Department of Health

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Guidelines for the BLOOD TRANSFUSION SERVICES *in the* UNITED KINGDOM 1989

VOLUME I

Guidelines for blood components prepared at regional transfusion centres

VOLUME II

Guidelines for the preparation of plasma fractions

VOLUME III

Guidelines for reagents for blood group serology and HLA typing

London: HMSO

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PREFACE

The three volumes have been prepared jointly between the UKBTS and NIBSC, to provide professional guidelines for the Transfusion Services in the United Kingdom. Since technology and legislation have both entered a period of rapid change, it is inevitable that revisions will be required and these Guidelines should be regarded, therefore, as a first edition. They will be circulated to all Centres of the UK Transfusion Services and to those Hospital Departments of Blood Transfusion who can be identified through the NEQAS Scheme in Blood Group Serology. It is considered that Volume III, in which reagents and techniques are specifically considered, will be of particular interest and use to Hospital Departments of Blood Transfusion.

Many of the guidelines have been assembled for the first time and some of the specifications are in a similar stage of infancy. With documents of this size and contents of such complexity it is imperative that the recommendations which they contain should be put into practice as the only means of discovering whether these newer aspects reflect what is truly achievable. Prolonged and careful deliberation by the Working Groups responsible for the compilation of the data in the Guidelines has led to the conclusion that the recommendations can indeed be implemented. Comments and suggestions for a revised second edition, however, will not only be welcomed but solicited. Preferably, these should be based on experience on the application of the Guidelines.

Comments should be sent to me in my capacity as Chairman of the UKBTS/NIBSC Liaison Group. It is anticipated that work on a second edition will begin in approximately one year from publication, which should allow users adequate time to comment realistically on their experiences with the contents of the first edition. Your assistance is earnestly sought.

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VOLUME I

Guidelines for blood components prepared at regional transfusion centres

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COUNCIL OF EUROPE DRAFT 1990

COMPONENT: Those therapeutic constituents of blood that can be prepared by centrifugation using conventional blood bank methodology.

STORAGE.

12/12 < -30°C 6/12 between -25°C & -30°C 3/12 -18°C to -25°C

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Chapter 1

Introduction

1.1 The United Kingdom Blood Transfusion Services (UKBTS) comprise twenty Regional Transfusion Centres (RTCs). Those in England are managed by Regional Health Authorities (RHAs), in Wales and Northern Ireland by the respective Health Offices and in Scotland by the Common Services Agency.

1.2 The National Directors in England and Scotland are responsible for the implementation of national policies and co-ordination of the work of the RTCs. The Headquarters Unit for Scotland was established in 1974 and the National Directorate for England and Wales in 1988.

1.3 During 1987 representatives of the UKBTS formed a liaison with those of the National Institute for Biological Standards and Control (NIBSC) to identify and define Guidelines for all materials produced by UKBTS both for therapeutic and diagnostic use.

1.4 Working Groups were formed to consider:

(i) Blood components prepared at RTCs
(ii) Fractionated plasma products
(iii) Blood Grouping reagents
(iv) Microbiological aspects, providing advice to the other Working Groups

1.5 The resulting Guidelines give advice, guidance and where appropriate, general specifications. Details of methods have been included when relevant. Standards have been identified within the Guidelines. Not all are available at the present time; those which are required are listed in Annex 9.

1.6 The Guidelines have been published in three volumes; the topics correspond to those of the first three Working Groups stated in paragraph 1.4

1.7 The Guidelines relate to blood and blood products from voluntary, non-remunerated donors and to reagents produced within the NHS, i.e. both within the UKBTS and the Central Blood Laboratories Authority (CBLA).

Separate guidance for hospital departments is being prepared by the Blood Transfusion Task Force of the British Committee for Standardisation in Haematology.

However, both hospitals and the Pharmaceutical Industry may find the Guidelines helpful.

1.8 It is not intended that the Guidelines should replace detailed specifications and standard operating procedures (SOPs), but they should be used in the preparation of specifications and SOPs. In this context most of the recommendations within the Guidelines state that they should be observed. However, there are certain recommendations, which by common consent, must be observed and in these circumstances the word 'shall' or 'must' has been used.

1.9 The Guidelines, in general, should be used in conjunction with the current Guide to Good Pharmaceutical Manufacturing Practice (HMSO) and the Requirements for the Collection, Processing and Quality Control of Blood, Blood Components and Plasma Derivatives (WHO: Requirements for Biological substances, No. 27, revised 1988). Where other texts are relevant reference will be made to them.

1.10 There are many reasons why UKBTS should achieve and maintain the highest

standard of operations. That some uniformity should be engendered in the determination of those procedures which will ensure maximum safety of blood and its products has been highlighted, viz;

(i) It is intended that Crown Privilege, under which the UKBTS and its associated Fractionation Centres have operated will be withdrawn. Thus, fractionated plasma products will require to be licensed and whilst it is unlikely that licensing procedures will be required for cellular products, licences could not be approved for fractionated products if the RTC producing the source plasma was not operating within agreed standards.

(ii) A Directive of the Council of European Committees (85/374/EEC) bound member states to introduce product liability by July 1988. In the UK this became a legal requirement on 1st March 1988. (Consumer Protection Act 1987, Chapter 43, Part I).

Human blood and substances prepared from it are products within the terms of the Act.

The contents of the Guidelines are not mandatory, but the recommendations contained in them do reflect the current state of technology and therapy in transfusion medicine and as such are advisory. They will be revised as technology advances and new products become available.

1.11 Volume I contains guidelines for blood components prepared at RTCs.

1.111 After the Introduction, in Chapter 2 the general requirements for a quality assurance system suitable for the collection and processing of blood and blood products are described. Chapters 3 and 4 build on this framework with specific Guidelines for blood and plasma donor sessions.

1.112 Chapters 5, 6 and 7 contain recommendations for the selection of donors, in general, and specifically for apheresis and immune plasma respectively. Chapter 8 contains Guidelines for blood component specification.

1.113 Since many of the general specifications are common to procedures described in the text, these have been placed in Annexes 1 to 6 to avoid repetition.

1.114 In certain countries, routine tests of blood donations for viral markers are undertaken which are not yet performed in the U.K. These include tests for anti-HTLVI and surrogate tests for the detection of donations possibly capable of transmitting non A, non B hepatitis. (i.e. serum alaninine aminotransferase (ALT), anti-HBc). More recently the test for anti-HCV has been undergoing evaluation. Consideration is being given to the value of such tests in the U.K. Should any of these tests be included in routine screening of blood donations prior to publication of these Guidelines, an addendum will be included.

Chapter 2

Guidelines for a quality system for the collection and processing of blood and blood products

2.1 Introduction

2.11 **Quality Assurance** is a total scheme to ensure that the product meets specification. In terms of the UKBTS the objective is to ensure the availability of a sufficient supply of blood and components of sufficiently high quality with maximum efficiency and with minimum risk to both donors and patients.

2.12 In order to implement satisfactory quality assurance it is essential that there should be a structured and organised approach. This is the **quality system**.

2.13 In this chapter the Guidelines or principles for the establishment of a quality system will be presented. They are derived from the British Standard 5750 (Quality Systems; specification for design, manufacture and installation).

2.14 The recommendations are written in the imperative tense, since a quality system cannot be implemented otherwise.

2.15 Although written for industry in general the principles enunciated in BS 5750 apply to the UKBTS. Human blood is a biological substance and no two donations can be the same. This applies also to the products derived from it, e.g. platelets. Thus each donation represents a batch and testing of an individual donation can only be performed within limits that do not lead to its destruction.

2.16 The formal style of BS 5750 will be used in this chapter, but the implications of each recommendation will be interpreted when appropriate.

2.17 The word **materiel** is used frequently in this chapter. For those readers who may not be familiar with its use, it can be defined as 'all components, materials or other supplies which are to be incorporated or to be used in the testing or processing of the product.'

Thus, materiel may comprise a pack for the collection of blood or plasma, the donation of whole blood or plasma, components, plasma fractions, the test reagents used during their preparation or pieces of equipment. In using this word, lengthy explanations of the different component parts of products or materials can be avoided.

2.2 A quality system

2.21 Each RTC shall establish, document and maintain an effective and economical quality system to ensure and demonstrate that the materiel and services conform to specified requirements. The documented quality system shall include quality management objectives, policies, organisation and procedures to demonstrate compliance with the requirements of these Guidelines.

2.22 The documentation of the quality management system should be presented as a quality manual.

2.23 Regional Transfusion Directors (RTDs) have to consider the overall operational plan for their RTC. In conjunction with staff with specialised skills, the policy for the RTC should be compiled and recorded. The principles involved in the production of safe, efficacious products should be defined together with the principles involved in determining specifications for materiel and services. These should include general principles of quality control to ensure that the stated requirements can be met.

2.3 Organisation

2.31 Quality assurance manager

2.311 Each RTC shall appoint a management representative, independent of production and preferably of other functions, who shall have the necessary authority and the responsibility for ensuring that the requirements of these Guidelines are implemented and maintained.

2.312 The quality assurance manager should report directly to the RTD or to another person deputed by the RTD who is entirely independent of production.

2.313 In the event of conflict arising between the Q.A. manager and the RTD (or the person nominated by the RTD), the circumstances and the decision taken must be fully documented and discussed at the time of the next quality audit.

2.32 Staff responsible for functions affecting quality

2.321 Each member of the staff of the RTC has responsibility for functions affecting quality. The level of responsibility shall be defined for each group or individual members of staff. The degree of authority allocated to each member of staff to evaluate quality problems and to initiate, recommend and provide effective solutions shall be determined.

2.322 This Guideline calls for a thorough appraisal of the quality assurance programme of the RTC and the part played in this by all members of staff. It is often considered that quality assurance is exclusively a laboratory function. This is not so. Every person in the RTC who is involved with the collection, testing, processing and release of products for issue, the selection of venues for blood collection, the cleaning of premises and the maintenance of equipment is responsible for quality.

2.4 Review of the quality system (quality audits)

2.41 The quality system established in accordance with these Guidelines shall be periodically and systematically reviewed to ensure its continued effectiveness; records of the review shall be maintained.

2.42 The quality audits should be performed by trained RTC personnel who do not have direct responsibilities in the procedures being audited.

2.43 External assessments should also be part of quality audits.

2.44 When the audit demonstrates that a procedural change should be made, the revised procedure should be validated before introduction.

2.5 Standard operating procedures (work instructions)

2.51 Each RTC shall develop and maintain clear and documented instructions that set out for all staff involved in functions affecting quality, the procedures which they will use.

2.52 Each procedure which affects the quality of a product should have a standard operating procedure.

2.6 Records

2.61 Each RTC shall develop and maintain records that demonstrate that the required quality has been achieved and that the quality system has operated effectively.

2.62 Specific requirement: product history file.

The records or reference shall be maintained in a product history file for at least fifteen years. Records shall include or refer to the location of the following.

2.621 The unique donation number allocated to each donation of whole blood or plasma from which products are derived.

(Note: When plasma is collected by apheresis from a single donor into more than one pack RTCs should build in a security system to ensure that when more than one pack bears the same number that the total number of packs to trace in the event of a recall can be identified).

2.622 The session record of each donation of whole blood or plasma from which the products are derived.

2.623 The processing record incorporating the date performed, the designated individual or when appropriate, the names of team members performing each operation and, when applicable, the major equipment used.

2.624 The inspection checks and quality control tests performed, the methods and equipment used, results, the date and signature of the person carrying out the inspection or tests.

2.625 A record of the label or, if appropriate, the package insert and the control number (i.e. a distinctive combination of numbers or letters which uniquely identifies an individual product) for each product produced

2.626 The records for each product shall be such that the origin of the product can be traced to the donor of the whole blood or plasma.

2.7 Corrective action

Each RTC shall establish and maintain documented procedures to provide for.

2.71 A continuing analysis of production losses to determine the cause and the corrective action needed.

2.72 A continuing monitoring of processing and analysis of records to detect and eliminate potential causes of lost production.

2.73 Records which give assurance that the corrective actions are effective.

2.8 Control and changes of documentation

2.81 General guidelines.

Each RTC shall establish and maintain control of all documentation that relates to the requirements of these Guidelines.

To this end the RTC shall ensure that the following is carried out.

2.811 The pertinent parts of appropriate documents are available at all locations, including mobile collection sites, where operations are performed which are essential to the effective functioning of the quality system.

2.812 All changes to documentation are in writing, dated and signed by the designated person.

2.813 All changes to documentation shall be provided to specified staff responsible for the procedure and steps taken to ensure that the revised instructions are understood and will be acted upon promptly.

2.814 Documents are reissued after a practical number of changes have been made.

2.815 Provision is made for the prompt removal of obsolete documents from all points of issue or use.

2.82 Documentation for specific products **i.e., a product master file.**

Each RTC shall establish and maintain detailed documentation for each product. This document shall be prepared, dated and signed by a designated person(s). Any changes shall be dated and authorised in writing by the signature of the designated person.

The product master file shall include or refer to the location of the following information.

2.821 Specifications.

2.822 Standard operating procedures (work instructions) .

2.823 Quality control procedures and the apparatus used.

2.824 Full information concerning the selection of donors and the donations to be used for the preparation of the product.

2.825 Full information concerning suppliers of critical components such as blood packs, including the specification for those components and written copies of any agreements made with these suppliers.

2.826 Complete labelling procedures for the donations and products together with copies of all approved labels and other labelling.

2.827 Records or references shall be maintained in the product master file for at least fifteen years.

2.9 Control of inspection of test materiel

2.91 Each RTC shall ensure that there is provision of suitable equipment and reagents for the preparation and testing of products prepared from whole blood and plasma.

2.92 Each RTC shall control the calibration and maintenance of equipment used in the preparation of products prepared from whole blood and plasma; these procedures should be suitable to demonstrate the conformance of this materiel

to the specified requirements.

2.93 By means of suitable quality control, both by the use of internal and, where appropriate or available, external control material, each RTC shall ensure that all reagents conform to specified requirements.

2.10 Control of purchased materiel and services

2.101 Purchasing

2.1011 Each RTC shall be responsible for ensuring that all purchased materiel and services conform to specified requirements. The selection of sources and the type and extent of control exercised by the RTC will be dependent on the type of materiel and the suppliers demonstrated capability.

2.1012 In particular, when the purchase of a licenced product is involved, the RTC shall require the supplier to provide information to confirm that the product conforms to the terms of the licence.

2.102 Purchasing data

Each purchasing document shall contain a clear description of materiel and services ordered.

2.103 Inspection on receipt

Each RTC shall ensure that no purchased materiel is used until it has been inspected and verified as conforming to the manufacturers specifications.

2.11 Collection of whole blood and plasma and production control

2.111 Each RTC shall ensure that blood and plasma collection and processing are carried out under appropriately controlled conditions.

Controlled conditions includes, when appropriate, the use of suitable processing equipment and a special working environment, e.g. the use of laminar flow cabinets in controlled areas. It also includes documented standard operating procedures in which the manner of collection and processing are defined.

2.112 Each RTC shall ensure that after each process stage that affects quality there is either an inspection or a quality control test.

Many examples for this Guideline can be provided. For instance in the laboratory the preparation of platelets from donations of whole blood the following processing stages would require inspection and quality control:

- initial selection of the collected donations for processing e.g. elimination of donations for platelet preparation from persons who have taken aspirin in the past 7 days, those donations which have not been collected into appropriate packs or have not been transported to the RTC at ambient temperature
- verification of correct centrifugal speeds
- examination for pack defects during processing
- specified volumes of plasma used for the processing and final suspension of the platelets

- labelling of product
- release of platelets from quarantine after all mandatory tests have been completed on the whole blood donation
- conditions of transportation
- performance of quality control tests (Chapter 8)

2.113 Specific requirements

Each RTC shall ensure that requirements under the following headings in the current Guide to Good Pharmaceutical Manufacturing Practice are followed:

personal health and cleanliness

buildings

environmental control

cleanliness

equipment

equipment maintenance

limits and tolerances

processing materials and reagents

2.12 Finished product inspection

2.121 Each RTC shall perform all inspections and tests on the finished product to complete the evidence of full conformance to specified requirements.

2.122 Before a product is released for distribution, all test results and acceptance records shall be checked by a designated person(s). Release shall be authorised by the signature of a designated person.

2.123 Where release is subject to computer derived information the computer system must be shown to be fully secure against the possibility of uninspected or defective materials being released.

2.13 Quality control tests

2.131 The quality control test procedures used by the RTC shall be in accordance with the Guidelines given for each product (see Chapter 8).

2.132 Quality control test procedures shall be regularly reviewed in the light of the finding of products not conforming to specification or as a result of information obtained from quality audits. It must be recognised, however, that the product may be substandard and this alternative must not be ignored.

2.14 Control of non-conforming materiel

2.141 Each RTC shall establish and maintain procedures for controlling materiel that does not conform to specified requirements. This materiel may consist of all

materials used in the collection and processing of whole blood and plasma, the reagents used in testing the donations of whole blood and plasma, or the products which have not met specified requirements.

2.142 The procedures shall include provision for identification, segregation and disposal as appropriate. All non-conforming materiel shall be clearly identified to prevent unauthorised use or mixing with materiel which conforms to specified requirements.

2.15 Identification of processing status

2.151 Each RTC shall establish and maintain a system for the identification of product status during all stages of processing and for those tests which have been carried out.

2.152 Each RTC shall ensure by means of suitable identification, and preferably by storage in a separate location, that products which have not been released for issue can be identified from those which conform to specification and have received their final inspection.

2.16 Protection and preservation of product quality

2.161 Each RTC shall establish and maintain a system to control the packing and preservation of the products during their shelf life to the extent that it is necessary to ensure that during this period they conform to specified requirements. This includes any transportation that may be required.

2.162 The requirements under the following headings in the current Guide to Good Pharmaceutical Manufacturing Practice shall be followed.

Labelling

Storage

Transportation

(For specific Guidelines for products see Chapter 8 and for general Guidelines see Annex 4).

2.17 Training

2.171 Each RTC shall establish a system for identifying and implementing training needs and certification requirements for all staff.

2.172 Specific requirement

Personnel working in controlled environments shall be given training related to maintaining the integrity of that controlled environment.

2.18 Product recall and notification of defects

Each RTC shall establish a procedure for the recall of a product suspected or known to be defective or hazardous in accordance with the specific requirement of the current Guide to Good Pharmaceutical Manufacturing Practice.

