to be retained in the manufacturer's immediate container, a space should be available on the container label for the user to write the expiry date of the prepared product when stored as recommended by the manufacturer.
- The main panel of labels of enzyme-treated reagent red cells may be coloured pink in order to be distinguishable from non-enzyme-treated reagent red cells. Pantone colour reference 223 is recommended.

For other reagents, any colour appearing on the main panel of the label should comply with Food and Drug Administration regulations (21 CFR 660.28) as shown in Table 11.1.

Table 11.1 Label colour coding

Specificity	Colour	Code
anti-A	Blue	305C
anti-B	Yellow	102C
anti-C	Pink	204C
anti-D	Grey	429C
anti-E	Brown	465C
anti-CDE	Orange	151C
anti-c	Lavender	529C
anti-e	Green	577C

11.1.4.12: Instructions for use (package insert)

The instructions for use must conform to the requirements of BS EN 18113:2011 Information Supplied by the Manufacturer with *in vitro* Diagnostic Reagents for Professional Use.

In addition:

- For blood grouping reagents containing monoclonal antibodies, the identity of the cell line(s) from which the monoclonal antibodies have been derived.
- For reagent red cells for antibody screening and for «antibody» identification, the 'antigen profile' of the component cell samples ~~is part of the instructions for use and~~ should have the lot number and expiry date of the reagent to which it refers.
- A statement that loss of reactivity may occur during the stated shelf life of the red cells and that since this loss is partly determined by characteristics of individual blood donations or donors, which cannot be predicted or controlled, the conditions of storage and use recommended by the manufacturer should be rigidly applied.
- For enzyme-treated reagent red cells, information should be given concerning those antigens which are rendered inactive or less active by the enzyme treatment used.

11.2: Specifications, performance evaluation and quality control of blood grouping reagents

11.2.1: Blood typing antisera

11.2.1.1: General requirements

- It is essential that blood grouping reagents are prepared using reliable manufacturing procedures that are consistently capable of producing safe and efficacious products. The products must comply with requirements of the EU Directive (98/79/EC) on *in vitro* diagnostic medical devices and other relevant international standards detailed in section 11.3.
- The term weak D is used in these guidelines to indicate a weakened expression of a normal D antigen. The term partial D is used in these recommendations to indicate the expression of only a part of the normal D antigen. The reactivity of RhD blood grouping reagents against partial D red cells is determined by the nature of the D variant, the anti-D reagent and the technique used.
- «Red cell samples with partial antigen expression (e.g. partial D) or weak antigen expression (e.g. A_x) may not react with some reagents and, where this is known to be true, must be stated in the limitations.»
- The blood grouping reagent is satisfactory if an unequivocal positive result is obtained with all the red cell samples having the antigen corresponding to the blood grouping reagent being assessed, by all the methods recommended for use by the manufacturer.
- If reactivity is claimed by the manufacturer against weak variants or subgroups of a particular antigen, red cells from at least two confirmed/reference samples should be tested (see Table 11.3).

The grading system shown in Table 11.2 is used throughout these guidelines for manual tube/microplate serological testing.

Table 11.2 Grading system for serological tests

Reaction grade	Description
Grade 5	Cell button remains in one clump or dislodges into a few large clumps
Grade 4	Cell button dislodges into numerous large clumps
Grade 3	Cell button dislodges into many small clumps
Grade 2	Cell button dislodges into finely granular but definite, small clumps
Grade 1	Cell button dislodges into fine granules
Grade 0	Negative result
Unless otherwise stated, an unequivocal manual tube reaction is defined as a grade 3 or greater and for column tests as «1»2+ or greater	

11.2.1.2: Performance evaluation

Performance evaluation should be undertaken in accordance with:

- BS EN 13612:2002 Performance Evaluation of *In Vitro* Diagnostic Medical Devices.
- Reagents listed in Annex II, List A, of the EU *In Vitro* Diagnostic Medical Devices Directive must also comply with the Common Technical Specifications for *In Vitro* Diagnostic Medical Devices (2009/108/EC).

Stability testing should be performed in accordance with BS EN «ISO 23640 *In vitro* diagnostic medical devices. Evaluation of stability of *in vitro* diagnostic reagents» ~~13640:2002 Stability Testing of In Vitro Diagnostic Reagents.~~

Where appropriate, the following requirements should also be included in performance evaluation:

- In the case of polyclonal antibodies, contaminating antibodies to antigens having a prevalence of greater than 99% in the general population of the UK should be excluded. ~~by~~ Negative results in tests using samples of red cells from four different individuals who lack the antigen corresponding to the antibody specificity «of the reagent» ~~under test. «but have the antigens to the potential contaminating antibodies should be obtained.»~~
- Tests for the presence of contaminating ABO antibodies should be performed with red cells from a minimum of two individuals of group A₁ and two of group B who lack the antigen corresponding to the antibody specificity under test.
- If tests using all methods recommended for use by the manufacturer do not exclude the presence of antibodies to the following antigens, these antibody specificities should be stated in the package insert² as not having been excluded in specificity testing:

Xg^a, Do^a, Yt^{«b»~~a~~}, Co^b, Wr^a and V^w

- Blood grouping reagents which are chemically modified, and/or contain in their formulation a potentiator of agglutination, or require the user to add a potentiator, shall be tested, by all methods recommended by the manufacturer, with red cells lacking the antigen corresponding to the antibody specificity under test but sensitised with an IgG antibody to effect a grade 5 reaction in the anti-human globulin technique.
- Potentiated blood grouping reagents producing agglutination by those methods recommended by the manufacturer, should be supplied with a reagent control that has been shown to effect a degree of non-specific reaction with IgG-coated red cells similar to the corresponding blood grouping reagent.
- Blood grouping reagents recommended for use by a direct agglutination method should not contain antibodies reactive against red cells coated with IgG when used by direct agglutination methods recommended by the manufacturer.

11.2.1.3: Batch release testing requirements

Specificity tests

- The manufacturer «must provide a certificate of analysis to customers once evidence has been obtained by the manufacturer that the product achieves the specificity and reactivity claimed by the manufacturer for each method recommended by the manufacturer. Assurance of Specificity should be determined in accordance with the requirements in Table 11.3. The certificate of analysis should also ensure that the potency of the material meets the requirements of the final bullet point on Potency below.» ~~should test the blood grouping reagent as a final product, by all methods recommended by the manufacturer for the specificity and reactivity claimed. Specificity should be determined by testing the reagent in accordance with the requirements outlined in Table 11.3.~~
- If a range of incubation times or incubation temperatures is recommended by the manufacturer, the range(s) should be used in these test procedures.

Requirements

- Blood grouping reagents should not produce a positive reaction when tested with red cells lacking the antigen corresponding to the antibody specificity under test, by any method recommended for use by the manufacturer. Should reactivity to a low-frequency antigen be observed with subsequent batches of a reagent, this fact should be brought to the attention of all primary consignees of that reagent.
- Rouleaux formation, prozone or haemolysis should not occur in tests using any of the methods recommended by the manufacturer.

Potency tests – tube or microplate methods

- Potency titrations should be performed in accordance with the manufacturer's recommended method of use using an appropriate diluent.
- Manufacturers should compare the potency titre of each batch of reagent with an appropriate reference preparation (see section 11.3).
- Potency titrations for each batch tested should equal or exceed any existing British or International reference preparations.

Table 11.3 Requirements for conventional blood typing reagents

Antibody specificity	Specification	Performance evaluation «(as a minimum, two examples of the following reference cells should be included if available)»	Batch release testing					
			Specificity				Potency	
			Positive reactors		Negative reactors			
			Cell type	No.	Cell type	No.	Cell type	No.
anti-A	Normally blue coloured Should equal or exceed potency of reference preparation(s) Should detect variants and subgroups as detailed in the manufacturer's instructions for use	A _x ,A ₃	A ₁	2	B	2	See insert of reference preparation(s)	
		A cord cells	A ₂ B	2	O	2		
			A _x *					
anti-B	Normally yellow coloured Should equal or exceed potency of reference preparation(s) Should detect variants and subgroups as detailed in the manufacturer's instructions for use	B _x , B ₃ , B _y	B	2	A ₁	2	See insert of reference preparation(s)	
		B cord cells	A ₁ B	2	O	2		
anti-A,B	Normally clear coloured Should equal or exceed potency of reference preparation(s) Should detect variants and subgroups as detailed in the manufacturer's instructions for use	A ₁ , A ₂ , B, A ₁ B, A ₂ B	A ₁	1	O	4	See insert of reference preparation(s)	
		A _x , A ₃	A ₂	2				
		B _x , B ₃	B	2				
		A and B cord cells	A _x	2				
anti-A1	Normally clear coloured Should detect variants and subgroups as detailed in the manufacturer's instructions for use		A ₁	2	A ₂	2	A ₁	2
			A ₁ B	2	A ₂ B	2		
					B	2		
					O	2		
anti-D	Normally clear coloured Should equal or exceed potency of reference preparation(s) Should detect variants and subgroups as detailed in the manufacturer's instructions for use	Weak D (500 sites/cell)	R ₁ r	2	r'r	1	See insert of reference preparation(s)	
		C ^w , C ^x	R ₂ r	2	r'r	1		
		D ^{VI} type 1, D ^{VI} type 3, D ^{IV} , D ^V , D ^{VII} , DFR, DBT, R ₀ ^{Har}	Weak D*	2	rr	1		
anti-C	Normally clear coloured Potency titre greater than 4 vs by techniques detailed in manufacturer's instructions for use Should detect variants and subgroups as detailed in the manufacturer's instructions for use	C ^w , C ^x , r ^S	R ₁ r	1	R ₂ R ₂	1	R ₁ r	2
		R ₂ R ₂	R ₁ R ₂ R ₂ R ₂	1 1	r'r	1		
			r'r	1	rr	1		
anti-E	Normally clear coloured Potency titre greater than 4 vs by techniques detailed in manufacturer's instructions for use Should detect variants and subgroups as detailed in the manufacturer's instructions for use	R ₁ R ₂	R ₂ r	1	R ₁ R ₁	1	R ₂ r	2
		E ^w	R ₁ R ₂	2	r'r	1		
			r'r	1	rr	1		

anti-c	Normally clear coloured Potency titre greater than 4 vs by techniques detailed in the manufacturer's instructions for use Should detect variants and subgroups as detailed in the manufacturer's instructions for use	R ₁ R ₂ , R ₁ ^w R ₁	R ₁ r	2	R ₁ R ₁	3	R ₁ r	2
			R ₁ R ₂	1				
			r'r	1				
anti-e	Normally clear coloured Potency titre greater than 4 vs by techniques detailed in the manufacturer's instructions for use Should detect variants and subgroups as detailed in the manufacturer's instructions for use	R ₂ R ₂	R ₂ r	2	R ₂ R ₂	3	R ₂ r	2
			R ₁ R ₂	1				
			r''r	1				
anti-C ^w	Normally clear coloured Potency titre greater than 4 vs by techniques detailed in the manufacturer's instructions for use	R ₁ ^w R ₁ , r ^w r, R ₁ ^w r	R ₁ ^w r or	2	R ₁ r	1	R ₁ ^w r	2
			R ₁ ^w R ₂	2	R ₁ R ₁	1		
			r ^w r	1	r'r	1		
anti-K	Normally clear coloured Potency titre greater than 4 vs by techniques detailed in the manufacturer's instructions for use Should detect variants and subgroups as detailed in the manufacturer's instructions for use	K+k+ Kp(a+b+)	K+k+	4	K-k+	4	K+k+	2
		K+k+ Kp(a-b+)						
Anti-k	Normally clear coloured Potency titre greater than 4 vs by techniques detailed in the manufacturer's instructions for use Should detect variants and subgroups as detailed in the manufacturer's instructions for use	K+k+ Kp(a+)	K+k+	4	K+k-	4	K+k+	2
			Kp(a+b+)	2				
			K+k+ Kp(a-)	2				
anti-Fy ^a	Normally clear coloured Potency titre greater than 4 vs by techniques detailed in the manufacturer's instructions for use Should detect variants and subgroups as detailed in the manufacturer's instructions for use		Fy(a+b+)	4	Fy(a-)	4	Fy(a+b+)	2
anti-Fy ^b	Normally clear coloured Potency titre greater than 4 vs by techniques detailed in the manufacturer's instructions for use Should detect variants and subgroups as detailed in the manufacturer's instructions for use		Fy(a+b+)	4	Fy(b-)	4	Fy(a+b+)	2
anti-Jk ^a	Normally clear coloured Potency titre greater than 4 vs by techniques detailed in the manufacturer's instructions for use Should detect variants and subgroups as detailed in the manufacturer's instructions for use		Jk(a+b+)	4	Jk(a-)	4	Jk(a+b+)	2
anti-Jk ^b	Normally clear coloured Potency titre greater than 4 vs by techniques detailed in the manufacturer's instructions for use Should detect variants and subgroups as detailed in the manufacturer's instructions for use		Jk(a+b+)	4	Jk(b-)	4	Jk(a+b+)	2
anti-S	Normally clear coloured Potency titre greater than 4 vs by techniques detailed in the manufacturer's instructions for use Should detect variants and subgroups as detailed in the manufacturer's instructions for use	S+s-, S+s+, S-s+	S+s+	4	S-s+	4	S+s+	2

anti-s	Normally clear coloured Potency titre greater than 4 vs by techniques detailed in the manufacturer's instructions for use Should detect variants and subgroups as detailed in the manufacturer's instructions for use		S+s+	4	S+s–	4	S+s+	2
anti-M	Normally clear coloured Potency titre greater than «2»4 vs by techniques detailed in the manufacturer's instructions for use Should detect variants and subgroups as detailed in the manufacturer's instructions for use	M–N+ He+	M+N+	4	M–N+	4	M+N+	2
anti-N	Normally clear coloured Potency titre greater than 4 vs by techniques detailed in the manufacturer's instructions for use Should detect variants and subgroups as detailed in the manufacturer's instructions for use		M+N+	4	M+N–	4	M+N+	2
anti-P1	Normally clear coloured Potency titre greater than 4 vs by techniques detailed in the manufacturer's instructions for use Should detect variants and subgroups as detailed in the manufacturer's instructions for use		P1+ strong	4	P1–	4	P1+	2
			P1+ weak	4				
anti-Le ^a	Normally clear coloured Potency titre greater than 4 vs by techniques detailed in the manufacturer's instructions for use Should detect variants and subgroups as detailed in the manufacturer's instructions for use		Le(a+b–)	4	Le(a–)	4	Le(a+)	2
anti-Le ^b	Normally clear coloured Potency titre greater than 4 vs by techniques detailed in the manufacturer's instructions for use Should detect variants and subgroups as detailed in the manufacturer's instructions for use	A ₁ B Le(a–b+)	A ₁ B Le(a–b+)	4	Le(b–)	4	Le(b+)	2
«Other»	Normally clear coloured. Potency titre greater than 2 vs by techniques detailed in the manufacturer's instructions for use. Should detect variants and subgroups as detailed in the manufacturer's instructions for use		Heterozygous positive	4	Antigen negative	4	Heterozygous positive	2»
* For reagents where reactivity against the antigen is claimed								

«For batch acceptance testing the user must ensure that the typing reagent reacts with the weakest available antigen expressing cells (refer to batch release positive reactors in table 11.3) and does not produce false positives with cells negative for the antigen.»

11.2.2: Anti-human globulin reagents

11.2.2.1: Introduction

Monoclonal antibodies have been developed which necessitate revision of the optimal composition of anti-human globulin reagents. For example, because of the limitations imposed by the presence of C3d on normal red cells, particularly in stored blood, conventional polyclonal anti-complement reagents rely on anti-C3c to detect *in vitro* bound complement and limited amounts of anti-C3d to detect *in vivo* bound complement. However, some monoclonal IgM anti-C3d reagents can be used at concentrations adequate to detect both *in vitro* and *in vivo* bound complement without causing unwanted positive reactions with normal red cells and fresh, inert, group-compatible serum in routine tests.

11.2.2.2: General requirements

- anti-IgG is the essential component since the majority of red cell alloantibodies are non-complement binding IgG.
- anti-complement should be present in reagents recommended for use with serum test samples.
- anti-light chain activity is desirable in reagents recommended for use with plasma test samples in order to detect IgM antibodies at levels unable to be detected in direct agglutination tests, especially with washed red cells.
- anti-C4d must be avoided. It is accepted that very low titres of anti-C4c may occur in reagents of animal origin.
- Reagents should be tested for the presence of heterospecific antibodies which can cause haemolysis or agglutination of unsensitised red cells in the indirect antiglobulin test and for the presence of unwanted positive reactions.

11.2.2.3: Performance evaluation

Performance evaluation should be undertaken in accordance with:

- BS EN 13612:2002 Performance Evaluation of *In Vitro* Diagnostic Medical Devices.
- Reagents listed in Annex II, List A, of the EU *In Vitro* Diagnostic Medical Devices Directive must also comply with the Common Technical Specifications for *In Vitro* Diagnostic Medical Devices (2009/108/EC).

Stability testing should be performed in accordance with:

- BS EN «ISO 23640 *In vitro* diagnostic medical devices. Evaluation of stability of in vitro diagnostic reagents.» ~~13640:2002 Stability Testing of In Vitro Diagnostic Reagents.~~

11.2.2.4: Batch release testing requirements

Specificity testing

Tests for IgM or IgG red cell heterospecific antibodies

- Heterospecific antibodies can cause haemolysis or agglutination of unsensitised red cells in the indirect antiglobulin test. Details of tests for heterospecific antibodies are outlined in section 11.4.

Requirements

- The anti-human globulin reagent should not agglutinate or haemolyse washed unsensitised red cells from two individuals of group A1 RhD positive, two individuals of group B RhD positive and two individuals of group O RhD positive, whether or not treated with proteolytic enzyme (e.g. papain, bromelain or ficin).

Tests for unwanted positive reactions

- These test for excess anti-C3d and anti-C3c, which can cause unwanted positive reactions in the indirect antiglobulin test, and for the presence of any undesirable antibodies in the reagent. Details of tests are outlined in section 11.4.

Requirements

- All reactions should be negative on macroscopic examination.

anti-IgG potency: polyspecific anti-human globulin and anti-IgG reagents for use in tube or microplate techniques

The anti-human globulin reference reagent should be tested in parallel with the test reagent, each being titrated against red cells sensitised with potent IgG anti-D antibody.

Requirements

- The potency titre of the test anti-human globulin or anti-IgG reagent should be at least equal to that of the reference reagent.

Potency tests

anti-IgG potency by chequerboard titration studies with red cells sensitised with weak IgG antibodies (anti-D, anti-K and anti-Fy^a)

- Test anti-human globulin or anti-IgG reagents against a selection of weak antibodies to determine the optimum potency. Antibody preparations should not be diluted and the use of single-donor antibody preparations is preferred. Antibodies should include:
 - an IgG anti-D to give an anti-human globulin potency titre of 8–32 using a pool of group O R₁r red cells from four individuals
 - an IgG to give an anti-human globulin potency titre of 8–32 using K+k+ red cells
 - an IgG anti-Fy^a, to give an anti-human globulin potency titre of 8–32 using Fy(a+b+) red cells.

Details of tests are outlined in section 11.4.

Requirements

- The anti-human globulin reagent or anti-IgG reagent is satisfactory if the reaction grade at all dilutions attains or exceeds that of the reference reagent without significant prozone, against red cells sensitised with all dilutions of the anti-D, anti-K and anti-Fy^a. In this context, a significant prozone is more than one grade difference between the reaction of the anti-human globulin reagent undiluted and 1 in 2.

anti-complement potency; polyspecific anti-human globulin reagents for use in tube tests

- Test anti-human globulin or anti-complement reagents against a selection of complement-coated red cells to determine the optimum potency. C3 and C4 complement-coated red cells should be prepared as described in section 11.4. In addition, anti-complement activity may be evaluated by tests with complement-fixing antibodies, such as anti-Jk^a.

Requirements

- The anti-human globulin reagent should have an anti-C4c titre of 1 in 2 or less.
- The anti-human globulin reagent should not affect a macroscopic reaction with EC4d red cells.
- The reagent should attain the potency titre of the reference reagent.
- Conventional (polyclonal) anti-human globulin or anti-human globulin containing monoclonal IgG anti-C3d that attain adequate reactivity with an optimal incubation period different from that recommended for the detection of IgG antibody, should state in the instructions for use the appropriate incubation period required for the optimum detection of red cell bound C3c/d complement components.

Tests for unwanted positive reactions

- These test for excess anti-C3d and anti-C3c, which can cause unwanted positive reactions in the indirect antiglobulin test, and for the presence of any undesirable antibodies in the reagent. Details of tests are outlined in section 11.4.
- All test results should be negative as defined by the manufacturer in the 'instructions for use'.

Instructions for use

The instructions for use for anti-human globulin reagents used in tube and microplate tests should also include a statement that:

- Inadequate washing of red cells in the anti-human globulin test may result in neutralisation of the anti-human globulin reagent.
- Following completion of the wash phase in the anti-human globulin test, excess residual saline may dilute the anti-human globulin reagent, when added, beyond that in the manufacturer's assessment.
- No single test is capable of detecting all clinically significant antibodies.
- For each batch of antibody screening being undertaken by an anti-human globulin test, a positive and negative control should be included. The positive control should be a weak anti-D (not more than 0.1 IU/mL); the negative control an inert serum, tested against the antibody screening cells being used.

11.2.3: Reagent red cells

11.2.3.1: Introduction

Reagent red cells prepared from human blood are essential in ensuring safe transfusion practice. They are used in the determination of ABO blood groups, in the control of blood grouping reagents and of the anti-human globulin technique, and in the detection and identification of «atypical» ~~irregular~~ red cell alloantibodies.

11.2.3.2: General guidelines for reagent red cell manufacture

- When testing reagent red cells, in order to confirm the presence or absence of antigens listed in the antigen profile, a sample from each individual should «be tested whenever possible, with a minimum of two antisera for each specificity prepared from different donors/cell lines.» ~~have the phenotype confirmed either by duplicate testing on this sample or by confirming a historical type by single testing on the current sample.~~
- «Where such testing produces conflicting results, repeat and further testing with at least one additional example of the relevant antibody(ies) should be undertaken to confirm the antigenic status of that cell.
- Where such testing has been performed with only one example of any blood grouping reagent, this information should be stated in the antigen profile included within the package insert.»
- Reagent red cells should be shown not to produce unwanted positive reactions by the methods recommended for use by the manufacturer.
- Except for IgG-sensitised and C3-sensitised red cells, reagent red cells should be negative in the direct anti-human globulin technique with anti-IgG, ~~anti-complement~~ and polyspecific anti-human globulin reagents.
- With the exception of umbilical cord blood, «alloabsorption and quantification cells,» red cells used to test a patient's samples for «atypical» ~~irregular~~ antibodies should not be pooled.
- Reagent red cells should be processed by a method and suspended in a medium that consistently ensures stability of the antigens specified in the antigen profile included within the package insert.
- «With the exception of controls for automated systems representing whole blood,» all red cell reagents should be free of ABH-specific blood group substances and blood group antibodies, including anti-A and anti-B, demonstrable by the manufacturer's recommended methods of use
- The method of manufacture should ensure that white cells are removed from donations of red cells before the white cells lyse and release enzymes, which may adversely affect the properties of the red cells.

11.2.3.3: Immediate container label and «/or» instructions for use sheet

The immediate container and instructions for use sheet for reagent red cells should also meet the following criteria:

- Include «a» ~~the~~ statement «regarding the use of» 'pooled cells', if cells are prepared from pooled material.
- Where reagent red cells are intended for use in ABO grouping or control of ABO or D blood grouping reagents, only the ABO and D group need be stated.
- When the reagent red cells are a multi-container product such as a red cell panel, the label on the immediate containers and packaging should be assigned the same identifying batch reference and carry a number or symbol to distinguish one container from another. This number or symbol should also appear in the antigenic profile.
- The date of expiry of reagent red cells should be stated on the antigenic profile.
- Where reagent red cells are provided suspended in preservative medium, the components of the medium should be stated in the instructions for use.
- The concentration and limits of the red cell suspension (~~e.g. 3 ± 0.2%~~) should be stated in the instructions for use.
- For enzyme-treated reagent red cells, information should be given in the instructions for use concerning those antigens which are rendered inactive or less active by the enzyme treatment used.

11.2.3.4: Reagent red cells for use in ABO and RhD grouping

- Reagent red cells should be groups A₁ and B. In addition, A₂, ~~B~~ or O red cells may be included.
- At least one of the set should be RhD positive and one RhD negative.

11.2.3.5: Reagent red cells for use in antibody screening

The detection of «atypical» ~~irregular~~ antibodies in the serum of a patient is of greater clinical significance than if such antibodies are detected in blood donors. Reagent red cells of a lesser specification may be used when performing antibody screening tests on blood donor samples.

In general the following should apply:

- Reagent red cells for use in antibody screening should be confirmed as group O by an ABO blood grouping procedure that is capable of demonstrating the A_x phenotype.
- Where practicable, reagent red cells known to express antigens having a frequency of less than 1% in the general population of the UK should not be included in reagent red cells for antibody screening.
- Where practicable, red cells from individuals known consistently to effect troublesome reactions with HLA antibodies should not be used as reagent red cells for antibody screening «of patients».

11.2.3.6: Reagent red cells for use in antibody screening of patient samples

- As a minimum the following antigens should be expressed on the reagent red cells for antibody screening:
C, c, D, E, e, K, k, Fy^a, Fy^b, Jk^a, Jk^b, S, s, M, N, P1, Le^a and Le^b.
- As a minimum, reagent red cells from two individuals should be provided. These red cells should not be pooled. One reagent red cell should be R₂R₂; the other R₁R₁ (or R₁^WR₁).
- Apparent homozygous expression of the following antigens is desirable:
Fy^a, Fy^b, Jk^a, Jk^b, S and s.