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Chapter 3

Quality assurance at blood donor sessions

3.1 General specifications

3.11 General comments

3.111 This section applies to the collection of donations of whole blood at permanent sites or by mobile blood collection teams.

3.112 The ultimate responsibility for the correct safe procedure for the collection of blood is that of the Regional Transfusion Director; the immediate responsibility for the operation of the blood collection session is that of the medical practitioner or senior nurse in attendance.

3.113 Each RTC must prepare its own procedures manual, covering all phases of activity of blood collection. Numbered copies of the procedures manual should be issued to all staff involved in sessional procedures and measures should be instituted to ensure that every copy is regularly updated.

3.12 Guidance for the following procedures is given in Annex 1.

Haemoglobin or haematrocrit screening

Copper sulphate haemoglobin screen
Copper sulphate storage
Copper sulphate for routine use
Copper sulphate procedure: fingerprick blood sample
Spectrophotometric method for haemoglobin screening
The microhaematocrit method for haemoglobin screening

Donor identification

Preparation of the venepuncture site

Preparation of the blood pack

Performance of the venepuncture

Blood donation

Blood anticoagulation
Blood flow
Blood volume monitoring
Sample collection
Completion of the donation
Final inspection
Safety related defects

3.13 Guidance for laboratory testing procedures is given in Annex 5.

3.2 Records

3.21 Donation identification

3.211 Sessional reception staff must ensure that a unique number set is assigned to each donation. Great caution is necessary to avoid crossover or duplication of numbers.

3.212 Sets of numbers not used should be placed in a container for destruction and must be accounted for.

3.213 If there is need to renumber a blood pack system, new numbers should be used; labels which have been discarded shall not be retrieved.

3.22 Labelling

3.221 Donor session staff must ensure that the unique number assigned to the donation appears on the donor session record, the primary and secondary collection packs and all the sample tubes used.

3 221 The organisation should be such as to avoid the possibility of errors in the labelling of blood containers and blood samples; for example, the taking of samples at the end of a donation should be directly linked with the cessation of the donation with the minimum possible time interval and the blood bag and the corresponding samples should not be removed from the donor's couch until a satisfactory check on correct labelling has been carried out.

For this purpose it is recommended that each donor couch has its own individual facilities for the handling of samples during donation and labelling.

3.23 Donor session records

3.231 A record of the sessional venue, the date, the donation number and the identity of all donors attending must be maintained.

For any donors who are deferred, rejected or retired the full details must be recorded and the reasons given for the action taken.

3.232 The records of blood donation sessions should allow identification of each important step associated with the donation.

All successful donations must be recorded; unsuccessful donations must be recorded together with the reason why they were unsuccessful, all adverse reactions must be recorded together with the action taken; full details of any other incidents, including those involving only staff must be recorded.

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

3.3 Documentation

3.31 Selection of donors - see Chapter 5.

3.32 Specification of blood and blood products - see Chapter 8.

3.4 Control of inspection of test materiel

Guidance on the calibration and control of laboratory tests is given in Annex 5.

3.5 Control of purchased materiel and services

3.51 Specification and inspection of blood bags

3.511 Blood 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 and measures must be taken to prevent unsterile air entering the system.

3.512 Blood shall be collected into containers that are pyrogen free and sterile, containing sufficient licensed anticoagulant for the quantity of blood to be collected.

3.513 The container label shall state the kind and amount of anticoagulant, the amount of blood that can be collected and the required storage temperature. Blood packs may be supplied in containers holding 1 - 12 bags.

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

3.515 Batch numbers of the blood packs used should be recorded.

3.516 The donation number on the pack and sample tubes should be checked at the end of the donation to ensure that those for a given donation are identical.

3.517 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 for future analysis and action (see Annex 1, 6.8 and 6.9).

3.52 Inspection of labels for printing errors

All donor records and labels should be checked for printing errors. Duplicate number sets shall not be used and these and missing numbers shall be reported via a designated senior manager to the printer concerned and to the Chairman of the National Working Party on machine-readable labels.

3.6 Collection control

Guidance on the selection of premises for donation sessions is given in Annex 3.

3.7 Protection and preservation of product quality

Guidance on requirements for labelling is given in Annex 4.

Guidance on requirements for storage is given in Annex 4.

Guidance on requirements for transportation is given in Annex 4.

Chapter 4

Quality assurance at plasma donor sessions

4.1 General specifications

4.11 General comments

4.111 This section only applies to manual plasmapheresis and automated machine plasma and platelet apheresis sessions involving single arm procedures with minimal return of anticoagulant.

4.112 These are the only type of apheresis procedures that should be undertaken at sites significantly removed from intensive care facilities.

4.113 A medically qualified consultant fully experienced in apheresis procedures must be ultimately responsible for the selection, health and welfare of the apheresis donors: responsible for the organisation of satisfactory staff training programmes and proficiency testing either in his/her own unit or by secondment to a unit where there is greater experience.

The consultant should ensure that constantly updated written protocols are prepared for each procedure undertaken and that these are always available at the session.

4.12 Guidance for the following procedures is given in Annex 2.

Donor identification
Preparation of the venepuncture site
Machine/manual plasmapheresis set
Performance of the venepuncture
Plasmapheresis equipment
Anticoagulation
Blood flow
Blood flow monitoring
Blood volume monitoring
Sample collection
Completion of the donation
Adverse donor reactions

4.13 Guidance for laboratory testing procedures is given in Annex 5.

4.14 Plasma and platelet donations

4.141 A written protocol shall be prepared for each apheresis procedure in use. These instructions shall be adhered to and any deviations from the standard operating procedure recorded.

4.142 In particular the return of red blood cells to donors undergoing manual plasmapheresis should be subjected to stringent controls.

The biggest inherent danger is confusion between two packs of concentrated red cells during their centrifugation and return to individual donors. A proper identification system to avoid this is essential; e.g. the donor may be asked to sign the label on the pack and to confirm his/her signature before the return of the red cells. In addition use can be made of the integral numbering system on the pilot tube of plastic bags, perhaps by transferring this number to the wrist of the donor.

4.143 Particular attention should also be given to the donor's extracorporeal blood volume when employing machine apheresis. The written protocol for each type of machine and procedure shall state that the maximum allowable extracorporeal volume is not exceeded (see current Guidelines for the use of automated machine plasma and platelet apheresis of volunteer donors; UK Working Party).

4.15 Final donation inspection

4.151 As in Annex 1, but in addition the plasma donation should be inspected for the presence of any clots and any colour change that might suggest the presence of haemolysis. Such changes may require a review of the apheresis procedures and/or equipment.

4.152 Any suspected abnormality should also be recorded on the donor record card and if the sessional staff are in doubt as to whether the donation is suitable for the purpose for which it was intended, it should be marked for inspection by the blood product laboratory staff before further processing.

4.2 Donor session records

As in Chapter 3, but in addition to the standard procedure for whole blood donation, a potential apheresis donor must give their informed consent by signing a specific consent form (see current Guidelines for use of automated machine plasma and platelet apheresis of volunteer donors; UK Working Party).

4.3 Documentation

4.31 Selection of donors - see Chapter 5.

4.32 Specification of blood and blood products - see Chapter 8.

4.4 Control of inspection of test materiel

4.41 Guidance on the calibration and control of laboratory tests is given in Annex 5.

4.5 Control of purchased materiel and services

4.51 Specification and inspection of blood packs.

4.511 The complete harness/plasmapheresis set should be thoroughly inspected for faults prior to use and during the setting up procedure. The set must be in date and particular attention must be given to searching for any kinks or occlusions or points of weakness or actual leaks that may become more detectable during the setting up and priming procedure before the donor is attached to the set.

4.512 If an occlusive kink or a leak becomes apparent during a machine procedure then that procedure should be abandoned and any remaining blood constituents should not be returned to the donor.

4.513 Any faults detected before or during a procedure should be reported on the daily worksheet for in house quality control of the sets in use.

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

4.6 Collection control

4.61 Premises

Guidance on the selection of premises for donation sessions is given in Annex 3.

4.62 Equipment

Guidance on the specification and care of equipment for plasmapheresis is given in Annex 2.

4.7 Protection and preservation of product quality

4.71 Sessional storage of blood donations

As in Annex 4, but in addition, plasma donations may be frozen on site at static donor centres. If a freezer is installed on site, there shall be a system to monitor temperature as well as an alarm system to warn personnel before storage temperatures reach unacceptable limits, as described for blood bag refrigerators in compliance with BS 4376:1982.

4.72 Guidance on requirements for labelling is given in Annex 4.

4.73 Guidance on requirements for storage is given in Annex 4.

4.74 Guidance on requirements for transportation is given in Annex 4.

Chapter 5

Selection of donors

5.1 General considerations

5.11 Donations of whole blood or some of its components provide the material from which all blood products are derived. The criteria for selection of blood donors apply equally to donors of whole blood and of cellular or plasma components collected by apheresis.

5.12 The Guidelines for selection of suitable blood donors have the purpose of ensuring that the potential donor is in good health for two reasons.

5.121 To protect the recipient from any ill-effect through transmission of disease or drugs by blood transfusion.

5.122 To protect the volunteer from any harm to his/her health.

5.13 As this document is concerned with the quality of the final product, issues relating solely to donor safety, donor ill-effects and donor care are largely excluded, although these considerations form an important part of donor selection.

5.14 Only persons in good health should be accepted as donors of blood for therapeutic use.

5.141 The prospective donor's medical history should be evaluated on the day of donation by a suitably qualified person who has been trained to utilize accepted guidelines for the selection of blood donors.

5.142 If there is doubt about the suitability of a prospective donor, a donation should not be taken and the details should be referred to a medical practitioner for a decision.

5.143 The ultimate responsibility for the selection of donors rests with the RTD; the immediate responsibility is that of the medical practitioner or senior nurse in attendance at the session.

5.15 Patients referred for therapeutic venesection should not be accepted at donor sessions.

5.16 The Guidelines in this Chapter do not apply to donors wishing to give blood for autologous transfusion. Specific guidance for autologous transfusion is given in Clin. Lab. Haemat. 1988, 10, 193-201.

5.2 Medical assessment

5.21 In practice it is impossible to perform a complete medical and physical examination of every prospective donor. A significant part of the assessment procedure will usually rely on answers to simple standard questions relating to general health, past medical history and medication. This is combined with

simple visual assessment of the donor and selected testing of samples collected at the time of donation.

5.22 In order to obtain relevant information about medical history a standard set of questions must be put to a first-time prospective donor. (Example of a simple questionnaire is given in ISBT Guide No. 1 or Annex 1, section 7).

5.23 Age

5.231 Donors should generally be between the ages of 18 and 65 i.e. from their eighteenth to sixty-sixth birthday. It is general practice to set an upper age limit of 60 for first-time donors in view of the increased incidence of cardiovascular disease over that age, and potential adverse effects in first-time donors.

5.232 Donors may be accepted, subject to national policy, from the age of 17 years with appropriate consent.

5.233 The medical practitioner in charge may authorise continuation of donation beyond the age of 65, up to the donor's seventieth birthday, but in these cases due regard should be made of the increased likelihood of coincident events which might be precipitated by or associated with the act of blood donation.

5.24 Frequency of donations

Usually 2 donations are given in a 12 month period. Many donors are able to give blood more frequently without developing iron deficiency so that an interval of 16 weeks between donations is considered a reasonable minimum.

5.25 Volume of donation

No more that 13% of the estimated blood volume should be taken during one blood donation. For an individual weighing over 50 kgs (7 stones 12lb), a donation of 450 ml is usually taken.

5.3 Medical history of donors

5.31 General considerations

5.311 All donors should be specifically questioned about the conditions listed on NBTS 110 (or equivalent document) and every donor should sign NBTS 110 (or equivalent document) Any condition declared should be discussed with the medical practitioner or senior nurse in attendance at the blood collection session unless clear, unequivocal instructions regarding the responses are available to the member of staff conducting the questioning.

It may be helpful if new donors, in addition, fill in a short questionnaire (an example of which is given in ISBT Guide No. 1 and Annex 1, Section 7).

5.312 Donors whose serum or plasma or cells are to be used for laboratory as opposed to therapeutic purposes should be submitted to the same routine as other donors, but obviously some decisions regarding their suitability to donate may be different (e.g. treatment with certain medications, medical history or allergy) .

5.313 Individuals who attend a session and give the information that they are currently undergoing medical investigations or have been referred for a specialist opinion should be advised not to donate blood until investigations are complete, even if perfectly asymptomatic on the day.

5.314 Donors should be made aware that recipients experience risk from transfusion, and donors should therefore be asked to report any illness developing subsequent to the donation.

5.315 Information which is, or may be, of relevance to the health of the recipient and which arises subsequent to the transfusion of the blood, should be reported to the appropriate party (i.e. National Fractionation Centre or Consultant in charge of the hospital blood transfusion laboratory) so that further action may be taken if deemed necessary.

5.316 The record of physical assessment and medical history of the donor must be identified by the examiner's signature. Any reason for exclusion should be recorded. Lists of conditions necessitating permanent or temporary exclusion from blood donation follow.

5.317 If a donor is following his/her normal meal pattern they may be accepted, if a donor presents having missed his/her normal meal a cup of fluid and biscuits should be consumed at the session prior to collection of the blood.

5.4 Examples of conditions necessitating permanent exclusion

This list is not necessarily exhaustive, other conditions which arise may be added.

In cases of doubt, the donor should be asked for written permission to contact his/her General Practitioner, and donation postponed until further information is available.

5.41 Cardiovascular diseases

Individuals with circulatory disorders are especially subject to cardiovascular and cerebrovascular disturbances resulting from sudden haemodynamic changes. Thus, all such donors are excluded.

5.42 Central nervous system diseases

In general, these conditions are contra-indications to donation, as the individual may well be unduly susceptible to sudden haemodynamic changes. In addition, those conditions known or suspected to be of viral origin (e.g. Creutzfeld-Jakob, multiple sclerosis), should be reason for permanent exclusion.

5.43 Gastrointestinal diseases

All diseases which may be of immune origin, or which render the individual liable to iron deficiency through impaired iron absorption or blood loss, should be reason for exclusion.

5.44 Haematological disease

Any disorder which may be of viral or immune origin, and all those which may be of malignant potential (e.g. polycythaemia and other myeloproliferative disorders) should be reason for permanent exclusion.

5.45 Infectious diseases which are reasons for permanent exclusion

AIDS, HIV infection

Brucellosis

Granuloma Inguinale

Kala-azar

Lymphogranuloma venereum

Q fever

Syphilis

Trypanosmiasis cruzi (Chaga's disease)

5.46 Metabolic diseases

In general, individuals who are receiving continual therapy which might adversely affect a transfusion recipient should be permanently excluded. Replacement therapy with some hormones (e.g. thyroxine) need not be reason for permanent exclusion, but there may need to be consultation with the donor's medical adviser before a decision can be made.

5.47 Renal diseases

All chronic renal diseases are a reason for permanent exclusion.

5.48 Respiratory diseases

Individuals who have significant chest disease should not be accepted as blood donors.

5.49 Recipient of human growth hormone

Individuals who have received human growth hormone are permanently excluded. Potential donors who have received (usually after 1985) recominent derived human growth hormone need not be debarred.

5.410 Miscellaneous

Any active and/or chronic diseases which may be auto-immune or viral origin, or whose basis is unknown, should be a reason for permanent exclusion. Diseases which are known to relapse should also be considered as contraindications to blood donation. All diseases of malignant origin should be cause for permanent exclusion, although exception may be made for localised conditions such as carcinoma in situ of the cervix and rodent ulcer.

5.5 Conditions necessitating temporary deferral

Condition	Comment
Abortion, (see pregnancy)	gestation > 6/12–wait 1 year gestation < 6/12–wait 6 months
Accident, minor	wait 3 months
Accident major	wait 6 months
Acupuncture–performed by a registered medical practitioner – performed by others	accept wait 6 months
Allergy, including – hay fever – desensitising injections – drug allergy	wait until asymptomatic wait 72 hours after last injection generally defer permanently
Anaemia	wait until cause is determined; assess each potential donor
Blood donation within 3 months	wait until 16 weeks since last donation
Blood transfusion (including cellular and plasma products)	wait 6 months
Contact with infectious fevers	wait for duration of incubation period–4 weeks if this is unknown

Dental treatment – complicated surgery – uncomplicated extraction	 wait 3 months wait 24 hours
Drugs – prescribed by physician or self-medication (eg aspirin)	individual assessment – see 5.7
Ear-piercing	wait 6 months
Electrolysis	wait 6 months
Epilepsy	individual assessment – some individuals with epilepsy react to minor stress by having fits and it is important that additional risks should be avoided. Anyone on regular medication for epilepsy should not be bled. A known epileptic who has not required anticonvulsant therapy and has not been subject to fits for 3 years may with discretion be considered as a possible donor.
Fractures	wait until recovered
General anaesthetic	wait 3 months (in view of underlying condition – if for uncomplicated dental extraction then 24 hrs)
Genital herpes	wait until lesions are beginning to heal
Glandular Fever	wait 2 years from recovery
Heart operations	may be accepted after appropriate consultation when corrective surgery was carried out for congenital defects
Hepatitis	wait 12 months from recovery
Herpes simplex (cold sore)	wait until lesions are beginning to heal
Hereditary Hb disorders	may be accepted. Providing the donor is well and has acceptable Hb concentration there is little risk to donor or recipient, but it may be preferred to use the cells for laboratory purposes only (with the donor's consent)
Infections – boils, sore throat etc	wait until recovered and off antibiotics
Infectious diseases – recent measles mumps etc	wait until 4 weeks after recovery

Infectious mononucleosis	wait 2 years from recovery
Inoculations and immunisations	see 5.11
Jaundice	wait 12 months
Legionnaire's Disease	wait until fully recovered
Leptospirosis	wait until fully recovered
Malaria	(see 5.9)
Meningitis	wait until fully recovered
Peptic ulcer – on active therapy	wait 6 months after completion of treatment
Petit mal	wait until off all therapy and asymptomatic for 3 years (see epilepsy)
Pregnancy	wait until 1 year after delivery (see 5.6)
Renal disease (acute)	normally wait 5 years after recovery but seek advice from donor's medical adviser
Renal disease (chronic)	see 5.47
Respiratory tract	wait until acute symptoms resolve
Surgery minor e.g – tonsillectomy – herniorraphy – appendicectomy	wait until fully recovered
Surgery major e.g. – hysterectomy – cholecystectomy	wait 6 months
Tattooing	wait 6 months
Thrombosis deep venous (of calf)	wait 6 months
Thrombophlebitis – unrelated to venepuncture	wait 6 months
Thyroid disease	wait until consultation with donor's medical adviser
Toxoplasmosis	wait 2 years from recovery, absence of IgM antibodies
Tropical diseases	see 5.10
Tuberculosis	wait until off all therapy for 2 years
Venereal diseases	
– gonorrhoea	wait until 1 month after the end of treatment
– non-specific urethritis (NSU)	wait until symptoms resolved
genital herpes	wait until symptoms resolved

5.6 Pregnancy

Pregnant and lactating women should not give blood in view of their high iron requirements at this time. Exceptions to this rule may be made with the consent of the woman's medical practitioner in the case of women whose blood contains antibodies.