«11.2.3.7: Reagent red cells for use in antibody screening of patient samples who have received prophylactic anti-D

- For pregnant patients who have received prophylactic anti-D, as a minimum the following antigens should be expressed:
c, e, K, k, Fy^a, Fy^b, Jk^a, Jk^b, S, s, M, N, P1, Le^a and Le^b.
- The cells must be RhD negative
- As a minimum, reagent red cells from two individuals should be provided. These cells should not be pooled.
- Apparent homozygous expression of the following antigens is desirable:
Fy^a, Fy^b, Jk^a, Jk^b, S and s

These cells cannot be used in place of standard antibody screening cells (see 11.2.3.5 and 11.2.3.6).»

«11.2.3.8» ~~11.2.3.7~~: Reagent red cells for use in antibody screening of donor samples

- Reagent red cells may be:
 - provided unpooled from a minimum of two individuals
OR
 - as a pool of red cells in equal proportions from no more than two donors
OR
 - red cells from a single donor.

- Pooled reagent red cells for antibody screening should be used only for testing samples from blood donors, not samples from patients.
- As a minimum the following antigens should be expressed:
D, C, c, E, e and K.
- «To enhance the antigens of these screening cells they may be treated by proteolytic enzymes.»

«11.2.3.9» ~~11.2.3.8~~: Reagent red cells for use in antibody identification

- Reagent red cells for use in the identification of «atypical» ~~irregular~~ antibodies should be confirmed as group O by an ABO blood grouping procedure which is capable of demonstrating the A_x phenotype.
- Where practicable, red cells from individuals known consistently to effect troublesome reactions with HLA antibodies should not be used in reagent red cells for antibody identification.
- The antigen profile of reagent red cells for antibody identification should permit the identification of frequently encountered antibodies (e.g. anti-D, anti-E, anti-K and anti-Fy_a), and of commonly encountered alloantibody mixtures (e.g. anti-D+K).
- A red cell antibody identification panel comprises cells from eight or more individuals which should between them express the following antigens:
C, C^W, c, D, E, e, K, k, Kp^a, Fy^a, Fy^b, Jk^a, Jk^b, S, s, Le^a, Le^b, M, N, P1 and Lu^a.
- Red cells from one individual should be R₁R₁ and from another R₁^WR₁ and between them should express the antigens:
K, k, Fy^a, Fy^b, Jk^a, Jk^b, S and s.
- Red cells from one individual should be R₂R₂, another r⁺r and those from another r⁺r.
- Red cells from a minimum of three individuals should lack the Rh antigens C, E and D. One of these three individuals should be K positive. Between them, red cells from these individuals should exhibit apparent homozygous expression of the antigens:
c, k, Fy^a, Fy^b, Jk^a, Jk^b, S and s.

«11.2.3.10» ~~11.2.3.9~~: Reagent red cells (IgG-coated) for use in the control of the anti-human globulin technique

- To ensure that the anti-IgG activity in negative antiglobulin tests has not been fully or partially neutralised, control red cells 'sensitised' with IgG antibody are added to negative tests.
- Group O RhD positive red cells are sensitised with sufficient anti-D to render an indirect antiglobulin test negative when a volume of these sensitised red cells and a volume of serum diluted 1 in 1000 are added, but remains positive if a volume of saline instead of diluted serum is added.

«11.2.3.11: Reagent red cells for use in antibody in antibody strength determination (other than anti-D and anti-c)

Reagent red cells for use in the identification of atypical antibodies should be confirmed as group O by an ABO blood grouping procedure which is capable of demonstrating the A_x phenotype.

- As a minimum, reagent red cells from two individuals should be provided
- These red cells should not be pooled
- One D+ C+ E+ c- e+ (R₁R₂)
- One D+ C+ E+ c+ e+ (R₁R₂)
- Between them will show heterozygous expression of the following antigens: M, N, S, s, K, k, Fy^a, Fy^b, Jk^a and Jk^b
- They will be negative for W^r^a

11.2.3.12: Reagent red cells for use in patients with pan-reactive autoantibodies to determine the presence of underlying alloantibodies

Reagent red cells

- may be provided un-pooled OR as a pool of red cells from more than one donor
- should be selected to ensure the ID of underlying clinically significant antibodies to the following specificities;
C, C^W, c, D, E, K, Fy^a, Fy^b, Jk^a, Jk^b, S, s, M, N
- an R₂R₂ cell may be included to exclude the presence of an underlying allo anti-e in e negative patients
- Rh (D, C, E, c, e), K, Jk^a and Jk^b phenotypes must be provided for the users as a minimum.
- The cells may be enzyme treated which would result in them being negative for the following red cell antigens:
M, N, S, s, Fy^a and Fy^b

11.2.3.13: Reagent red cells for quantification of anti-D and anti-c antibody strength

Reagent red cells for use in quantification techniques should be confirmed as group O by an ABO blood grouping procedure which is capable of demonstrating the A_x phenotype.

Reagent red cells

- may be provided unpooled OR as a pool of red cells from more than one donor
- For anti-D quantification have the following phenotype: D+ C+ E– c– e+ (R₁R₁)
- For anti-c quantification have the following phenotype: D– C– E– c+ e+ (rr)
- The cells should be negative for C^W and K antigens
- The cells should be enzyme treated to enhance the antibody-antigen reaction»

«11.2.3.14» ~~11.2.3.10~~: Other reagent red cells

These reagent red cells should be manufactured in accordance with the «general» ~~relevant~~ guidelines «in section 11.2.3.2.» ~~above.~~

11.2.4: Miscellaneous reagents

11.2.4.1: Fetal calf serum and bovine serum albumin

When used in the formulation of reagents, fetal calf serum and bovine serum albumin should be obtained from a closed herd in the female line since 1980, in which no animal has been clinically suspected of having bovine spongiform encephalopathy (BSE), and which has not been fed rations containing ruminant-derived protein during that period.

Bovine albumin, usually supplied as a 20% or 30% solution, can be used as a constituent of a diluent for use in automated blood grouping antibody detection machines, for antibody quantification or as a potentiator in antisera, monoclonal reagents and anti-human globulin. When diluted and used in the system prescribed it should not cause:

- red cells to become T/Tk etc. transformed
- inhibition of antigen:antibody reactions
- false-positive reactions or rouleaux.

11.2.4.2: Proteolytic enzyme preparations

The activity of each batch of proteolytic enzyme should be assessed to ensure batch-to-batch consistency using a biochemical assay (e.g. azo-albumin technique).⁵

For manual antibody detection techniques, red blood cells treated with the enzyme should achieve activity comparable to that of the reference enzyme preparation 92/658 «used with an anti-D of 2.5 to 3.5 IU/mL.» ~~and associated reference anti-D 91/562.~~

For automated antibody detection techniques for patient pre-transfusion samples red blood cells treated with the enzyme should readily detect a weak anti-D of no more than 0.1 IU/mL (e.g. NIBSC anti-D standard for assessing operator and test performance as described at «www.nibsc.org» ~~www.nibsc.ac.uk~~).

For automated antibody detection techniques for donation testing the red blood cells treated with the enzyme should readily detect a weak anti-D of 0.5 IU/mL.

11.2.4.3: Water

The quality of water used in the production of a reagent should be adequate for that reagent. Ionic and non-ionic contaminants of water may interfere with components of reagents or may result in a conductivity or osmolality other than that intended. Water should have a conductivity of 1.0 µS/cm or less or a resistivity of 1.0 Mohm/cm or greater.

11.2.4.4: Saline

Saline is an isotonic solution containing 8.5 to 9.0 g/L NaCl (0.145–0.154 M) and should contain sufficient buffer to maintain pH 7.0 ±0.2 at 22 ±1°C during its shelf life.

11.2.4.5: Low ionic strength solution

The term low ionic strength solution (LISS) should not be used to denote a low ionic strength formulation other than that described by Moore and Mollison.⁶ LISS should not be used in place of preparations designed for a particular technology. LISS has the following properties:

- pH 6.5–7.0 at 22 ±1°C
- conductivity 3.4–4.0 mS/cm
- osmolality 285–305 mOsmol/kg.

The reactions obtained by an indirect antiglobulin test (IAT) with a weak anti-D and D positive cells suspended in LISS should be equal to, or better than, those obtained with the same cells suspended in saline and incubated at 37°C for 15 minutes.

11.2.4.6: Weak antibodies for use as controls in antibody «investigation» ~~detection~~ techniques

Weak antibodies, such as anti-D, -K, -Fy^a can be used to control antibody «investigation» ~~detection~~ techniques using indirect antiglobulin methods.

To act as a wash control the weak anti-D positive control could be diluted in serum or plasma. If the diluent is saline/bovine serum albumin, the control test could be positive, even though the cell washing was sub-optimal and this should be noted in the package insert.

These weak antibodies should:

- when used undiluted give a grade 2–4 reaction with red cells with homozygous antigen expression and have a mean IAT titre of 4 with the same cells.

For weak anti-D the antibody activity should be expressed in IU/mL.

«11.2.4.7: Antibodies and cells representative of patient samples i.e. whole blood controls to control automated systems

As a minimum two vials containing red cells and plasma combined:

- One sample to be RhD positive, the other RhD negative with anti-D in the RhD negative plasma
- Red cells of the same ABO/D group may be pooled
- The red cells must give an unequivocal positive reaction with the appropriate ABO/Rh D grouping reagents

- One sample will contain anti-K or other non-Rh antibody (the red cells for this sample must be antigen negative for the corresponding antibody)
- Anti-D and anti-K (or other non-Rh antibody) must give an unequivocal positive reaction with antigen positive red cells by IAT at 37°C
- The plasma component of the whole blood controls should be free of other blood group antibodies unless stated in the instructions for use

11.2.4.8: AB Serum

The reagent should be

- prepared from a pool of human group AB plasma or serum
- IAT antibody screen negative
- Negative for rouleaux inducing properties by direct agglutination at room temperature and by IAT and enzyme techniques at 37°C

11.2.4.9 Dithiothreitol (DTT)

DTT can be used to alter the red cell membrane and/ or reduce the disulphide bonds of IgM molecules and can be supplied at different concentrations to treat red cells and plasma samples or reagents.

11.2.4.10 Reagents for use in assessing the amount of D positive red cells in a suspected fetomaternal haemorrhage (FMH) by flow cytometry

Fluorescently labelled monoclonal antibodies used as a group of reagents to accurately determine the size of an RhD positive fetal bleed in an RhD negative person.

11.3: Reference preparations

11.3.1: Introduction

One of the major «regulatory requirements» ~~components of the EU Directive (98/79/EC) on In Vitro Diagnostic Medical Devices~~ is a requirement for traceability to reference materials of higher order. In the case of blood grouping reagents, ~~which come under Annex II of the Directive~~, there are several national and international reference preparations already available to manufacturers to ensure adequate potency of anti-A, anti-B and anti-D grouping reagents and the potency and/or performance of a number of other serology reagents or procedures, ~~for compliance with the Directive and the Guidelines for the Blood Transfusion Services in the United Kingdom.~~

As batch identifiers may change during the lifetime of these guidelines please refer to www.nibsc.org for guidance.

11.3.2: International Standards for minimum potency of anti-A and anti-B blood grouping reagents

These anti-A and anti-B preparations are the lyophilised residues of culture supernatants from murine monoclonal hybridomas BRIC 131 and ES4 respectively. The preparations, when reconstituted and diluted according to the supplied instructions, define the minimum acceptable potency of manufactured anti-A, anti-B, anti-A,B and anti-A+B blood grouping reagents, i.e. the titre of the grouping reagent should be at least equal to that of the appropriate minimum potency reference preparation.

11.3.3: International Standard for minimum potency of anti-D blood grouping reagents for use in direct tests

This preparation is the lyophilised residue of culture supernatant from a human-murine monoclonal heterohybridoma secreting an IgM anti-D (RUM-1). When reconstituted and diluted according to the supplied instructions, this material defines the minimum acceptable potency of anti-D grouping reagents in direct tube tests, i.e. the titre of the grouping reagent should be at least equal to that of the minimum potency reference preparation in tube tests using unmodified red cells and without additional agents.

11.3.4: International Council for Standardization in Hematology/International Society of Blood Transfusion (ICSH/ISBT) reference preparations for papain and anti-D

The intended use of these preparations is to ensure adequate sensitivity combined with freedom from false-positive reactions associated with some manufacturers' enzyme preparations and techniques. The recommended procedure is to test the papain reference material in conjunction with «a suitable anti-D preparation of 2.5 to 3.5 IU/mL» ~~the anti-D for use with papain standard~~ using a titration series for sensitivity, and a series of inert sera for false-positive reactions, according to the specified two-stage reference method in the product insert and to compare the titration scores with those obtained from testing the manufacturer's enzyme preparation in its recommended technique with the anti-D reference preparation and the inert sera.

~~11.3.5: ICSH/ISBT standard for anti-human globulin~~

~~This preparation consists of lyophilised rabbit antisera against human IgG blended with murine monoclonal anti-C3d. This is intended for use in the evaluation of anti-human globulin reagents containing either of these components, or polyspecific reagents containing them both.~~

«11.3.5» ~~11.3.6~~: UKBTS/NIBSC anti-D reference preparation for assuring operator and test performance

The current preparation («98/540» ~~07/304~~) consists of lyophilised human plasma with a reconstituted anti-D potency of «1.8» ~~1.0~~ IU/mL. At 1 in «20» ~~10~~ dilution, it is intended to be used to assure the efficacy of red cell washing prior to the addition of an antiglobulin reagent. At 1 in «40» ~~20~~ dilution, it is intended to be used in intra-laboratory monitoring to assess test operator variability in the detection of weak, macroscopic agglutination in the spin-tube antiglobulin test «or equivalent reaction grades using automated methods».

11.4: Recommended serological techniques for reagent testing

1.4.1: Potency titrations

11.4.1.1: Introduction

The use of a semi-automatic pipette is recommended; one volume being in the order of 40 µL. A separate pipette tip should be used for each reagent.

If the reagent is formulated with a medium to enhance its reactivity then the diluent for the determination of the potency titre should be a formulation identical to the reagent but with antibody protein replaced by non-antibody protein, e.g. fetal calf serum or bovine serum albumin. Otherwise, dilutions may be prepared in saline containing a final concentration of 20 g/L bovine serum albumin that has not been deliberately polymerised or otherwise potentiated.

Beginning with the undiluted blood grouping reagent, doubling dilutions (1 in 2, 1 in 4, 1 in 8 etc.) should be prepared. When preparing doubling dilutions, after the addition of the reagent or diluted reagent to an equal volume of the diluent, the tip of the pipette is emptied and blotted before the dilution is mixed and a volume transferred to prepare the subsequent dilution.

The potency titre is the reciprocal of the highest dilution of the reagent that effects a grade 2 reaction using «tube and microplate or a grade 1 endpoint in column agglutination technologies.» ~~the required technique.~~

The dilution caused by the addition of the cell suspension should not be considered in determining the potency titre.

11.4.1.2: Potency test methods for manual and microplate blood grouping reagents

Manual method – direct test

- Add one volume of each dilution of the reagent to a separate tube.
- Add one volume of 2–3% test red cell suspension to each tube.
- Mix thoroughly and incubate for the appropriate temperature and duration.
- Centrifuge and determine the reaction grade.

Manual method – indirect anti-human globulin test

- Add two volumes of each dilution of the reagent to a separate tube.
- Add one volume of 2–3% test red cell suspension in saline, or two volumes of 1.5–2% test red cell suspension in LISS.
- Mix thoroughly and incubate at 37°C for 45 minutes if the red cells are suspended in saline, or for 15 minutes if suspended in LISS.
- Wash the red cells four times.
- Add two volumes of anti-human globulin reagent to the button of test red cells. Mix. Centrifuge and determine the reaction grade.

Microplate method

Equipment

- Rigid polystyrene microplates with 'U'-shaped wells.
- Centrifuge with microplate carriers having a radius of at least 10 cm.
- Microplate shaker.
- Concave microplate reading mirror or automated plate reader.
- Red cells for microplate use, bromelain-treated if required.

Method

- Using a microplate, add one volume (25–50 µL) of each dilution of the reagent to one volume of 2–3% test red cells.
- Mix the contents of the wells using a microplate shaker. Incubate at 19–25°C for 15 minutes.
- Centrifuge the microplate at 100g for 40 seconds. Gently dislodge the red cells from the bottom of the wells using a microplate shaker.
- Determine the reaction grade using a concave mirror or automatic plate reader.

11.4.1.3: Avidity determination

- Mix over an oval area of approximately 20 mm × 40 mm on a glass slide, one volume of the undiluted reagent and one volume of a 30–45% red cell suspension in allogeneic serum or ABO group-compatible plasma.
- Maintain the slide at the recommended temperature for a slide test. If a range of incubation temperatures is given, for those blood grouping reagents where the antibody-antigen reaction is favoured by a colder temperature, the higher temperature should be used; for other blood grouping reagents, the lower temperature should be used.
- Determine the time from mixing at which macroscopic agglutination first appears and record the reaction grade at 1 minute.

11.4.1.4: Test used in performance evaluation and batch release testing of anti-human globulin

Tests for IgM and IgG red cell heterospecific antibodies

- These test for heterospecific antibodies which can cause haemolysis or agglutination of unsensitised red cells in the indirect antiglobulin test.

Method

- Divide 12 test tubes into two sets of six.
- Into each of the first set of tubes, add one volume of washed 2–3% untreated red cells in saline from two group A₁ RhD positive, two group B RhD positive and two group O RhD positive individuals.

- Into each of the second set of tubes add one volume of washed 2–3% enzyme-treated red cells (papain, bromelain or ficin) in saline from the same group A₁ RhD positive, group B RhD positive and group O RhD positive individuals.
- Add two volumes of the anti-human globulin reagent, as intended to be supplied for use, to each test tube. Mix thoroughly. Incubate the reactants for five minutes at 19–25°C.
- Centrifuge the tubes.
- Determine the reaction grade.

Control of enzyme treatment

- Weak IgG anti-D known to be reactive with enzyme-treated red cells should effect a positive reaction with each washed, enzyme-treated, red cell sample by the following method:
- To separate tubes, add one volume of the weak IgG anti-D to one volume of each of the washed, 2–3% suspension of enzyme-treated, RhD positive red cell samples. Mix thoroughly. Incubate for five minutes at 37°C. Centrifuge the tubes. Determine the reaction grade.
- The weak anti-D used for this purpose must be absorbed to remove anti-A or anti-B.
- Each of the enzyme-treated RhD positive red cell samples should be agglutinated by the weak IgG anti-D.

Tests for unwanted positive reactions

These test for excess anti-C3d and anti-C3c, which can cause unwanted positive reactions in the indirect antiglobulin test, and for the presence of any undesirable antibodies in the reagent.

Method for preparation of the red cell suspensions from segmented bleed line samples

- Select integral segment lines from two packs of group A₁, two packs of group B and two packs of group O blood stored at 2–6°C for at least 10 days.
- Wash each of the red cell samples with saline sufficient to remove serologically reactive traces of plasma.
- Prepare suspensions of each red cell sample as 2–3% in saline and as 1.5–2% in LISS.

Incubation of red cells and fresh group-compatible serum

- Each of the six red cell samples described above is tested as a saline and a LISS suspension with a different, fresh, group-compatible serum.
- For each anti-human globulin reagent to be assessed, prepare two sets of six tubes.
- To the first tube of the first set of six tubes and the first tube of the second set of six tubes, add 1 mL of a fresh, single-donor group-compatible serum. Add 1 mL of a second fresh, single-donor group-compatible serum to the second tube of each set, and so on for the six different, fresh, group-compatible sera.
- To the first tube of the first set of six tubes, add 0.5 mL of a red cell sample as a 2–3% suspension in saline. Add 1 mL of the same red cell sample as a 1.5–2% suspension in LISS to the first tube of the second set of six tubes. Add 0.5 mL of the second red cell sample as a 2–3% suspension in saline to the second tube of the first set of tubes and 1 mL of the same red cell sample as a 1.5–2% suspension in LISS to the second tube of the second set of tubes, and so on for each of the six different, red cell samples.
- Incubate the first set of tubes (saline suspended red cell samples) for 45 minutes at 37°C. Incubate the second set of tubes (LISS suspended red cell samples) for 15 minutes at 37°C.
- Wash the red cell samples with saline sufficient to remove serologically reactive traces of serum. Resuspend the red cells to 2–3% in saline.

Tests with anti-human globulin reagents

- For each anti-human globulin reagent, prepare two sets of six tubes. To each of the first set of six tubes, add in sequence one volume of the 2–3% suspension of washed red cells from the saline test above.
- To each of the second set of six tubes, add in sequence one volume of the washed 2–3% suspension of washed red cells from the LISS tests above.

- Add two volumes of undiluted anti-human globulin, as supplied for use, to each of the 12 tubes. Mix thoroughly.
- Centrifuge the tubes.
- Determine the reaction grade.

anti-IgG potency: polyspecific anti-human globulin and anti-IgG reagents for use in tube or microplate techniques

The anti-IgG reference reagent (see section 11.3.5) should be tested in parallel with the test reagent, each being titrated against red cells sensitised with potent IgG anti-D **antibody**.

Method

Test cells

- A 2–3% suspension in saline of washed pooled group O R₁r red cells is prepared from four individuals.

anti-D

- anti-D suitable for use in this application should have a potency titre of greater than 512.
- To 4 mL of the potent IgG anti-D add 2 mL of the 2–3% suspension of pooled group O R₁r red cells.
- Mix and incubate at 37°C for 45 minutes.
- Wash the red cell sample with saline sufficient to remove serologically reactive traces of serum. Prepare suspensions of each red cell sample as 2–3% in saline.

Technique

- Prepare 1 mL volumes of twofold serial dilutions of the test anti-human globulin reagent and anti-IgG reference preparation from 1 in 8 to 1 in 4096 (ten tubes).
- Prepare a set of ten tubes for each anti-human globulin reagent to be assessed.
- Place two volumes of each dilution into each of the series of ten tubes.
- Add one volume of the 2–3% suspension of pooled sensitised R₁r red cells to each tube, mix and centrifuge.
- Determine the potency titre.

Controls

The washed, strongly sensitised 2–3% suspension of R₁r red cells gives a negative result when centrifuged and gives negative results using the direct anti-human globulin technique with anti-complement (anti-C3c, anti-C3d, anti-C4c and anti-C4d) reagents and with anti-human globulin diluent in place of the anti-human globulin reagent. (The anti-complement specificities may be present as mixtures in one or more reagents.)

Test for anti-IgG potency by chequerboard titration studies with red cells sensitised with weak IgG antibodies (anti-D, anti-K and anti-Fy^a)

Selection of weak IgG antibody preparations

Antibody preparations should not be diluted to attain the following potency requirements. The use of single-donor antibody preparations is preferred.

The following are selected:

- an IgG anti-D to give an anti-human globulin potency titre of 8–32 using a pool of group O R₁r red cells from four individuals
- an IgG anti-K containing a final concentration of 0.014M EDTA neutralised to pH 7, to give an anti-human globulin potency titre of 8–32 using Kk red cells
- an IgG anti-Fy_a containing a final concentration of 0.014M EDTA neutralised to pH 7, to give an anti-human globulin potency titre of 8–32 using Fy(a+b+) red cells.

Test cells

Prepare 10 mL of a 2–3% suspension of washed R₁r red cells pooled in equal proportions from four individuals. Similarly, prepare 10 mL of a 2–3% suspension of washed K+k+ red cells and 10 mL of a 2–3% suspension of washed Fy(a+b+) red cells.

Sensitisation of test cells

anti-D

- Using a set of five containers each of 20 mL to 25 mL volume, prepare 4 mL volumes of serial twofold dilutions of the anti-D from undiluted to 1 in 16.
- Add 2 mL of the 2–3% suspension of pooled R₁r red cells in saline to each container. Mix and incubate at 37°C for 45 minutes.
- Wash the red cells four times with 20 mL volumes of saline at each wash and remove the last supernatant.
- Add 2 mL of saline to the packed washed red cells to prepare the 2–3% suspensions of sensitised red cells.

anti-K

As above, but using the anti-K with the K+k+ red cells.

anti-Fy^a

As above, but using the anti-Fy^a, with the Fy(a+b+) red cells.

Preparation of anti-IgG and/or anti-human globulin dilutions

For each anti-IgG and/or anti-human globulin under test and the anti-IgG reference preparation, prepare 2 mL volumes of twofold serial dilutions from undiluted, that is as supplied for use, to 1 in 16.

Test method for anti-IgG or antiglobulin potency by chequerboard titration

anti-D sensitised red cells

- Prepare five sets of five tubes for each anti-human globulin reagent under test and the anti-IgG reference reagent.
- Place two volumes of the anti-human globulin reagent, undiluted to 1 in 16, in the appropriate tubes for each of the five sets of five tubes.
- Using the 2–3% suspension of red cells sensitised with the undiluted anti-D for the first set of five tubes, the 2–3% suspension of red cells sensitised with the anti-D diluted 1 in 2 for the second set of five tubes, and so on, finishing with the 2–3% suspension of red cells sensitised using the anti-D diluted 1 in 16 for the fifth set of five tubes, add one volume of the washed red cells to each of the sets of anti-human globulin dilutions (see Table 11.4).
- Mix thoroughly. Centrifuge the tubes, appropriately.
- Determine the reaction grade.

Table 11.4 Chequerboard test format

Set	anti-D used to coat red cells	Dilution of anti-human globulin reagent				
		N	2	4	8	16
1	Undiluted					
2	1 in 2					
3	1 in 4					
4	1 in 8					
5	1 in 16					

anti-K sensitised red cells

As above, but using the anti-K sensitised K+k+ cells.

anti-Fy^a sensitised red cells

As above, but using the anti-Fy^a sensitised Fy(a+b+) cells.

Controls

The unwashed 2–3% red cell suspensions sensitised with the undiluted anti-D, anti-K and anti-Fy^a give negative results in a spin-tube test. The washed sensitised cells should not react with the diluent or the anti-complement components of the anti-human globulin reagents.

Test for anti-complement potency; polyspecific anti-human globulin reagents for use in tube tests⁷

Preparation of the complement sensitised red cells

Various very low ionic strength medium techniques are used to prepare the iC3b, C4b, C3d and C4d sensitised red cells that are necessary for the assessment of anti-complement activity.

The C3 and C4 activation states produced on red cells by the various methods are shown in Table 11.5.