5.7 Donors on treatment with drugs

5.71 Donors receiving a course of prescribed medication should be deferred until at least one week after treatment is complete. This is to ensure that the blood collected is as near normal as possible, and to minimise risks to donors themselves.

5.72 Donors taking drugs which are proven or potentially teratogenic (e.g. vitamin A derivatives) or who are taking drugs which accumulate in tissues over long periods, should not be accepted for blood donation.

5.73 Sporadic self-medication with some drugs (e.g. vitamins, aspirin, sleeping tablets) need not prevent a donation being accepted, provided the donor is fit and well.

5.74 If the donor has taken drugs affecting platelet function (aspirin, anti-inflammatory drugs) within the last 7 days, the donation should not be used for the preparation of platelets. A list of all such drugs should be made available to staff at blood donor sessions.

5.75 Other drugs/tablets may be acceptable. The taking of some drugs may indicate a disease which would automatically make a donor ineligible.

5.76 It is recommended that each RTC should prepare a list of the commonly used drugs with rules for acceptability of donors, and that in any doubtful situation it is wiser to defer than to take a donation.

5.8 Infectious diseases

5.81 HIV infections

5.811 All potential donors must be provided with information on AIDS so that those at risk of HIV infection will refrain from donation.

Note: see special Department of Health (DH) leaflet for self-exclusion of persons at risk of HIV infection.

5.812 Donors must be asked to read the notices regarding the testing of donations for anti-HIV so that consent for this test is obtained.

5.813 Potential donors who are blind, partially sighted or illiterate should be informed of the contents of the AIDS literature and the notices regarding testing of donations for anti-HIV.

5.814 There is no evidence to suggest that hospital staff involved in caring for AIDS patients, or working in hospital laboratories, are at any greater risk with respect to HIV infection than the general public. Such persons may be accepted as donors, providing that they have not suffered an inoculation injury or suffered contamination of non-intact skin with blood from an individual infected with HIV.

5.82 Hepatitis B

Individuals with a history of jaundice or hepatitis may be accepted as blood donors 12 months after recovery from the illness provided an approved test for HBsAg is negative at this stage. Persons whose blood gives a positive reaction for the presence of HBsAg are excluded. The presence of anti-HBs does not debar.

5.821 Risk groups

All persons who have received a transfusion of blood or blood products, acupuncture (other than by a registered medical practitioner), tattooing, electrolysis and ear-piercing should be deferred for six months, as should those who have been in close contact with a case of hepatitis. Hospital staff involved in caring for patients with hepatitis B, or working in hospital laboratories, may be accepted as donors provided they have not suffered an inoculation injury with blood from an individual infected with hepatitis B and have not received hepatitis B vaccination and/or immunoglobulin as prophylaxis against a possible exposure within the previous 9 months.

5.822 Circumstantial involvement

Donors without demonstrable markers of hepatitis who have donated blood to two patients strongly suspected of having transfusion-transmitted hepatitis should be permanently excluded. The only donor of blood to a recipient with transfusion-transmitted hepatitis should also be excluded.

5.823 Donors from areas endemic for hepatitis B

Although hepatitis B is not strictly a tropical disease, its causative virus is far more prevalent in tropical and subtropical areas than in the UK. However, they may be accepted as donors, provided their blood is shown to be negative for hepatitis B surface antigen by a test which detects the nationally agreed level of HBsAg.

5.9 Malaria

5.91 Donors should be asked if they have visited places abroad (other than in Western Europe, Australia, New Zealand or North America) or have lived in such places for a period of 3 months within the past five years.

5.92 Individuals who have had a pyrexia of unknown origin during or after a visit to a tropical area may be infected with the causative agent of hepatitis B, lassa fever, malaria or other dangerous infections. Therefore, blood should not be collected from such persons until one year after resolution of the pyrexia or return to the U.K., whichever is the longer.

5.93 **Visitors to malarial areas acceptable as cell donors**

5.931 The quarantine periods listed below should operate whether or not the donor has taken anti-malarial prophylaxis. A non-immune individual who has omitted prophylaxis and has become infected with malaria is likely to become unwell very quickly, well within the one year quarantine period. On the other hand, if appropriate prophylaxis has been taken by a non-immune individual, who remains well after discontinuation of the prophylaxis, then infection with malaria is unlikely.

5.932 The questioning of donors as to the country(ies) in which they were born, brought up, or visited, is essential for the determination of potential transmission of malaria by transfusion. It is recommended that RTCs should provide maps and lists of the countries concerned for staff to consult. W.H.O. regularly produces comprehensive information on malaria risk in individual

countries from which a composite list of countries with a risk of malaria may be prepared. An example of such a list is given in Annex 1, Section 8.

5.933 No febrile episodes during or following stay	one year after return to UK
5.934 Residents or former residents of endemic malarial areas (no recent febrile episodes)	acceptable as cell donors one year after return to UK if a validated antibody test is negative at the end of this period; otherwise 3 years
5.935 Visitor to endemic malarial areas who have febrile episodes during or after their stay	acceptable as cell donors one year after recovery or return, whichever is the later, and provided a validated antibody test is negative; otherwise 3 years
5.936 Individuals with a history of proven malaria	acceptable as cell donors one year after becoming asymptomatic or the cessation of anti-malarial therapy, whichever is the later, and provided a validated antibody test is negative; otherwise 3 years.

5.94 Visitors to malarial areas acceptable as plasma donors.

5.941 Individuals in all categories may be accepted as donors of plasma for fractionation only without a quarantine period, provided they satisfy all other criteria of acceptability. This would exclude those with a recent, and possibly undiagnosed, febrile episode.

5.942 Products such as plasma for clinical use and cryoprecipitate may contain red cell debris and should not be prepared from donations which are not acceptable for red cell use.

5.10 Other tropical diseases

5.101 Trypanosomiasis (Cruzi)

This may lead to an acute or chronic, incurable, and even fatal illness. Blood from donors who have visited or lived in rural S. America or Central America including Southern Mexico should ONLY be used for preparing plasma fractions (*not* plasma for clinical use or cryoprecipitate). Donations from such persons may be used for normal purposes provided they have been shown by suitable test to be free of antibodies to Trypanosma Cruzi.

5.102 Filariasis, Kala Azar, Q Fever and Yaws

These are contra-indications to blood donations even after recovery has occurred.

5.103 Amoebic dysentery, Schistosomiasis; and Arthropod-borne encephalitides

These are not contra-indications for donation once complete recovery has taken place.

A period of 2 years should be allowed after recovery from relapsing fever.

5.11 Inoculations and immunisations

5.111 Prospective donors who have been immunised recently and are symptom-free may be accepted after the following

Live vaccines	BCG, measles, mumps (oral) polio, yellow fever	3 weeks
	rubella	3 months
Killed vaccines	anthrax, cholera, common cold, diphtheria, (Salk) polio, rabies, tetanus, typhoid	48 hours
	hepatitis B	1 week
(providing there has been no known exposure)		
	hepatitis B (given with immune globulin)	9 months

Donors who have recently been actively immunised may have suitable levels of immune antibodies to merit donation for specific immune plasma.

5.112 Immunoglobulins administered after a known exposure can prolong the incubation period of a disease, hence the deferral period should be as follows:

anti-tetanus Ig	4 weeks
normal human Ig	6 weeks
hepatitis B Ig	9 months

5.113 Normal human immunoglobulin administered prophylactically prior to going abroad does not in itself merit deferral although the country visited may do so.

5.12 Physical examination of donors

5.121 General considerations

A complete medical examination including X-ray examination, electrocardiogram and extensive haematological tests is impractical for normal donors. Most donors may be accepted on the basis of medical history, general appearance and haemoglobin estimation, although it is advisable to examine the pulse and check the blood pressure where there are any doubts, particularly in new donors.

This procedure, used skilfully, will lead to rejection or deferment of most donors who are unfit to be bled and it should be carried out meticulously. When in doubt it is better to reject or defer, and the Medical Officer or Nurse should ensure that an appropriate entry is made on the donor's record.

5.122 Inspection of the donor

The donor should appear to be in good health. Note should be taken of poor physique, debilitation, undernutrition, plethora, anaemia, jaundice, cyanosis, dyspnoea and mental instability. Suggestion of intoxication either by alcohol or narcotic drugs should be a reason to exclude that donor. The skin at the venepuncture site should be free from lesions.

5.123 Weight

Healthy individuals can generally donate up to 450 ml of blood (plus small laboratory sample) without any deleterious effect on their health. A standard blood donation is 450ml±10% with an optimum blood/ anticoagulant ration of 7:1. (See Chapter 8).

Those who weigh less than approx 50 kg (7 stone 12 lb) are more likely to suffer adverse effects (in particular dizziness and fainting) after a standard blood donation as this represents a greater proportion of their blood volume. Potential donors who weigh less than 48-50 kg may give a smaller donation with the anticoagulant content adjusted accordingly, but all such donors should be assessed carefully to ensure that the low body weight is not due to illness. The minimum weight for donation is set arbitrarily at 41 kg (6 stone 6 lbs).

5.124 Haemoglobin estimation

The haemoglobin concentration should be determined each time a potential donor presents. The acceptable lower limits are *female* donors 12.5 g/dl, or male donors 13.5 g/dl. The type of test used is left to the discretion of the Regional Transfusion Director.

Potential donors whose haemoglobin appears to be below the appropriate concentration should not be bled. It is recommended that a check of the concentration is made using other methods. The reason for deferral should be explained to such donors and they should be advised to see their own General Practitioner if this is considered to be appropriate. Precise details may vary between Centres according to individual arrangements.

Chapter 6

Donors for plasmapheresis

6.1 Supervision and medical care

This should be the direct responsibility of a medical practitioner specially trained in apheresis procedures. The relevant DH Code of Practice and BTS Guidelines should be followed.

6.2 Criteria for acceptance

6.21 Other than in exceptional circumstances (to be decided by a designated medical practitioner), donors for apheresis procedures shall meet the usual criteria for ordinary whole blood donations. They should preferably have given at least 2 routine blood donations without untoward effect.

6.22 In addition the following criteria should be observed for apheresis donors donating by one-arm technique only.

6.221 Ordinarily, the donors should be between 18 and 60 years of age. First time donors should not normally be accepted over the age of 50 years.

6.222 First time donors should not normally be less than 50 kgs in weight. The donor weight should be assessed to ensure that the maximum extracorporeal volume during plasmapheresis does not exceed 15% of the total blood volume.

6.3 Pre-donation screening

6.31 A donor recruited to a continuing programme of apheresis should have the following blood tests in addition to a haemoglobin screen: haematocrit, white blood cell count, platelet count, blood film and serum proteins.

6.32 The results obtained should be within the normal range for the age and sex for the donor. The pre-donation platelet count at recruitment must be greater than 150 x 10^9/L. On subsequent donations the platelet count should be measured retrospectively.

6.33 Follow up investigations should be carried out on all regular apheresis donors. Detailed haematological and serum protein assays including immunoglobulin levels should be carried out at regular intervals during the year and a consultant in the RTC should assess the donor's fitness to continue on the apheresis programme in the light of these laboratory investigations.

The regularity of the investigations are dependent on the frequency and volume of donations and are specified in the BTS Guidelines.

6.4 Medical examination

For donors under 45 years of age, a minimum requirement would be the examination of blood pressure and pulse. For donors over 45 years of age it may be necessary to extend the examination, e.g. ECG prior to recruitment to an apheresis programme.

6.5 Volume of plasma donated

6.51 Not more than 15 litres of plasma should be donated by one donor in a year.

6.52 The quantity of plasma donated per donation and per week are specified in the BTS Guidelines.

6.53 Erythrocyte loss should preferably be kept below 20 ml of packed red cells per week.

6.54 The interval between one apheresis procedure and a whole blood donation should be at least 48 hours. If a plasma donor donates a unit of whole blood or does not have the erythrocytes returned during a plasma donation then further donation should be delayed for a period decided by the designated medical practitioner.

6.6 Donors for plateletpheresis and leukopheresis

In general, donors for plateletpheresis and leukopheresis must meet the criteria for whole blood and plasmapheresis donors. The BTS Guidelines should be followed.

6.7 General specifications for plasma donor sessions

These can be found in Annex 2.

Chapter 7

Donors for immune plasma and/or immunisation

7.1 General considerations

The need for certain specific immunoglobulins, in particular anti-hepatitis B, anti-tetanus and anti-Rh D is such that an adequate supply is unlikely to be obtained from the general population. In these circumstances, deliberate immunisation of suitable donors has to be undertaken. The donors must be fully informed of the procedure and the risks involved. Suitable donors may be selected by random screening in order to detect those with pre-existing but low levels of the particular immune antibody required; this may be preferable to immunisation de novo. Reference should be made to the relevant MRC Guidelines concerning the use of volunteers.

7.2 Immune plasma donors

7.21 Donors may be identified by random screening of routine donors or selected screening of those with an appropriate history. Immunity may have been acquired through natural infection or through active immunisation. Immunisation may have been performed for the donor's own protection or may be purposely employed for the production of immune plasma in a suitable and willing donor. Donation of plasma following natural infection should take place within a period of 1-12 months following disappearance of symptoms.

7.22 Whenever a donor with a suitable level of an immune antibody is identified, further donations should preferably be by plasmapheresis in order to obtain maximum yields of the antibody. Before any planned immunisation of a willing donor, the suitability of that individual to donate by plasmapheresis should be assessed.

7.23 In general, plasma obtained by therapeutic plasmapheresis should not be used for the preparation of blood products; an exception is made in the case of anti-Rh D plasma.

7.3 Immunisation of donors

7.31 Immunisation of donors with antigens should be carried out only when sufficient supplies of material of suitable quality cannot be obtained by the selection of appropriate donors from donations identified as suitable by screening.

7.32 Donors must be fully informed of the risk of any proposed immunisation procedure, and pressure should not be brought to bear on a donor to agree to immunisation.

7.33 Donors of blood and those undergoing plasmapheresis should, if necessary, undergo investigations that may reveal hypersensitivity to a proposed antigen. Immunisation should be performed using licensed vaccines. The choice of erythrocytes is discussed below.

7.34 The three main preparations used for immunisation in the U.K. are hepatitis B vaccine (for the production of anti-hepatitis B), tetanus toxoid (for the production of anti-tetanus) , and Rh D positive red cells (for the production of anti-Rh D).

7.35 Where erythrocyte or other cellular antigens are used, they should be appropriately selected and tested so as to reduce as much as is reasonably possible any additional risk to the recipient of the antigen, in particular with respect to disease transmission and production of other (unwanted) antibodies.

7.36 Criteria for the selection of donors of erythrocytes for immunisation are established by the Immunoglobulin Working Party of the U.K. Transfusion Directors, who also hold a register of suitable erythrocyte donors.

Only erythrocytes obtained from donors who fulfil the criteria of the Immunoglobulin Working Party should be used for immunisation, and the recommendations of the Working Party should be followed. (See Annex 6).

7.37 In general, the number and dose of injections of antigen should be restricted to the minimum required to obtain a satisfactory response. The criteria for a satisfactory response, number and frequency of doses, total dosage for the antigen and definition of a non-responder should be established for each antigen.

7.38 A donor who has been immunised with one particular antigen should not subsequently be immunised with the purpose of producing a second (different) immune antibody.

7.39 When immunisation is intended, the donor must be given the following information.

7.391 Informed of the procedures by a qualified physician and encouraged to take part in a discussion. This may be achieved by discussing the procedures with small groups of potential donors.

7.392 Encouraged to seek advice from his/her family doctor before agreeing to immunisation.

7.393 Informed that any physician of his/her choice will be sent all information about the proposed immunisation procedure.

7.394 Required to indicate his/her agreement by signing an informed consent form.

7.395 Informed and understands that he/she is free to withdraw consent at any time.

Chapter 8

Guidelines for blood componant specification

8.1 General considerations

8.11 These guidelines apply to single donor and small pool components (made from 12 donations or fewer), prepared either from units of whole blood or from the products of apheresis.

8.12 Specific tests are indicated, although not an exhaustive list, to indicate the minimum level of testing which is acceptable.

8.2 Frequency of tests

8.21 In many cases, because components are produced in a sealed sterile system which should not be breached, the testing of a component implies destructive testing. Several components are produced with sufficient frequency that it is possible to specify a statistically meaningful number of items to test as a percentage of the total output. Some components are made only infrequently, so it is not appropriate to set a percentage of the output for testing.

8.22 In some instances it is possible to develop a non-invasive method for testing a component and avoid waste (e.g. by stripping and refilling the blood collection tubing and sampling by removal of a sealed section). Such a procedure must be fully validated and monitored to ensure that the results may truly reflect on the contents of the pack.

8.23 Some tests may be performed on units which have passed their expiry date, thus reducing wastage; this method however, does not sample a cross section of the total population of units produced. For example, a laboratory worker may issue platelets with a low erythrocyte contamination and leave the less satisfactory products, which will tend to be the ones which then become outdated. The QC sample is then the worst of the products, but does not give any information about how good the product is.

8.24 As a result of biological variability it is acceptable if 80% of the results of testing lie within the target figure. It is therefore essential that sufficient samples are tested; for example, if only 4 samples are tested per month and one fails this only gives a pass of 75% with no indication of whether this is a true reflection of the sample population.

8.25 A testing frequency of 1% of the annual output should be the target, wherever practical. Where this is impractical because it represents too high a wastage of a particularly valuable product, or the production frequency is too low, then the actual testing frequency should be set and documented. If possible a minimum of six units tested per month should be attained if conclusions about trends on a month by month basis are to be obtained.