As a minimum, red cell samples from two individuals are to be prepared and tested as described below.

anti-C4b potency

Method

- Prepare a set of three tubes for each anti-human globulin reagent under test.
- Prepare doubling dilutions of the anti-human globulin reagent from undiluted to 1 in 4.
- Place two volumes of each anti-human globulin dilution in the appropriate tubes.
- Add one volume of 2–3% EC4b red cells to each tube. Mix thoroughly. Centrifuge the tubes.
- Determine the reaction grade.

Controls

The EC4b cells do not react with anti-C3c, anti-C3d, anti-IgG or saline or the inert anti-human globulin diluent using the direct anti-human globulin technique. They react with anti-C4c and anti-C4d reagents.

anti-C4d potency

Method

- Place two volumes of undiluted anti-human globulin in a tube.
- Add one volume of 2–3% EC4d red cells. Mix thoroughly. Incubate for 5 minutes at 19–25°C.
- Centrifuge the tubes. Determine the reaction grade.

Controls

The EC4d cells do not react with anti-C3c, anti-C3d, anti-C4c, anti-IgG or saline or the inert anti-human globulin diluent using the direct anti-human globulin technique. The undiluted anti-human globulin does not agglutinate unsensitised red cells that have been trypsin-treated, using the direct anti-human globulin technique.

anti-C3d potency

Method

- Prepare a set of seven tubes for each anti-human globulin under test and the anti-C3d reference reagent (see section 11.3.5) which is tested in parallel, at the dilution for the 'immediate test' stated in its accompanying instructions for use.
- Place two volumes of each anti-human globulin dilution in each of the tubes (undiluted, that is as intended to be supplied for use, to 1 in 64).

- Add one volume of the 2–3% EC3d/EC4d red cells to each tube. Mix thoroughly and centrifuge the tubes, appropriately.
- Determine the reaction grade.

Controls

The EC3d/EC4d cells do not react with anti-C3c, anti-C4c, anti-IgG, saline or anti-human globulin diluent using the direct anti-human globulin technique. They do react with anti-C3d.

Table 11.5 Complement C3 and C4 activation

Method of preparation	Initial state	State after trypsin treatment
Very low ionic strength medium* 37°C	iC3b/C4b	iC3d/C4d
Very low ionic strength medium* 37°C	C3dg	C3d
Very low ionic strength medium* 37°C with EDTA	C4b	C4d
* These media are not to be confused with low ionic strength solution (LISS)		

11.5: References

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Chapter 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 he or she donates.

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 (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» ~~be validated and assured for~~ specificity and potency as per Table 11.3.

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 of these guidelines.

All mandatory tests must be performed using an automated test system in the first instance (see section 12.13). Any persistent failures may be resolved using manual methods (see section 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 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 guidelines in section 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, two anti-D blood grouping reagents should be used capable of detecting between them D^{IV}, D^V and D^{VI} antigens. If two monoclonal anti-Ds 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 one 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

- Quality control 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 section 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 11.2.3.7) may be:
 - provided from a minimum of two individual donations (not pooled); or
 - as a pool of red cells in equal proportions from no more than two 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	Antibody screen for blood for neonates	Donation plasma sample diluted 1 in 10	Donation plasma sample diluted 1 in 50
For neonatal use	Negative	Not applicable	Not applicable
Red cells in SAGM	Not applicable	Not applicable	Negative
All other components	Not applicable	Negative	Not applicable

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 one 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 1/128, 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 two 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

Blood grouping reagent	Test monitor red cell samples	
	Positive	Negative
anti-C	R ₁ r	ff-0f R ₂ r «or rr»
anti-E	R ₂ r or r''r	ff-0f 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 specialities	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.

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.

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

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)

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.

Chapter 13 Patient testing (red cell immunohaematology)

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13.1: Scope

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

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

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

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

And also comply with:

- «UKAS Medical Laboratory accreditation (ISO15189)⁵»
~~*The Clinical Pathology Accreditation (CPA-UK) Standard for Medical Laboratories⁵*~~
- The Blood Safety and Quality Regulations 2005⁶

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

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

13.2: Sample acceptance and labelling

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

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

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

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

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

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

13.3: Pre-transfusion testing

13.3.1: Resolution of anomalous grouping

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

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

Missing agglutinins in reverse grouping:

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

Unexpected additional reactions in the reverse group:

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

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

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

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

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

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

13.3.2: Antibody identification

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

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

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

~~When patients with rare phenotypes are encountered, laboratories should, when practicable, exchange material with other RCI departments via the UK Rare Red Cell Exchange to ensure continued supply of valuable materials.~~

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

13.3.2.1: Complex antibody mixtures

When investigating complex antibody mixtures RCI laboratories should consider:

- extended phenotyping of the patient, e.g. C, c, D, E, e, K, M, N, S, s, P₁, Le^a, Le^b, Fy^a, Fy^b, Jk^a, Jk^b
- if this is «not possible» impossible due to previous transfusion or heavy IgG sensitisation, «blood group» genotyping «should be considered» offers an alternative source of information
- extending the range of techniques and incubation temperature to identify component antibodies
- using cells matching the patient's phenotype/genotype to confirm the presence of multiple antibodies rather than an antibody to a high-frequency antigen
- careful use of alloadsorption «/ autoadsorption» techniques to confirm the specificity of elements of the mixture.

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

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

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

13.3.2.3: Antibodies to high-frequency antigens (HFA)

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

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

13.3.2.4: Antibodies to low-frequency antigens (LFA)

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

- testing the patient's plasma with a panel of red cells expressing LFA
- phenotyping the incompatible unit(s)«/cells» for LFA.

13.3.3: Autoantibodies

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

13.3.3.1: ABO and Rh grouping in the presence of autoantibodies

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

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

13.3.3.2: Alloantibody detection and identification in the presence of autoantibodies

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

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

13.3.4: Management of patients with autoantibodies

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

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

«13.3.5: Therapeutic monoclonal antibodies

A number of conditions are treated using therapeutic monoclonal antibodies (TMABs). These therapies have the potential to adversely interfere with serological investigations and compatibility testing in the blood bank, potentially causing unnecessary delays in providing blood components for transfusion. This may delay treatment of these patients, many of whom are transfusion dependent. Monoclonal antibody therapies may affect serological testing methods in a variety of ways, with the monoclonal antibody induced reactivity persisting for up to 6 months after the last treatment infusion.

The following testing protocols should be undertaken:

13.3.5.1 Management of patients on therapeutic monoclonal antibodies

Before TMAb therapy has started:

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

Once TMAb therapy has been commenced:

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

13.4: Antibody quantification and titration

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

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

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

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

13.4.2: Antibody titration of antibodies capable of causing HDFN

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

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

13.4.3: Antibody titration in ABO mismatched transplant

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

- the use of the organ donor's cells for titration

- inactivation of the IgM component of ABO antibodies (e.g. dithiothreitol (DTT) treatment)
- «participate in NEQAS BTLP ABO titration scheme, regularly review results and act on the findings»

«13.5: Fetomaternal haemorrhage estimation by flow cytometry

13.5.1 Indications for testing

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

13.5.2 Procedures for testing in the RCI laboratory

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

Flow Cytometry procedure

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

«13.6»~~13.5~~: Post-examination

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

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

Results reported by «email should be to a secure NHS email address» ~~fax should be to a designated fax number~~. The sender should confirm the «designated email address» ~~telephone number of the receiving fax machine and the designated member of staff to whom the report is to be addressed~~. The sender should indicate when the report will be sent and, following «email» ~~fax~~ transmission of the report, confirmation that the «email» ~~fax~~ has been received should be obtained from the intended recipient.

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

«13.7: References

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- ~~7. Daniels G, Poole J, de Silva M, Callaghan T, MacLennan S, Smith N (2002). The clinical significance of blood group antibodies. *Transfusion Medicine*, 12, 287–295.~~
- ~~8. Caldicott Report. Available at www.dh.gov.uk/prod_consum_dh/groups/dh_digitalassets/@dh/@en/documents/digitalasset/dh_4068404.pdf.~~
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Chapter 14 Guidelines for the use of DNA/PCR techniques in Blood Establishments [top](#)

14.1: Safety precautions

All human cells should be treated as potentially infectious. Materials should be handled and discarded according to in-house documented procedures for potentially infectious biological materials.

Operators working with ultraviolet (UV) light should wear opaque gloves and a UV protective visor, appropriate to the wavelength emitted. Exposure should be kept to a minimum.

~~Operators should wear nitrile gloves when handling~~ «Alternatives to» ethidium bromide «for gel-based detection of PCR products should be used where possible». ~~Liquid preparations of ethidium bromide are available from commercial sources and are preferable to handling the powder form.~~

14.2: Avoidance of contamination

DNA should be purified by a standard method that has been reported to the scientific literature and validated in the laboratory. DNA should be suitably stored to protect the integrity of the material.

During the preparation of genomic DNA, great care should be taken to avoid contamination from any other source of DNA. Pre-polymerase chain reaction (PCR) and post-PCR procedures should be undertaken in separate areas and using separate laboratory coats in each area. The laboratory should have documented procedures which have been constructed to eliminate potential causes of contamination, including training of the operator. If contamination does occur, all procedures should be reviewed and appropriate corrective action taken. Proposed change to procedures should be validated prior to their introduction.

In order to avoid contamination, the use of separate working stations or clearly defined work areas is beneficial for each stage of the PCR process. For example:

- One to prepare reagents. This is particularly important to avoid contamination of primers.
- One dedicated to pre-PCR manipulation (e.g. DNA isolation). A Class II laminar flow cabinet should avoid contamination of the sample with DNA from the operator.
- One dedicated to setting up PCRs.
- One for manipulation of PCR-amplified DNA. PCR-amplified products should be kept away from areas used for pre-amplification manipulation and reagent preparation.

Each ~~working~~ station should be adequately and independently equipped. However, the use of such ~~working~~ stations should not absolve the laboratory from «ensuring» procedures «are» constructed to eliminate contamination.

Examples of measures which will help to minimise contamination include:

- the use of new sterilised, disposable plastic tubes or glassware for handling DNA
- the use of freshly prepared and sterilised materials and reagents when making up solutions for DNA samples, particularly dH₂O and ~~Tris~~ buffers
- «~~aliquoting~~» ~~aliquotting~~ reagents in small amounts to minimise the number of repeat samplings
- the «~~changing~~» ~~change~~ of gloves and coats when moving between the areas dedicated for pre- and post-PCR manipulations
- the use of positive displacement dedicated pipettes or plugged tips to carry out PCR preparations
- routine wipe-tests of pre-amplification work areas should be performed. If an amplified product is detected, the area must be cleaned to eliminate the contamination, re-tested and measures taken to prevent future contamination
- reagents used for amplification must not be exposed to post-amplification work areas.

14.3: Working practices

- DNA should be as intact as possible. ~~Degraded DNA should be avoided.~~
- An archival record (e.g. photograph «or electronic image») of each «post-PCR» ~~electrophoretic~~ run should be retained.
- The performance of «non-commercial kit based» probes and primers «must» ~~should~~ be fully validated and characterised before they are put into use. ~~Others should be used only for research purposes.~~
- Reagents (e.g. chemicals, enzymes) «must» ~~should~~ be stored and utilised under conditions recommended by the manufacturer, including, for example, storage temperature, test temperature, shelf life, diluent buffer and concentration for use.
- Each lot of reagents must be tested before use in routine typing.
- For reagents and kits, the source, lot number, expiration date and storage conditions should be documented.
- Users should have procedures to ensure that periodic checks of probes and primers are carried out to detect their deteriorating performance or contamination.
- Thermal cyclers should be serviced at least annually according to the manufacturer's recommendations and a temperature calibration should be performed. A record of the service and calibration checks should be maintained.
- «When using non-commercial kit testing methods» laboratories should regularly check their primer sequences for newly discovered single nucleotide polymorphisms. This can be done «via the website for the National Genetics References Laboratories (ngri.org.uk).» ~~by checking the National Genetic Reference Laboratory website at <https://ngri.manchester.ac.uk/SNPCheckV3/snpcheck.htm>~~
- «Software used for analysis of results must be validated before use and updated regularly with appropriate allele sequences.»

Chapter 15 Molecular typing for red cell antigens

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15.1: Introduction

Genes for all of the blood group systems have been isolated and the molecular bases for most of the clinically important blood group antigens are known. So it is now possible to predict, with a high level of accuracy, most blood group phenotypes from genomic DNA.

This technology is generally applied when:

- we need to know a blood group phenotype, but do not have a suitable red cell sample
- molecular testing will provide more or better information than serological testing
- molecular testing is more efficient or more cost-effective than serological testing.

15.2: Clinical applications of blood group molecular typing

Various clinical applications of blood group molecular typing are listed below:

- **Fetal typing:**¹ Typing of fetuses, usually for D, but also K, C, c or E, of alloimmunised women, to assess whether the fetus is at risk of haemolytic disease of the fetus and newborn (HDFN). The DNA source is cell-free fetal DNA in the mother's plasma. ~~In the future~~ This technology ~~«is now also»~~ ~~may be~~ applied to ~~«high-throughput non-invasive prenatal testing (NIPT) for fetal RHD genotype of»~~ ~~all~~ D negative pregnant women~~«2»~~ to determine their requirement for antenatal anti-D prophylaxis.
- **Transfused patients:** Typing of multiply transfused patients, where serological testing cannot be used because of the presence of transfused red cells.
- **Immunoglobulin-coated red cells:** Typing of red cells giving a positive direct antiglobulin test (DAT), usually in patients with autoimmune haemolytic anaemia, to help in the identification of underlying alloantibodies.
- **Determining Rh variants:** Molecular methods are used for identifying Rh variants, especially the weak and partial variants of D, to assist in the provision of the most suitable blood for transfusion.
- **Confirmation of D negative:** Detection of *RHD* in an apparently D negative donor could signal very weak D expression, which could immunise a D negative patient.
- ***RHD* zygosity:** Quantitative PCR can reveal whether a D positive person is homozygous or hemizygous for *RHD*. This cannot be done by serological methods. Testing fathers of fetuses at risk of HDFN provides limited information on the D type of the fetus.
- **Testing when suitable reagents are not available:** Molecular methods can replace serological methods when suitable serological reagents are unreliable or not available, e.g. Dombrock typing of donors.
- **Supporting the serological reference laboratory:** Molecular methods are valuable for supporting the serological reference laboratory in sorting out difficult problems.

15.2.1: Testing donors for multiple blood groups

It is probable that molecular methods will replace serology in the near future for testing donors for multiple blood groups. The new high-throughput molecular technology will be more accurate than serological methods and will probably be more cost-effective. Molecular tests could also be applied to screening for donors with rare blood group phenotypes such as S–, U–, Lu(b–), k–, Js(b–), Yt(a–), ~~or~~ Co(a–) ~~«and»~~ ~~–plus~~ Vel– ~~when the molecular basis has been elucidated~~.

For prediction of blood group phenotypes from DNA of donors, results should either be confirmed by serological testing or by testing twice by molecular methods. This does not apply to ABO and RhD, which are always determined by serological testing.

15.3: ABO typing by molecular genetics

Whereas ABO typing by serological means is straightforward and extremely accurate, the genetics of ABO is complex, rendering ABO molecular typing by available methods unreliable. This is particularly so in people of African origin, where

hybrid ABO alleles are present. As it is never acceptable to obtain a false ABO typing, prediction of ABO phenotype by molecular methods is not currently recommended.

15.4: Methods available for molecular blood grouping

15.4.1: Fetal typing

The usual technology employed for fetal blood group typing, in which the mother lacks the antigen to be tested, is real-time quantitative PCR (RQ-PCR) on cell-free DNA isolated from the maternal plasma. For D, probes and primers are designed to detect two ~~«to four»~~ ~~or three~~ regions of *RHD*. ~~«There are numerous variants of D that could give rise to a false answer. Any test for D must reveal the D negative genes *RHD* Ψ and *RHD-CE-D^S*, which are common in people of African origin. Testing for at least *RHD* exons 5 and 7, with the test for the former being designed to give a negative result with *RHD* Ψ , is the minimum required.~~

Tests for fetal C, c, E and K involve RQ-PCR with allele-specific primers.

A test for the housekeeping gene *CCR5* is also included to confirm that DNA is present and that there is not an excess of maternal DNA.

The following DNA controls for fetal *RHD* are used: *RHD* positive; *RHD* negative and *RHD* pseudogene positive. The control DNA can be cell-free fetal DNA from maternal plasma, or cell-derived DNA appropriately diluted to simulate fetal DNA. In addition, an International Reference Reagent is available to purchase from the National Institute for Biological Standards and Control (see Annex 1 and www.nibsc.org), to use as a standard for minimum acceptable potency for the detection of *RHD/SRY* in cell-free plasma DNA³.»

~~Tests for fetal C, c, E and K involve RQ-PCR with allele-specific primers. A test for the housekeeping gene *CCR5* is also included to confirm that DNA is present and that there is not an excess of maternal DNA.~~

~~Three controls for fetal *RHD* are used: DNA from D negative pregnant women containing DNA from a D positive fetus, a D negative fetus, and from a D negative fetus with *RHD* Ψ .~~

Positive and negative ~~fetal~~ DNA controls are used for C, c, E and K.

15.4.2: Typing from DNA obtained from peripheral blood

There ~~«are»~~ ~~is a large~~ variety of platforms for detecting single nucleotide polymorphisms for the purpose of predicting blood group phenotypes of donors and patients from genomic DNA isolated from blood. ~~«4.5»2.3~~ These include low-throughput methods involving allele-specific primers and gel electrophoresis, a very comprehensive DNA microarray platform that identifies many D variants, and higher throughput platforms such as allelic discrimination technology and platforms involving the application of fluorescent beads coated with oligonucleotide probes. Those platforms that offer the possibility of high-throughput testing do not include testing for ABO or D.

The usual tests for blood group polymorphisms that would be required for testing donors and patients would be D, C, c, E, e, M, N, S, s, K, k, Fy^a, Fy^b, Fy-null, Jk^a, Jk^b, Do^a and Do^b. Often some others are also included. See Table 15.1.

Homozygous positive, homozygous negative and heterozygous controls are used when available. In addition, International Reference Reagents which can be purchased from the National Institute for Biological Standards and Control (see Annex 1 and www.nibsc.org) ~~www.nibsc-ae-uk~~ could be useful in the standardisation of blood group genotyping.

There are certain precautions that are required for all molecular testing and they are described in Chapter 14. In addition, there are certain tests in molecular blood grouping that must be carried out to ensure a reasonable level of accuracy. The hazards of ABO grouping are described above. There are numerous variants of D that could give rise to a false answer. Any test for D must reveal the D negative genes *RHD* Ψ and *RHD-CE-D^S*, which are common in people of African origin. Testing for at least *RHD* exons 5 and 7, with the test for the former being designed to give a negative result with *RHD* Ψ , is the minimum required. C typing should not depend on the *RHCE* nucleotide 48 polymorphism; testing for the *RHCE* intron 2 insert is more reliable. Duffy typing must include a test for the *GATA* mutation to detect the common silent allele.

15.5: External quality assurance

~~Although there is no National External Quality Assurance Scheme (NEQAS) for molecular blood grouping,~~ It is important that any laboratory performing this testing for clinical purposes participates in some sort of external quality assurance scheme. ~~«There is a National External Quality Assurance Scheme (NEQAS) available for molecular blood grouping.»~~ The International Society of Blood Transfusion (ISBT) organises workshops every two years in which DNA samples from 'patients' and plasma from D negative pregnant women are distributed. In addition, it ~~«may be»~~ ~~is~~ possible to set up sample-exchange schemes with other laboratories carrying out similar work.

Table 15.1 Some blood group polymorphisms and associated gene sequence changes

System	Gene	Antigens	Molecular test
MNS	<i>GYP A</i>	M/N	59 C/T, 71 G/A
Rh	<i>RHD</i>	D	Presence/absence
	<i>RHCE</i>	C	Intron 2 insertion
		c	307 C
		E/e	676 G/C
Lutheran	<i>LU</i> or <i>BCAM</i>	Lu ^a /Lu ^b	230 A/G
Kell	<i>KEL</i>	K/k	578 T/C
		Kp ^a /Kp ^b	841 T/C
		Js ^a /Js ^b	1790 C/T
Duffy	<i>FY</i> or « <i>ACKR1</i> » <i>DARC</i>	Fy ^a /Fy ^b	125 G/A
		Fy-null	-67t>c
Kidd	<i>JK</i> or <i>SLC14A1</i>	Jk ^a /Jk ^b	838 G/A
Diego	<i>DI</i> or <i>SLC4A1</i>	Di ^a /Di ^b	2561 T/C
Yt	<i>YT</i> or <i>ACHE</i>	Yt ^a /Yt ^b	1057 C/A
Dombrock	<i>DO</i> or <i>ART4</i>	Do ^a /Do ^b	793 A/G
Colton	<i>CO</i> or <i>AQP1</i>	Co ^a /Co ^b	134 C/T

15.6: References

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Chapter 16 HLA typing and HLA serology

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16.1: Preamble

~~For the eighth edition we have made significant changes in both the structure and content to the previous version of this chapter concerning human leucocyte antigen (HLA) typing and antibody testing. This obviously reflects the continuing scientific and technical development in the field.~~ DNA-based testing for HLA alleles ~~«has now completely replaced»~~ **now predominates over** serological phenotyping and antibody detection/characterisation ~~mostly~~ involves non-cell-based methods. This HLA section is constructed of three main parts, concerning reagents (section 16.4), testing (sections 16.5, 16.6 and 16.7), and application to donor and patient investigations (section 16.8). For certain patient or donor investigations there is, of course, an overlap with the guidance given in this and the granulocyte ~~«/»-or~~ platelet immunology chapters (Chapters 17 and 18 respectively). This is particularly relevant to the laboratory investigations of platelet refractoriness and transfusion-related acute lung injury (TRALI), so diagrams are included (Figures 16.1 and 16.2) to indicate how the different guidelines relate to each other.

16.2: Introduction

The transfusion or transplantation of blood components bearing allogeneic HLA can stimulate clinically significant immunological responses. All cellular components except erythrocytes express HLA and any plasma-containing product may include HLA-specific antibodies which are potentially harmful to the recipient.

Prospective HLA typing of platelet donors is undertaken for transfusion of immune refractory patients and those with disorders of platelet function and structure. Potential haematopoietic progenitor cell (HPC) donors are HLA typed to be placed on one of the national donor registries.

HLA typing or antibody investigations may be undertaken for diagnostic purposes or to investigate harmful consequences of transfusion. Thus the diagnosis of immune refractoriness requires the demonstration of HLA-specific antibodies (or other platelet-specific antibodies) in the patient. As part of the investigation of TRALI, implicated donors are screened for HLA (and human neutrophil antigen, HNA)-specific antibodies and the patient is HLA typed if possible.

The European Federation for Immunogenetics (EFI) has established standards¹ (at the time of writing, version ~~«8.0»~~ **5.7**) for histocompatibility testing and where appropriate ~~«the relevant EFI Standards must be followed.»~~ **these guidelines will refer to the relevant EFI Standard, which will be stated in the text. In general, guidance for practice is indicated by the term 'should'. The use of the term 'must' is mostly limited to circumstances where an EFI Standard applies.**

16.3: Terminology and nomenclature

All HLA assignments, irrespective of the method, must comply with the ~~«latest report of the»~~ **current** WHO Nomenclature Committee for Factors of the HLA System² ~~Report 2 and Nomenclature for Factors of the HLA System, 20103 (and see EFI Standards D1.000–D1.320, inclusive). Examples of acceptable HLA assignments are as follows: HLA-B12, HLA-B44, HLA-B 44(12), HLA-B*44, HLA-B*44:09.~~

HLA ~~«typing is now performed»~~ **may be serologically typed (to determine the phenotype) or typed** by DNA molecular analysis. ~~The term genotype is properly used to describe the genetic (DNA) constitution determined by the pattern of inheritance (EFI Standard D1.230).~~

HLA typing by DNA-based ~~molecular~~ techniques, ~~which~~ employ ~~«s»~~ either ~~«sequencing or»~~ DNA-based probes ~~«/»-or~~ primers ~~«to»~~, type for the presence or absence of sequence motifs. Kits using this technology are able to define the HLA alleles present in an individual to a variable level of resolution dependent on a number of factors. These include the number of probes or primers employed, the number of alleles defined for a given locus and the HLA alleles present in the individual. Although it is possible to achieve a high resolution or allele level typing using molecular methods, it is not a clinical requirement in transfusion practice. ~~Therefore, patients and donors are typed to a low or medium level of resolution, and may present HLA typing results that include some ambiguity in interpretation.~~

Each serologically defined HLA antigenic specificity may be encoded by a number of different HLA alleles. Conversely many HLA alleles have no determined serologically defined antigen. Thus it is not always possible to assign a serological equivalent to each HLA allele.⁴ ~~One consequence of this is that it is not practical to subject serological and DNA-based typing to the same standard as this would need to be unacceptably low (i.e. the lowest common denominator). Both techniques are in general use, each having specific advantages and disadvantages, and under these circumstances professional judgement together with the following guidelines should be used to deliver an appropriate standard of HLA typing.~~

«HLA typing results must conform to the recommendations of the WHO Nomenclature committee. Examples of suitable reporting formats as referenced in EFI Standards v.8.0 include the following:

- Single alleles: HLA-B*07. Single antigens: HLA-B7
- DNA assignment: HLA-A*02,*30; B*07,*44; C*07,*16; DRB1*01,*04; DQB1*05,*03:01
- Serological assignment: HLA-A2,30; B7,44; Cw7; DR1,4; DQ5,7

If an HLA typing is performed using DNA methods, it is acceptable to report an HLA serological assignment if required for the purposes of e.g. HLA matched platelet allocation. The translation of alleles to serological equivalence must be performed according to a documented protocol.»

Caution should ~~therefore~~ be exercised if an HLA type assigned using DNA-based molecular techniques is converted into a serological equivalent and such conversion must always be avoided with alleles for which the phenotype has not been unequivocally defined.