8.26 The setting of 1% testing rate should not be interpreted to mean that every one hundredth sample is to be tested, rather it is a guide to the total number to be examined. There should be a protocol for taking samples to be tested which takes into account all variables, such as different staff members at different times of the day, and samples taken across these variables.

8.27 Certain tests are performed on every component unit. Red cell serology and virology screening are mandatory quality control procedures, since the results have a direct bearing on the release of the final products. Each component must be visually inspected at each stage of processing and immediately prior to issue. The component must not be issued if there is any evidence of leakage, damage to the container, excessive air (over approximately 10ml), suspicion of microbial contamination or any other contra-indication such as unusual turbidity, haemolysis, or other colour change.

8.3 Setting of requirements

8.31 The wide variability of the source materials used to make blood components makes it difficult to set stringent limits within which a production laboratory should operate. Nevertheless, realistic minimum requirements should be set and complied with.

8.32 In general the minimum requirement should consist of figures for a range or a threshold value. The targets set must be validated taking into account intentional differences introduced by the choice of procedure; for example there may be differences in the final volume of frozen/deglycerolized blood depending upon the freezing protocol and where the final pack is centrifuged to increase its haematrocrit. A second example could be the degree of contamination of a red cell concentrate with platelets and white cells. A buffy coat depleted preparation where >70% of the white cells and >90% of the platelets have been removed is useful for the majority of recipients. However, if the patient has antibodies against leucocyte antigens white cell depletion will need to be more efficient.

8.33 Results should be subjected to statistical analysis, such as the Cusum or other test, so that trends can be identified.

8.34 If the results of analysis show a consistent trend away from the minimum requirements the cause should be investigated. The criteria to investigate will be detailed in the relevant Standard Operating Procedures (SOPs) together with the corrective action to be taken, although the quality assurance manager may also initiate investigations beyond the scope of written procedures. The steps taken should include the following.

8.341 An investigation of the testing procedure and protocols (abnormal results should be repeated as a matter of course).

8.342 Checking that SOPs are up to date and followed in practice.

8.343 Checking the operation of equipment and storage conditions.

If these steps do not reveal causative factors, then management procedures and training should be reviewed.

8.4 Processing

8.41 The starting material

The starting material for blood and component preparation is supplied from blood or plasma donations; thus it is possible only to specify this material with respect to the general good health of the donor. Certain properties of the starting materials can be checked by laboratory tests, such as virological screening and haematocrit; others have to be assumed. It is not possible to

define the starting material in a more rigorous fashion. (See Chapter 5 and Annex 5).

8.42 Separation of components

8.421 The timing and method of separation depends upon the components to be prepared from a given donation. When platelets and coagulation factors are prepared from the same donation, separation of the components should be performed as soon as possible after donation, preferably within 4-6 hours.

8.422 If platelets are to be prepared from a unit of whole blood, the unit should be kept as close as possible to 20°C - 24°C until the separation is effected.

8.423 Separation of components should be performed in a manner which ensures that the temperature of the pack contents are within designated storage and handling temperatures (see below).

8.424 Red cell concentrates should be prepared from whole blood collected in plastic packs designed for the purpose. Multiple plastic pack assemblies minimise the risk of microbial contamination by providing a closed system.

8.425 If an open system is used for the preparation of cells, separation should be effected with positive pressure on the original container which is maintained until it is sealed. This procedure must be undertaken in a designated clean environment as defined in the current Guide to Good Pharmaceutical Manufacturing Practice (HMSO).

8.43 Sterility

8.431 The introduction of presterilised multiple blood pack assemblies has greatly reduced the incidence of infection associated with bacterial contamination of blood and blood components. Occasional reports of such events still occur. Although there is no evidence to suggest that routine sterility testing of blood packs diminishes or eliminates such incidents of infections, it is likely that the following measures will ensure a higher degree of safety.

8.4311 Creating and maintaining the highest level of awareness amongst all personnel of the constant care and attention to detail needed to prevent microbial contamination. Well planned training and refresher programmes are an essential element of this effort.

8.4312 Establishment of well-conceived and validated procedures designed to prevent risks of microbial contamination of both environment and products.

8.4313 Environmental monitoring of the microbial load in specific blood processing areas and equipment, such as clean benches, clean rooms, centrifuge buckets, water baths.

8.432 It is important that data derived from such monitoring exercises are accumulated and regularly examined with a view to taking appropriate action when the recorded minimum requirements are exceeded.

8.433 A 'closed system' is a system, (such as a multiple pack assembly), where the assembly is manufactured under clean conditions, sealed to the external environment and thoroughly sterilised by an approved method. Apart from the act of blood collection, where a needle is exposed and enters the donor's arm, the integrity of this assembly must not in any way be breached.

Accidental breaching of its integrity renders the unit unusable.

When a sterile 'docking device' is used to join two packs it can be regarded as a closed system providing that it has been shown that the process of joining and sealing the two packs does not lead to the possibility of microbial contamination of the products in either pack.

8.434 An 'open system' is one where the system has been breached but where every effort is made to maintain sterility by using sterilised materials and operating in a clean environment, e.g. a procedure where the transfer container

is not integrally attached to the blood pack and the blood pack is breached after collection.

Components prepared in an open system should be monitored for sterility, following methods outlined in the European Pharmacopoeia.

8.435 Blood components prepared by an open system should be used in accordance with the following guidelines:

Storage/Transport Temperature	Time Limit
Ambient	6 hours
1-10°C	24 hours

8.436 All surfaces that come into contact with blood products intended for transfusion must be sterile and pyrogen-free.

8.437 Any new development in component preparation involving a change in preparative procedures or storage conditions or involving an open system or the breaching of a closed system in any way, must be subjected to intensive testing during the development stage to ensure the maintenance of sterility.

8.438 Each donation intended for transfusion and each component preparation constitutes a single batch. It must not be tested for sterility by a method that entails breaching the final container before the unit is transfused.

8.5 Product release

8.51 All components should be appropriately labelled in conformity with Guidelines outlined in Annex 4.

8.52 There should be a system of quarantine for all blood products to ensure that they cannot be released for issue until approved documentation indicates that they have undergone mandatory testing with satisfactory results.

8.53 In exceptional circumstances, blood and/or blood components may have to be issued when they do not conform to minimum requirements.

Each RTC must have written instructions on the procedure which details the circumstances under which such issues can be made. These instructions should, as a minimum, include the following.

8.531 That products which have not been subjected to all the tests required for release or those which fail to meet minimum requirements are only issued by a senior member of the medical staff of the RTC to a registered medical practitioner.

8.532 That the reason for the issue is fully documented.

8.533 That a warning indicating the suspected level of risk is given by a senior member of the medical staff of the RTC to the receiving doctor who should sign a statement indicating that he/she is willing to accept these risks.

8.534 That the name of the recipient is entered on the issue documents.

8.535 That the product is clearly identified with a label indicating which tests have not been performed or which minimum requirements have not been observed, together with a warning that the product should only be used in an emergency until the appropriate tests can be completed or a product complying with the minimum requirements can be provided.

8.6 Product recall

8.61 There must be a system available in each RTC whereby adverse effects caused by the administration of any component of a donation of blood or plasma can result in the recall of all unused components derived from that donation of blood or plasma or from a set of donations of blood or plasma if this is necessary.

8.62 Any recall of a product should lead to a thorough investigation of the causative factors of the adverse effects with a view to their elimination.

8.7 Whole blood

8.71 General description

A unit of blood collected into a licensed anticoagulant and not further processed.

8.72 Technical description

8.721 A unit of whole blood consists of 450 ml ± 10% of blood from a suitable donor (see Chapter 5) in an approved container containing anticoagulant at a ratio of approximately 7 parts of blood to 1 of anticoagulant and an erythrocyte volume fraction (EVF) between 0.35 and 0.45.

8.722 For paediatric purposes it may be considered necessary to produce a unit of lesser volume than 450ml. This may be achieved by dividing in a closed system the unit of whole blood, or by collecting a smaller quantity of blood whilst maintaining the same ratio of blood to anticoagulant. This procedure must be documented and validated and the unit labelled as 'paediatric'.

8.723 If the unit does not contain a 7:1 ratio of blood to anticoagulant it is not suitable for transfusion, although the plasma may be acceptable for fractionation with the agreement of the Plasma Fractionation Centre.

8.73 Labelling, (for general guidelines see Annex 4)

The following shall be included on the label.

- whole blood and volume
- the producer's name
- the donation number
- the ABO group
- the Rh D group stated as positive or negative (reference may also be made to tests for the C or E antigens of Rh D negative units)
- the composition and volume of the anticoagulant solution
- the date of collection and expiry date
- the temperature of storage
- that the blood must not be used if there is visible signs of deterioration.
- that the blood must be filtered before administration

8.74 Storage (for general guidelines see Annex 4)

8.741 Whole blood may be stored for a maximum of 35 days at 4° ± 2°C if an adenine supplemented anticoagulant is used, otherwise the maximum period of storage is 28 days.

The blood must not be allowed to reach a core temperature greater than 10°C during storage or further processing.

8.742 If platelet products prepared from whole blood are to be stored at 22°C they should be separated before the blood cools below 16°-18°C.

8.75 Testing

8.751 This should be performed as described in Annex 5.

8.752 Additional test

One percent of all units of whole blood should be weighed. From the tared weight of the pack assembly and anticoagulant the actual volume of blood can be calculated using the nominal specific gravity of 1.058.

PARAMETER	FREQUENCY	SPECIFICATION
Donation Volume	1%	450 ± 10%ml

8.76 Transportation (for general guidelines see Annex 4)

8.761 The temperature of units of whole blood should be maintained at 4° +/- 2°C during transportation from the RTC to the place that they are intended for use.

8.762 A despatch note detailing the unit numbers of each unit of whole blood by blood group should accompany the units during transportation.

This despatch note should contain the signature(s) of the designated person(s) responsible for the issue, and of the person receiving the consignment.

8.8 Modified whole blood

8.81 General description

8.811 Whole blood which has been modified by the removal of certain components.

8.812 The following types of modified whole blood may be prepared.

8.8121 Platelet depleted - a unit of whole blood from which the majority of platelets have been removed in a closed system and most of the plasma has been returned to the pack containing red cells.

8.8122 Leucocyte depleted - a unit of whole blood from which the majority of the leucocytes have been removed either by a centrifugal process in a closed system and most of the plasma has been returned to the pack containing red cells, or by a filtration process which retains 85 per cent of red cells and less than 5% of the original leucocytes.

8.8123 Cryoprecipitate depleted - a unit of whole blood from which the cryoprecipitate has been removed in a closed system and most of the plasma has been returned to the pack containing red cells.

8.82 Technical description

A unit of whole blood originally containing 450ml ± 10% from which some components have been removed. The red cells are reconstituted with plasma, allowing for the maximum removal of 50ml plasma.

8.83 Labelling (for general guidelines see Annex 4)

The following shall be included on the label.

- description of the modification, e.g. platelet depleted whole blood, leucocyte

depleted whole blood or cryoprecipitate depleted whole blood

- other details as for whole blood (8.73)

8.84 Storage (for general guidelines see Annex 4)

As for whole blood (8.74).

8.85 Testing

8.851 This should be performed as described in Annex 5

8.852 Additional test

One percent of the products derived from the whole blood weighed and from the tared weight the actual volume of plasma removed should be determined using the nominal specific gravity 1.030

PARAMETER	FREQUENCY	SPECIFICATION.
Product Volume	1%	1ml ±10% less than for whole blood

8.86 Transportation (for general guidelines see Annex 4)

As for whole blood (8.76).

8.9 Red cells

8.91 General description

A product prepared by removing most of the plasma after centrifugation, leaving behind most of the platelets and leucocytes, unless otherwise labelled.

8.92 Technical description

8.921 A red cell product with an EVF between 0.55 to 0.75. 0.65 ± 5%.

8.922 For paediatric purposes it may be necessary to prepare a unit of smaller volume than that of a single donation. In this case the unit must be labelled "paediatric".

8.93 Labelling (for general guidelines see Annex 4)

The following should be included on the label
red cells and volume
other details as for whole blood (8.73)

8.94 Storage (for general guidelines see Annex 4)

As for whole blood (8.74).

8.95 Testing

8.951 This should be performed as in Annex 5.

8.952 Additional test

1% of all units of red cells should have the EVF determined.

PARAMETER	FREQUENCY	SPECIFICATION
EVF	1%	0.65 ± 0.1

8.96 Transportation (for general guidelines see Annex 4)

As for whole blood (8.76).

8.10 Red cells supplemented

8.101 General description

A red cell product from which most of the plasma has been removed leaving behind most of the platelets and leucocytes, unless otherwise labelled.

The resulting red cell mass is resuspended in a licensed isotonic nutrient supplement solution to aid the storage and rheological properties of the red cells.

8.102 Technical description

A red cell product supplemented with a licensed isotonic nutrient solution (with or without a secondary anticoagulant), such as Saline, Adenine, Glucose, Mannitol, SAG(M) and with an erythrocyte volume fraction (EVF) OF 0.5 - 0.7.

8.103 Labelling (for general guidelines see Annex 4)

– the name and volume of the preparation

– the composition and the volume of the suspending solution

– other details as for whole blood (8.73)

8.104 Storage (for general guidelines see Annex 4)

As for whole blood (8.74).

8.105 Testing

8.1051 This should be performed as described in Annex 5.

8.1052 Additional test

1% of all units of red cells should have the EVF determined.

PARAMETER	FREQUENCY	SPECIFICATION
EVF	1%	0.60 ± 0.1

8.106 Transportation (for general guidelines see Annex 4)

As for whole blood (8.76).

8.11 Red cells, leucocyte depleted, filtered

8.111 General description

A RED CELL PRODUCT from which most of the plasma has been removed by separation and most of the leucocytes have been removed by a filtration method. The degree of leucocyte removal will depend upon the filtration method used.

8.112 Technical description

A RED CELL PRODUCT that has been passed through an approved leucocyte filter. This product contains more than 85% of the original erythrocytes and less than 5% of the original leucocytes.

8.113 Labelling (for general guidelines see Annex 4)

- the name and volume of the product

- other details as for whole blood (8.73)

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8.114 Storage (for general guidelines see Annex 4)

As for whole blood (8.74) if prepared in a closed system; 24 hours at 4° ± 2°C, if prepared by an open process.

8.115 Testing

8.1151 This should be performed as in Annex 5.

8.1152 Additional test

PARAMETER	FREQUENCY	SPECIFICATION
Residual leucocytes	1% or 6/ month (whichever is higher)	$<0.1 \times 10^9$ /unit
Erythrocyte loss	ditto	<15%

8.116 Transportation (for general guidelines see Annex 4)

As for whole blood (8.76).

8.12 Red cells, washed

8.121 General description

A RED CELL PRODUCT from which most of the plasma, leucocytes and platelets have been removed by one or more stages of washing with an isotonic saline solution. Washing can be performed by interrupted or continuous flow centrifugation.

8.122 Technical description

A RED CELL PRODUCT which has been washed to remove 97% of plasma protein.

8.123 Labelling (for general guidelines, see Annex 4)

- name and volume of the product
- the composition and volume of the suspending solution
- other details as for whole blood (8.73)

8.124 Storage (for general guidelines, see Annex 4)

Since this product will have been prepared by an open process, storage shall be for 24 hours at 4° ± 2°C.

8.125 Testing

8.1251 This should be performed as described in Annex 5.

8.1252 Additional tests

PARAMETER	FREQUENCY	SPECIFICATION
Residual protein	6 units/month or every unit, if prepared infrequently	<0.5g/unit
Erythrocyte loss	ditto	<20%

8.126 Transportation (for general guidelines see Annex 4)

As for whole blood (8.76).

8.13 Red cells, thawed and washed

8.131 Description

A RED CELL PRODUCT to which has been added a cryoprotective agent (usually glycerol) . The unit has then been frozen and is intended for storage in the long term at very low temperatures in liquid nitrogen or electrical ultra low temperature refrigerator. This preparation has subsequently been thawed and washed to remove the cryoprotectant.

8.132 Technical description

A RED CELL PRODUCT which has been frozen, thawed and deglycerolised by a validated method. The washed final product should not show evidence of gross haemolysis and erythrocyte recovery in vivo should be greater than 80%.

8.133 Labelling (for general guidelines see Annex 4)

- name and volume of the product

- the composition and volume of the suspending solution

- other details as for whole blood (8.73)

8.134 Storage (for general guidelines see Annex 4)

Storage of the original frozen cells will be dependent upon cyroprotectant content, but should be 10 years.

Thawed and washed cells shall be stored for a maximum of 24 hours at 4°C +/- 2°C.

8.135 Testing

8.1351 This should be performed as described in Annex 5.

8.1352 Additional tests

PARAMETER	FREQUENCY	SPECIFICATION
Unit volume	6/month	dependent upon protocol, ± 10% stated volume
EVF	ditto	ditto
Supernatant haemoglobin	ditto	<0.2 g/dl
Erythrocyte loss	ditto	<20%

8.136 Transportation (for general guidelines see Annex 4)

As for whole blood (8.76).

8.14 Platelets

8.141 General description

A product prepared from WHOLE BLOOD within 6-8 hours of collection, which contains platelets as the major cellular product suspended in 50-60 ml of anticoagulated plasma or in an appropriate volume of a licensed isotonic suspension medium.

8.142 Technical description

50-60 ml plasma (or an appropriate volume of a licensed isotonic suspension medium) containing at least 60 per cent of the platelets in a unit of WHOLE BLOOD. For paediatric or neonatal purposes, the volume of resuspension medium may be reduced to .20 ml immediately before issue. The product, if it is to be stored at 22°C must be prepared at ambient temperature before the WHOLE BLOOD is cooled to storage temperature (see Annex 4).

8.143 Labelling (for general guidelines see Annex 4)

- the name of the product
- the producer's name
- the donation number
- date of collection and expiry
- recommended storage temperature
- ABO and Rh D blood group
- volume of contents
- constitution of additive solution, if used

8.144 Storage (for general guidelines see Annex 4)

8.1441 The storage period depends upon the nature of the container, dependent upon, for instance, the gas permeability of the plastic used. This will be for 3 or 5 days at 22° ± 2°C.

8.1442 Continuous gentle agitation either vertical or side-to-side of the platelets must be maintained during the storage period.

8.1443 After pooling, using an open procedure, maximum storage prior to infusion is 6 hours.

8.145 Testing

8.1451 This should be performed as described in Annex 5.

8.1452 Additional tests

PARAMETER	FREQUENCY	SPECIFICATION
Volume	1%	50-60 ml
Platelet count	1%	>55 x 10^9/unit
Leucocyte count	1%	<0.12 x 10^9/unit
Erythrocyte count	1%	<1.2 x 10^9/unit
pH at end of shelf life	6/month	6.4-7.4

8.146 Transportation (for general guidelines see Annex 4)

Containers for transporting platelets should be kept at room temperature for 30 minutes before use. During transportation the temperature of platelets must be kept as close as possible to the recommended storage temperature and, on receipt, unless intended for immediate therapeutic use they should be transferred to storage under recommended conditions.

8.15 Platelets, pooled and filtered

8.151 General description

A pool of platelets which have been filtered to remove most of the contaminating leucocytes.

8.152 Technical description

A pool of platelets which have been filtered to remove more than 90% of leucocytes with a loss of platelets of less than 15%.

8.153 Labelling

As for platelets (8.143).

8.154 Storage

A maximum of 6 hours at 20°-24°C.

8.155 Testing

8.151 This should be performed as in Annex 5.

8.1552 Specifications require to be set and validated depending on the number of units of platelets contributing to the pool based on the technical specification of greater than 90% reduction of leucocytes with a loss of platelets no greater than 15% and based upon the equivalent values for single donor platelets.

An appropriate table can be constructed similar to that shown in 8.1452.

8.156 Transportation (for general guidelines see Annex 4)

As for platelets (8.146).

8.16 Platelets, cytapheresis

8.161 General description

A product prepared by removing anticoagulated whole blood from a donor, separation into components by a suitable apheresis machine, retention of the platelets and a varying proportion of the plasma, with return of the recombined remaining elements to the donor. There may or may not be an intermediate product of platelet rich plasma from which the platelets may be concentrated either manually or automatically.

The platelets remain suspended in the donor's own citrated plasma with a variable number of residual leucocytes, but with minimal red cell contamination.

8.162 Specification

Technical description

Platelets may be collected by a variety of apheresis machines using different protocols. The yields may vary between 150 - 600 x 10^9 depending upon whether platelet rich plasma or platelet concentrates are collected. Each procedural protocol must therefore be fully validated and specifications set accordingly.

8.163 Labelling (for general guidelines see Annex 4)

As for platelets (8.143).

8.164 Storage (for general guidelines see Annex 4)

If the apheresis harness is not preconnected prior to use, it must be regarded as an 'open' system and the platelet preparation must be used within 24 hours. If a 'closed' preconnected harness system is used the platelet preparation may be stored for 3 or 5 days at 22° C ± 2° C. Storage life depends on the nature and size of the container, the suspension volume of plasma, or other licensed suspension medium used, and the total number of cellular elements present.

The storage time must be validated and specifications set for each type of apheresis protocol used.

8.165 Testing

8.1651 This should be performed as described in Annex 5.

8.1652 Additional testing

Specifications require to be set and validated for each cytapheresis protocol in use, based on equivalent values for single donor units.

An appropriate table can be constructed, similar to that shown in 8.1452.

8.166 Transportation (for general guidelines see Annex 4)

As for platelets, 8.46.

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8.17 Granulocytes (Buffy coats)

8.171 General description

A product prepared from WHOLE BLOOD by separation of the 'buffy coat' fraction. Leucocytes are the major 'cellular' fraction of interest suspended in anticoagulated plasma or a licenced isotonic suspension medium, but this component may also contain large numbers of erythrocytes and platelets.

8.172 Technical description

Leucocytes separated from WHOLE BLOOD within 6 hours of collection by one of a variety of procedures. Yield of leucocytes can vary between 30% and 90% of the whole blood value depending upon the procedure used, thus each procedure must be fully validated and specifications set accordingly.

8.173 Labelling

- the name of the product
- the producer's name
- the donation number
- the date and time of the preparation
- the date and time of expiry
- the presence and type of suspension and additive media
- the ABO and Rh D group
- the volume of the suspension and the expected number of granulocytes
- to be transfused immediately

8.174 Storage (for general guidelines see Annex 4)

This product is not suitable for storage but if this is unavoidable it should be limited to 24 hours at 22° ± 2°C. If the product has been pooled by an open procedure there should be a maximum 6 hour storage period.

(Storage at 4°C will reduce granulocyte and platelet viability and cellular aggregation will occur.)

8.175 Testing

8.1751 This should be as described in Annex 5.

8.1752 Additional testing

PARAMETER	FREQUENCY	SPECIFICATION
Leucocyte yield	1% or 6/month (each unit if infrequent)	0.5 to 1.5 x 10^9 per buffy coat unit (depending upon procedure)

8.176 Transportation

As for platelets (8.46).

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8.18 Granulocytes, cytapheresis

8.181 General description

A product prepared by removing anticoagulated whole blood from a donor, separation into components by a suitable apheresis machine, retention of the leucocyte layer with a varying proportion of platelets, red cells and plasma, with return of the recombined remaining elements to the donor. The leucocytes remain suspended in the donor's own citrated plasma with a varying degree of residual platelets and red cells.

8.182 Technical description

Leucocytes can be collected by a variety of apheresis machines using different protocols. The yields can vary between 5-30 x 10^9 depending on the technique employed and the individual donor. A combined leucocyte and platelet product can be collected with a platelet count varying between 100 - 400 x 10^9. Each procedural protocol must therefore be fully validated and specifications set accordingly.

8.183 Labelling (for general guidelines see Annex 4)

As for granulocytes (8.173).

8.184 Storage (for general guidelines see Annex 5)

As for granulocytes (8.174).

8.185 Testing

8.1851 Tests should be performed as described in Annex 5.

8.1852 Additional tests

PARAMETER	FREQUENCY	SPECIFICATION
Volume	each unit,	needs to be set and
Granulocyte count	because of	validated for each
Lymphocyte count	infrequency	apheresis protocol
Platelet count	of	but granulocytes should be greater or
Red cell count	production	equal to 10 x 10^9
Haematocrit		10 x 10^9

8.186 Transportation

As for platelets (8.46).

RB/ 53

8.19 Plasma, frozen fresh

8.191 General description

Plasma is the liquid fraction of WHOLE BLOOD containing anticoagulant. It may be obtained by separation from whole blood or, by plasmapheresis and frozen as quickly as possible. This product may be kept for clinical use (or despatched to a Fractionation Centre for further processing (see Volume II).

8.192 Technical description

8.1921 Plasma, frozen fresh for clinical use

The plasma should be frozen quickly following collection of the whole blood or by plasmapheresis to a core temperature of minus 30°C or below within 8 hours of collection.

8.1922 The method of preparation should ensure the maintenance of the maximum level of factor VIII and minimum contamination with cellular material.

8.1923 For paediatric use it may be necessary to produce a !unit of smaller volume than that derived from a unit of WHOLE BLOOD. In this instance the unit must be labelled 'paediatric'.

8.1924 Plasma, frozen fresh for fractionation

See Volume II, 3.22.

8.193 Labelling (for general guidelines see Annex 4)

8.1931 Plasma, frozen fresh for clinical use

- the name of the product
- the producer's name
- the nature of the product, i.e. whether derived from WHOLE BLOOD or by PLASMAPHERESIS
- the donation number
- ABO and Rh D group
- the date of preparation
- the storage temperature
- expiry date
- instructions for thawing

8.1932 Plasma, frozen fresh for fractionation

See Volume II, 3.13.

8.194 Storage (for general guidelines see Annex 4)

8.1941 Plasma, frozen fresh for clinical use

The plasma should be stored at a temperature of -30°C or lower for a maximum period of 12 months.

Note: once thawed, plasma,frozen fresh should not be refrozen.

8.1942 Plasma, frozen fresh for fractionation

See Volume II, 3.127.

8.195 Testing

8.1951 Tests should be performed as described in Annex 5.

8.2041 Plasma, 18 hour for clinical use

As for plasma, frozen fresh (8.1941).

8.2042 Plasma, 18 hour for fractionation

See Volume II, 3.127.

8.205 Testing

8.2051 Tests should be performed as described in Annex 5.

8.2052 Additional tests

PARAMETER	FREQUENCY	SPECIFICATION
Volume	1%	Stated volume ±10%
Factor VIII: C	1%	>0.5 iu/ml. (If used for Factor VIII replacement)

8.206 Transportation (for general guidelines see Annex 4)

8.2061 Plasma, 18 hour for clinical use

As for plasma, frozen fresh (8.1961).

8.2062 Plasma, 18 hour for fractionation

See Volume II, 3.127.

8.21 Plasma, single donor

8.211 General description

Plasma separated from WHOLE BLOOD later than 18 hours after collection. This product is not suitable as a source of factor VIII or other labile coagulation factors and has limited clinical use as a source of protein and other stable products.

8.212 Technical description

Plasma separated from WHOLE BLOOD and frozen quickly to a core temperature of minus 30°C or lower at a time later than 18 hours after collection.

8.213 Labelling

As for plasma, 18 hour for clinical use (8.2031).

8.214 Storage (for general guidelines see Annex 4)

As for plasma, frozen fresh for clinical use (8.1931).

8.215 Testing

8.2151 Tests should be performed as described in Annex 5.

8.2152 Additional test

PARAMETER	FREQUENCY	SPECIFICATION
Volume	1%	stated volume ±10%

8.216 Transportation (for general guidelines see Annex 4)

As for plasma, frozen fresh for clinical use (8.196).

Note: This product should not be confused with outdated plasma which may be used for fractionation under certain circumstances. For details of outdated plasma see Volume II, 3.24.

8.22 Cryoprecipitate

8.221 General description

The coagulation fraction of plasma obtained by thawing PLASMA, FROZEN FRESH at low temperature and concentrated to a final volume of 10-20 ml. The product contains the major portion of factor VIII, together with von Willebrand factor, fibrinogen and fibronectin.

8.222 Technical description

The cryoprecipitated fraction of PLASMA, FROZEN FRESH containing more than 70 iu factor VIII in 10-20 ml volume.

8.223 Labelling (for general guidelines see Annex 4)

- the name of the product
- the producer's name
- the donation number
- ABO and Rh D group
- the date of preparation
- the storage temperature
- expiry date
- instructions for thawing

8.224 Storage (for general guidelines see Annex 4)

8.2241 As for plasma, frozen fresh for clinical use (8.1941).

8.2242 After thawing and pooling, if performed by an open procedure, the maximum storage at ambient temperature is 4 hours.

Note: Once thawed, cryoprecipitate should not be refrozen.

8.225 Testing

8.2251 Tests should be performed as described in Annex 5.

8.2252 Additional tests

PARAMETER	FREQUENCY	SPECIFICATION
Volume	1%	10-25 ml
Factor VIII: C	1%	>70 iu/unit

8.226 Transportation (for general guidelines see Annex 4)

As for plasma, frozen fresh for clinical use (8.1961).

8.23 Plasma, cryoprecipitate depleted

8.231 General description

The supernatant plasma following the removal of cryoprecipitate. The content of albumin, immunoglobulins and coagulation factors with the exception of factor VIII, are the same as PLASMA, FROZEN FRESH. Its content of fibrinogen and fibronectin will also be reduced.

8.232 Technical description

The plasma supernatant after removal of croprecipitate frozen to a core temperature of minus 30°C or less within one hour of the separation of cryoprecipitate.

8.233 Labelling (for general guidelines see Annex 4)

As for plasma, frozen fresh for clinical use (8.1931).

8.234 Storage (for general guidelines see Annex 4)

As for plasma, frozen fresh for clinical use (8.1941).

8.235 Testing

8.2351 Tests should be performed as described in Annex 5.

8.2352

PARAMETER	FREQUENCY	SPECIFICATION,
Volume	1%	Stated volume ±10%

8.236 Transportation (for general guidelines see Annex 4)

As for plasma, frozen fresh for clinical use (8.1961).

Note: Plasma, cryoprecipitate depleted may be acceptable for fractionation by agreement with the National Fractionation Centres; in this instance the Guidelines in Volume II should be followed.

8.24 Irradiated components

Whole blood, red cell concentrates or platelets may be gamma-irradiated for specific clinical indications. The usual dose is 15 to 50 Gy, but whichever dose the unit receives must be validated and documented. All such units must be labelled as 'irradiated'.

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Annex 1

General specifications for blood donor sessions

1. Haemoglobin or haematocrit screening

1.1 Copper sulphate haemoglobin screen

Aqueous copper sulphate, coloured blue, with a specific gravity of 1.053, equivalent to 12.5g/dl haemoglobin is used to test female donors. Copper sulphate, coloured green, with a specific gravity of 1.055, equivalent to 13.5g/dl is used to test male donors. These stock solutions should be colour coded and labelled accordingly.

1.2 Copper sulphate storage

Stock solutions should be stored at room temperature in tightly capped, dark glass containers to prevent evaporation and contamination. Copper sulphate solutions must not be frozen or exposed to high temperatures. The specific gravity of each batch in the stock solution should be checked at least weekly by designated staff with a calibrated hydrometer. The date, the result and the name of the individual who carried out the check must be recorded on the bottle.

1.3 Copper sulphate for routine use

Designated staff should be responsible for dispensing the stock solutions for sessional use. The solution shall be well mixed before dispensing the required amount of each solution into appropriately labelled clean dry tubes or bottles. These solutions shall be changed daily or after 25 tests, depending on the volume of solution dispensed, otherwise contamination of the solution will affect the accuracy of the test. Any used solution at the end of a session shall be discarded. The working temperature of the copper sulphate should be that specified by the manufacturer to provide the correct specific gravity – e.g. cupric sulphate MAR, (material conforming to the AnalaR specification) has the correct specific gravity for Hb estimations at 15.5^{0}C.

1.4 Copper sulphate procedure : fingerprick blood sample

1.41 The skin at the chosen site on the finger must be cleaned with antiseptic solution and wiped clean with sterile gauze or cotton wool. The skin must be punctured firmly, near the end but slightly to the side of the finger, with a sterile disposable lancet, or spring loaded disposable needle system. A good free flow of blood must be obtained.

1.42 The first drop of blood should be discarded and the finger should not be squeezed repeatedly as this may dilute the blood with tissue fluid and give falsely low results.

1.43 Blood from ear lobe puncture should not be used as it has a higher haemoglobin and haematocrit than blood from a finger prick sample and may allow donors with unsuitably low levels to give blood.

1.44 The blood is collected into a capillary tube without any air entry as this may prevent or delay the delivery of the drop.

1.45 One drop of blood is allowed to fall by unassisted gravity from the tube from a height of lcm above the surface of the copper sulphate solution. The drop is observed for 15 seconds. If the drop of blood has a higher specific gravity than the solution, it will sink within 15 seconds. If not, the sinking drop will

hesitate, remain suspended, or rise to the top of the solution.

1.46 Results are recorded as greater or less than 12.5g/dl for females and greater or less than 13.5g/dl for males.

1.5 Spectrophotometric method for haemoglobin screening

1.51 If a haemoglobin photometer is used to provide a quantitative measurement of haemoglobin at the donor session, standard operating procedures for the use of the instrument must be available in the sessions procedure manual.

1.52 They should include a technique where a haemoglobin working standard is tested every 10th sample, a calibration standard is tested daily and a haemoglobin quality control sample is tested monthly. Unless such calibration and controls are used, quantitative measurement of Hb by Hb photometers may produce more inaccurate results than the more simple indirect screening tests such as the copper sulphate techniques or the micro haematocrit which are more easily controlled.

1.6 The microhaematocit method for haemoglobin screening

1.61 The micro haematocrit centrifuge should be calibrated when first placed in service, after repairs, and annually thereafter.

1.62 The time and speed should be checked at a minimum of 6 months and preferably every 3 months by an appropriately qualified person using a precision RPM meter and a stopwatch to check speed, acceleration and retardation.

1.63 A calibration method that provides quality control and allows selection of optimal centrifugation time, is examination of replicate specimens of red cell suspensions within, below and above the acceptable haematocrit range.

1.64 The time selected for routine use should be the minimum time at which maximum packing occurs. Deviation of 2% between replicates is acceptable.

1.65 If a micro haematocrit method is employed for Hb screening, standard operating procedures for the use of this instrument must be available in the session procedure manual.

2. Donor identification

Before the venepuncture the identity of the donor must be checked. The identically numbered labels must be checked to ensure that those on the blood packs and sample tubes are identical.

3. Preparation of the venepuncture site

3.1 Blood should 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 a blood pressure cuff inflated to 40-60 mm Hg and by asking the donor to open and close his/her hand a few times.

3.2 A prolonged cuff pressure of greater than 60 mm Hg should not be employed as this could alter some blood constitutents and reduce the quality of the blood collected, particularly with regard to the number of functional platelets obtained and Factor VIII recovery.

3.3 Although it is not possible to guarantee sterility of the skin surface for

venepuncture a strict standardised procedure for the preparation of the venepuncture site should be in operation to achieve surgical cleanliness to provide maximum assurance of a sterile product.

3.4 The antiseptic solution used should be allowed to dry completely or wiped dry with sterile gauze before venepuncture and the prepared area must not be touched with fingers before the needle is inserted.

4 Preparation of the blood pack

4.1 The blood collection pack must be in date and inspected for any defects. These may be hidden behind the label attached to the container so careful inspection is required.

4.2 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, all the packs in that container should be rejected.

4.3 The anticoagulant solution must be clear, if not the pack should be rejected.

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

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

5. Performance of the venepuncture

5.1 Venepuncture should only be undertaken by authorised and trained personnel according to the policy of the RTC.

5.2 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 (eg using individual disposable syringes and needles). A record of the batch number(s) should be recorded at each blood collection session.

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

5.4 Unused material should be discarded at the end of each donor session.

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

5.6 Items used for venepuncture should be obtained in a sterile, single use disposable form. If the dry outer wrapping of sterile packs becomes wet the contents must not be used. Containers of bulk sterilised items should be labelled and dated when they were sterilised and when opened. Unopened sterilised containers may be stored for 2 or 3 weeks provided the outer package is sealed.

5.7 Prior to use, sessional staff must ensure that the materiel used for venepuncture are sterile, in date and suitable for procedure to be undertaken. The sterile donor needle should not be uncovered and its tamperproof cover checked for integrity immediately prior to the venepuncture.

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

5.9 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.

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

6. Blood donation

6.1 If necessary the donor should be asked to open and close their hand, over a suitable hand grip slowly every 10-12 seconds to encourage a free flow of blood.

6.2 The donor should never be left unattended during or immediately after donation and should be kept under observation throughout the phlebotomy.