16.4: Reagents

16.4.1: General guidelines

~~HLA reagents prepared from human source material should comply with the guidelines in section 11.1.4.10.~~

~~Exceptionally, reagents not tested at source as required in section 11.1.4.10, and for which no alternative exists, may be supplied for use with the expressed approval of the user and with the understanding that the reagent must be regarded as potentially infectious.~~

~~These reagents should be marked 'Potentially infectious— not tested at source for...'; as appropriate, both on the immediate container label or multi-well tray or reservoir, and the outer packaging.~~

~~The instructions for use of these reagents should indicate that the reagent(s) has not been tested at source as required in section 11.1.4.10, and that the reagents are to be considered as potentially infectious. In addition, the package insert should give information on the safe disposal of the material and the container, multi-well tray or reservoir.~~

16.4.1.1: Immediate container label

~~HLA reagents issued separately: The label should conform to the requirements of EN ISO 18113:2009. In addition, the body of a container presented in sealed bags or foiled pouches should be marked with a unique identifier to enable identification and traceability.~~

~~Multi-component test systems: In addition to the label information required above, a test system comprising multiple components should be marked to ensure identification and traceability of all components, for example multi-well trays or reservoirs, strips and pre-prepared membranes.~~

~~The instructions for use should contain the information required by EN ISO 18113:2009, and should comply, where applicable, with the requirements of section 11.1.4.12.~~

16.4.2: Serological typing reagents

~~The following information must be provided for each individual serological HLA typing reagent or HLA typing set:~~

- ~~The claimed HLA specificity(ies) of the reagent, the percentage of specific reactions giving a cytotoxicity score of 80% to 100% cell death, the values of the correlation coefficient r obtained by the pre-testing of the reagent against a well-characterised cell panel, and the reaction score.~~
- ~~The manufacturer should provide information of the incidence of equivocal cytotoxicity scores within the package insert.~~
- ~~HLA typing sets should include a representation of the multi-well tray or reservoir layout indicating the position, HLA specificity(ies) and batch (or sub-batch) reference of the HLA typing reagent contained in each well.~~
- ~~Monoclonal antibodies should be identified as such.~~
- ~~An instruction that thawing and refreezing of the HLA typing reagents should be kept to the absolute minimum from the date of manufacture to the date of use. HLA typing sera frozen in micro-well trays should be used within 1 hour of thawing. Sera supplied freeze-dried in micro-well trays should be used within 1 hour of their reconstitution; unused trays should not be refrozen for later use.~~

- ~~When reagents are supplied as an HLA typing set for the detection of a single antigen, the instructions for use should indicate which controls are appropriate to demonstrate specificity and cross-reactivity.~~
- ~~For HLA typing sets, a list should be provided of those specificities that cannot be adequately defined in the presence of other specified specificities.~~
- ~~Each HLA typing set for Class I or Class II phenotyping should contain at least one positive control antibody preparation, previously shown to react with all target cells, and should include at least one negative control preparation, previously shown to lack antibody activity or be from a male with no history of blood transfusion.~~

16.4.2.1: Preservation

~~HLA typing reagents may be preserved in the liquid or in the dried state. Reagents should be stored as recommended by the manufacturer.~~

~~HLA typing reagents, after being thawed or reconstituted, should be transparent and should not contain any sediment, gel or particles visible on microscopy ($\times 200$).~~

16.4.2.2: Stability and expiry date

~~Manufacturers should ensure that HLA typing reagents have a shelf life of at least 1 year, when stored as recommended.~~

~~Any extension by the user of the expiry date stated by the manufacturer should be supported by documented test data.~~

~~Manufacturers should notify all primary users if an HLA typing reagent or a constituent reagent of an HLA typing set stored as recommended fails to perform satisfactorily within the expiry date allotted by the manufacturer.~~

16.4.3: Requirements for phenotype assignment

~~HLA Class I and Class II serological typing must comply with EFL Standards E1.000 to E2.740 inclusive. HLA reagents and kits to be used for phenotyping lymphocytes by cytotoxicity should comply with the following:~~

- ~~HLA typing reagents, when used by all methods recommended by the producer, should react with all lymphocyte samples with the corresponding antigen(s) when tested against a panel of lymphocyte samples bearing those antigen(s) collected from at least 25 individuals. HLA typing reagents should not react with any lymphocyte samples when tested against a panel of lymphocyte samples known not to bear the corresponding antigen(s) collected from at least 100 individuals. Reagents that conform to the requirements of this paragraph are termed operationally monospecific.~~
- ~~Not more than half of the HLA typing reagents used together to detect an antigen should have the same extra claimed specificity.~~
- ~~None of the HLA typing reagents used together should have been shown to react with more than 5% of the separate samples of a lymphocyte panel which do not express any of the antigen(s) that the reagent is claimed to detect.~~
- ~~Manufacturers should indicate in the instructions for use those specificities whose detection does not comply with the requirements of any of the above.~~

16.4.4: Rabbit complement for use in HLA serology

16.4.4.1: General guidelines

~~Rabbit complement supplied for use in HLA serology should be stored as recommended by the manufacturer.~~

~~Manufacturers should be aware that highly active complement can cause unwanted specificities to become apparent in HLA typing reagents that have been characterised on less active but adequate complement.~~

16.4.4.2: Immediate container label

~~The label of the immediate container of rabbit complement for use in HLA serology should conform to the specifications in section 11.1.4.11.~~

16.4.4.3: Instructions for use

~~The instructions for use supplied with rabbit complement for use in HLA serology should conform to the specifications in section 11.1.4.12.~~

~~The instructions for use should offer guidance on the method of thawing. In addition, they should contain an instruction that the complement, once thawed from the immediate container or reconstituted from the freeze-dried state, should not be refrozen.~~

~~The instructions for use should state whether the rabbit complement has been tested and found suitable for use with monoclonal HLA typing reagents.~~

16.4.4.4: Potency tests on rabbit complement for use in HLA serology

~~Rabbit complement should be tested prior to use, in accordance with EFI Standards E2.700–E2.740.~~

«16.4.1:» 16.4.5: DNA typing reagents

Methods available for HLA typing of DNA samples rely on identification of polymorphic HLA gene sequence motifs. In all widely used methods, the polymerase chain reaction (PCR) is utilised, either through the use of sequence-specific primers as in PCR-SSP, or to produce a locus-specific DNA template (e.g. HLA-A) which can subsequently be typed using a panel of sequence-specific oligonucleotide probes (PCR-SSOP). The locus-specific template may also be directly sequenced ~~using locus or allele group-specific sequencing primers.~~

DNA can be prepared from various tissues by a variety of methods. The laboratory should prepare DNA by a standard method that has been reported in the scientific literature and validated in the laboratory for the HLA typing method to be used.

«16.4.1.1:» 16.4.5.1: Instructions for use

In addition to section 11.1.4.12 of these guidelines, the instructions for use must adhere to the «relevant» EFI Standards ~~for Nucleic Acid Analysis (Section L)~~ and should include the following:

- a statement explaining the test and intended application of the kit
- the principle of the procedure
- reagents and equipment required to perform the test
- detailed instructions for all components of the test
- the gene targeted as a PCR amplification control (PCR-SSP)
- the specificity and nucleotide sequence of all primers and probes used in the HLA typing kit
- a table or diagram indicating the location of the probes and/or primers utilised in the test
- a list of ambiguous combinations of alleles defined for each test kit – this may also be given as part of interpretative software
- the HLA alleles which are claimed to be detected by the HLA typing kit, further divided into the following groups:
 - those HLA alleles which have been detected in appropriately controlled validation tests
 - those HLA alleles which have not been directly detected in validation tests but where the reactivity of the allele is expected to be detected
 - those HLA alleles which have not been directly detected in validation tests and whose reactivity cannot be assumed to be detected by the kit
 - those HLA alleles that are known to produce weak or unreliable signals in the output systems
- the date and the source of the sequence information used in the kit design and a statement that new alleles described following the date of design may not be detected by the kit
- the control tests to be performed to check for contamination (negative control) of the test system
- the control DNA to be included to check for quality of sample DNA used

- the control test to be performed to generate a true positive signal
- acceptable limits of signal intensity should be specified for positive and negative results
- all computer software assisted interpretation of results should be validated on control DNA
- the chemical components of the kits should be listed and reference made to any toxic substances included in the kit with recommendations for their safe disposal. Reference to material safety data sheets should be given.

«16.4.1.2:» ~~16.4.5.2:~~ **Requirements**

Manufacturers should inform all primary users of a DNA-based HLA typing kit when any changes to a kit's ability to perform are detected. All users of DNA-based HLA typing kits should report any kit-related problems directly to the manufacturer and maintain records of such events.

«16.4.2: HLA Antibody Testing Reagents

All commercial HLA antibody test kits should be CE and in vitro device (IVD) marked and validated for use. Each batch of commercial test kit or in-house panel should be evaluated against a minimum of three sera of known HLA specificity from different cross-reacting groups.

HLA-specific antibodies may be detected by solid-phase bound, purified HLA molecules, or particle bound, purified HLA molecules. If such techniques are used for screening (i.e. not characterisation of specificity) the following apply:

- There should be discrimination between HLA Class I and Class II-specific antibodies.
- Overall the target cells or molecules should cover either all the known HLA immunogenic epitopes or all HLA specificities (Class I, Class II, or both as appropriate) found in the population at over 0.5%.

16.4.2.1: Instructions for use

The instructions for use must comply with the requirements of EN ISO 18113:2009 and the information required in section 16.6. In addition, the instructions for use should include the following information on each individual preparation or component of a set of HLA screening product:

- the HLA antigens represented in each container
- the expiry life of the HLA screening product following reconstitution or preparation and subsequent storage in conditions recommended by the manufacturer should be stated
- when components of an HLA screening product contains preservatives the name of the chemical preservatives and the components which contain them should be stated.

16.4.3: External Quality Assessment (EQA) Schemes

Laboratories should take part in regular external quality assessment exercises such as the UK NEQAS for Histocompatibility and Immunogenetics schemes. Effective mechanisms should be in place to correct poor performance in EQA schemes.»

16.5: Testing of HLA genes and gene products

DNA-based methods must identify all HLA alleles included in the most recent WHO Nomenclature Committee for Factors of the HLA System Report² and Nomenclature for Factors of the HLA System, 2010³. Alleles should be reported either as individual alleles or as allele groups with two digits (first field). Definitions of allelic, high, intermediate and low resolution molecular HLA typing are available through EFI.

The minimum level of resolution by serological typing is given in Table 16.1. Typing to the level of broad specificities is acceptable but the higher level to include the split specificities, as indicated, is recommended. HLA-C types Cw12 and Cw14 to Cw18 have not been formally designated as recognised antigens and may not be identified serologically.

Table 16.1 HLA antigens that are defined by serological typing (with broad specificities shown in brackets)

HLA-A	HLA-B	HLA-C	HLA-DR	HLA-DQ
A1	B7	Cw1	DR1	DQ5 (1)
A2	B8	Cw2	DR15 (2)	DQ6 (1)
A3	B13	Cw9 (3)	DR16 (2)	DQ2
A11	B18	Cw10 (3)	DR17 (3)	DQ7 (3)
A23 (A9)	B27	Cw4	DR18 (3)	DQ8 (3)
A24 (A9)	B35	Cw5	DR4	DQ9 (3)
A25 (A10)	B37	Cw6	DR11 (5)	DQ4
A26 (A10)	B38 (B16)	Cw7	DR12 (5)	
A29 (A19)	B38 (B16)	Cw8	DR13 (6)	
A30 (A19)	B41	Cw12*	DR14 (6)	
A31 (A19)	B42	Cw14*	DR7	
A32 (A19)	B45	Cw15*	DR8	
A33 (A19)	B44	Cw16*	DR9	
A34 (A10)	B46	Cw17*	DR10	
A36	B47	Cw18*	DR103	
A43	B48			
A66 (A10)	B49 (B21)		DR51	
A68 (A28)	B50 (B21)		DR52	
A69 (A28)	B51 (B5)		DR53	
A74 (A19)	B52 (B5)			
A80	B53			
	B54 (B22)			
	B55 (B22)			
	B56 (B22)			
	B57 (B17)			
	B58 (B17)			
	B59			
	B60 (B40)			
	B61 (B40)			
	B62 (B15)			
	B63 (B15)			
	B64 (B14)			
	B65 (B14)			
	B67			
	B71 (B70)			
	B71 (B70)			
	B73			
	B71 (B70)			
	B72 (B70)			
	B73			
	B75 (B15)			
	B76 (B15)			
	B77 (B15)			
	B78			
	B81			
	Bw4			
	Bw6			

* The products of the Cw12 and Cw14 to Cw18 genes have not been formally designated as recognised antigens and might not be identified serologically.

16.6: Testing for HLA-specific antibodies

16.6.1: General guidance

HLA-specific antibody screening and characterisation must comply with «the relevant» EFI Standards ~~Section F (Antibody Screening and Crossmatching) and Section M (Flow Cytometry)~~.

~~All commercial HLA antibody test kits should be CE and in vitro device (IVD) marked and validated for use. Each batch of commercial test kit or in-house panel should be evaluated against a minimum of three sera of known HLA specificity from different cross-reacting groups.~~

~~HLA-specific antibodies may be detected using reagent lymphocytes (or cell lines), solid-phase bound, purified HLA molecules, or particle-bound, purified HLA molecules. If such techniques are used for screening (i.e. not characterisation of specificity) the following apply:~~

- ~~• There should be discrimination between HLA Class I and Class II specific antibodies.~~
- ~~• Overall the target cells or molecules should cover either all the known HLA immunogenic epitopes or all HLA specificities (Class I, Class II, or both as appropriate) found in the population at over 0.5%.~~

16.6.2: Characterisation of antibody specificity

Sera containing HLA-specific antibodies may be interpreted in terms of specific antigens (i.e. whole gene products), cross-reactive groups, single epitopes, or any combination of these as long as standard and unequivocal nomenclature is used. Specificity characterisation may be helped by computer analysis but a final result must involve manual interpretation.

«Solid phase techniques have now superseded cellular based methods for HLA antibody detection and identification. Commercial kits are available which consist of beads impregnated with differing ratios of two fluorochromes resulting in a unique signal for each bead and which have one or several types of HLA molecules attached.

The assay involves:

- incubation of a patient's serum with the beads
- if the patient has HLA antibodies the serum will react with the bead expressing the appropriate HLA molecule
- after washing, the beads are incubated with a secondary antibody, usually with a phycoerythrin (PE)-labeled anti-human IgG

Three levels of testing are possible depending on requirements:

1. The first level provides a positive/negative result with respect to a patient's antibody status. In this instance, the beads are bound with a large number of HLA class I or class II molecules derived from lymphoblastoid cell lines.
2. Beads used in second level testing are bound with molecules derived from a single cell line and hence express two HLA molecules for each of the HLA loci (HLA-A, -B, -C for class I and HLA-DR, -DQ and -DP for class II).
3. The third level of testing involves the use of beads bound with single HLA molecules produced by recombinant technology, so called single antigen beads (SAB). These beads provide a real advantage of this technology as complex mixtures of antibodies can be characterized and HLA specificities accurately determined. This technology is now considered essential for the pretransplant testing of sensitized patients.

~~Panels of HLA typed cells or purified HLA molecules are used for identification.~~ The composition of the panel should be sufficient to discriminate the specificities (Class I, Class II, or both as appropriate) given in Table 16.2. The full list of antigens comprising a panel should be supplied and typed to the higher level of resolution shown in Table 16.1.

~~There are many techniques available for the detection of HLA antibodies such as those developed for the detection and identification of HLA antibodies utilising Luminex microspheres. These assays are highly sensitive, leading to the detection of very low levels of HLA antibodies.~~ «The detector reagent should be able to identify IgG and discriminate between IgG, IgA and IgM.» Cut-off values for HLA antibody detection should be set in accordance with manufacturer's instructions and local clinical evaluation.

For DNA typed reagents the types should be supplied at the four-digit (second field) level (e.g. HLA-A*02:01) and null alleles identified.

Table 16.2 Characterisation of HLA-specific antibodies

HLA-A broad specificities	Splits	HLA-B broad specificities	Splits	HLA-C broad specificities	Splits	HLA-DR broad specificities	Splits	HLA-DQ broad specificities	Splits
A1		B5	B51	Cw1		DR1		DQ1	DQ5
A2		B5	B52	Cw2		DR103		DQ1	DQ6
A3		B7		Cw3	Cw9	DR2	DR15	DQ2	
A9	A23	B8		Cw3	Cw10	DR2	DR16	DQ3	DQ7
A9	A24	B12	B44	Cw4		DR3	DR17	DQ3	DQ8
A10	A25	B12	B45	Cw5		DR3	DR18	DQ3	DQ9
A10	A26	B13		Cw6		DR4		DQ4	
A10	A34	B14	B64	Cw7		DR5	DR11		
A10	A66	B14	B65	Cw8		DR5	DR12		
A11		B15	B62	Cw12		DR6	DR13		
A19	A29	B15	B63	Cw14		DR6	DR14		
A19	A30	B15	B75	Cw15		DR7			
A19	A31	B15	B76	Cw16		DR8			
A19	A32	B15	B77	Cw17		DR9			
A19	A33	B16	B38	Cw18		DR10			
A19	A74	B16	B39						
A28	A68	B17	B57						
A28	A69	B17	B58			DR51			
A36		B18				DR52			
A43		B21	B49			DR53			
A80		B21	B50						
		B22	B54						
		B22	B55						
		B22	B56						
		B27							
		B35							
		B37							
		B40	B60						
		B40	B61						
		B41							
		B42							
		B46							
		B47							
		B48							
		B53							
		B59							
		B67							
		B70	B71						
		B70	B72						
		B73							
		B78							
		B81							
		Bw4							
		Bw6							

16.6.2.1: HLA antibody characterisation by complement dependent cytotoxicity

Rabbit complement

~~Rabbit complement used for detection of HLA antibodies by complement dependent cytotoxicity should comply with guidelines in section 16.4.4.~~

Instructions for use

~~In addition to the information required in section 16.6.2, the instructions for use should include the following information on each individual preparation of HLA reagent lymphocytes or set of HLA reagent lymphocytes:~~

- ~~• the HLA phenotype of the reagent lymphocytes~~
- ~~• the nature of the HLA reagent lymphocytes (e.g. normal peripheral lymphocytes, separated peripheral B lymphocytes, separated peripheral T lymphocytes, chronic lymphocytic leukemia (CLL) cells, splenic lymphocytes, lymph node lymphocytes, lymphoblastoid cell line)~~
- ~~• the concentration of the lymphocyte suspension should be stated in the instructions for use for HLA reagent lymphocytes issued in individual immediate containers, or on the phenotype listing of batches issued as multi-immediate container products~~
- ~~• HLA reagent lymphocyte sets issued in multi-well trays should include a representation of the tray or reservoir layout indicating the location of the various HLA reagent lymphocytes in the wells of the tray~~
- ~~• for HLA reagent lymphocyte sets issued in multi-well trays or reservoirs the phenotype information may take the form of a listing of the phenotypes of each of the individual donations comprising the set~~
- ~~• the shelf life of the HLA reagent lymphocytes following recovery from long-term storage and subsequent storage in conditions recommended by the manufacturer should be stated in the instructions for use~~
- ~~• when HLA reagent lymphocytes are provided suspended in preservative or medium, the components of the preservative or the name of the medium should be stated in the instructions for use.~~

Reagent lymphocytes

~~Freshly isolated or previously frozen lymphocytes should have a viability of at least 80% and should contain less than 1% platelets or granulocytes.~~

~~Reagent B lymphocytes isolated for the identification of Class II antibodies should contain less than 10% of non-B cells.~~

~~The background incidence of spontaneous cell death, as assessed by a negative control serum, should be less than 30%.~~

~~Reagent lymphocytes supplied as previously frozen in test trays should contain 1000 to 2000 lymphocytes per well, after recovery following manufacturer's instructions.~~

~~The manufacturer should specify in the instructions for use those antigens known to be present or absent, and those for which no testing has been performed. HLA-A, HLA-B, HLA-C, HLA-DR and HLA-DQ serologically defined specificities should be included in this statement.~~

16.6.2.2: HLA antibody characterisation by solid-phase and particle bound methods

~~Purified HLA captured onto a microtitre well, nylon membrane or microparticles can be used as sensor molecules for characterising sera containing HLA-specific antibodies.~~

~~Antibody binding can be detected by ELISA or fluorescence. The detector reagent should be able to identify IgG and discriminate between IgG, IgA and IgM.~~

Human material

~~If a product is prepared from human source material then the guidance in section 11.1.4.10 must be followed.~~

Instructions for use

~~The instructions for use must comply with the requirements of EN ISO 18113:2009 and the information required in section 16.6.1. In addition, the instructions for use should include the following information on each individual preparation or component of a set of HLA screening product:~~

- ~~• the HLA antigens represented in each container~~
- ~~• the concentration of any cells or particles in suspension should be stated in the instructions for use of HLA screening product issued in individual immediate containers or on the antigen information table of batches issued as multi-immediate container products or multi-well trays or reservoirs~~
- ~~• HLA screening products issued in multi-well trays should include a representation of the tray or reservoir layout indicating the location of the HLA antigens in the wells of the tray~~
- ~~• the expiry life of the HLA screening product following reconstitution or preparation and subsequent storage in conditions recommended by the manufacturer should be stated~~
- ~~• when components of an HLA screening product contains preservatives the name of the chemical preservatives and the components which contain them should be stated.~~

Validation

~~Kits for the detection of HLA antibodies should be validated for sensitivity and specificity on a batch basis using a panel of clinically representative HLA antisera. A panel of sera shown to be inert for HNA and HLA antibodies should also be used.~~

16.7: Leucocyte crossmatching in blood transfusion

Crossmatching may be used in the diagnosis of TRALI and the treatment of HLA- or HNA-sensitised patients with granulocyte transfusions. Unusually it may also be used in the management of patients refractory to random donor platelet transfusion.

A patient's serum should be comprehensively screened for HLA-specific antibodies prior to the crossmatch being performed. The ~~chosen~~ crossmatch technique should be of similar or greater sensitivity than the screening technique.

The presence of HLA-specific antibodies in a current patient serum sample that gives rise to a positive crossmatch excludes that donor providing platelets or leucocytes for that particular patient.

16.7.1: Lymphocytotoxic crossmatch

~~Assessment of leucocyte crossmatches must comply with EFI Standards within F6.000 and the standards for serological investigation given above.~~

16.7.1.1: Cytotoxic crossmatch requirements

~~A policy to determine which sera should be crossmatched should be established and based on local clinical data, where possible, before a crossmatch service is provided.~~

~~A negative control serum derived from a pool of sera that has been previously shown not to react with lymphocytes by complement dependent cytotoxicity (CDC) should be used.~~

~~At least one positive control serum reacting with all lymphocytes or a mixture of anti-HLA-Bw4 and anti-HLA-Bw6-specific reagents should be used to confirm the activity of complement and HLA expression on the cell surface.~~

~~The crossmatch should be performed with and without dithiothreitol (DTT) to distinguish between IgM and IgG antibodies. An IgM control reagent should be included in the crossmatch test as a control for DTT activity.~~

~~Each patient's serum should be tested in triplicate to control for unusual reactions in individual wells of the microplate.~~

«16.7.1:» 16.7.2: Flow cytometric crossmatch

The flow cytometric crossmatch (FCXM) offers greater sensitivity than the microlymphocytotoxicity test for the detection of HLA-specific antibodies in patients receiving blood products. The FCXM may be performed with platelets, lymphocytes and/or granulocytes from the donor.

A two- or three-colour FCXM should be used with one antibody directed against human IgG conjugated to a fluorochrome (e.g. fluorescein isothiocyanate (FITC)). Antibody conjugated to different fluorochromes (e.g. anti-CD3 (T cells) and phycoerythrin (PE) and anti-CD19 (B cells) and allophycocyanin (APC)), should be used to identify the cell lineage under investigation, unless a purified cell population is used, to distinguish between anti-HLA Class I and II reactivity. Testing must be in compliance with «relevant» EFI Standards **M4.000**.

«16.7.1.1:» ~~16.7.2.1:~~ **FCXM requirements**

A policy to determine which sera should be crossmatched should be established and based on local clinical data, where possible, before a crossmatch service is provided.

A negative control serum derived from a pool of sera that has been previously shown not to react with lymphocytes by flow cytometry should be used.

At least one positive control serum reacting with all lymphocytes or a mixture of anti-HLA-Bw4 and anti-HLA-Bw6-specific reagents should be used to confirm the activity of complement and HLA expression on the cell surface.

Each patient's serum should be tested in duplicate to control for unusual reactions.

An additional weak positive control, which gives a fluorescent intensity just greater than the cut-off point between positive and negative, may also be included to evaluate assay performance.

16.8: Application of HLA/HPA testing to patients and donors «investigations

16.8.1: Investigation of refractoriness

Please refer to the relevant EFI Standards for Transfusion.

The most common cause of immunological refractoriness to random donor platelet transfusion is the presence of HLA-specific antibodies in the patient receiving platelet transfusions. The management of this group of patients may involve the provision of HLA-compatible platelets

HLA Class I typed platelets should normally be provided for refractory patients with the aim of minimising exposure to mismatched Class I antigens. In the absence of a zero mismatched donor, a compatible donor can be selected on the basis of a lack of antigens or alleles corresponding to the antibody specificities identified in the patient.

The investigation of refractoriness (see Figure 16.1) and the provision of selected platelets in such cases should comply with the British Committee for Standards in Haematology (BCSH) Guidelines for the Use of Platelet Transfusions.⁵ Serological investigation of suspected immune refractoriness requires screening for HLA Class I-specific antibodies only, but the screening technique must detect HLA-A, HLA-B, and HLA-C-specific antibodies. Any screen-positive patient should be tested further for specificity to include all the Class I antigens listed in Table 16.2.

If a patient has HLA-specific antibodies that cannot be completely characterised, or a specificity corresponding to any of the donor's HLA Class I antigens cannot be excluded, then a crossmatch between donor and patient may be performed as described above.

16.8.2: Investigation of TRALI

Please refer to the relevant EFI Standards for Transfusion.