6.3 Blood anticoagulation

The blood and anticoagulant should be mixed gently and periodically (approximately every 30 seconds) during collection. Manual mixing should be achieved by inversion of the blood pack every 30 seconds, or automatically by placing the blood pack on a mechanical agitator or by using a rocking device.

6.4 Blood flow

6.41 Blood flow should be constantly observed to ensure that the flow remains fairly brisk so that blood coagulation is not initiated.

If a 450 ml blood donation takes longer than 12 minutes to obtain, the donor session record of that blood pack should be marked accordingly as it is inadvisable to use this donation for the preparation of platelet concentrates, plasma frozen fresh, cryoprecipitate or for plasma for fractionation.

6.5 Blood volume monitoring

6.51 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.

6.52 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; a unit containing

405-495 ml should therefore weigh 425-520g plus the weight of the container and its anticoagulant.

6.53 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.

6.54 Several kinds of weighing equipment may be used and such devices should be used according to the manufacturers instructions for weighing blood into its plastic pack and periodically calibrated by appropriate techniques.

6.6 Sample collection

At the end of the donation, the tubing can be temporarily clamped with a haemostat. The donor samples can then be collected by a method that precludes contamination of the donor unit. Any reusable equipment must be cleaned between donations, e.g. scissors and haemostat. The methods employed must be clearly defined in the sessional procedures manual.

6.7 Completion of the donation

6.71 The pressure cuff should be deflated and the needle then removed from the arm. Immediate pressure should then be applied to the venepuncture site with a sterile cotton wool ball or gauze.

6.72 The needle must be discarded into a special container designed to prevent any risk to personnel.

6.73 The bag should be inverted several times to mix the contents thoroughly.

6.74 The free end of the tubing should be sealed immediately. The blood contained in the collection tube should be expressed into the pack containing the blood donation and allowed to flow back into the tube to ensure anticoagulation.

The sealed off tubing left attached to the bag may be further sealed into segments for crossmatching purposes preferably using a heat sealer. If this is done the segment number must be clearly and completely readable on each segment and it must be possible to separate the segments from the container without breaking the sterility of the container.

6.8 Final donation inspection

All bag defects e.g. pinhole leaks, must be recorded and all defects should be reported to the QA manager and to the Chairman of the National Equipment Monitoring Group. If the defect appears to be batch related, all packs and blood collected in them, must be set aside for further investigation.

6.9 Safety related defects

Any safety related defects in equipment including single use items must be reported via the head of department to the DH in accordance with the requirements of the Health Notice HN(83)21.

7. Declaration of health forms

7.1 These forms are meant to be used at the sessions. The donor is asked to circle the correct answer.

7.2 The Medical Officer should be able to pick out at a glance which donors need further questioning.

7.3 For accepted donors the forms may be destroyed at the session. If further details are necessary the forms may be returned to the RTC with the Enrolment Form and follow up continued.

7.4 An example of a form for a new donor (i. e. first time) is given below:

DECLARATION OF HEALTH

Name:	Date:	
D.O.B:		
Are you in good health?	Yes	No
Are you over 50 Kg (7 3/4) stone in weight?	Yes	No
Are you taking any medicines?	Yes	No
Have you consulted a doctor in the last year?	Yes	No
Have you attended hospital either as an in-patient or out-patient in the last 10 years?	Yes	No
Have you had a serious illness or operation?	Yes	No
Have you had any vaccinations or immunisations in the last year?	Yes	No
If so, which ones?		
Have you been abroad in the last year?	Yes	No
If Yes, where?		

8.

Countries with a risk of malaria

AFRICA

Algeria
Angola
Benin
Botswana
Burkina Faso (formerly Upper Volta)
Burundi
Cameroon,United Republic of
Cape Verde
Central African Republic
Chad
Comoro
Congo
Cote d'Ivoire (Ivory Coast)
Djibouti
Egypt
Equatorial Guinea
Ethiopia
Gabon
Gambia
Ghana
Guinea
Guinea-Bissau
Ivory Coast
Kenya
Liberia
Madagascar
Mali
Mauritania
Mauritius
Morocco: only in rural areas
Mozambique
Namibia
Niger
Nigeria
Rwanda
Sao Tome and Principe
Senegal
Sierra Leone
Somalia
South Africa
Sudan
Swaziland
Tanzania, United Republic of
Togo
Uganda
Upper Volta (now Burkina Faso)
Zaire
Zambia
Zimbabwe

AMERICAS

Argentina
Belize
Bolivi
Brazil
Colombia
Costa Rica
Dominican Republic
Equador
El Salvador
French Guiana
Guatemala
Guyana
Honduras
Mexico
Nicaragua
Panama
Paraguay
Peru
Surinam
Venezuela

OCEANIA

Papua New Guinea

Solomon Islands

Vanuata (formerly New Hebrides)

ASIA

Afghanistan

Bahrain

Bangladesh

Bhutan

Burma

China, People's Republic of

Democratic Kampuchea (formerly Cambodia)

India

Indonesia

Iran

Iraq

Lao People's Democratic Republic

Malaysia

Maldives

Nepal

Oman

Pakistan

Phillippines

Saudi Arabia

Sri Lanka (formerly Ceylon)

Syrian Arab Republic

Thailand

Turkey

Anatolia

United Arab Emirates

Vietnam

Yemen

Yemen, Democratic

Annex 2

General specifications for plasma donor sessions

1. These operating procedures provide guidance only. Further specific definition is required by individual RTCs.

2. Donor identification

As in Annex 1, section 2.

3. Preparation of the venepuncture site

As in Annex 1, section 3.

4. Preparation of the machine/manual plasmapheresis set

The apheresis harness/manual plasmapheresis set should be for single use, preferably preconnected by the manufacturer to ensure a safe sterile pathway once the venepuncture needle is in place and designed in such a way that whole blood can be collected, separated and cellular elements safely returned to the donor.

5. Performance of the venepuncture

As in Annex 1, section 5, but once the venepuncture has been performed subsequent procedures such as releasing the clamp on the bleed line should follow the protocol for the particular type of apheresis procedure being undertaken.

6. Plasmapheresis equipment

6.1 On sites where manual plasmapheresis is performed, a blood bag centrifuge is required for plasma separation. The operating procedure for using this centrifuge must be available at the session and the centrifuge must be checked every 2 months by a designated engineer using a precision RPM meter and stopwatch to check speed, acceleration and retardation.

6.2 Automated plasmapheresis machines should have the following features.

6.21 A manual override system.

6.22 A blood flow monitor.

6.23 An in-line air detector.

6.24 control system to ensure any automatic pressure cuff is deflated during the donor return cycle.

6.25 A. blood filter integral with the harness to prevent any aggregates formed during the procedure from being returned to the donor.

6.26 An anticoagulant flow indicator.

6.27 A device for presetting the volume of plasma required and monitoring the volume of plasma collected.

6.28 A means of preventing unauthorised personnel from making procedural changes on a machine capable of more than one type of donor apheresis procedure.

6.29 Automated plasma Filtration machines must be provided with a monitoring device to record the transmembrane pressure. If the transmembrane pressure falls outside the preset safe operating range, an alarm should be activated.

6.30 In the event of a power failure the machine should automatically enter a standby mode once power returns, and a manual system should exist for the return of the remaining donor cells if the extracorporeal red cell volume is in excess of 200 ml.

6.4 Care and cleaning of apheresis machines

6.41 Apheresis machines should be serviced in accordance with the manufacturer's instructions and an entry made into the machine log that this has occurred.

6.42 A planned maintenance scheme should be followed (see also DH Engineering data, reference EU.26, Electronic Medical Equipment Guidance on documentation required for maintenance).

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

6.44 Apheresis machines should be routinely cleaned daily with a suitable decontaminating agent and a standard procedure for dealing with blood spillage should be in operation.

7. Anticoagulation

As in Annex 1, section 6.3 for manual plasmapheresis, occurs automatically in machine apheresis.

8. Blood flow

8.1 As in Annex 1, section 6.4 for manual plasmapheresis. This occurs automatically in machine apheresis but the procedure should abort if a satisfactory flow rate cannot be maintained.

8.2 Instructions are needed for the machine operator of the action to be taken in the event of a low or not flow situation. Particular care is needed when monitoring the return flow rate in machine apheresis since most machines operate a pumped red cell return such that haematomas can rapidly form unless appropriate action is taken by the machine operator to prevent this from occurring.

9. Blood volume monitoring

As in Annex 1, section 6.5 for manual plasmapheresis. This occurs automatically in machine apheresis.

10. Sample collection

10.1 As in Annex 1, section 6.6 for manual plasmapheresis, but in machine procedures sampling usually takes place at the beginning of the donation. The methods employed shall ensure an aseptic technique with no risk of contaminating the donation and be clearly defined in the sessional procedures manual.

10.2 Extra samples are required at specified intervals from regular apheresis donors to ensure their continued suitability and safety. The samples required should be recorded on the donor session record together with information as to when they were last collected.

10.3 A system should be in operation for regular review of these results organised by the consultant in charge of apheresis, together with a written protocol of the action to be taken in the light of any abnormal findings.

11. Completion of the donation

11.1 As in Annex 1, section 6.7 except that further segmentation of the sealed off tubing is unnecessary. A length of tubing should only be left attached to the pack of plasma if samples of the plasma are required for testing purposes as any side tubing left attached to the plasma bag is more likely to fracture when the plasma bag is frozen.

11.2 Any tubing containing samples required for testing should be separated from the plasma pack prior to freezing without compromising the sterility of the container.

11.3 Regular quality control of the plasma and platelets collected should be undertaken on these samples.

11.4 All disposable used equipment must be discarded in such a way as to prevent any risk to personnel, according to the Health and Safety regulations of the RTC.

12. Adverse donor reactions

12.1 For donor apheresis procedures any potentially serious procedural problem should be reported to the Central Collator designated by the Regional Transfusion Directors who will ensure that all other practising centres are informed of the potential hazard without delay. The appropriate department of the DH should also be informed.

12.2 A National Register of untoward incidents in donor apheresis is being maintained for statistical purposes by the Central Collator.

12.3 A system should be in operation for accurate recording of apheresis data and a method for onward transmission of this data to the Central Collator who should provide regular reports of the data analysis to all RTCS.

Annex 3

Premises

1. General considerations

1.1 Premises used for the preparation of components from blood and plasma will be subjected to scrutiny by the Medicines Inspectorate. Such facilities must comply with the principles embodied in the current Guide To Good Pharmaceutical Manufacturing Practice (HMSO).

1.2 Notwithstanding the fact that premises used for mobile donor sessions may often be accepted, from necessity, as the only local venue available, they must be of sufficient size, construction and location to allow proper operation, cleaning and maintenance, in accordance with accepted rules of hygiene and in compliance with revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories, WHO Technical Report Series No. 323, 1966).

1.3 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.

2. Activities to be borne in mind when accepting a venue

2.1 Registration of donors and all other necessary data processing. If possible, access to a telephone should ideally be immediate, and certainly 'on-site'.

2.2 Laboratory and medical examination of donors, as appropriate, to determine fitness to donate.

2.3 Withdrawal of blood from donors without risk of contamination and errors.

2.4 Performance of apheresis, where applicable, by single-arm techniques only. When apheresis machines are to be used, the flooring should conform to, the appropriate manufacturers recommendations. Flooring should be nonslip, whether for a routine or an apheresis session.

2.5 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.

2.6 Storage of equipment, reagents and disposables. At mobile sessions, this may involve allocation of inadequate space for transit containers and other equipment.

2.7 Storage during the session of blood and components, if they are not to be transferred immediately to the RTC or to appropriate storage in the team vehicle.

2.8 Access to an adequate electrical supply for any onboard refrigerator of the sessional vehicle, and for all electrical equipment used on the session.

2.9 The space required for these activities will obviously depend on the workload and rate.

3 Health and safety factors to be considered

3.1 The requirements of the Health and Safety at Work Act should be taken into account when selecting sessional venues.

3.2 In particular, the following points should be borne in mind.

3.21 The venue should be 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. The ground to be covered by staff carrying equipment should be even and well lit. Preferably, the space to be used should not entail carriage of equipment on stairs. A similar safe approach should be ensured for donors, with as much provision as possible for the parking of their cars. Notices should be displayed, directing donors to the appropriate entry point of the building, and to the room being used.

3.22 Arrangement of furniture and equipment within the available space should be such as to minimise crowding (with its increased possibility of mistake or accident), enabling adequate supervision and ensuring a smooth and logical work-flow.

3.23 Fire exits should be unobstructed and operational. All session staff must be aware of their location and that of the fire extinguishers.

3.24 Lighting should be adequate for all the required activities. Provision must be made for the use of emergency lighting in the event of interruption of the electricity supply.

3.25 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, too cold or stuffy. Subsidiary cooling fans and heating should be carried on sessional vehicles, and used as necessary. This equipment should be subjected to a planned maintenance programme at the RTC.

3.26 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.

3.27 Toilet facilities for male and female donors and staff should be provided. Separate washing facilities are desirable for those staff involved in 'clean' procedures.

3.28 Adequate facilities should be available for the disposal of waste. on mobile sessions, solid waste should be collected and contained in a suitable manner for return to the RTC and subsequent disposal.

3.29 The premises should be free from vermin.

Annex 4

General specifications for labelling, storage and transportation of blood and blood products

1. General considerations

1.1 These operating procedures provide guidance only. Further specific definition is required by individual RTCs.

1.2 The guidance given in this Chapter sets out the minimum requirements for labelling, storage and transportation of materiel, i.e.raw materials, intermediate and finished products at any facility involved in the collection, separation or processing of blood, blood products or plasma fractions.

2. Labelling

2.1 The use wherever possible of machine readable symbology and associated technology is recommended.

2.2 The design and use of labels must comply with specifications set out in appropriate sections of the British and European Pharmacopoeias. The requirements for labelling set out in Part A, section 8, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories). No. 323 of the WHO Technical Report Series should also be followed, together with the additional recommendations in Part D.9 of the revised Requirement for the Collection, Processing and Quality Control of Blood, Blood Components and Plasma Derivatives, WHO 1988.

2.3 Specific guidelines on labels for single donor and small pool products, plasma derivatives and reagents for blood group serology are given, where appropriate, in the relevant section of these guidelines and in the BTS document 'Specifications for Uniform labelling of Blood and Blood Products (1988)' compiled by the UK Working Party for the Supervision of Introduction of Machine Readable Labels in the UK.

3. Storage

3.1 Procedures

3.11 Written procedures for the storage of materiel must be established and followed. These include at least the following.

3.12 Definition of the designated storage areas including the conditions which should be achieved, the status of materials to be stored in each area and the persons authorised as requiring access to each area.

3.13 Specific directions on stacking units of stock such that storage does not jeopardise the identity, integrity or quality of individual units.

3.14 A procedure for stock control allowing batch differentiation and rotation of stocks.

3.15 A procedure for the quarantine of materiel before release by the designated person.

3.16 A procedure for validating the conditions of storage achieved in any given storage area.

3.17 A procedure for regular verification of the cleanliness and good order of all storage areas.

3.2 Operation

3.21 Storage areas should provide adequate space, suitable lighting, and be arranged and equipped to allow dry, clean and orderly placement of stored materiel under controlled conditions of temperature and humidity.

3.22 Storage areas should provide for suitable and effective separation of quarantined and released materiel. There should be clear demarcation in the storage of similar materials.

3.23 Segregated areas should also be available for rejected or returned materiel.

3.3 Recommended Storage Conditions (and expiry dates).

3.31 At all stages of the manufacturing process, materiel should be stored at temperatures and under conditions shown to be adequate to prevent contamination by the proliferation of microorganisms and to preserve biological activity as appropriate.

3.32 Expiry dates shall be assigned to materiel as appropriate, indicating the maximum period of storage under a given set of conditions.

3.33 The expiry time of red cell concentrates prepared in a closed system is not influenced by the type of separation or the time of separation. The expiry time of the separated component depends upon its designation.

3.34 Storage conditions and expiry dates for individual Storage conditions and materials and products are presented in the appropriate sections of these Guidelines.

4. Transportation

4.1 General considerations

Blood and blood products should be transported under conditions as similar as possible to the recommended conditions of storage. Transit times should be minimised, and on receipt materiel should be transferred to storage under the recommended conditions, unless for immediate use.

4.2 Procedures

4.21 Written procedures for the transportation of materials and products should be established and followed. These should include at least the following.

4.211 Definition of the approved systems of transportation, and of the conditions it is required to be maintained in each case.

4.212 Specific directions on the packing of materiel such that transportation does not jeopardise identity, integrity or quality.

4.213 A procedure for identifying the contents of a given vehicle load by a system of consignment notes.

4.214 A procedure for validating the condition of storage achieved during transportation.

4.215 A procedure for regular verification of the cleanliness and good order of all vehicles and containers used for the transport of materials and products.

5. Operations

5.1 Materiel should be transported both within and between sites by means which ensure the following.

5.11 Identity is maintained.

5.12 Status (quarantined, released or rejected) is maintained.

5.13 Integrity is maintained.

5.14 Materiel is not adversely affected by the conditions of storage during transportation.

Annex 5

General specifications for laboratory test procedures and their calibration

1. General considerations

1.1 These operating procedures provide guidance only. Further specific definition is required by individual RTCs.

1.2 Before blood products are labelled and/or released from quarantine, all the laboratory tests designed to show that the individual donations have been tested and conform to specifications should have been completed and documented. In addition to these mandatory tests, other specific tests are performed on blood donations when components are used, either (a) to immunise volunteers or (b) to increase the safety for transfusion to susceptible patients.

1.3 As a matter of principle all tests shall be conducted by nationally agreed methods or, failing this, by well validated and documented methods giving equivalent results.

1.4 Reagents used shall be shown to conform to national standards of performance when used in large scale by the method adopted. There is also a set of minimum controls which must give satisfactory results with every series of tests.

1.5 Laboratory testing is part of a complex procedure that includes proper donor identification, sample labelling, sample storage, sample processing, quality control and reporting.

1.6 The report from the laboratories should indicate the result of each and every test, preferably by a system that provides specific sample identification with direct recording of individual test results either manually or ideally in a computer. Reporting series of tests, particularly those of a microbiological nature, as being 'negative' is potentially dangerous and should be discouraged. A complete result profile of all laboratory tests is a pre-requisite of individual product issue.