HLA and/or granulocyte-specific antibodies present in donor plasma have been implicated in nearly 80% of TRALI cases (patient leucocyte antibody or inter-donor reactions in pooled products have also been reported as causes of TRALI). The identification of leucocyte-specific antibodies in implicated donors provides support for the diagnosis of TRALI.

Sera from all implicated donors must be screened for both HLA Class I and Class II specific antibodies and HNA antibodies (see section 16.6.2) Any screen positive serum should be further characterised for HLA Class I and Class II to identify the antibody specificity

Sera from all implicated donors must be screened for both HLA Class I and Class II-specific antibodies and HNA antibodies (see section 16.6.2).

Any screen-positive serum should be further characterised for HLA Class I and Class II to identify the antibody specificity.

If any of the implicated donors are shown to have HLA-specific antibodies the patient should be typed for HLA Class I and Class II to determine the presence of alleles/antigens corresponding to the antibody specificities found in the donor(s).

If a donor serum has HLA-specific antibodies that cannot be completely characterised, or a specificity corresponding to any of the patient's HLA antigens cannot be excluded, then a crossmatch between donor and patient should be performed.

See Figure 16.2 which gives an algorithm for laboratory investigation of TRALI.

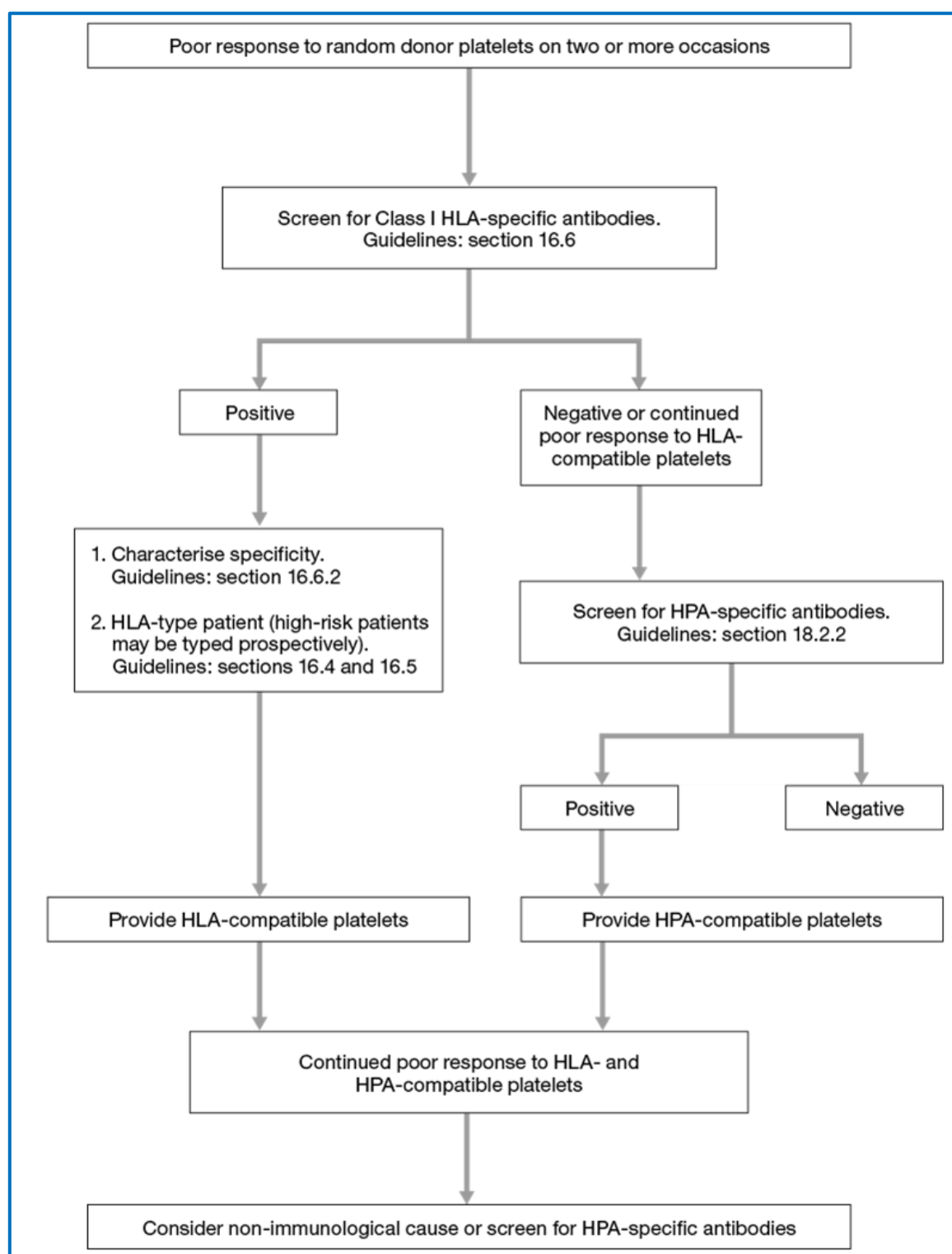


Figure 16.1 Algorithm for laboratory investigation of platelet refractoriness

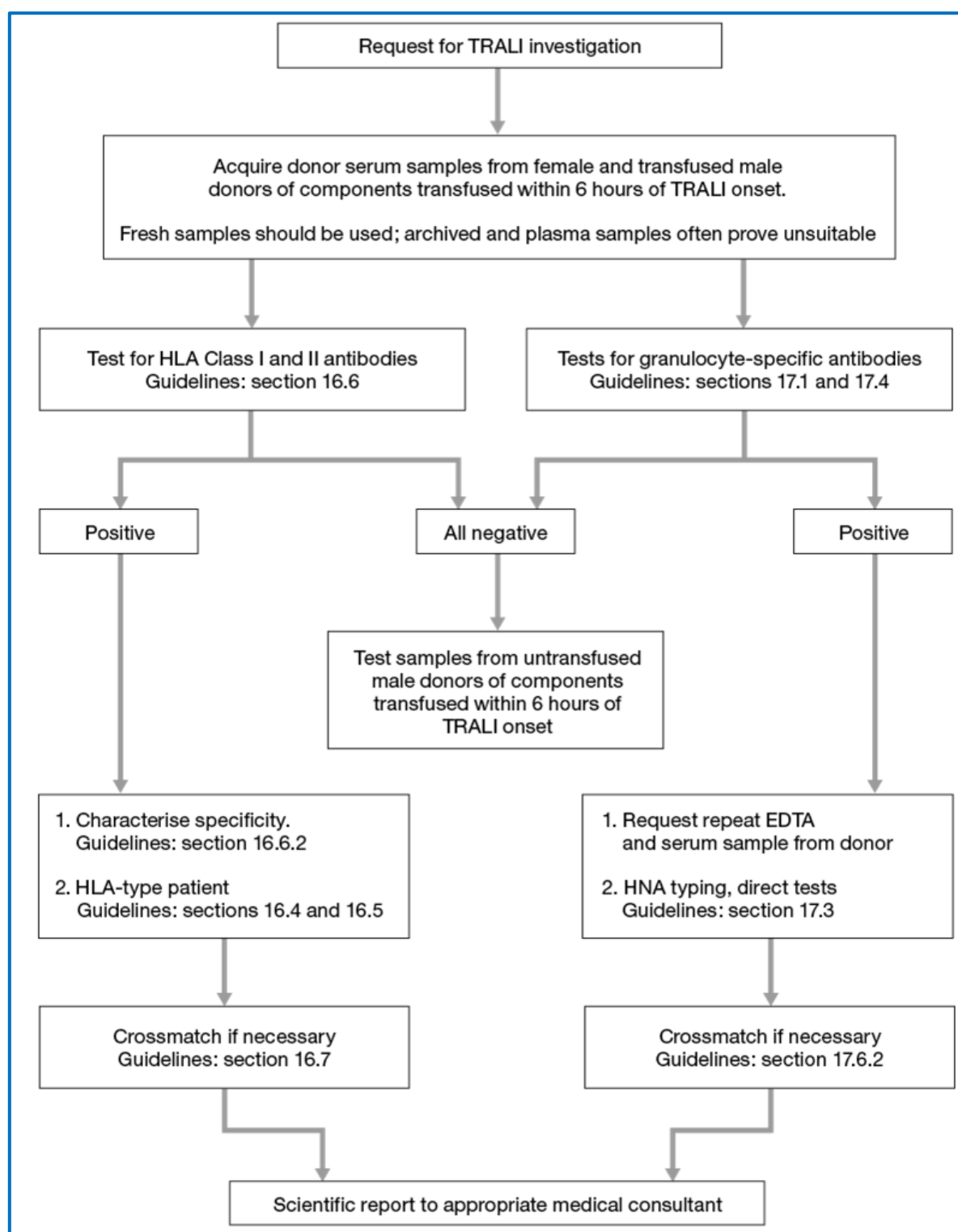


Figure 16.2 Algorithm for laboratory investigation and reporting of TRALI case

16.8.3: Investigation of febrile transfusion reactions

Please refer to the relevant EFI Standards for Transfusion.

If an investigation is requested, sera from patients should be screened for both HLA Class I and Class II-specific antibodies. Any screen-positive serum should be further characterised for HLA Class I and Class II specificities to include all those listed in Table 16.2.»

~~16.8.1: Donor and patient testing~~

~~The most common cause of immunological refractoriness to random donor platelet transfusion is the presence of HLA-specific antibodies in the patient receiving platelet transfusion. The management of this group of patients may involve the provision of HLA-compatible platelets and/or crossmatch-negative donors.~~

~~HLA Class I typed platelets should normally be provided for refractory patients with the aim of minimising exposure to mismatched Class I antigens. In the absence of a zero mismatched donor, a compatible donor can be selected on the basis of a lack of antigens or alleles corresponding to the antibody specificities identified in the patient. Where a patient's antibodies have not been characterised, a crossmatch can be performed; however, it is best practice to establish the patient's antibody specificities if long-term platelet support is envisaged.~~

~~There are several crossmatch techniques for the detection of donor reactive antibodies that may involve the use of donor lymphocytes or donor platelets. The basic principle is the same for most of the techniques in that serum or plasma from the patient is incubated with donor cells and reactivity is detected by flow cytometry or cytotoxicity. Platelets from donors negative in the crossmatch testing may be used for transfusion of the patient whose serum has been crossmatched.~~

~~HLA and/or granulocyte-specific antibodies present in donor plasma have been implicated in nearly 80% of TRALI cases (patient leucocyte antibody or inter-donor reactions in pooled products have also been reported as causes of TRALI). The identification of leucocyte-specific antibodies in implicated donors provides support for the diagnosis of TRALI.~~

«16.8.4:» ~~16.8.2: Apheresis platelet donors~~

«Please refer to the relevant EFI Standards for Transfusion.»

All potential «~~apheresis platelet~~» ~~plateletpheresis~~ donors used for the provision of HLA selected platelets should be typed for HLA-A, HLA-B and HLA-C. ~~If serological typing is used the minimum level of typing should be for the HLA Class I specificities listed in Table 16.1. For all donors HLA-Bw4 or HLA-Bw6 should be assigned.~~

~~If DNA-based typing is performed on donors a typing strategy should «allow» be employed that allows for HLA alleles to be defined to at least the two-digit (first field) level of resolution. Typing should also be capable of determining the presence of the Bw4 and Bw6 epitopes.~~

Each donor should be HLA typed twice using samples collected on separate occasions, such that only if the second test confirms the first should the donor provide platelets for clinical use.

«16.8.5:» ~~16.8.3: Testing of donors/cord units for related haematopoietic stem cell transplant (EFI Standard I1.000)~~

«Please refer to relevant EFI standards for Haematopoietic Stem Cell Transplantation.»

DNA-based HLA typing, to at least the two-digit (first field) level of resolution, should be performed on donors. High-resolution typing may also be necessary as detailed below.

Initially, all potential related stem cell donors must be typed for at least HLA-A, HLA-B and/or HLA-DR to assess compatibility. Further testing must then be undertaken to establish a phenotypic match for HLA Class I and II loci, as described in local protocols. HLA types of the matched patient and donor must be confirmed on a second sample. If HLA haplotype inheritance can be established by typing family members, then high-resolution typing is not required to establish a genotypic match. However, if haplotype inheritance is not established, high-resolution typing of HLA Class I and/or Class II should be undertaken as required by the local transplant protocol. Intra-familial donors who are not HLA identical siblings require both Class I and Class II high-resolution typing as required by the local transplant protocol.

As a minimum related cord units must be typed at low resolution for HLA-A, -B and -DRB1. Extended typing must be undertaken if required by the transplant protocol.

Prior to cord unit transplant, confirmatory typing at low resolution must be performed for HLA-A, -B and -DRB1. Typing must be performed on a segment of the tubing integrally attached to the unit, on a satellite vial or on the content of the thawed unit.

«16.8.6:» ~~16.8.4:~~ **Testing of donors/cord units for unrelated haematopoietic stem cell transplant** (~~EFI Standard I2-000~~)

«Please refer to relevant EFI standards for Haematopoietic Stem Cell Transplantation.»

DNA-based HLA typing, to at least the two-digit (first field) level of resolution, should be performed on donors. High-resolution typing may also be necessary as detailed below.

As a minimum all potential unrelated donors should be typed for HLA-A, -B, -C and -DRB1. HLA types of patient and donor should be confirmed, although the original type from the unrelated donor registry is acceptable for this purpose. The need for high-resolution typing of HLA Class I and II will depend upon local transplant protocols.

As a minimum cord units must be typed at low resolution for HLA-A and -B and high resolution for -DRB1. Extended typing must be performed if required by the local transplant protocol. Prior to commencement of patient conditioning, a minimum low-resolution confirmatory type of at least HLA-A, -B and -DRB1 must be performed upon receipt of the shipped unit. Typing must be performed on a segment of the tubing integrally attached to the unit, on a satellite vial or on the content of the thawed unit.

16.8.5: Investigation of refractoriness

~~The investigation of refractoriness (see Figure 16.1) and the provision of selected platelets in such cases should comply with the British Committee for Standards in Haematology (BCSH) Guidelines for the Use of Platelet Transfusions.5 Serological investigation of suspected immune refractoriness requires screening for HLA Class I-specific antibodies only, but the screening technique must detect HLA-A, HLA-B, and HLA-C-specific antibodies. Any screen-positive patient should be tested further for specificity to include all the Class I antigens listed in Table 16.2.~~

~~If a patient has HLA-specific antibodies that cannot be completely characterised, or a specificity corresponding to any of the donor's HLA Class I antigens cannot be excluded, then a crossmatch between donor and patient may be performed as described above.~~

16.8.6: Investigation of TRALI

~~Sera from all implicated donors must be screened for both HLA Class I and Class II specific antibodies and HNA antibodies (see section 16.6.2).~~

~~Any screen-positive serum should be further characterised for HLA Class I and Class II to identify the antibody specificity.~~

~~If any of the implicated donors are shown to have HLA-specific antibodies the patient should be typed for HLA Class I and Class II to determine the presence of alleles/antigens corresponding to the antibody specificities found in the donor(s).~~

~~If a donor serum has HLA-specific antibodies that cannot be completely characterised, or a specificity corresponding to any of the patient's HLA antigens cannot be excluded, then a crossmatch between donor and patient should be performed.~~

~~See Figure 16.2 which gives an algorithm for laboratory investigation of TRALI.~~

~~Figure 16.1 Algorithm for laboratory investigation of platelet refractoriness~~

~~Figure 16.2 Algorithm for laboratory investigation and reporting of TRALI case~~

16.8.7: Investigation of febrile transfusion reactions

~~If an investigation is requested, sera from patients should be screened for both HLA Class I and Class II-specific antibodies. Any screen-positive serum should be further characterised for HLA Class I and Class II specificities to include all those listed in Table 16.2.~~

«16.8.7:» ~~16.8.8:~~ **Investigation of female donors to reduce the incidence of TRALI**

Many transfusion services «screen» ~~have introduced screening~~ for HLA or HLA and HNA antibodies to reduce the incidence of TRALI. An initial screen for HLA antibodies may be followed by a screen for HNA antibodies to further reduce the potential incidence of TRALI (see section 16.8.6). Female blood donors should be investigated for HLA antibodies following the guidelines set out in section 16.6. There is no requirement to determine the specificity of any HLA antibodies detected or type the donor for HLA.

16.9: References

«

1. The European Federation for Immunogenetics (EFI) Standards. Available at www.efiweb.eu
2. WHO Nomenclature Committee for Factors of the HLA System Report. Available at <https://hla.alleles.org/nomenclature/index.html>
3. Marsh SGE (2010). Nomenclature for Factors of the HLA System. *Tissue Antigens*, 65, 291–455.
4. Holdsworth R et al. (2009). The HLA dictionary 2008: a summary of HLA-A, -B, -C, -DRB1/3/4/5, and -DQB1 alleles and their association with serologically defined HLA-A, -B, -C, -DR, and -DQ antigens. *Tissue Antigens*, 73, 95–170.
5. British Committee for Standards in Haematology (2017). Guidelines for the Use of Platelet Transfusions. *British Journal of Haematology*, 176(3):365-394.

»

- ~~1. The European Federation for Immunogenetics (EFI) Standards. Available at www.efiweb.eu.~~
- ~~2. WHO Nomenclature Committee for Factors of the HLA System Report. Available at www.anthonynolan.org.uk.~~
- ~~3. Marsh SGE (2010). Nomenclature for Factors of the HLA System. *Tissue Antigens*, 65, 291–455.~~
- ~~4. Holdsworth R et al. (2009). The HLA dictionary 2008: a summary of HLA-A, -B, -C, -DRB1/3/4/5, and -DQB1 alleles and their association with serologically defined HLA-A, -B, -C, -DR, and -DQ antigens. *Tissue Antigens*, 73, 95–170.~~
- ~~5. British Committee for Standards in Haematology (2003). Guidelines for the Use of Platelet Transfusions. *British Journal of Haematology*, 122, 10–23.~~

Chapter 17 Granulocyte immunology

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17.1: Reagent manufacture/reference preparations/cell panels

17.1.1: HNA typing reagents

There are several human neutrophil antigen (HNA) genotyping and phenotyping techniques. The latter are generally based on the use of polyclonal HNA alloantibodies obtained from immunised donors or patients or monoclonal antibodies. HNA typing techniques that do not require polyclonal antibodies derived from donors or patients are the techniques of choice.

HNA typing reagents prepared from human source material should comply with the guidelines in section 11.1.4.10.

An 'Instructions for use' sheet (package insert) should be prepared and supplied with antibody typing reagents, see section 11.1.4.12. Information in the instructions for use sheet should further indicate the immunoglobulin class of the antibodies and the presence of any other contaminating antibodies reactive by the recommended methods.

HNA typing reagents used in genomic DNA and polymerase chain reaction (PCR)-based techniques should comply with the guidelines in Chapter 14.

17.1.2: Composition of granulocyte cell panel for HNA antibody detection

It is recommended that laboratories make all reasonable efforts to include cells in their panel that will aid the detection and identification of clinically significant HNA antibodies. The panel should consist of granulocytes typed for HNA-1a, 1b, 1c, 2, 3a, 3b, 4a, 4bw, 5a and 5bw by validated HNA typing techniques. «A minimum» ~~ideally, the~~ panel should «include» ~~contain~~ granulocytes that are homozygous for HNA-1a and HNA-1b and «preferably» be from Group O donors. The panel can be expanded to include granulocytes homozygous for other HNA as indicated by the results of laboratory testing.

HNA typing of a granulocyte panel donor should «ideally» be based on two concordant «typing results» ~~typings~~ performed on samples obtained on different occasions. Wherever possible, both phenotyping and genotyping should be performed for the above antigens.

17.1.3: The preparation of granulocytes/lymphocytes

Granulocytes and lymphocytes for use in serological investigations should be prepared with regard to the following criteria:

- Granulocytes/lymphocytes should be prepared from donors/patients within 24 hours of venesection. Precautions must be taken to minimise activation of granulocytes during isolation.
- Granulocyte/lymphocyte preparations should be essentially free from red cells that would otherwise interfere with the technique or its reading.
- The viability of isolated granulocytes should be sufficient as to not interfere in the technique «used» ~~or its reading~~.

17.1.4: Selection of normal control sera

Normal control sera should be taken from untransfused male blood donors. The sera should be screened and found negative for granulocyte-reactive antibodies (e.g. clinically non-significant autoantibodies are occasionally detected in apheresis donors). An appropriate number of normal sera should be used, so that in any given assay a statistically relevant normal range can be determined.

17.1.5: Selection of positive control sera

At least one positive control should be included in each assay. The selection and number of positive control sera will depend on the technique and the HNA type of the granulocytes being used. In glycoprotein-specific assays, a positive control for each glycoprotein used should be included as a minimum. If different capture monoclonal antibodies are used, the positive control selected should be reactive with the monoclonal antibody selected.

17.1.6: Reference preparations

Sensitivity of techniques should be monitored on the basis of the inclusion of a 'weak positive' control. For anti-HNA-1a, the internal sensitivity control should be calibrated against the WHO International Reference Reagents for anti-HNA-1a (NIBSC code 09/284) when diluted as instructed by the manufacturer.

In-house sensitivity standards, with similar reaction strengths to the above reagent, should be prepared for other HNA antibodies.

17.1.7: Quality control schemes

Laboratories should take part in regular external quality control exercises such as the International Granulocyte Immunology Workshops for HNA antibody detection and for HNA genotyping. Effective mechanisms should be in place to correct poor performance in the quality scheme.

17.2: Nomenclature

The current nomenclature for HNA and corresponding antibodies [«is based on epitopes and allelic typing and this»](#) must be used for recording granulocyte-specific alloantigen and alloantibody specificities¹ (see Table 17.1).

Table 17.1 Current nomenclature for HNA «alleles, epitopes» and corresponding antibodies

System	«Allele»	«Epitopes» <i>Antigen</i>	Original name	Glycoprotein	CD
HNA-1	«FCGR3B*01»	HNA-1a	NA1	FcγRIIIb	CD16
	«FCGR3B*02»	HNA-1b «, HNA-1d»	NA2	FcγRIIIb	CD16
	«FCGR3B*03»	«HNA-1b, HNA-1c» <i>HNA-1e</i>	SH	FcγRIIIb	CD16
	«FCGR3B*04»	«HNA-1a»		«FcγRIIIb»	«CD16»
	«FCGR3B*05»	«HNA-1bv»		«FcγRIIIb»	«CD16»
HNA-2	«Unknown»	HNA-2	NB1	GP56-64kDa	CD177
HNA-3	«SLC44A2*01»	HNA-3a	5b	CTL-2	
	«SLC44A2*02»	HNA-3b	5a	CTL-2	
	«SLC44A2*03»	«HNA-3av»		«CTL-2»	
HNA-4	«ITGAM*01»	HNA-4a	MART ^a	CD11/18	CD11b
	«ITGAM*02»	HNA-4bw		CD11/18	CD11b
HNA-5	«ITGAL*01»	HNA-5a	OND ^a	CD11/18	CD11a
	«ITGAL*02»	HNA-5bw		CD11/18	CD11a

v: indicates a variant epitope that may have different serological characteristics with some antisera.

w: indicates a workshop designation – no antibodies against this epitope have currently been described.

17.3: HNA typing methods

HNA types should be determined using antibody-based and [«/or»](#) DNA/PCR-based techniques that have been validated in the laboratory.

Polyclonal human anti-HNA antisera used in serological techniques should be well characterised. There is no requirement to use typing antisera that are ABO compatible with the granulocytes since the available evidence suggests that granulocytes either do not express Blood Group A or B antigens or do so very weakly. [«However, human HNA antisera may contain HLA class I antibodies that may confound results.»](#)

[DNA based HNA typing techniques should be capable of distinguishing the different allelic forms described in Table 1 or, where ambiguities are recognised in distinguishing different alleles, these should be clearly identified in any reports.»](#)

17.4: HNA antibody detection methods

There are several techniques for the detection of HNA-reactive antibodies. These techniques can be divided into non-specific (where intact granulocytes are used, e.g. granulocyte immunofluorescence test, granulocyte agglutination test) and specific assays (where glycoprotein capture, or purified glycoproteins or recombinant antigens are used, e.g. monoclonal antibody immobilisation of granulocyte antigen test). Laboratories should use tests with adequate sensitivity for the detection and identification of HNA-reactive antibodies. It is recommended that more than one technique is used to detect HNA-specific antibodies.

The combination of chosen technique(s) and the composition of the cell panel cells (if applicable) must ensure:

- The detection of clinically significant HNA-reactive alloantibodies to the antigens of the HNA-1, HNA-2, HNA-3, HNA-4 and HNA-5 systems.
- The detection and identification of HNA-reactive antibodies in samples containing a mixture of both HNA and HLA-reactive antibodies, including antibodies to HNA-3 system antigens, which ~~are~~ **is** expressed on both granulocytes and lymphocytes.
- The identification of the individual HNA specificities in samples containing mixtures of alloantibodies against several HNA antigens (e.g. masking of certain HNA specificities by composition of the panel).
- Techniques should be available to detect cytotoxic and non-cytotoxic anti-lymphocyte antibodies and thereby aid the distinction between granulocyte-specific, lymphocyte-reactive and HLA Class I and Class II antibodies.
- Assays for the detection of granulocyte antibodies, which utilise glycoproteins isolated from human cells, soluble recombinant antigens attached to a solid phase or recombinant cell lines expressing HNA should be used in parallel with established human granulocyte-based tests, either 'in house' or at a reference laboratory, while further data on the performance of these tests is gathered. An antibody specificity determined on the basis of reactivity with a single recombinant antigen or single isolated membrane glycoprotein should be viewed as indicative rather than definitive. Further work should be undertaken to confirm the antibody specificity using other sources of the implicated antigen. If the 'indicative' antibody specificity is confirmed by other techniques the original result can be used as supporting evidence to satisfy the requirements in 17.5.2. The existing advice that, wherever possible, a patient or donor with suspected HNA specific alloantibodies should either be genotyped to determine if they are negative for the allele encoding the implicated antigen or be phenotyped to ensure the absence of the antigen (17.5.2) should be applied.

Where granulocyte-specific antibodies are detected, which appear to have allo-specificity, but the specificity cannot be determined, the samples should be referred to a reference laboratory for further antibody specificity investigations. However, laboratories should make all reasonable efforts to screen against the widest possible range of HNA antigens.

17.4.1: Validation of laboratory kits

- Kits for the detection of HNA-reactive antibodies should be validated for sensitivity and specificity on a batch basis using a panel of clinically representative HNA antisera. It is recommended that the sensitivity of HNA antibody detection should be monitored using a panel of antisera containing 'weak' reactive HNA antibodies (not obtained by dilution of strongly reactive HNA typing sera). A panel of sera shown to be inert for HNA and HLA antibodies should also be used.
- Kits for HNA typing should be validated for specificity on a batch basis using nine donors (three donors homozygous for each HNA allele together with three heterozygotes).

17.5: Donor testing

17.5.1: HNA typing

HNA typing of donors whose granulocytes may be transfused to support HNA-immunised recipients should, wherever possible, be typed twice using samples collected on different occasions. However, it may be necessary to issue HNA-selected products on the basis of a single or 'unconfirmed' type.

17.5.2: Investigation of HNA antibodies

HNA antibody specificities should only be assigned when the sample investigated has been tested and a minimum of three positive and three negative reactions obtained with a single technique or a minimum of two positive and two negative reactions with two techniques. A report identifying the antibody can be issued at this stage. A donor with an HNA alloantibody

should receive an HNA antibody card and an information leaflet, wherever this is available. However, before an HNA antibody card and information leaflet is issued, the donor should be typed (on one occasion but ideally by two methods) and found negative for that antigen.

17.5.3: Investigation of female donors to reduce the incidence of TRALI

Many transfusion services have introduced screening for HLA or HLA and HNA antibodies to reduce the incidence of transfusion-related acute lung injury (TRALI). An initial screen for HLA antibodies may be followed by a screen for HNA antibodies to further reduce the potential incidence of TRALI. Female blood donors should be investigated for HNA antibodies following the guidelines for donor investigation, except that there is only a requirement to test for IgG antibodies. The screening techniques used should «, as a minimum,» enable detection of HNA-1a, -1b, -2 and -3a antibodies which are known to be implicated in causing TRALI.

HLA antibodies should be investigated using the guidelines set out in section 16.6.

17.6: Patient testing

17.6.1: HNA typing

Patients should be typed for HNA following the guidelines for donor HNA typing. A provisional type can be issued on the basis of a phenotype/genotype performed on one occasion. However, it is recommended that, if possible, a second typing technique be used on the first occasion of testing, especially where quality exercises or routine practice have revealed technical problems in typing for particular polymorphisms.

17.6.2: Investigation of HNA antibodies

Patients should be investigated for HNA antibodies following the guidelines for donor investigation. «The investigation of neonatal alloimmune neutropenia (NAIN) should include HNA typing of the parents and affected baby(ies) as this will help identify any potential HNA incompatibilities and can be used to direct antibody screening if the father and baby both have a low frequency HNA that is absent in the mother.» ~~However, laboratories providing diagnostic tests for neonatal alloimmune neutropenia (NAIN) are advised to investigate~~ Cases with a clinical diagnosis of possible NAIN and a negative HNA antibody screen «against common HNA should be investigated for the presence of» ~~for~~ antibodies against low-frequency or 'private' antigens. An effective approach is to use granulocytes from the child's father as an additional panel cell (paternal granulocytes should be HNA typed as a 'patient sample'). Alternatively, laboratories may refer such cases to a reference laboratory.

In the investigation of TRALI, implicated donor samples should be investigated for the presence of both HNA and HLA Class I and Class II antibodies (see also section 16.6). There is usually no requirement to investigate the patient's serum for HNA or HLA antibodies, but if this is necessary both pre- and post-transfusion samples (where available) should be investigated. Where antibody specificities are identified, the donor and patient should be typed to determine the presence or absence of the cognate antigen. If required, a crossmatch may be performed between the implicated donor serum samples and granulocytes/lymphocytes from the patient to determine the clinical relevance of any antibodies and the presence of any low-frequency antibodies. When 'pooled' platelet products are implicated in a case of TRALI, consideration should also be given to the possibility of the formation of inter-donor immune complexes. In such cases, all the donors who contributed to the pool should also be HNA and HLA typed. In a small proportion of TRALI cases, patient antibodies may react with infused donor cells/antigens and it may be necessary to incubate the patient's serum with granulocytes/lymphocytes from the donor.

Crossmatch studies in both suspected NAIN and TRALI cases require that the granulocytes/lymphocytes are isolated from the patient's blood samples within 24 hours of venesection.

A patient or donor with HNA alloantibodies should receive an HNA antibody card and an information leaflet wherever this is available.

17.6.3: Controls for direct tests for granulocyte bound immunoglobulins

Anticoagulated blood samples, less than 24 hours old, from a sufficient number of different normal donors to give a statistically valid normal range, should be used as control samples for the determination of granulocyte-bound immunoglobulins.

17.7: References

»

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»

- ~~1. Bux J (1999). Nomenclature of granulocyte alloantigens. ISBT Working party on Platelet and Granulocyte Serology, Granulocyte Antigen Working Party. International Society of Blood Transfusion. *Transfusion*, 39(6), 662–663.~~

Chapter 18 Platelet immunology

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18.1: Reagent manufacture/reference preparations

18.1.1: HPA typing reagents

- There are several human platelet antigen (HPA) genotyping and phenotyping techniques. The latter are generally based on the use of polyclonal HPA alloantibodies obtained from immunised donors or patients, or monoclonal antibodies. HPA typing techniques that do not require polyclonal antibodies derived from donors or patients are the techniques of choice.
- HPA typing reagents prepared from human source material should comply with the guidelines in section 11.1.4.10. An 'Instructions for use' sheet (package insert) should be prepared and supplied with antibody typing reagents. Information in the 'Instructions for use' should further indicate the immunoglobulin class of the antibodies and the presence of any other contaminating antibodies reactive by the recommended methods.
- HPA typing reagents used in genomic DNA and «polymerase chain reaction (»PCR«)»-based techniques should comply with the guidelines in Chapter 14.

18.1.2: Composition of platelet cell panel for HPA antibody detection

- It is recommended that laboratories make all reasonable efforts to include cells in their panel that will aid the detection and identification of clinically significant HPA antibodies. The panel should consist of platelets typed at a minimum for HPA-1, -2, -3, -5 and -15 by validated HPA typing techniques. Ideally, the panel should contain platelets that are homozygous for HPA-1a, -1b, -2a, -2b, -3a, -3b, -5a, -5b, -15a and -15b and be from Group O donors.
- HPA typing of a platelet panel donor should be based on two concordant typing results «using» ~~performed on~~ samples obtained on different occasions. ~~Wherever possible, phenotyping for the above antigens should also be performed on one occasion.~~

18.1.3: Selection of normal control sera

Normal control sera should be taken from non-transfused group AB male or ABO compatible blood donors. The sera should be screened and found negative for platelet-reactive-antibodies (e.g. clinically non-significant autoantibodies or EDTA-dependent antibodies are occasionally detected in apheresis donors). An appropriate number of normal sera should be used so that a statistically relevant normal range in a given assay can be determined.

18.1.4: Selection of positive control sera

At least one positive control should be included in each assay. The selection and number of positive control sera will depend on the technique and the HPA type of the platelets being used.

In glycoprotein-specific assays a positive control for each glycoprotein used should be included as a minimum.

18.1.5: Reference preparations

- Sensitivity of techniques should be monitored on the basis of the inclusion of a 'weak positive' control. For anti-HPA-1a, -3a and -5b, the internal sensitivity control should be calibrated against the WHO International Reference Reagents for anti-HPA-1a (NIBSC code 05/106), anti-HPA-3a (NIBSC code 03/190) «,» ~~and~~ anti-HPA-5b (NIBSC code 99/666) «and anti-HPA-15b (NIBSC code 18/220)» when diluted as instructed by the manufacturer.
- In-house sensitivity standards, with similar reaction strengths to the above reagents, should be prepared for anti-HPA-1, -3 and -5, and, if possible, for anti-HPA-2 and -15 antibodies.

Table 18.1 Current HPA nomenclature

System	Antigen	Original names	Glycoprotein	CD
HPA-1	HPA-1a	Zw ^a , Pl ^{A1}	GP1IIa	CD61
	HPA-1b	Zw ^b , Pl ^{A2}		
HPA-2	HPA-2a	Ko ^b	GP1balpha	CD42b
	HPA-2b	Ko ^a , Sib ^a		
HPA-3	HPA-3a	Bak ^a , Lek ^a	GP1Ib	CD41
	HPA-3b	Bak ^b		
HPA-4	HPA-4a	Yuk ^b , Pen ^a	GP1IIa	CD61
	HPA-4b	Yuk ^a , Pen ^b		
HPA-5	HPA-5a	Br ^b , Zav ^b	GP1a	CD49b
	HPA-5b	Br ^a , Zav ^a , Hc ^a		
	HPA-6bw	Ca ^a , Tu ^a	GP1IIa	CD61
	HPA-7bw	Mo ^a	GP1IIa	CD61
	HPA-8bw	Sr ^a	GP1IIa	CD61
	HPA-9bw	Max ^a	GP1Ib	CD41
	HPA-10bw	La ^a	GP1IIa	CD61
	HPA-11bw	Gro ^a	GP1IIa	CD61
	HPA-12bw	Iy ^a	GP1Ib beta	CD42c
	HPA-13bw	Sit ^a	GP1b	CD49b
	HPA-14bw	Oe ^a	GP1IIa	CD61
HPA15	HPA-15a	Gov ^b	CD109	CD109
	HPA-15b	Gov ^a		
	HPA-16b ^w	Duv ^a	GP1IIa	CD61
	HPA-17b ^w	Va ^a	GP1IIa	CD61
	HPA-18b ^w	Cab ^a	GP1a	CD49b
	HPA-19b ^w	Sta	GP1IIa	CD61
	HPA-20b ^w	Kno	GP1Ib	CD41
	HPA-21b ^w	Nos	GP1IIa	CD61
	HPA-22b ^w	Sey	GP1Ib	CD41
	HPA-23b ^w	Hug	GP1IIa	CD61
	HPA-24b ^w	Cab2 ^{a+}	GP1Ib	CD41
	HPA-25b ^w	Swi ^a	GP1a	CD49b
	HPA-26b ^w	Sec ^a	GP1IIa	CD61
	HPA-27b ^w	Cab3 ^{a+}	GP1Ib	CD41
	«HPA-28b	War	GP1Ib	CD41
	HPA-29b	Kha ^b	GP1IIa	CD61
	HPA-30b	Lab ^a	GP1Ib	CD41
	HPA-31b	Cab4 ^{b+}	GP1X	CD42a
	HPA-32b	Dom ^b	GP1IIa	CD61
	HPA-33b	Bl ^a	GP1IIa	CD61
	HPA-34b	Bzh ^a	GP1IIa	CD61
	HPA-35b	Efs ^a	GP1IIa	CD61»

18.1.6: Quality control schemes

Laboratories should take part in regular external quality control exercises such as the «UK NEQAS for Histocompatibility and Immunogenetics schemes» ~~National Institute for Biological Standards and Control (NIBSC) Platelet Immunology Quality Scheme for HPA antibody detection and~~ for HPA genotyping «and HPA antibody detection/specification». Effective mechanisms should be in place to correct poor performance in the quality scheme.

18.1.7: Nomenclature

The current HPA nomenclature must be used for recording platelet-specific alloantigen and alloantibody specificities¹ (see Table 18.1). Any subsequent additions can be found in the «Human Platelet Antigen Database (<https://www.versiti.org/products-services/human-platelet-antigen-hpa-database>)» ~~Immuno Polymorphism Database (IPD) website (www.ebi.ac.uk/ipd/hpa/).~~

18.2: Methods

18.2.1: HPA typing methods

- HPA types should be determined using antibody-based and«/or» DNA/PCR-based techniques that have been validated in the laboratory.
- Polyclonal human anti-HPA antisera used in serological techniques should be well characterised. When used in techniques with 'intact' platelets the antisera should be ABO compatible with the platelets to be typed. Alternatively, anti-A and anti-B antibodies may be removed by absorption or neutralisation. This is not a requirement when using human antisera in glycoprotein capture assays, but reactivity against ABO incompatible platelets should be assessed. Sera shown to contain anti-A/B activity in these assays should be subject to the same requirements as those used in 'intact' platelet assays.

18.2.2: HPA antibody detection methods

- There are several techniques for the detection of HPA-reactive antibodies. These techniques can be divided into non-specific (where intact platelets are used, e.g. platelet immunofluorescence test, solid phase adherence assay) and specific assays (where glycoprotein capture, or purified glycoproteins or recombinant antigens are used, e.g. monoclonal antibody-specific immobilisation of platelet antigen assay). Laboratories should use tests with adequate sensitivity for the detection and «specification» ~~identification~~ of HPA-reactive antibodies.
- The combination of chosen technique(s) and the composition of the cell panel cells (if applicable) must ensure:
 - the detection of clinically significant HPA-reactive alloantibodies in the HPA-1, HPA-2, HPA-3, HPA-5 and HPA-15 systems
 - the identification of HPA-reactive antibodies and their specificity in samples containing a mixture of HPA and HLA-reactive antibodies
 - the identification of the specificities in samples containing mixtures of alloantibodies against several HPA antigens (i.e. avoiding the masking of certain HPA specificities by the composition of the panel).
- ~~Where HPA-reactive antibodies are detected, but the specificity cannot be determined, the samples should be referred to a reference laboratory for antibody specificity investigations. However, all reasonable efforts should be made to screen against the widest possible range of HPA antigens.~~
- Assays for the detection of platelet antibodies, which utilise:
 1. glycoproteins isolated from human cells or soluble recombinant antigens attached to a solid phase, or
 2. recombinant cell lines expressing HPA

should be used in parallel with established human platelet-based tests, either 'in house' or at a reference laboratory.
- ~~Assays for the detection of platelet antibodies, which utilise glycoproteins isolated from human cells, soluble recombinant antigens attached to a solid phase or recombinant cell lines expressing HPA should be used in parallel with established human platelet based tests, either in house or at a reference laboratory, while further data on the performance of these tests is gathered.~~

- An antibody specificity determined on the basis of reactivity with a single recombinant antigen or single isolated membrane glycoprotein should be viewed as indicative rather than definitive. Further work should be undertaken to confirm the antibody specificity using other sources of the implicated antigen. «If the 'indicative' antibody specificity is confirmed by other techniques the original result can be used as supporting evidence to satisfy the requirements in 18.3.2. The existing advice that, wherever possible, a patient or donor with suspected HPA specific alloantibodies should either be genotyped to determine if they are negative for the allele encoding the implicated antigen or be phenotyped to ensure the absence of the antigen (18.3.2) should be applied.» ~~In addition, the existing advice that, wherever possible, a patient or donor with suspected HPA specific alloantibodies should either be genotyped to determine if they are negative for the allele encoding the implicated antigen or be phenotyped to ensure the absence of the antigen, should be followed (18.3.2).~~
- Where HPA-reactive antibodies are detected, but the specificity cannot be determined, the samples should be referred to a reference laboratory for antibody specificity investigations. However, all reasonable efforts should be made to screen against the widest possible range of HPA antigens.

18.2.3: Validation of laboratory kits

- Kits for HPA typing should be validated for specificity on a batch basis «with samples that possess homozygosity and heterozygosity for the relevant HPA polymorphism that is included in the test kit.» ~~using nine donors (three donors homozygous for each HPA allele together with three heterozygotes).~~
- Kits for the detection of HPA-reactive antibodies should be validated for sensitivity and specificity on a batch basis using a panel of clinically representative HPA antisera. It is recommended that for monitoring of the sensitivity of HPA antibody detection the panel of antisera should contain 'weak' reactive HPA antibodies (not obtained by dilution of strongly reactive HPA typing sera). A panel of sera shown to be inert for HPA and HLA antibodies should also be used.

18.3: Donor testing

18.3.1: HPA typing

Donors whose products may be used for fetal/neonatal transfusions should be HPA typed twice using samples collected on different occasions. Further HPA typing at subsequent donations is not required after a confirmed type has been established. HPA typing of other donors need only be performed on one occasion and HPA-selected products may be issued on the basis of this 'unconfirmed' type.

18.3.2: Investigation of HPA antibodies

HPA antibody specificities should only be assigned when the sample investigated has been tested and a minimum of three positive and three negative reactions obtained. An antibody report can be issued at this stage. A donor with an HPA antibody should receive an HPA antibody card and an information leaflet «, [where this is available](#)». However, before an HPA antibody card and information leaflet is issued, the donor should be typed and found negative for that antigen.

18.4: Patient testing

18.4.1: HPA typing

Patients should be typed for HPA following the guidelines for donor HPA typing with the following exceptions:

- A provisional type can be issued on the basis of a genotype performed on one occasion. However, it is recommended that a second typing technique be used when quality exercises or routine practice have revealed technical problems when typing for particular polymorphisms. Typing of subsequent samples will allow a confirmed genotype to be reported.
- HPA typing of fetal amniocytes can be undertaken by molecular techniques using DNA isolated from non-cultured amniocytes and a provisional HPA genotype reported. The HPA genotype should be repeated on DNA extracted from cultured amniocytes and shown to be concordant with the first result.
- «HPA typing from cell-free fetal DNA can also be applied, similar to that for blood group typing described in section 15.4.1. Appropriate validation of HPA typing using cell-free fetal DNA against existing techniques using amniocytes should be in place.»

18.4.2: Investigation of HPA antibodies

Patients should be investigated for HPA antibodies following the guidelines ~~for donor investigation~~ with the following exceptions:

- Laboratories serving populations with non-Caucasoid patients are advised to include cells in their panels which will aid the detection and identification of additional clinically significant antibodies (e.g. HPA-4, Naka/GPIV). If the acquisition of GPIV negative cells is not possible, an alternative approach is to establish assays capable of identifying GPIV antibodies that are controlled by appropriate positive control sera.
- Laboratories providing diagnostic testing for Neonatal Alloimmune Thrombocytopenia (NAITP) «should include HPA typing of the parents and affected baby(ies). This testing will help identify any potential HPA incompatibilities and can be used to direct antibody screening if the father and baby both have a low frequency HPA that is absent in the mother. Laboratories» are advised to investigate cases with a clinical diagnosis suggestive of NAITP and «with» a negative ~~HPA-antibody~~ screen for «common HPA» antibodies «, for antibodies» against low-frequency or 'private' antigens. An effective approach is to use platelets from the child's father as an additional panel cell (paternal platelets should be HPA typed as a 'patient sample'). Alternatively, laboratories may refer such cases to a reference laboratory. In the event of a negative antibody screen in a case where NAITP is suspected and there is a potential HPA incompatibility between maternal and baby HPA types, laboratories are advised to repeat the antibody investigation one month after delivery.
- Laboratories providing diagnostic testing for platelet refractoriness should follow the algorithm for laboratory investigations of platelet refractoriness in Figure 16.1.

A patient with HPA antibodies should receive an HPA antibody card and, wherever possible, an information leaflet. However, before an HPA antibody card and information leaflet is issued, the patient should be typed and found negative for that antigen.

18.5: References

1. Metcalfe P, Watkins NA, Ouwehand WH, Kaplan C, Newman P, Kekomaki R, De Haas M, Aster R, Shibata Y, Smith J, Kiefel V, Santoso S. (2003). Nomenclature of Human Platelet Antigens. Vox Sanguinis, 85, 240–245.

Chapter 22 Haemopoietic progenitor cells

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22.1: Introduction

Cellular therapy is now covered by a variety of legislation. The EU Directive on Tissues and Cells (2004/23/EC) and its associated Commission Directives (2006/17/EC and 2006/86/EC) have been transposed into UK law as the Human Tissue (Quality and Safety for Human Application) Regulations, 2007 *«as amended»*. ~~For advanced therapy medicinal products there is EU Directive 2001/83/EC with its subsequent amendments and Regulation (EC) No. 1394/2007 on advanced therapy medicinal products.~~ The Human Tissue Act 2004, Human Tissue (Scotland) Act 2006 and Directions or Codes of Practice issued by the Human Tissue Authority also apply. In addition, there are a number of key international standards for haemopoietic stem cells, notably the FACT-JACIE and the NetCord-FACT Standards *«and the WMDA standards»*. The lists of publications in sections 22.1.1 *«and 22.1.2»* ~~to 22.1.5~~ have been grouped according to their origins.

The guidelines *«references»* in this chapter apply to the donation, collection, testing, processing, cryopreservation, storage and distribution of haemopoietic progenitor cells (HPC) *«and mononuclear cells (MNC)»* ~~and therapeutic cells (TC)~~ within the UK ~~Blood Transfusion Services (UKBTS). HPCs include bone marrow, peripheral blood and cord blood progenitor cells. The guidelines must be read in conjunction with the other sections of the book including those that apply to quality systems, quality assurance and testing of donors.~~ These guidelines are applicable to stem cell donor registries and to bone marrow, peripheral blood and cord blood collection and processing facilities *«, and importing facilities, hereafter mentioned as establishments»*.

«22.1.1: UK Regulation/Guidelines

1. Statutory Instrument 2007 No. 1523 The Human Tissue (Quality and Safety for Human Application) Regulations 2007, and subsequent amendments. Available at www.legislation.gov.uk
2. HTA Guide to Quality and Safety Assurance for Human Tissues and Cells for Patient Treatment (current edition), available at: <https://www.hta.gov.uk/guidance-professionals/regulated-sectors/human-application/hta-guide-quality-and-safety-assurance>
3. Human Tissue Act 2004 (except Scotland). Available at www.legislation.gov.uk
4. Human Tissue (Scotland) Act 2006. Available at www.show.scot.nhs.uk
5. Human Tissue Authority Codes of Practice:
 - i. Guiding principles and the fundamental principle of consent (Code A)
 - ii. Donation of Allogeneic Bone Marrow and Peripheral Blood Stem Cells for Transplantation (Code G).
 Available at www.hta.gov.uk
6. Human Tissue Authority Guidance document for establishments working with Umbilical cord blood. Available at: <https://www.hta.gov.uk/guidance-professionals/regulated-sectors/human-application/establishments-involved-cord-blood>
7. Data Protection Act 2018. Available at <https://www.gov.uk/data-protection>
8. BSHI Guidelines for HLA matching and donor selection for haematopoietic progenitor cell transplantation. Available at: <http://www.bshi.org.uk>
9. Joint UKBTS Professional Advisory Committee's (JPAC) Donor Selection Guidelines for either cord blood donors or bone marrow/peripheral blood stem cell donors. Available at www.transfusionguidelines.org.uk
10. Joint UKBTS Professional Advisory Committee's (JPAC) Geographical Disease Risk Index (GDRI). Available at: www.transfusionguidelines.org.uk
11. Advisory Committee on the Safety of Blood, Tissues and Organs (SaBTO) Microbiological Safety Guidelines. Available at <https://www.gov.uk/government/publications/guidance-on-the-microbiological-safety-of-human-organs-tissues-and-cells-used-in-transplantation>

22.2.2: European & International Directives/Guidelines

1. EDQM Guide to the quality and safety of tissues and cells for human application. Available at: <https://www.edqm.eu/en/guide-to-the-quality-and-safety-of-tissues-and-cells-for-human-application1>

2. EC Guidelines to Good Manufacturing Practice (Eudralex) Manufacture of Sterile Medicinal Products. Available at https://ec.europa.eu/health/medicinal-products/eudralex_en
3. International Standards for Cellular Therapy Product Collection, Processing, and Administration. From the Foundation for the Accreditation of Cellular Therapy (FACT) and the Joint Accreditation Committee of ISCT-Europe and EBMT (JACIE). Available at <https://www.ebmt.org/accreditation/jacie-standards>
4. International Standards for Cellular Therapy Product Collection, Processing, and Administration Accreditation Manual. Available at www.jacie.org
5. NetCord-FACT International Standards for Cord Blood Collection, Banking and Release for Administration. Available at www.factwebsite.org
6. NetCord-FACT Cord Blood Accreditation Manual. Available at <https://www.factweb.org/forms/store/CommercePlusFormPublic/search?action=Publications>
7. World Marrow Donor Association (WMDA) International Standards for Unrelated Haematopoietic Stem Cell Donor Registries - promotes a range of standards, guidelines and recommendations to facilitate the exchange of haematopoietic stem cells across international borders. Available at www.wmda.info
8. European Federation for Immunogenetics (EFI) 'Standards for histocompatibility testing' Available at <https://efi-web.org>
9. National Marrow Donor Program (USA) Standards. May be helpful in benchmarking for equivalent UK standards. Available at www.marrows.org
10. WMDA Donor Medical Suitability Recommendations. Available at: <https://share.wmda.info/display/DMSR/WMDA+Donor+Medical+Suitability+Recommendations+Main+page> »

22.1.1: European Union Directives/guidelines

- ~~1. Directive 2004/23/EC of the European Parliament and of the Council of 31 March 2004 on setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells. OJ, L 102, 07.04.2004, p48. Available at www.transfusionguidelines.org.uk~~
 - ~~2. Commission Directive 2006/17/EC of 8 February 2006 implementing Directive 2004/23/EC of the European Parliament and of the Council as regards certain technical requirements for the donation, procurement and testing of human tissues and cells. OJ, L 038, 09.02.2006, p40. Available at www.transfusionguidelines.org.uk~~
 - ~~3. Commission Directive 2006/86/EC of 24 October 2006 implementing Directive 2004/23/EC of the European Parliament and of the Council as regards traceability requirements, notification of serious adverse reactions and events and certain technical requirements for the coding, processing, preservation, storage and distribution of human tissues and cells. OJ, L 294, 25.10.2006, p32. Available at www.transfusionguidelines.org.uk~~
- ~~(Note: The EU Directive on Tissues and Cells and its associated Commission Directives are referred to collectively as the EU Directives on Tissues and Cells in this chapter.)~~
- ~~4. Regulation (EC) No. 1394/2007 of the European Parliament and Council on advanced therapy medicinal products and amending Directive 2001/83/EC and Regulation (EC) No. 726/2004. Available at <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2007:324:0121:0137:EN:PDF>~~
 - ~~5. Directive 2001/83/EC of the European Parliament and Council on the Community code relating to medicinal products for human use. Available at <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2001:311:0067:0128:EN:PDF>~~
 - ~~6. Commission Directive 2003/63/EC amending Directive 2001/83 on the Community code relating to medicinal products for human use. Available at <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2003:159:0046:0094:EN:PDF>~~
 - ~~7. Regulation (EC) No. 726/2004 of the European Parliament and of the Council laying down Community procedures for the authorisation and supervision of medicinal products for human and veterinary use and establishing a European Medicines Agency. Available at <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2004:136:0001:0033:EN:PDF>~~