2. Specific requirements

2.1 ABO blood grouping

2.11 The ABO blood group shall be determined on each donation of blood and the blood and blood components labelled appropriately.

2.12 The ABO blood group should be determined by examination of both the red cells and serum or plasma of the donor. The national specification for the performance of ABO grouping reagents is given in Volume III.

2.13 The sample of blood from a donor whose ABO blood group is unknown, e.g. first time donor, should be tested twice and the ABO blood group only accepted when the results are in agreement. For those donors whose ABO blood group is known, a single set of tests can be used provided these tests show agreement with the previously recorded ABO blood group of the donor.

2.14 The quality control requirements for blood grouping reagents used in ABO

blood grouping should be applied to manual, microplate and automated techniques. Manufacturers of reagents or grouping machines may recommend the use of additional controls; these should be used.

2.15 Quality control of ABO reagent red cells (A_1 A_2 and B)

Control Test	*Acceptable Criteria*	*Frequency of Tests*
Appearance	No visual haemolysis or turbidity in supernatant	Each Series
Specificity and Sensitivity	Clear-cut appropriate reactions with anti-A anti-B and anti-A, B or anti-A+B	of tests

2.16 Quality control of ABO blood grouping antibodies (anti-A, anti-B, anti-A,B or anti-A+B)

Control Test	*Acceptable Criteria*	*Frequency of Tests*
Appearance	No visual haemolysis, precipitate particles or gel formation	Each Series
Specificity and Sensitivity	Clear-cut appropriate reactions with: Pos & Neg Controls Anti-A A_2B B, O Anti-B A_1B A_1, O Anti-A+B A_2B O Anti-A,B A_2B O	of tests
	A_X	each batch

2.2 Rh grouping

2.21 The Rh blood group shall be determined on each donation of blood and the blood and blood components labelled appropriately.

2.22. The Rh blood group should be determined by testing the red cells of the donor against Rh grouping reagents. The national specification for the performance of Rh grouping reagent is given in Volume III.

2.23 Initially, the red cells of donors should be tested against two different examples of anti-D grouping reagents, at least one of which should be IgG. If found to give a positive reaction with both, the donor should be regarded as Rh positive.

2.24 Donors whose blood is clearly negative for the D antigens should be described as Rh D negative. If the results with the anti-D grouping reagents are discordant, the result should be checked by repeating the tests and then, if still discordant or ambiguous, by further testing with blood grouping reagents which will detect the presence of Rh D, C and E antigens.

2.25 The quality control requirements for blood grouping reagents used in Rh typing should be applied to manual, microplate and automated techniques. Manufacturers of reagents or grouping machines may recommend the use of additional controls; these should be used.

2.26 Quality control Rh blood grouping reagents

Control Test	*Acceptable Criteria*	*Frequency of Tests*
Appearance	No visual haemolysis, precipitate, particles or gel formation	Each Series of tests (pre-
Specificity and Sensitivity	Clear-cut appropriate reactions with known:	acceptance testing as in Vol III)
	Pos. and Neg. Controls R_1r A_1rr Brr Or'r Or"r	

2.27 For reagents with other Rh antibody specificities, the pre-acceptance testing should follow that given in Volume III. These reagents should be free from anti-A and anti-B and their daily controls should be as follows for each series of tests.

	Positive	*Negative*
Anti-C	Or'r or R_1r	Orr
Anti-E	Or"r or R_1R_2	Orr
Anti-CD	OR_0 and r'r	Orr
Anti-CDE	OR_0 r'r and r"r	Orr

2.3 Red cell antibody screen

2.31 The release from quarantine of blood components containing an irregular antibody shall be determined by the documented policy of the RTC.

2.32 The presence or absence of clinically important red cell antibodies should be determined by examination of the serum or plasma of the donor using recognised manual or automated techniques. The testing method used in the screening should be validated and capable of detecting anti-D at 0.5 iu/ml by automated techniques and 0.25 iu/ml by manual and microplate techniques.

2.4 Hepatitis B surface antigen (HBsAg)

2.41 Products from a donation should not be released from quarantine unless they have been tested and found HBsAg negative.

2.42 The presence or absence of HBsAg should be determined by examination of the serum of the donor. If plasma is used for testing, it should be treated according to the instructions accompanying the test kit or the technique should be validated with similarly treated plasma to ensure it meets the required specificity and sensitivity criteria.

2.43 The national specification for the minimum level of sensitivity for the performance of HBsAg screening is defined as 1 iu/ml, though present tests will consistently detect 0.5 iu/ml or less.

2.44 In addition to the test kit manufacturer's validation controls, quality control measures should be taken in the laboratory to demonstrate acceptable specificity and sensitivity of the testing method. Separate tests should be set up to confirm the sensitivity and should include, when available, a national weak positive control (or a local control calibrated against such a national standard) at its defined potency of 1 iu/ml. No set of results should be considered acceptable unless the manufacturer's and the national and/or local quality control tests have satisfactorily passed the criteria laid down.

2.5 Quality control of HBsAg testing

Control Test	*Acceptance Criteria*	*Frequency Tests*
	Clear cut positive reactions with a panel of known low titre HBsAg positive sera of subtypes ad and ay	Each batch of test kits
Specificity and Sensitivity	Clear cut positive reaction with at least one sample of a serum whose HBsAg concentration is equal to or less than 1 iu/ml	Each series of tests

2.6 Antibody to human immunodeficiency virus, type 1 (anti-HIV 1)

2.61 Products from a donation should not be released from quarantine unless it has been tested and found to be anti-HIV1 negative.

2.62 The presence or absence of anti-HIV1 should be determined by examination of the serum of the donor. If plasma is used for testing, it should be treated according to the instructions accompanying the test kit or the technique should be validated with similarly treated plasma to ensure it meets the required specificity and sensitivity criteria.

2.63 The national specification of the minimum level of sensitivity for the performance of anti-HIV1 screening has not yet been defined beyond the requirement that positive results must be obtained on a sample of the national working standard, when it becomes available. Until this is established agreed control sera should be used.

2.64 n addition to the test kit manufacturers' validation controls, quality control measures should be taken in the laboratory to demonstrate an acceptable specificity and sensitivity of the testing method.

2.65 Although the United Kingdom is a low prevalence area for anti-HIV1 markers, separate tests should be set up to confirm sensitivity and should include the nationally distributed working standard, when this is available.

2.66 No set of results should be considered acceptable unless the manufacturer's, and the national and local quality control tests have satisfactorily passed the laid down criteria.

2.67 Quality control of anti-HIV-1 testing

Control Test	*Acceptable Criteria*	*Frequency of tests*
	Clear cut reactions with a panel of known anti-HIV-1 positive and negative sera	Each batch of test kits
Specificity and Sensitivity	Clear cut positive reactions with the nationally distributed anti-HIV-1 weak positive working standard. Where possible other local 'weak' positive quality control samples should be included.	Each series of tests

2.7 Syphilis antibody testing

2.71 Products from a donation should not be released from quarantine unless it has been tested and has been shown to be negative in a test for syphilis antibodies.

2.72 The presence or absence of an antibody to syphilis should be determined by examination of the serum of plasma of the donor.

2.73 The national specification of the minimum level of sensitivity for the

performance of a syphilis screen has not yet been defined but will entail the detection of antibody levels present in a nationally distributed working standard when this is available.

2.74 In addition to test kit manufacturer's validation controls, quality control measures should be taken in the laboratory to demonstrate an acceptable specificity and sensitivity of the testing method. Although the UK is a low prevalence area for syphilis markers, separate tests should be set up to confirm sensitivity and, when available, should include the nationally distributed working standard.

2.75 No set of results should be considered acceptable unless the manufacturer's and the national and/or local quality control tests have satisfactorily passed the laid down criteria.

2.76 Quality control of syphilis antibody testing

Control Test	*Acceptable Criteria*	*Frequency of tests*
	Clear cut positive reactions with a panel of known positive sera	Each batch of test kits
Specificity and Sensitivity	Clear cut positive reaction with the nationally distributed working standard. Where possible, other local 'weak' positive quality control samples should be included.	Each series of tests

3. Additional guidelines for red cells for the immunisation of volunteers

3.1 Red cells for the immunisation of volunteers shall not be issued from quarantine until they have been tested for HBsAg, anti-HIV1 and syphilis antibodies and negative results obtained.

3.2 In addition the following tests must be performed for the accreditation of red cells for the immunisation of volunteers.

3.21 Human immunodeficiency viral antigen, type 1 (HIV-1 antigen)

3.211 The detection of HIV-1 antigen should be determined by examination of the serum of the donor using recognised techniques. Such assays should be performed by a specialist laboratory that employs an established testing method on a regular and routine basis.

3.212 The red cells from a donation shall not be released from quarantine for immunisation purposes unless it has been tested and found HIV-1 antigen negative.

3.22 Antibody to hepatitis B surface antigen (anti-HBs).

3.221 The presence or absence of anti-HBs should be determined by examination of the serum or plasma of the donor using recognised techniques. If plasma is used for testing, it should be treated according to the instructions accompanying the test kit or the technique should be validated with similarly treated plasma to ensure it meets required specificity and sensitivity.

3.222 The national specification of the minimum level of sensitivity for the performance of an anti-HBs screen has not yet been defined but will entail the detection of antibody levels present in a nationally distributed working standard when this is available.

3.223 In addition to the test kit manufacturers' validation controls, quality control measures should be taken in the laboratory to demonstrate acceptable specificity and sensitivity of the test method. Separate tests should be set up to confirm the sensitivity and, when available, should include the nationally distributed working standard.

3.224 A set of results should not be considered acceptable unless both the manufacturer's and the national and/or local quality control tests have satisfactorily passed the laid down criteria.

3.225 Products from a donation shall not be released from quarantine for immunisation purposes unless it has been tested and found to be anti-HBs negative.

3.226 Quality control of anti-HBs testing

Control Test	*Acceptable Criteria*	*Frequency of tests*
	Clear cut positive reactions with a panel of known low titre anti-HBs positive sera	Each batch of test kits
Specificity and Sensitivity	Clear cut positive reactions with the nationally distributed anti-HBs working standard. Where possible other local 'weak' positive quality control samples should be included.	Each series of tests

3.23 Antibody to hepatitis B core antigen (anti-HBc)

3.231 The presence or absence of anti-HBc should be determined by examination of the serum or plasma of the donor using recognised techniques. If plasma is used for testing, it should be treated according to instructions accompanying the test kit or the technique should be validated with similarly treated plasma to ensure it meets required specificity and sensitivity.

3.232 The national specification of the minimum level of sensitivity for the performance of an anti-HBc test has not yet been defined but will entail the detection of antibody levels present in a nationally distributed working standard when this is available.

3.233 In addition to the test kit manufacturers' validation controls, quality control measures should be taken in the laboratory to demonstrate acceptable specificity and sensitivity of the testing method. Separate tests should be set up to confirm the sensitivity and, when available, should include the nationally distributed working standard.

3.234 No set of results should be considered acceptable unless both the manufacturer's and the national and/or local quality control tests have satisfactorily passed the criteria laid down.

3.235 Products from a donation shall not be released from quarantine for immunisation purposes unless it has been tested and found to be anti-HBc negative.

3.236 Quality control of anti-HBc testing

Control Test	*Acceptable Criteria*	*Frequency of tests*
	Clear cut positive reactions with a panel of known low titre anti-HBc positive sera	Each batch of test kits
Specificity and Sensitivity	Clear cut positive reactions with the nationally distributed anti-Hbc working standard. Where possible, other local 'weak' positive quality control samples should be included	Each series of tests

3.24 Serum alanine aminotransferase (ALT)

3.241 The estimation of the alanine aminotransferase level should be determined by examination of the serum or plasma of the donor using recognised techniques. Such assays should be performed by a laboratory that employs an established testing method on a regular and routine basis. The normal levels should be clearly defined and validated against the donor population.

3.242 The red cells from a donation shall not be released from quarantine for immunisation purposes unless it has been tested and found to be within normal limits.

3.25 Characterisation of red cells

3.251 Red cells used for immunisation of volunteers should be tested for the following antigens.

ABO
Rh: D, C, C^w, E, c, e
M, N, S, s, Vw
P_1
K, k, Kp^a, Kp^b
Fy^a, Fy^b
Jk^a, Jk^b
Le^a, Le^b
Lu^a, Lu^b
Wr^a

3.252 Red cells should be typed by an external reference laboratory, e.g. MRC Blood Group Unit or Blood Group Reference Laboratory (BGRL), in addition to the Regional Transfusion Centre concerned. Recognised techniques should be used. Only those blood grouping reagents which meet national specifications (see Volume III) should be used and the technique used should be that for which the reagents are specifically recommended.

3.253 Two different examples of each blood grouping reagents should be used with controls which clearly identify the specificity and sensitivity of the reactions obtained.

3.254 Red cells from a donation shall not be released from quarantine and used to immunise a volunteer unless the donation has been tested and the test results meet the agreed criteria.

4. Additional testing for selected blood donors

4.1 In certain circumstances additional testing of donations may be necessary, e.g. characterisation of cellular antigens (HLA), the examination for cytomegalovirus antibody (IgG), and tests for antibodies to the malarial parasite.

4.2 Characterisation of red cells

4.21 Red cells selected for patients with irregular antibodies should be phenotyped to ensure the red cells do not contain antigen reactive against these antibodies. Only those blood grouping reagents which meet national specifications should be used and the technique used should be that for which the reagents are specifically recommended.

4.22 Two different examples of each blood grouping reagent should be used with controls which clearly identify the specificity and sensitivity of the reactions obtained.

However, if the donor has been previously typed, and the donor's record clearly marked, only one reagent need be used to check the results in subsequent donations.

4.23 The red cells from a donation should not be released, from quarantine for this purpose and labelled unless it has been tested and the results meet the agreed criteria.

4.3 HLA characterisation

4.31 HLA matched platelets, if necessary, are obtained from blood donors whose lymphocytes have been typed for HLA antigens at the A and B loci. The testing should be performed using the recognised methods described in Volume III.

4.32 The platelet concentrate from a donation should not be released for issue or labelled as HLA-matched for a specific patient unless the records of previous HLA typing have been examined and the typing found to match the HLA type of the recipient.

4.4 Antibody (IgG) to cytomegalovirus (IgG anti-CMV)

4.41 The presence or absence of IgG anti-CMV should be determined by examination of the serum or plasma of the donor. The national specification of the minimum level of sensitivity for the performance of an IgG anti-CMV screen has not yet been defined but will entail the detection of antibody levels present in a nationally distributed working standard.

4.42 In addition to the test kit manufacturers' validation controls, quality control measures should be taken in the laboratory to demonstrate acceptable specificity and sensitivity of the testing method. Separate tests should be set up to confirm the sensitivity and, when available, should include the nationally distributed working standard.

4.43 No set of results should be considered acceptable unless both the manufacturer's and the national and/or local quality control tests have satisfactorily passed the criteria laid down.

4.44 Although it is advisable to have panels of CMV seronegative donors, a donation should not be considered CMV negative, and be labelled accordingly, unless it has been tested and found to be IgG anti-CMV negative.

4.45 Quality control IgG anti-CMV

Control Test	*Acceptable Criteria*	*Frequency of tests*
Specificity and Sensitivity	Clear cut positive reactions with known low titre anti-CMV positive sera	Each batch of test kits
	Clear cut positive reactions with the nationally distributed anti-CMV working standard. Where possible other local 'weak' positive quality control samples should be included.	Each series of tests

4.5 Tests for malarial antibodies

4.51 The exclusion period for donors from malarial areas is given in 5.9 dealing with the selection of donors. Certain of these categories require to be tested for malarial antibodies and negative results obtained prior to the release of any blood product.

4.52 The presence or absence of malarial antibodies should be determined by examination of the serum or plasma of the donor. The national specification of the minimum level of sensitivity for the performance of these tests has not yet been defined but will entail the detection of antibody levels present in a nationally distributed working standard.

4.53 In addition to the test kit manufacturers' validation control, quality control measures should be taken in the laboratory to demonstrate acceptable specificity and sensitivity of the testing method.

4.54 No set of results should be considered acceptable unless both the manufacturer's and the national and/or local quality control tests have satisfactorily passed the criteria laid down.

4.55 Quality control malarial antibodies

Control Test	*Acceptable Criteria*	*Frequency of tests*
	Clear cut appropriate reactions with a panel of negative and known low titre malarial antibody sera	Each batch of test kits
Specificity and Sensitivity	Clear cut positive reactions with the nationally distributed malarial antibody working standard. Where possible, other local 'weak' positive quality control samples should be included.	Each series of tests

Annex 6

Accredited donors of red cells for Rh immunisation
(Recommendations of the UK Immunoglobulin Working Party, 1987)

Accredited donors are a special panel of donors who, because they have been tested in detail on a very regular basis, can be relied upon to provide extremely safe blood components which are very valuable for the immunisation and boosting of Rh negative donors for the collection of high titre plasma for the production of anti-D immunoglobulin. Accreditation normally takes one year and the requirements are that the donor should comply with the following.

OPTIONALLY

1. The donor should have donated regularly by plasmapheresis for one year and given at least 12 plasma donations per year at intervals of less than 28 days.

NECESSARILY

2. The donor must have donated blood or plasma on at least three occasions during the last two years and have blood samples tested at intervals of not greater than four weeks for one year. All samples should be negative for.

2. 1 Anti-HBs and anti-HBc by RIA or equivalent test prior to commencement of apheresis or to commencement of the accreditation period.

2.2 HBsAg by RIA (B.P.L. or equivalent assay) on each occasion.

2.3 Anti-HIV by an agreed test on each occasion.

2.4 Tests for HIV antigens should be performed at the end of the accreditation period and found negative.

2.5 On each occasion given serum values within the normal range as determined locally for the following indices : total protein, albumin, alanine anmotransferase, and optionally, gamma-glutamyl transpeptidase and aspartate transaminase.

3. The donor must have remained free of serious illness and be found clinically normal.

4. The donor must have no history of jaundice or hepatitis, and not knowingly been in contact with a case within the last year.

5. The donor must complete a questionnaire and a personal interview stating that they do not belong to a group of the population at high risk of contracting HIV infection.

6. The donor must not have been transfused or injected with blood or any components other than that emanating from themselves since 1977.

7. The donor must not have had acupuncture, ear piercing, tattooing or hair electrolysis performed since the start of regular sampling.

8. In addition to the full red cell typing done by the RTC concerned, red cells from the accredited donors will be grouped for all clinically relevant red cell antigens by an external laboratory, e.g. MRC Blood Group Unit, or BGRL. The white cells will be HLA typed by the lymphototoxicity test.