- ~~8. EC Guidelines to Good Manufacturing Practice Volume 4, Annex 1 (2008 revision): Manufacture of Sterile Medicinal Products. Available at http://ec.europa.eu/health/documents/eudralex/vol-4/index_en.htm~~
- ~~9. Directive 2001/20/EC of the European Parliament and of the Council on the approximation of the laws, regulations and administrative provisions of the member states relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use. Available at <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2001:121:0034:0044:en:PDF>~~
- ~~10. Commission Directive 2005/28/EC laying down principles and detailed guidelines for good clinical practice as regards investigational medicinal products for human use, as well as the requirements for authorisation of the manufacturing or importation of such products. Available at <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2005:091:0013:0019:EN:PDF>~~
- ~~11. The European Medicines Agency Committee for Medicinal Products for Human Use (CHMP) prepares scientific guidelines, in consultation with the Competent Authorities of the EU member states, to help applicants prepare marketing authorisation applications for medicinal products for human use. Guidelines are intended to provide a basis for practical harmonisation of the manner in which the EU member states and the Agency interpret and apply the detailed requirements for the demonstration of quality, safety and efficacy contained in the EU Directives. Available at www.ema.europa.eu/ema/index.jsp~~

22.1.2: International Standards

- ~~12. International Standards for Cellular Therapy Product Collection, Processing, and Administration. Fifth edition (2012) from the Foundation for the Accreditation of Cellular Therapy (FACT) and the Joint Accreditation Committee of ISCT-Europe and EBMT (JACIE). Available at www.jacie.org~~
- ~~13. International Standards for Cellular Therapy Product Collection, Processing, and Administration Accreditation Manual. Fifth edition (2012). Available at www.jacie.org~~
- ~~14. NetCord-FACT International Standards for Cord Blood Collection, Processing and Release for Administration. Fourth edition (2010). Available at www.factwebsite.org~~
- ~~15. NetCord-FACT Cord Blood Accreditation Manual. Fourth edition (2012). Available at www.factwebsite.org~~
- ~~16. World Marrow Donor Association (WMDA) promotes a range of standards, guidelines and recommendations to facilitate the exchange of haemopoietic stem cells across international borders. Available at www.worldmarrow.org~~

22.1.3: Human Tissue Authority

- ~~17. Human Tissue Act 2004 (except Scotland). Available at www.legislation.gov.uk. The Human Tissue Act 2006 (Scotland). Available at www.show.scot.nhs.uk~~
- ~~18. Statutory Instrument 2007 No. 1523 The Human Tissue (Quality and Safety for Human Application) Regulations 2007, implementing the EU Directives on Tissues and Cells. Available at www.legislation.gov.uk/uksi/2007/1523/made~~
- ~~19. Human Tissue Authority Guide to Quality and Safety Assurance for Human Tissues and Cells for Patient Treatment (implemented by HTA Directions: 003/2010), explains the requirements under the EU Tissues and Cells Directives. Available at www.hta.gov.uk~~
- ~~20. Human Tissue Authority (HTA). Codes of Practice for: Consent (Code 1); for Disposal of Human Tissue (Code 5); for Donation of Allogeneic Bone Marrow and Peripheral Blood Stem Cells for Transplantation (Code 6). Available at www.hta.gov.uk~~

22.1.4: Histocompatibility, donor selection and microbiology documents

- ~~21. Hurley CK (1999). Histocompatibility testing guidelines for haematopoietic stem cell transplantation using volunteer donors: report from the World Marrow Donor Association. Quality Assurance and Donor Registries Working Groups of the World Marrow Donor Association. Bone Marrow Transplant, 24(2), 119–121.~~
- ~~22. European Federation for Immunogenetics (EFI) ‘Standards for histocompatibility testing’ version 5.6.1 (2010). Available at www.efiweb.org~~

- ~~23. National Marrow Donor Program (USA) Standards, 20th edition, March 2009. Available at www.marrow.org~~
- ~~24. Joint UKBTS/NIBSC Professional Advisory Committee's (JPAC) Donor Selection Guidelines for either cord blood donors or bone marrow/peripheral blood stem cell donors are available at www.transfusionguidelines.org.uk~~
- ~~25. SaBTO (2011). Guidance on the microbiological safety of human organs, tissues and cells used in transplantation. Advisory Committee on the Safety of Blood Tissues and Organs. Available at <https://www.gov.uk/government/publications/guidance-on-the-microbiological-safety-of-human-organs-tissues-and-cells-used-in-transplantation>~~

22.1.5: UK legislation

- ~~26. Caldicott Report (1997). Available at http://webarchive.nationalarchives.gov.uk/20130107105354/http://www.dh.gov.uk/prod_consum_dh/groups/dh_digitalassets/@dh/@en/documents/digitalasset/dh_4068404.pdf~~
- ~~27. Data Protection Act 1998. Available at www.legislation.gov.uk/ukpga/1998/29/contents~~

22.2: Terminology

«This chapter aligns with the terminology described at FACT-JACIE International Standards for Haemopoietic Cellular Therapy, Product Collection, Processing, and Administration, as below.

https://www.ebmt.org/sites/default/files/2021-12/STS_5_2_041_FACT-JACIE%20Standards%20Eighth%20Edition_8_1_R2_12142021_ForWeb.pdf »

~~For the purposes of these guidelines, the terms shall, will, or must mean that the guideline is to be complied with at all times. The terms may and should indicate an activity that is recommended or advised, but for which there may be effective alternatives.~~

~~The cellular therapy products described in these guidelines are referred to as haemopoietic progenitor cells HPC-A, HPC-M and HPC-C to denote their collection by apheresis or from marrow and cord blood respectively or as therapeutic cells (TC), the most commonly used of which is TC T cells (T), often referred to as donor lymphocyte infusions (DLI).~~

22.3: Policy and procedure requirements «and Safety

22.3.1: HTA licensing and requirements

The procurement and testing of human tissues or cells in UK should only be carried out by establishments holding an appropriate HTA licence or by individuals or organisations working under the authority of a third-party agreement with an establishment holding an appropriate HTA licence.

The below requirements apply to the establishments and third parties which carry out the procurement, testing, processing, distribution, or export of tissues and cells for human application, and for licensed establishments which store or import tissues and cells for human application.

<https://content.hta.gov.uk/sites/default/files/2021-06/HTA%20guide%20to%20Quality%20and%20Safety%20Assurance%20for%20Human%20Tissue%20and%20Cells%20for%20Patient%20Treatment%20-%20Jan%202021.pdf>

22.3.2: FACT-JACIE and NetCord-FACT standards

The FACT-JACIE and NetCord-FACT standards are available for the clinical and laboratory facilities who wish to conform to the FACT-JACIE and NetCord-FACT Standards as appropriate.

https://www.ebmt.org/sites/default/files/2021-12/STS_5_2_041_FACT-JACIE%20Standards%20Eighth%20Edition_8_1_R2_12142021_ForWeb.pdf »

~~Policies and procedures must include all aspects of the operation including donor selection, assessment, consent, microbiological testing, collection, labelling, system of numbering, processing, quality management and improvement, proficiency testing, storage, including alternative storage strategies if the primary storage device fails, transportation,~~

~~outcome analysis, audits, expiry dates, emergency and safety procedures, equipment and supplies, maintenance and monitoring, cleaning procedures, personnel training, disposal of medical and biohazard waste, release procedures, including criteria for exceptional release, references, tolerance limits, corrective actions, recall, returns and discard policy. A risk management approach must be demonstrated.~~

~~The medical director/advisor and laboratory director/manager must review and approve all policies, procedures and research protocols annually to determine that they are clinically appropriate and consistent with the requirements of users of the service. They should seek to maximise safety for both donors and recipients.~~

~~Procedures carried out by third parties (e.g. donor assessment and harvesting centres, clinical transplant units and testing laboratories) must be described by written agreements. These must define and document relationships between the facility and the third party. The details of the agreement including responsibilities must be clearly specified, documented and agreed between parties. The agreement must include an option for audit of procedures carried out by the third party. Documented procedures to review these agreements should be in place.~~

~~All clinical and laboratory facilities should conform to the relevant EU Directives and both FACT-JACIE and NetCord-FACT Standards as appropriate. Laboratories must participate in appropriate recognised external quality assurance schemes. All clinical and laboratory facilities must be compliant with the requirements of the EU Clinical Trials Directive. Documentation of all research protocols performed by the facility must be maintained. This must include copies of research and ethics committee approvals for all relevant procedures.~~

22.4: Safety requirements

~~Each HPC processing facility must be operated in a manner to minimise risks to the health and safety of employees, donors and recipients. Suitable facilities and equipment must be available to maintain safe operations.~~

~~There must be procedures for microbiological, chemical and radiation safety, as appropriate, and a system for monitoring training and compliance.~~

~~HPC and TC collections must be handled and discarded with precautions that recognise the potential for exposure to infectious agents.~~

«22.4:» 22.5: Adverse events and reactions

«22.4.1: HTA Guide for the management of serious adverse events (SAEs) and reactions (SARs)

All licensed establishments must have a system in place for reporting, investigating, registering and recording information about SAEs and SARs which may influence the quality and safety of tissues and cells, and which may be associated with any licensable activity, as well as any SAR observed during or after clinical application which may be linked to the quality and safety of tissues and cells.

<https://content.hta.gov.uk/sites/default/files/2021-06/HTA%20guide%20to%20Quality%20and%20Safety%20Assurance%20for%20Human%20Tissue%20and%20Cells%20for%20Patient%20Treatment%20-%20Jan%202021.pdf>

22.4.2: WMDA reporting system

WMDA maintains a voluntary central global reporting system to report Serious (Product) Events and Adverse Reactions – S(P)EARs as below

<https://share.wmda.info/pages/viewpage.action?pagelId=297107627> »

~~Facilities must ensure that there is a system in place to detect, report, investigate, document and follow up all errors, adverse events and reactions affecting donors and those which could affect the quality of HPC components and which may be attributable to their collection and processing.~~

~~These systems must also apply to any serious adverse events and reactions observed after administration of HPC components.~~

~~Documentation of these events shall be reviewed by the facilities' directors as appropriate.~~

~~The Designated Individual must ensure that these events are notified to the HTA within 24 hours of discovery.~~

~~Facilities must ensure that appropriate corrective actions are taken and that recall procedures are in place to enable it to recall any component(s) related to serious adverse events and reactions.~~

«22.5:» **22.6: Donor selection, consent and testing**

«Establishments must have detailed policies and procedures for the testing and assessment of donors of stem cells. These must be in accordance with the requirements of the Human Tissue (Quality and Safety for Human Application) Regulations 2007 (as amended), UK-JPAC standards, FACT-JACIE Standards and the WMDA standards.

Anonymity must be maintained between donors and recipients in accordance with the requirements of EU Directive 2004/23/EC (Northern Ireland) and the UK information governance regulations.

<https://www.transfusionguidelines.org/dsg/bm>

<https://share.wmda.info/display/DMSR/WMDA+Donor+Medical+Suitability+Recommendations+Main+page>

https://www.ebmt.org/sites/default/files/2021-12/STS_5_2_041_FACT-JACIE%20Standards%20Eighth%20Edition_8_1_R2_12142021_ForWeb.pdf

<https://fact.policytech.com/dotNet/documents/?docid=534&public=true>

<https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2004:102:0048:0058:en:PDFhttps://ico.org.uk/for-organisations/>»

22.6.1: Allogeneic HPC-M donors

22.6.1.1: General principles

- ~~Registries must have detailed policies and procedures for the testing and assessment of donors of HPC and TC. These must be in accordance with the requirements of the EU Directives on Tissues and Cells, FACT-JACIE Standards and the WMDA.~~
- ~~Counselling: Relevant information must be given to potential donors at appropriate times. This shall include an explanation of the risks of the procedure; benefits for the intended recipient; tests to be performed to protect the health of the donor and recipient; the policy of informing donors of significant abnormal results; the possible need for second donations of HPC or TC; the right to withdraw from the donation; the risk of death for the recipient if the donor withdraws after the recipient's conditioning therapy has started; anonymity policy; insurance arrangements; reimbursement of expenses.~~
- ~~Consent: The donor must be competent to give and have given valid consent before conditioning therapy is initiated in the recipient. Consent must be obtained in accordance with the requirements of the Human Tissue Act and the HTA's Codes of Practice on consent, and donation of allogeneic bone marrow and peripheral blood stem cells for transplantation.~~

22.6.1.2: Medical history, physical examination and testing

- ~~The donor medical assessment must be performed according to the requirements of the EU Directives on Tissues and Cells, FACT-JACIE Standards and the WMDA.~~
- ~~Anonymity must be maintained between donors and recipients in accordance with the requirements of EU Directive 2004/23/EC. The British Bone Marrow Registry (BBMR) and the Welsh Bone Marrow Donor Registry (WBMDR) must have robust policies for donor anonymity and follow-up in accordance with the requirements of the WMDA, FACT-JACIE and NetCord-FACT Standards and the relevant EU Directives.~~

22.6.2: Allogeneic HPC-A donors

~~HPC-A may be collected after mobilisation with a licensed G-CSF preparation.~~

~~The requirements of section 22.6.1 also apply. A donor of HPC-A must be found fit for both apheresis and G-CSF administration and may also be assessed for fitness to undergo bone marrow harvest in the event of failure to mobilise stem cells.~~

22.6.3: Autologous HPC-M and HPC-A donors

~~The assessment and counselling of patients is not within the scope of these guidelines. However, consent must be obtained in accordance with the requirements of the HTA. The requirements for processing, preservation, storage and testing of autologous donations are described in sections 22.9, 22.10 and 22.11.~~

22.6.4: Repeat donations of allogeneic HPC-A, HPC-M or first or repeat donations of TC

~~These are requests either for further donations of HPC, for the same or a different patient, from donors who have in the past given an HPC donation, or for a TC donation for the same patient where an HPC donation has already been given. Individual assessment of each request is required. This must include further medical assessment with appropriate testing, counselling and consent.~~

22.6.5: Allogeneic HPC-C donors

- ~~• HPC facilities/cord blood banks must have detailed policies and procedures for the assessment and testing of donor mothers and infant donors of HPC-C. These must be in accordance with the requirements of the EU Directives on Tissues and Cells, NetCord-FACT Standards and the WMDA.~~
- ~~• Maternal assessment must be performed by appropriately trained staff, according to the requirements of the EU Directives on Tissues and Cells, NetCord-FACT Standards and the WMDA.~~
- ~~• Infant assessment must be performed by appropriately trained staff, according to the requirements of the EU Directives on Tissues and Cells, NetCord-FACT Standards, SaBTO and the WMDA.~~
- ~~• Testing requirements, see section 22.11 Maternal samples taken at time of collection of the HPC-C (Day 0 to +7) shall be tested in accordance with the requirements of the EU Directives on Tissues and Cells, NetCord-FACT Standards and the WMDA.~~
- ~~• Consent. Detailed information must be provided to potential donor mothers prior to requesting consent, in terms and translations relevant to the mother. Consent for collection must be obtained prior to harvest of the cord blood. Consent must be obtained in accordance with the requirements of the Human Tissue Act, the HTA's Codes of Practice for consent and donation of organs, tissue and cells for transplantation, the EU Directives on Tissues and Cells and NetCord-FACT Standards.~~

«22.6: Collection processing and storage

Stem Cells and Therapeutic Cells should only be collected in a hospital facility or Blood Service apheresis unit with appropriate experience (see section 5.8) and which meets the standards required by the Human Tissue (Quality and Safety for Human Application) Regulations 2007 (as amended), FACT-JACIE Standards and NetCord-FACT Standards as appropriate.

HTA Guide to Quality and Safety Assurance for Human Tissues and Cells for Patient Treatment- procurement

<https://content.hta.gov.uk/sites/default/files/2021-06/HTA%20Guide%20to%20Quality%20and%20Safety%20Assurance%20for%20Human%20Tissue%20and%20Cell%20for%20Patient%20Treatment%20-%20Jan%202021.pdf>

FACT-JACIE standards Part CM and Part C

<https://www.ebmt.org/sites/default/files/2018-06/FACT-JACIE%207th%20Edition%20Standards.pdf> »

22.7: Collection facilities for HPC-A, HPC-M, HPC-C and TC

22.7.1: General

~~HPC-A, HPC-M, HPC-C and TC should only be collected in a hospital facility or Blood Service apheresis unit with appropriate experience (see section 5.8) and which meets the standards required by the EU Directives on Tissues and Cells, FACT-JACIE Standards and NetCord-FACT Standards as appropriate. The facility will be headed by a medical director/advisor and a collection facility director with appropriate experience as described in the above standards. The collection facility shall have an organisational structure and operational procedures appropriate for the activities carried out. There must be an organisational chart which clearly defines accountability and reporting relationships. The medical director/advisor shall have responsibility and authority for all clinical aspects of the programme including compliance with national and local guidelines as well as ensuring compliance with regulatory requirements.~~

~~The collection facility director is responsible for the operational management and technical aspects of the service. The medical director/advisor may also act as the collection facility director. There shall be adequate numbers of staff whose training and competency to perform the assigned procedures must comply with the requirements of the EU Directives on Tissues and Cells, FACT-JACIE Standards and NetCord-FACT Standards.~~

~~There must be a documented quality management system applied to all activities, and a designated quality manager.~~

~~There must be a Designated Individual as defined by the EU Directives on Tissues and Cells/Human Tissue Act.~~

22.7.1.1: HPC-M donors

~~HPC-M donors should be assessed and managed in accordance with the aforementioned guidance. Specific points of importance are:~~

- ~~• A consultant anaesthetist should take responsibility for the care of the donor during the harvest procedure.~~
- ~~• There should be intensive care (or equivalent) and resuscitation facilities on-site.~~

22.7.1.2: HPC-A donors

~~HPC-A donors should be assessed and managed in accordance with the aforementioned guidance. Specific points of importance are:~~

- ~~• Physicians prescribing human growth factors must be experienced in their use.~~
- ~~• Donors and recipients undergoing progenitor cell mobilisation must have access to advice and medical supervision 24 hours a day.~~

Venous access

- ~~• Peripheral veins should ordinarily be used for venous access for donors.~~
- ~~• Where access via peripheral veins is not feasible and appropriate consent is obtained, central venous catheterisation (e.g. via the femoral or other route) may be considered.~~
- ~~• The placing of central catheters should only be undertaken in hospital facilities with access to intensive care and radiology facilities by highly trained staff who regularly perform this procedure.~~
- ~~• Collection centres must ensure that the adequacy of central venous catheterisation has been confirmed.~~

22.7.1.3: HPC-C collections

~~HPC-C collections should be managed in accordance with the aforementioned guidance. Specific points of importance are:~~

- ~~• For unrelated collections there must be a written agreement defining the responsibilities and expectations between the cord blood bank and the obstetric department of the collection hospital.~~
- ~~• For directed allogeneic or autologous collections, harvested in a non-fixed collection facility, there must be a written agreement related to HPC-C collection, transport, processing, testing, storage and release, between the referring consultant and the HPC facility.~~
- ~~• Delivery practices must not be modified in an attempt to facilitate HPC-C collections.~~
- ~~• There must exist a documented system for identification of the HPC-C product and for confirming the link with the mother.~~

22.8: Components definitions

22.8.1: Definitions

Unmanipulated: ~~HPC as obtained at collection and not subject to any manipulation.~~

Manipulated: ~~Subjected to an ex vivo process that selectively removes/enriches, expands or functionally alters HPCs.~~

Minimally manipulated: ~~Processing that does not alter the relevant biological characteristics of cells or tissues.~~

More than minimally manipulated: ~~Processing that does alter the relevant biological characteristics of cells or tissues.~~

Investigational medicinal product (IMP): ~~A pharmaceutical form of an active substance or placebo being tested or used as a reference in a clinical trial, including a product with a marketing authorisation when used or assembled in a way different~~

~~from the authorised form, or when used for an unauthorised indication, or when used to gain further information about the authorised form. These products require a separate IMP manufacturer's licence from the Medicines and Healthcare products Regulatory Agency (MHRA).~~

Advanced therapy medicinal product (ATMP): ~~An ATMP is a medicinal product which is:~~

- ~~• a gene therapy medicinal product as defined in Part IV of Annex 1 to Directive 2001/83/EC; or~~
- ~~• a somatic cell therapy medicinal product as defined in Part IV of Annex 1 to Directive 2001/83/EC; or~~
- ~~• a tissue engineered product as defined in Article 2 1 (b) of the ATMP Regulation.~~

~~The MHRA is the Competent Authority for the assessment of applications for clinical trial authorisations and the associated manufacturer's licence for investigational ATMPs. It is also the Competent Authority for ATMPs which are prepared and used under the hospital exemption scheme (laid down in Article 3 (7) of the ATMP Regulation) and made and supplied under the 'specials' scheme.~~

22.8.2: Products

HPC, apheresis (HPC-A): ~~HPC collected from the peripheral blood using an apheresis technique usually after receiving a haemopoietic growth factor.~~

HPC marrow (HPC-M): ~~HPC aspirated from the iliac crests, sternum or other bones.~~

HPC cord blood (HPC-C): ~~HPC from umbilical cord ± placenta at time of delivery.~~

Therapeutic cells (TC): ~~Cell products harvested or manufactured for the purpose of providing therapeutic benefit.~~

- ~~• TC, T cells (TC)~~
- ~~• TC, dendritic cells (DC)~~
- ~~• TC, marrow~~
- ~~• TC, whole blood~~
- ~~• TC, apheresis~~
- ~~• TC, T regulatory cells (T-Reg)~~
- ~~• TC, tumour derived~~
- ~~• TC, mesenchymal stem cell (MSC)~~
- ~~• TC, natural killer (NK)~~
- ~~• TC, cytotoxic lymphocytes (CTL)~~
- ~~• Other therapeutic cells~~

22.8.3: Product modifications

B-cell depleted: ~~Cells processed by negative selection for B lymphocytes.~~

Buffy coat enriched: ~~Cells remaining after depletion of mature erythrocytes and plasma by sedimentation or centrifugation using devices, supplies and techniques validated for this purpose.~~

CD34 selected: ~~Enriched cells processed by positive selection for CD34 antigen-bearing cells.~~

Cryopreserved: ~~Cells frozen using devices, supplies and techniques validated to maintain viability.~~

Density enriched: ~~Primarily mononuclear cells remaining after depletion of mature erythrocytes, polymorphonuclear cells and plasma by separation of the cell on the basis of density. This is achieved using devices or reagents validated for the separation of cells based on density.~~

Ex vivo expanded: ~~Cells that have been cultured in vitro for the purpose of producing and/or enriching for a specific functional subset. Note: Ex vivo expanded cells may require an IMP or ATMP manufacturer's licence from the MHRA.~~

~~**Gene-manipulated:** Cells that have been processed to alter their own genes or introduce new genetic material.~~

~~**Plasma and RBC-reduced:** Cells remaining after depletion of mature erythrocytes and a portion of plasma by sedimentation and/or centrifugation using devices, supplies and techniques validated for this purpose~~

~~**Plasma-reduced:** Cells remaining after a portion of plasma has been depleted by sedimentation or centrifugation using devices, supplies and techniques validated for this purpose.~~

~~**RBC-reduced:** Cells remaining after depletion of mature erythrocytes by sedimentation and/or centrifugation using devices, supplies and techniques validated for this purpose.~~

~~**T-cell-depleted:** Cells processed by negative selection for T lymphocytes.~~

~~**Tumour cell-depleted:** Cells processed by negative selection for tumour cells.~~

22.9: Haemopoietic progenitor cell processing standards

22.9.1: Personnel and facilities

~~Processing facilities must comply with the requirements of the EU Directives on Tissues and Cells, FACT-JACIE Standards and NetCord-FACT Standards. There shall be a medical director/advisor who will have responsibility and authority for all clinical aspects of the programme including compliance with national and local guidelines as well as ensuring compliance with regulatory requirements.~~

~~There will be a laboratory director/manager who is responsible for the operational management and technical aspects of the service. There should be adequate numbers of staff whose training and competency to perform the assigned procedures must comply with the requirements of appropriate regulations and standards.~~

~~There must be a Designated Individual as defined by the EU Directives on Tissues and Cells/Human Tissue Act.~~

~~The HPC processing facility shall have an organisational structure and operational procedures appropriate for the activities carried out. There must be an organisational chart which clearly defines accountability and reporting relationships. There must be a documented quality management system applied to all activities, and a designated quality manager.~~

22.9.2: Procedures

- ~~• Before processing there should be a written request from the transplant physician. This is not required for unrelated cord blood collections.~~
- ~~• Processing should be performed according to written procedures and policies. All procedures must be validated prior to implementation. Aseptic techniques must be employed. Any deviation from such written procedures shall be documented and reviewed.~~
- ~~• Documented process simulation must be routinely undertaken to demonstrate that all processes are adequate and staff and facilities are fit for purpose.~~
- ~~• Before material is accepted from a third party, including receipt from abroad, the laboratory accepting the donation should, wherever possible, ensure that standards equivalent to those in UK guidelines have been met. Material should be inspected upon receipt and the condition of the product recorded.~~
- ~~• Where appropriate the HPC donation should be passed through a sterile non-reactive aggregate filter to remove fat, clots or bone spicules that may be present. A closed system must be used wherever practical.~~
- ~~• Processing and transplant facilities must agree and validate the adequacy of dose (total nucleated cells, mononuclear cells, CD34 positive cells and/or CFU-GM (colony forming unit—granulocyte/macrophage) as appropriate for each source of HPC) required to achieve reliable and sustainable engraftment. Tests for cell dose and viability should be performed as in section 22.11.~~

22.10: Storage of cellular therapy products

~~Policies must be in place for the storage of all material whether or not destined for cryopreservation, e.g. HPC-M undergoing red cell depletion and for other HPCs prior to cryopreservation. Details should be specified for all types of storage conditions. These should cover:~~

- ~~• labelling~~

- ~~primary and secondary containers~~
- ~~storage temperature and duration~~
- ~~cell concentration~~

~~It is recommended that donations with a nucleated cell concentration above $200 \times 10^9/L$ are diluted to less than $200 \times 10^9/L$, preferably with autologous plasma. HPC-A donations must be placed at $4 \pm 2^\circ C$ if they are for liquid storage and/or are not being processed immediately. It is recommended that the final concentration after addition of the cryoprotectant is less than $100 \times 10^9/L$.~~

- ~~transport if appropriate.~~

~~Where donations of known virology or bacteriology positive material are stored, appropriate risk assessments ensuring adequate controls are in place must be completed.~~

22.10.1: Duration

~~Facilities storing HPC components shall establish policies for the duration and conditions of storage and indications for discard. Patients, donors and associated transplant centres should be informed about these policies and consent obtained where appropriate.~~

22.10.2: Alarm systems

- ~~Storage devices shall have alarm systems that are continuously active.~~
- ~~Alarm systems shall have audible signals.~~
- ~~If laboratory personnel are not always present in the immediate area of the storage device, a remote alarm device shall be required at a location staffed 24 hours a day. Alternatively an auto-dial facility connecting to an on-call member of staff may be satisfactory.~~
- ~~Alarms shall be set to activate at temperatures, or an unsafe level of liquid nitrogen, to allow time to salvage components.~~
- ~~There shall be a written procedure to be followed if the storage device fails.~~
- ~~A procedure for notifying laboratory personnel should be in place.~~
- ~~Alarm systems shall be checked periodically for function.~~
- ~~Additional storage devices of appropriate temperature shall be available for component storage if the primary storage device fails.~~

22.10.3: Inventory control

~~There shall be an inventory control system to enable component and quality control vials to be located. It should include the donor name or unique identifier, date of collection, type of storage device and location within it, and state the number of containers and vials and number issued, dates of issue and numbers of containers and vials remaining.~~

22.10.4: Cryopreservation

- ~~Archive samples Aliquots of the HPC component, processed and stored under the same conditions as the HPC component, must be available for additional testing as necessary.~~
- ~~Methods should be validated, taking into account critical pre-freeze variables such as temperature, duration of storage, cell density and type of cryoprotectant.~~
- ~~A secondary container, 'double bagging', must always be used to prevent cross-contamination between donations and to effectively quarantine the unit.~~
- ~~The containers must be clearly and unambiguously labelled using labels that have been validated for use under the required storage conditions. The data on the labels must be in accordance with FACT-JACIE and NetCord-FACT Standards.~~

- ~~• Cryopreservation of the HPC product must be with an established cryoprotectant (e.g. 10% DMSO), used in a validated procedure with defined times and temperatures of exposure to specified concentrations.~~
- ~~• Established conditions of time and temperature of exposure of the HPC component to the cryoprotectant must be observed. These must be specific to the cryoprotectant system used. Validated storage conditions for the cryoprotectant must be observed.~~

~~Frozen HPCs should be stored at a sufficiently low temperature to ensure recovery of living cells after the intended preservation period. HPC donations are generally stored for named patients in low volumes using containers with a high surface area. To minimise the risk of transient warming events that may reduce viability and to maximise the time available to salvage donations should a storage device fail a temperature below –150°C should be used.~~

~~It is recommended that the vapour phase of liquid nitrogen is used to reduce the risk of cross-contamination. It is recognised, however, that this is associated with a greater temperature fluctuation and measures should be taken to ensure that the paragraph above applies. Some facilities may employ total or partial immersion in liquid phase to store HPC donations. Whatever method of storage is used it must always be assumed that liquid nitrogen is microbially contaminated and secondary enclosure must be employed.~~

~~For vapour phase the storage vessels should be fitted with a minimum of two temperature probes that are linked to a remote central monitoring system manned continuously. For liquid phase storage the vessel should be fitted with a minimum of a single probe. Records must be kept of these temperatures.~~

~~If liquid nitrogen refrigeration is used an automatic filling mechanism or a standardised manual procedure must be provided to ensure and document that adequate levels of liquid nitrogen are maintained.~~

«22.7:» 22.11: Testing of haemopoietic progenitor cell donors and components *including therapeutic cells*

«Infectious disease marker testing, ABO and RhD typing and clonogenic assays must be done in accordance with the HTA guide to quality and safety assurance for tissues and cells for patient treatments -updated on January 2021, and the SaBTO guidance.

Additional information and guidance available with FACT-JACIE, WMDA and NetCord-FACT standards.

The minimum current requirements for mandatory and additional microbiology testing (serology and/or NAT) are described in Chapter 9.

Annex 7 indicates the requirements for the timing of testing for each type of HPC.

22.8: requirements for the timing of testing

Please see Annex 7 for guidance relating to the timing of testing for different categories of HPC.»

22.11.1: Infectious disease marker testing

~~This must be done in accordance with the requirements of the EU Directives on Tissues and Cells, SaBTO, FACT-JACIE and NetCord-FACT.~~

~~The minimum current requirements include testing for HIV, HTLV I/II, HBV, HCV and syphilis. Additional testing may be required in some cases, e.g. for malaria and toxoplasmosis. Table 22.1 indicates the requirements for the timing of testing for each type of HPC, while Chapter 9 contains further information on microbiology testing procedures.~~

Table 22.1 Requirements for the timing of testing

Test	Allo-HPC-A/ HPC-M/TC	Auto-HPC-A/ HPC-M	HPC-C donor-mother	HPC-C
ABO + RhD	Test at each donation	Test at first donation		Day 0 to Day +7
anti-HIV 1/2 antibody	Day –30 to Day 0	Day –30 to Day 0	Day –7 to Day +7	Prior to release
anti-HCV antibody	Day –30 to Day 0	Day –30 to Day 0	Day –7 to Day +7	Prior to release
anti-HTLV I/II (pooled) antibody	Day –30 to Day 0	Day –30 to Day 0	Day –7 to Day +7	Prior to release

HCV-RNA (pooled)	Day –30 to Day 0	Day –30 to Day 0	Day –7 to Day +7	Prior to release
HBsAg	Day –30 to Day 0	Day –30 to Day 0	Day –7 to Day +7	Prior to release
anti-HBc antibody	Day –30 to Day 0	Day –30 to Day 0	Day –7 to Day +7	Prior to release
CMV	Day –30 to Day 0	Day –30 to Day 0	Day –7 to Day +7	Prior to release
Pregnancy test	–7 days preconditioning			
Malaria	Where clinical indication	Where clinical indication	Where clinical indication. All mothers where there is risk	
Haemoglobinopathy i.e. sickle cell	Where clinical indication	Where clinical indication		Prior to release
Syphilis screen*	Day –30 to Day 0	Day –30 to Day 0	Day –7 to Day +7	
Bacteriology testing	If manipulation	If manipulation		On final product
FBC	Before each apheresis procedure	Before each apheresis procedure		Pre and post product
* Confirmatory tests should be performed if screen positive				

Additional tests must be undertaken for quarantined HPC-C products where a Day 180 repeat test has not been performed on the mother. The following tests should be performed on the mother's initial sample to permit release:

- HIV PCR pooled/single
- HCV PCR pooled/single
- HBV PCR single

Mechanisms should be in place to ensure that archived material/samples can be re-tested at the time of issue of donation for all current markers of infection including the latest generation of assays.

22.11.2: HLA typing

- At initial registration: HLA-A, -B, -DR type by a Clinical Pathology Accreditation (CPA) and European Federation for Immunogenetics (EFI) accredited laboratory. As a minimum these antigens should be defined at low/medium resolution level using DNA techniques.
- Confirmatory typing: Must be performed on a sample drawn independently of that used for initial registration. HLA-A, -B, -C, -DRB3, -DRB4, -DRB5 and -DQB1 types should be defined, at a minimum, to medium resolution using DNA techniques. HLA-DRB1 should be defined to the allele level by DNA techniques. High/allele resolution typing for HLA-A, -B, -C, -DRB3, -DRB4, -DRB5, -DQB1 and -DPB1 can also be performed as required by the transplant protocol.

For cord blood donations it is recommended that a maternal sample is HLA typed to confirm identity. High resolution typing of cord blood units shall take place when requested by a transplant centre. In cord blood banking, prior to the release of a cord blood unit for transplantation a sample obtained from a contiguous segment of the cryopreserved cord blood unit must be tested to verify HLA type or short tandem repeat (STR) can be performed according to NetCord-FACT Standards.

22.11.3: ABO and RhD typing

For allogeneic donors of HPC-A and HPC-M, ABO and RhD typing must be performed on samples taken from the donor or cell therapy component at the time of each collection. For autologous donors of HPC-A and HPC-M, ABO and RhD typing must be performed on samples taken from the donor or cell therapy component at the time of first collection. For HPC-C the ABO and RhD type of each donation shall be determined.

22.11.4: Clonogenic assays

~~Clonogenic assays (e.g. CFU-GM) may be undertaken as part of a quality programme or when specifically indicated or requested by the transplant physician. Consideration should be given to performing surrogate tests for viability prior to conditioning on a representative archive sample of any cryopreserved HPC components. For cord blood units CD34+ cells should be enumerated according to NetCord-FACT Standards and progenitor cell assays should be assessed on a thawed sample before release of the unit for transplant.~~

22.11.5: Sterility

~~Bacteriological and fungal screening employing aerobic and anaerobic conditions must be performed on the final HPC component after processing and before cryopreservation, unless validation studies demonstrate that bacteriological screening of waste processing material, such as plasma or erythrocytes, are equivalent to screening of the final product. All positive cultures should be subsequently identified and antibiotic sensitivities performed if the material is to be put to clinical use.~~

22.11.6: Test samples

~~Archival samples must be stored for reference and any future testing that may be required as described in the EU Directives on Tissues and Cells, FACT-JACIE Standards and NetCord-FACT Standards. Documentation must be kept to ensure security and accurate retrieval of the stored samples when required. Storage conditions must:~~

- ~~• maintain cell viability (below –150°C)~~
- ~~• be suitable to obtain material for the preparation of 50 mg DNA.~~

«22.9:» ~~22.12:~~ Labelling, packaging, transportation and «release» ~~temperature controls~~

«The mandatory requirements for these are described in the HTA's Guide to Quality and Safety Assurance for Human Tissue and Cells for Patient Treatment.

The FACT-JACIE Standards and NetCord-FACT Standards will also apply as appropriate.

The requirement for an EU Single European Code (SEC) still applies in Northern Ireland.

https://health.ec.europa.eu/blood-tissues-cells-and-organs/implementation/single-european-code-sec-tissues-and-cells_en#:~:text=The%20%22Single%20European%20Code%E2%80%9D%20or,type%20of%20tissue%20or%20cells%20»

~~The requirements for these are described in the HTA's Guide to Quality and Safety Assurance for Human Tissue and Cells for Patient Treatment, FACT-JACIE Standards and NetCord-FACT Standards and the requirements for labelling are summarised in Tables 22.2 and 22.3.~~

Table 22.2 Label content adapted from FACT-JACIE

Element	Partial label	Label at completion of collection	Label during processing	Label at completion of processing	Label at distribution	Inner and outer shipping container
Unique identifier of product	AF	AF	AF	AF	AF	
Proper name of product	AF	AF	AF	AF	AF	
Recipient name and identifier	AF (if applicable)	AF (if applicable)	AF (if applicable)	AF (if applicable)	AF (if applicable)	
Date, time collection ends and (if applicable) time zone		AF		AG	AG	
Approximate volume		AF		AG	AG	
Name and volume or concentration of anticoagulant and other additives		AG		AG	AG	
Donor identifier and (if applicable) name		AF		AT	AT	

Identify and address of collection facility or donor registry		AC		AC	AC	
Recommended storage temperature		AT		AT	AT	
Biohazard label		AC (if applicable)		AC (if applicable)	AC (if applicable)	AC (if applicable)
Identity and address of processing facility				AF	AF	
ABO and Rh of donor				AC	AC	
Red blood cell compatibility testing results				AC	AC (if applicable)	
Statement 'Properly identify intended recipient and product'				AC	AC	
Statement 'Warning: this product may transmit infectious agents'				AF	AF	
Expiration date				AF (if applicable)	AF (if applicable)	
Expiration time				AF (if applicable)	AF (if applicable)	
Statement 'For autologous use only' or Statement 'For use by intended recipient only'				AF (if applicable) AF (if for allogeneic recipient)	AF (if applicable) AF (if for allogeneic recipient)	
Statement 'Do not irradiate'				AT	AT	
Statement 'Not for infusion' including reason				AT (if applicable)	AT (if applicable)	
Name and address of receiving institution						AT
Name and telephone number of contact person at receiving institution						AT
Statement 'Medical specimen'						AT
Statement 'Do not X-ray'						AT
Name, address and telephone number of shipping facility						AT
AF = affixed, AT = attached or affixed, AC = accompanying or attached or affixed						

22.13: Release

~~Prior to HPCs being cleared for issue, all relevant records, including donor records, processing and storage records, and post-processing quality control tests must have been reviewed, approved and documented as acceptable by the individual(s) responsible according to the relevant local standard operating procedures. Responsibility for setting policies for exceptional release and for issuing products on concession resides with the medical director/advisor.~~

~~Records must demonstrate that before cells are released the product specification is met and verified according to a written procedure by a person authorised by the Designated Individual.~~

~~For clinical use of a product that has not met its specification, exceptional release-specific authorisation must be given by the facility medical director or designee.~~

~~For cord blood donations release occurs at two stages:~~

- ~~• Following completion of testing and donor selection when donations are formally banked and made available for search.~~
- ~~• At issue for transplantation.~~

Table 22.3 Label content for HPC-C adapted from NetCord-FACT

Label element	Partial label	Label at completion of collection	Shipping container labelling for transport from collection	Label at completion of processing	Label at cord blood unit release	Dry shipper labelling at issue
Unique numeric or alphanumeric identifier	AF	AF		AF	AF	
Proper name HPC, Cord Blood	AF	AF	AF	AF	AF	
Product modifiers				AC	AC	
Statement 'Directed donor' (directed allogeneic and autologous HPC-C units)	AF	AF		AF	AF	
Collection centre identifier		AF	AT			
Date of collection		AF		AC	AC	
Time of collection		AC				
Name and volume or concentration of anticoagulant and other additives		AF		AC	AC	
Recommended storage temperature		AT		AF	AF	
Donor name (directed allogeneic and autologous HPC-C units)		AF		AF	AF	
Recipient's name, unique identifier or family (directed allogeneic and autologous HPC-C units) — if applicable		AF			AF	
Volume or weight of the HPC-C unit at the end of collection				AC	AC	
Volume or weight of the HPC-C unit at the end of processing				AC	AC	
Date of cryopreservation				AC	AC	
ABO group and Rh type				AC	AC	
HLA phenotype				AC	AC	
Number of nucleated cells post-processing				AC	AC	
Gender of HPC-C infant donor				AC	AC	
Identity of the cord blood bank				AF	AF	
Statement 'Properly identify intended recipient and product'					AT	

Statement 'For use by intended recipient only' (allogeneic HPC-C units)					AT	
A statement indicating that leucoreduction filters should not be used					AT	
Statement 'Do not irradiate'					AT	
Statement 'For non-clinical use only' (if applicable)					AT	
Biohazard labels – if applicable		AF	AF	AT	AT	
Date of distribution					AG	AF
Shipping facility name, address, telephone number				AF		AF
Receiving facility contact details				AF		AF
Identity of person or position responsible for receipt of shipment				AF		AF
Statement 'Do not X-ray'						AF
Statement 'Medical specimen', 'Handle with care'						AF
Statement indicating HPC-C for transplantation						AF
Shipper handling instructions						AF
AF = affixed, AT = attached or affixed, AC = accompanying or attached or affixed						

22.14: Transportation

~~The methods used to transport frozen components to the hospital must have been shown to maintain integrity of the component and to provide the temperature specified for storage. Liquid nitrogen dry shippers are suitable. Only components that were stored either partially or completely submerged in liquid nitrogen may be submerged in liquid nitrogen for transport.~~

22.15: Thawing and infusion

- ~~The units should be thawed in a manner that has been established as appropriate for the overall preservation technique.~~
- ~~Infusion documentation shall facilitate tracking of the product from the donor to recipient. A component infusion form shall be issued with the product and completed for each component infused. A copy should be returned to the processing laboratory.~~
- ~~There must be an effective recall procedure in place defining responsibilities and actions to be taken including notification to the Competent Authority (HTA).~~
- ~~Procedures must be in place for the documentation of returned products, defining acceptance criteria into the inventory.~~

«22.10:» 22.16: Disposal of haemopoietic progenitor cells

«Disposal of cellular therapy products shall include the following requirements shall meet the EBMT guideline requirements (Chapter D12: Disposal)

https://www.ebmt.org/sites/default/files/2021-12/STS_5_2_041_FACT-JACIE%20Standards%20Eighth%20Edition_8_1_R2_12142021_ForWeb.pdf »

- ~~Appropriate prospective consent for discard should have been obtained. Prior to collection there shall be a written agreement between the processing facility and the donor defining the length of storage and circumstances for disposal or transfer of cellular therapy products to an alternative facility.~~
- ~~The medical director/advisor of the processing facility, in consultation with the patient's transplant physician, must approve of component discard and method of disposal.~~
- ~~There must be written documentation of the recipient's death or no further need for any component before it is discarded. Written instructions from the transplant physician should be obtained. The records for discarded components must indicate the component discarded, date of discard and method of disposal.~~
- ~~The method of disposal and decontamination must meet the UK laws, current codes, rules and regulations for disposal of biohazardous materials.~~

«22.11:» ~~22.17: Records~~ «Maintenance»

«All patient records and results should be maintained to comply the requirements of the GDPR.

<https://ico.org.uk/for-organisations/guide-to-data-protection/guide-to-the-general-data-protection-regulation-gdpr/accountability-and-governance/documentation/#:~:text=The%20UK%20GDPR%20contains%20explicit,to%20the%20ICO%20on%20request>

Establishments must comply with SaBTO advice in record keeping and maintenance.

https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/876161/SaBTO-microbiological-safety-guidelines.pdf

The requirements for these are described in the Human Tissue (Quality and Safety for Human Application) Regulations 2007 (as amended), FACT-JACIE Standards, NetCord-FACT Standards and WMDA standards as above.»

~~22.17.1: General requirements~~

- ~~All patient records and results should be maintained to the requirements of the Caldicott Report (1997) and the Data Protection Act (1998).~~
- ~~Records shall be accurate, legible and indelible.~~
- ~~Records must be made concurrently with each step of the harvesting, processing, testing, cryopreservation, storage, issue and transplant or disposal of each component in such a way that all the steps may be accurately traced from donor to recipient.~~
- ~~All records and communications between the collection, processing and transplant centres must be regarded as privileged and confidential. Safeguards to assure this confidentiality must be established and followed.~~
- ~~Records required for full traceability must be kept for a minimum of 30 years after clinical use, in an appropriate and readable storage medium.~~
- ~~Records including raw data, such as original temperature monitoring records, which are critical to the safety and quality of the tissues and cells, must be kept for at least 10 years after any expiry date, clinical use or disposal of the tissues and cells.~~

~~22.17.2: Records to be maintained~~

~~The requirements for these are described in the EU Directives on Tissues and Cells, FACT-JACIE Standards and NetCord-FACT Standards. Records of the following must be kept:~~

- ~~donor and patient details~~
- ~~collection and processing~~
- ~~storage, issue and administration~~

- ~~compatibility testing~~
- ~~quality control~~
- ~~personnel, training, continued education, competency testing~~
- ~~incidents, errors and corrective action taken.~~

~~22.17.3: Records in cases of divided responsibility~~

~~If two or more facilities participate in the collection, processing or distribution of the product, the records of the processing facility shall show clearly the extent of its responsibility.~~

Annex 7 Requirements for the timing of testing for HPCs

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«Annex 7 - Requirements for the timing of testing for Hematopoietic Progenitor Cells (HPCs): Minimum standards and good practice

Terminology:

HPC-A – Peripheral Blood (stem cells, collected by apheresis)

HPC-M – Bone marrow (stem cells, collected from bone marrow)

MNC-A – Mononuclear cells (collected by apheresis, including starting material for ATMP manufacture and DLIs)

HPC-CB – Umbilical cord blood

Mandatory – The test is either a regulatory requirement or deemed necessary to ensure regulatory requirements relating to the assessment of donor suitability are met

Discretionary – The test must be performed on certain donors/donations if indicated by medical, social or travel history

Recommended – This test is recommended by an advisory committee or a professional body, but is not a regulatory requirement

Optional – The test may be done at timepoints outside of the mandatory testing timeline

Table 1 – Allogeneic HPC-A, HPC-M

Test	Performed on donor, product or both?	Test mandatory, discretionary, recommended or optional?	Timing of test	Notes
ABO + RhD	Donor	Mandatory	Prior to donation	Using two independently collected samples; different needlesticks
Mandatory infectious markers	Donor	Mandatory	Within 30 days prior to the donation episode	See Table 9.2
		Optional	At the time of donation or within seven days post donation	
Discretionary Additional infectious markers (e.g. Malaria, WNV, T.cruzi)	Donor	Discretionary	Prior to donation, depending on travel history	Align with JPAC donor selection guidelines
CMV	Donor	Recommended	At donor selection, and Within 30 days prior to the donation episode	
Toxoplasma, Epstein Barr Virus	Donor	Recommended	Within 30 days prior to the donation episode	
Pregnancy test	Donor	Discretionary	7 days prior to starting donor mobilisation regime, and, as applicable, within 7 days prior to the initiation of the recipient's preparative regime	Applies to all donors of childbearing potential
Haemoglobinopathies	Donor	Discretionary	At the time of donor assessment	Applies to those donors thought to be at risk of sickle cell disease and compound haemoglobinopathies

Bacteriology testing	Product (processed)	Optional	Pre-processing	
		Mandatory	Post-processing	
	Product (fresh)	Mandatory	Post collection	
FBC	Donor	Mandatory	Immediately before every collection for HPC-A; prior to first donation for HPC-M	

Table 2 – Autologous HPC-A, HPC-M

Test	Performed on donor, product or both?	Test mandatory, discretionary or optional?	Timing of test	Notes
ABO + RhD	Donor	Optional	Prior to donation	Due to autologous nature of product, not essential
Mandatory infectious markers	Donor	Mandatory	Within 30 days prior to the donation episode	April 2023: Sample timing currently under review by HTA
		Optional	At the time of donation or within seven days post donation	See Table 9.2
Discretionary Additional infectious markers (e.g. Malaria, WNV, T.cruzi)	Donor	Discretionary	Prior to donation, depending on travel history	In selected circumstances based on individual risk assessment, testing may be requested/required. Align with JPAC donor selection guidelines
Pregnancy test	Donor	Discretionary	7 days prior to starting donor mobilisation regime, and, as applicable, within 7 days prior to the initiation of the recipient's preparative regime	Applies to all donors of childbearing potential
Haemoglobinopathies	Donor	Discretionary	At the time of donor assessment	Applies to those donors thought to be at risk of sickle cell disease and compound haemoglobinopathies
Bacteriology testing	Product (processed)	Optional	Pre-processing	
		Mandatory	Post-processing	
	Product (fresh)	Mandatory	Post collection	
FBC	Donor	Mandatory	Immediately before every collection for HPC-A; prior to first donation for HPC-M	

Table 3 – Autologous & Allogeneic MNC-A

Test	Performed on donor, product or both?	Test mandatory, discretionary, recommended or optional?	Timing of test	Notes
ABO + RhD	Donor	Mandatory	Prior to donation	Using two independently collected samples; different needlesticks

Mandatory infectious markers	Donor	Mandatory	At the time of donation or within seven days post donation	See Table 9.2
Discretionary Additional infectious markers (e.g. Malaria, WNV, T.cruzi)	Donor	Discretionary	Prior to donation, depending on travel history	Align with JPAC donor selection guidelines
CMV	Donor	Recommended	At donor selection, and Within 30 days prior to the donation episode	
Toxoplasma, Epstein Barr Virus	Donor	Recommended	Within 30 days prior to the donation episode	
Pregnancy test	Donor	Discretionary	7 days prior to starting donor mobilisation regime, and, as applicable, within 7 days prior to the initiation of the recipient's preparative regime	Applies to all donors of childbearing potential
Haemoglobinopathies	Donor	Discretionary	At the time of donor assessment	Applies to those donors thought to be at risk of sickle cell disease and compound haemoglobinopathies
Bacteriology testing	Product (processed)	Optional	Pre-processing	
		Mandatory	Post-processing	
	Product (fresh)	Mandatory	Post collection	
FBC	Donor	Mandatory	Immediately before every collection	

Table 4 – HPC-CB

Test	Performed on mother, product or both?	Test mandatory, discretionary, recommended or optional?	Timing of test	Notes
ABO + RhD	Product	Mandatory	Prior to cryopreservation	
Mandatory infectious markers	Mother	Mandatory	At the time of donation or within seven days post donation	See Table 9.2
	Product	Recommended	Prior to release	Testing of the maternal sample at the time of donation, including NAT, may be used as a surrogate marker for the product. Testing of the product is recommended but not mandatory.
Discretionary Additional infectious markers (e.g. Malaria, WNV, T.cruzi)	Mother	Discretionary	0 to +7 days	Depending on travel history. Align with JPAC donor selection guidelines
	Product	Discretionary	Prior to release, as applicable	
CMV	Mother	Recommended	0 to +7 days	
	Product	Recommended	Prior to release	

Toxoplasma, Epstein Barr Virus	Mother	Recommended	0 to +7 days	
Haemoglobinopathies	Product	Discretionary	Prior to release	Sample from product or neonatal screen
Bacteriology testing	Product	Mandatory	Post processing, prior to cryopreservation	
FBC	Product	Mandatory	Between the end of collection and pre-processing	

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