9. The use of accredited red cells for immunisation/stimulation of volunteers

9.1 It is recommended that whenever possible reconstituted frozen red cells from an accredited donor should be used for immunisation/stimulation of donors for the production of anti-Rh immune plasma.

9.2 The criteria for accreditation should continue to be fulfilled for at least six months before an aliquote of frozen accredited red cells is used for immunisation/stimulation.

9.3 When frozen red cells from an accredited donor are used for immunisation/stimulation of anti-Rh every attempt should be made to ensure that the donor maintains the accredited status for at least a further six months.

Annex 7

Glossary of terms and nomenclature for blood and blood components

1. **Quality Assurance** is a total scheme to ensure that the product meets specification.

2. **Quality Control** is a part of a Quality Assurance programme and consists of end product tests which must be completed with satisfactory results before either the results of a set of tests are accepted or a product is released for issue.

3. **Quality Audit** is a review of the quality system.

4. **Quarantine** is the status of material or products set apart from others whilst awaiting a decision on their suitability for processing or issue.

5. **Sensitivity** is a term defining the limit of detectable specific reactions using reagents or test systems. The document specifies levels of sensitivity which must be achieved.

6. **Specificity** is a term defining the ability of a reagent or test system to react selectively. In practical terms, it represents the absence of false positive reactions.

7. **Validation of a test procedure** is a part of a Quality Assurance programme and consists of those steps which are taken in advance to ensure that the test results provide the required information.

8. **Validation of a manufacturing method** is a part of a Quality Assurance programme and consists of those steps which are taken in advance 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.

9. **A Working Standard** is a preparation prepared nationally or locally containing a known or agreed concentration of the activity being measured and it should be assayed with each group of tests to establish the sensitivity or calibration of the unknown tests in the group.

COMPONENT CATEGORY	MAIN LABEL
A. Whole Blood	Whole Blood
B. Modified Whole Blood	Whole Blood, Platelet, Leucocyte Depleted, Cryoprecipitate Depleted
C. Red Cell Components	Red Cells Red Cells, Supplemented Red Cells Leucocyte depleted Depleted, Filtered Red Cells, Washed Red Cells, Thawed and Washed
D. Platelet Components	Platelets Platelets, pooled and Filtered Platelets, Cytapheresis
E. Leucocyte Components	Granulocytes Granulocytes, Cytaphersis

F. Plasma Components	Plasma, Frozen Fresh Plasma, 18 Hour Plasma, Single Donor Plasma, Cryoprecipitate Depleted Cryoprecipitate

OPTIONAL SUBSIDIARY LABELS

All	Irradiated
All	Paediatric
A	Cryoprecipitate Depleted
A + C	Leucocyte Depleted
A + C	Platelet Depleted

Annex 8

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Mr. R. Knight		RTC, N. London
Dr. P. Minor		NIBSC
Dr. T. Wallington		RTC, Bristol

Annex 9

Standards Required

HBsAg:	Weak positive control
anti-HBs:	National working standard
anti-HBc:	National working standard
HIV:	National working standard
Syphilis:	National working standard
anti-CMV:	National working standard
Malarial antibodies:	National working standard

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Annex 10

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Guidelines for the preparation of plasma fractions

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Chapter 1

Introduction

1.1 The United Kingdom Blood Transfusion Service (UKBTS) comprises twenty Regional Transfusion Centres (RTCs).Those in England are managed by the Regional Health Authorities (RHAs), in Wales and Northern Ireland by the respective Health Offices and in Scotland by the Common Services Agency.

1.2 The National Directors in England and Scotland are responsible for the implementation of national policies and co-ordination of the work of the RTCs. The Headquarters Unit for Scotland was established in 1974 and the National Directorate for England and Wales in 1988.

1.3 During 1987 representatives of the UKBTS formed a liaison with those of the National Institute for Biological Standards and Control (NIBSC) to identify and define guidelines for all materials produced by UKBTS both for therapeutic and diagnostic use.

1.4 Working Groups were formed to consider:

(i) RTC derived blood components

(ii) Fractionated plasma products

(iii) Blood grouping serology and HLA typing

(iv) Microbiological aspects, providing advice to the other Working Groups

1.5 The resulting Guidelines give advice, guidelines and, where appropriate, general specifications. Details of methods have been included when relevant. Standards have been identified within the Guidelines. Not all are available at the present time; those which are available and required are listed in Annex 3.

1.6 The Guidelines have been published in three volumes with topics corresponding to the first three Working Groups stated in paragraph 1.4.

1.7 The Guidelines relate to blood and blood products from voluntary, non-remunerated donors and to reagents produced within the NHS, i.e. both within the UKBTS and the Central Blood Laboratories Authority (CBLA).Separate guidance for hospital departments is being prepared by the Blood Transfusion Task Force of the British Committee for Standardisation in Haematology. However, both hospitals and the Pharmaceutical Industry may find parts of the Guidelines helpful.

1.8 It is not intended that the Guidelines should replace detailed specifications, and standard operating procedures (SOPS), but they should be used in the preparation of specifications and SOPs. In this context most of the recommendations within the Guidelines state that they should be observed. However, there are certain recommendations, which by common consent, must be observed and in these circumstances the word 'shall' or 'must' has been used.

1.9 The Guidelines, in general, should be used in conjunction with the current Guide to Good Pharmaceutical Manufacturing Practice (HMSO) and the Requirements for the Collection, Processing and Quality Control of Blood, Blood Components and Plasma Derivatives (WHO: Requirements for Biological substances, No. 27, revised 1988). Where other texts are relevant reference will be made to them.

1.10 There are many reasons why UKBTS should achieve and maintain the highest

standard of operations. That some uniformity should be engendered in the determination of those procedures which will ensure maximum safety of blood and its products has been highlighted by two events, viz;

(i) It is intended that Crown Privilege, under which the UKBTS and its associated Fractionation Centres have operated is to be withdrawn. Thus, fractionated plasma products will require to be licensed and whilst it is unlikely that licensing procedures will be required for cellular products, licences could not be approved for fractionated products if the RTC producing the source plasma was not operating within agreed standards.

(ii) A Directive of the Council of European Committees (85/374/EEC) bound member states to introduce product liability by July 1988.In the UK this became a legal requirement on 1st March 1988. Consumer Protection Act 1987 Chapter 43, Part I).

Human blood and substances prepared from it are products within the terms of the Act.

The contents of the Guidelines are not mandatory, but the recommendations contained in them do reflect the current state of technology and therapy in transfusion medicine and as such are advisory. They will be revised as technology advances and new products become available.

1.11 Volume II contains Guidelines for the preparation of plasma fractions from human source plasma although the word human will not be included in the text.

1.111 After the Introduction, Chapter 2 sets out general requirements for a quality assurance system suitable for the collection and processing of source plasma and plasma products.

1.112 Chapter 3 contains general specifications for plasma intended for fractionation and this has been divided for convenience into those features common to all types of plasma and those specific for defined plasma types.

Detailed plasma specifications have been agreed between the NBTS and the SNBTS and their respective Fractionation Centres.

1.113 Product characteristics, the assignment of potency to batches of Factor VIII and IX concentrates and steps to achieve viral inactionation can be found in chapters 4,5 and 6.

Chapter 2

Guidelines for a quality system for collection and processing of source plasma and plasma products

2.1 Introduction

2.11 **Quality assurance** is a total scheme to ensure that the product meets specification. In terms of the UK Blood Transfusion Services and the National Fractionation Centres the objective is to ensure the availability of a sufficient supply of plasma suitable for fractionation into plasma products of sufficiently high quality, maximum efficacy and with minimum risk to patients.

2.12 In order to implement satisfactory quality assurance it is essential that there should be a structured and organised approach. This is the **quality system.**

2.13 In this chapter, the guidelines or principles for the establishment of a quality system will be presented. They are derived from the British Standard 5750 (Quality Systems; specification for design, manufacture and installation).

2.14 The recommendations are written in the imperative tense since a quality system cannot be implemented otherwise.

2.15 Although written for industry in general the principles enunciated in BS5750 apply to the Transfusion Services and to the National Fractionation Centres. Because human plasma is a biological product no two donations can be the same. Nevertheless, certain tests to determine the suitability of source plasma are applicable (see Vol. I, Chapter 8).

2.16 Plasma fractions are prepared from large plasma pools and whilst there may be a variation in the composition of the starting material, each batch of final product should be sufficiently homogenous and samples from this batch should meet defined criteria when tested.

2.17 The word **materiel** is used frequently in this chapter. For those readers who may be unfamiliar with its use it can be defined as 'all components, materials or other supplies which are to be incorporated or to be used in the testing or processing of the product.'Thus, materiel may be the pack for the collection of the plasma, the plasma itself, reagents used during the testing of the plasma, and the intermediate or final plasma fractions. In using this word lengthy explanations of the different component parts of products or materials can be avoided.

2.2 A quality system

2.21 Each RTC and each National Fractionation Centre shall establish, document and maintain an effective and economical quality system to ensure and demonstrate that materiel and services conform to the specified requirements. The documented quality system shall include quality management objectives, policies, organisation and procedures to demonstrate compliance with these guidelines.

2.22 The documentation of the quality management system should be presented as a

quality manual.

2.23 The Regional Transfusion Directors (RTDs) and the Directors of the National Fractionation Centres have to consider the overall operation plan for their Centres. In conjunction with staff with specialised skills the policy for the Centres should be compiled and recorded. The principles involved in the production of safe, efficacious products should be defined together with the principles involved in determining specifications for materiel and services. These should include general principles of quality control to ensure that the stated requirements can be met.

2.3 Organisation

2.31 Quality assurance manager

2.311 Each RTC and each National Fractionation Centre shall appoint a management representative, preferably independent of other functions, who shall have the necessary authority and the responsibility for ensuring that the requirements of the guidelines are implemented and maintained.

2.312 The quality assurance manager should report directly to the RTD or the Director of the Fractionation Centre or another deputed person who is entirely independent of production.

2.313 In the event of conflict arising between the QA manager and the RTD or the Director (of the Fractionation 'Centre, the circumstances and the decision taken must be fully documented and discussed at the time of the next quality audit.

2.32 Staff responsible for functions affecting quality.

Each staff member has responsibility for functions affecting quality. The level of responsibility shall be defined for each group or individual member of staff. The degree of authority allocated to each member of staff to evaluate quality problems and to initiate, recommend and provide solutions shall be determined.

2.4 Review of the quality system (quality audits)

2.41 The quality system established in accordance with these Guidelines shall be periodically and systematically reviewed to ensure its continued effectiveness; records of the review shall be maintained.

2.42 The quality audits should be performed by trained personnel who do not have direct responsibilities in the procedures being audited.

2.43 External assessments should also be part of such audits.

2.5 Standard operating procedures (work instructions)

2.51 Each RTC and each National Fractionation Centre shall develop and maintain clear and complete documented instructions that set out for all staff involved in functions affecting quality, the procedures which they will use.

2.52 Each procedure which affects in the quality of a product should have a standard operating procedure.

2.6 Records

2.61 Each RTC and each National Fractionation Centre shall develop and maintain records that demonstrate achievement of the required quality and the effective operation of the quality system.

2.62 Specific requirement(1) : product history file for source plasma.

The records or references shall be maintained in a product history file for at least fifteen years. Records shall include or refer to the location of the following.

2.621 The unique donation number allocated to each donation the plasma.

(Note; when plasma is collected by apheresis from a single donor into more than one pack, RTCs should build in a security system to ensure that where more than one pack bears the same number that the total number of packs to trace in the event of a recall can be identified).

2.622 The session record of the donation of whole blood or plasma from which source plasma is obtained.

2.623 The processing record, incorporating the date performed, the designated individual or where appropriate, the names of team members performing each operation and, when applicable, the major equipment used.

2.624 The inspection tests and the quality control tests performed, the methods and equipment used, the results, the date and signature of the person carrying out the inspection or tests.

2.625 A record of the label or, if appropriate, the package insert and the control number for each product produced (i.e. a distinctive combination of numbers or letters which uniquely identifies an individual product).

2.626 The records for each product shall be such that the origin of the product can be traced to the donor of the whole blood or plasma..

2.63 Specific requirement(2): product history file for plasma fractions

The records or references shall be maintained in a product history file for at least fifteen years. Records shall include or refer to the location of the following.

2.631 The unique donation numbers of each unit of plasma which contributes to a pool of plasma constituting a batch to be fractionated.

2.632 Records of each intermediate fraction produced during processing.

2.6331 The recommendations given in 2.623 to 2.626 inclusive, shall apply.

2.7 Corrective action

Each RTC and each National Fractionation Centre shall establish and maintain documented procedures to provide for the following.

2.71 A continuing analysis of production losses to determine the cause and the corrective action needed.

2.72 A continuing monitoring of processes and work operations and analysis of records to detect and eliminate potential causes of lost production.

2.73 An assurance that corrective actions are effective.

2.8 Control and changes of documentation

2.81 General guidelines

Each RTC and each National Fractionation Centre shall establish and maintain control of all documentation that relates to the requirements of these Guidelines.

To this end each RTC and each National Fractionation Centre shall ensure that the following are observed.

2.811 The pertinent parts of all documents are available at all locations, including mobile collection sites, where operations are performed which are essential to the effective functioning of the quality system.

2.812 All changes to documentation are in writing, dated and signed by the designated person.

2.813 All changes to documentation shall be provided to specified staff responsible for the procedure, ensuring that the revised instructions are understood and will be acted upon promptly.

2.814 Documents are reissued after a practical number of changes have been made.

2.815 Provision is made for the prompt removal of obsolete documents from all points of issue or use.

2.82 Documentation for specific products (i.e. product master file)

Each RTC and each National Fractionation Centre shall establish and maintain detailed documentation for source plasma and for each product respectively, i.e. a product master file. This document shall be prepared, dated and signed by a designated person(s). Any changes shall be dated and authorised in writing by the signature of the designated person.

The product master file shall include or refer to the location of the following information.

2.821 Specifications

2.822 Standard operating procedures (work instructions).

2.823 Quality control procedures and the apparatus used.

2.824 For RTCs, full information concerning the selection of donors and the donations to be used for source plasma. For National Fractionation Centres, full information concerning the constituents of each plasma pool, intermediate products and final products.

2.825 Full information concerning the suppliers of critical components including the specification for such components and written copies of any agreements made with these suppliers.

2.826 Complete labelling procedures for plasma donations and products complete with copies of all approved labels and other labelling.

2.827 Records or references shall be maintained in the product master file for at least fifteen years.

2.9 Control of inspection of test materiel

2.91 Each RTC and each National Fractionation Centre shall ensure that there is provision of suitable equipment and reagents for the preparation and testing of the source plasma and the products prepared from it.

2.92 Each RTC and each National Fractionation Centre shall control the calibration and maintenance of equipment used in the provision of source plasma and the products prepared from it; these procedures should be suitable to demonstrate the conformance of this materiel to the specified requirements.

2.93 By means of suitable quality assessment, both by the use of internal and, where appropriate or available, external control materiel, each RTC and each National Fractionation Centre shall ensure that all reagents conform to specified requirements.

2.10 Control of purchased materiel and services

2.101 Purchasing

2.1011 Each RTC and each National Fractionation Centre shall be responsible for ensuring that all purchased materiel and services conform to specified requirements. The selection of sources and the type of control exercised by the RTCs and the National Fractionation Centres will be dependent on the type of materiel and the suppliers' demonstrated capability.

2.1012 In particular, when the purchase of a licensed product is involved, RTCs and National Fractionation Centres shall require the supplier to provide information to confirm that the product conforms to the terms of the licence.

2.102 Purchasing data

Each purchasing document shall contain a clear description of materiel and services ordered.

2.103 Inspection on receipt

Each RTC and each National Fractionation Centre shall ensure that no purchased materiel is used until it has been inspected and verified as conforming to the manufacturer's specifications.

2.11 Collection of whole blood and plasma production control

2.111 Each RTC shall ensure that blood and plasma collection and processing is carried out under appropriately controlled conditions.

Controlled conditions includes the use of suitable processing equipment and a special working environment when appropriate, e.g. the use of laminar flow cabinets in controlled areas. It also includes documented standard operating procedures in which the manner of collection and processing are defined.

2.112 Each RTC and each National Fractionation Centre shall ensure that after each process stage that affects quality there is either an inspection or a quality control test.

2.113 Specific requirements

Each RTC and each National Fractionation Centre shall ensure that requirements under the following headings in the current Guide to Good Pharmaceutical Manufacturing Practice are followed.

- Personal health and cleanliness
- Buildings
- Environmental control
- Cleanliness
- Equipment
- Equipment maintenance
- Limits and tolerances
- Processing materials and reagents

2.114 Each Fractionation Centre should agree specifications with the RTCs supplying their source plasma.

2.12 Finished product inspection

2.121 Each RTC and each National Fractionation Centre shall perform all inspections and tests on the finished product to complete the evidence of full conformance to specified requirements.

2.122 Before a product is released for distribution, all test results and acceptance records shall be checked by a designated person(s). Release shall be authorised by the signature of a designated person.

2.123 Where release is subject to computer derived information, the computer system must be shown to be fully secure against the possibility of uninspected or defective material being released.

2.13 Quality control tests

2.131 The quality control test procedures used by each RTC and each National Fractionation Centre shall be in accordance with the guidelines given for each product.

2.132 Quality control test procedures shall be regularly reviewed in the light of the finding of products not conforming to specification or as a result of information obtained from quality audits. It must be recognised, however, that the product may be sub-standard and this alternative must not be ignored.

2.14 Control of non-conforming materiel

2.141 Each RTC and each National Fractionation Centre shall establish and maintain procedures for controlling materiel that does not conform to specified requirements. This materiel may consist of all materials used in the collection and processing of whole blood and plasma and in the fractionation process, the reagents used in testing the donations of whole blood, plasma, intermediate fractions and finished plasma products which have not met specified requirements.

2.142 The procedures shall include provision for identification, segregation and

disposal as appropriate. All non-conforming materiel shall be clearly identified to prevent unauthorised use or mixing with materiel which conforms to specified requirements.

2.15 Identification of processing status

2.151 Each RTC and each National Fractionation Centre shall establish and maintain a system for the identification of the processing status of source plasma and plasma fractions during all stages of processing and for those tests which have been carried out.

2.152 Each RTC and each National Fractionation Centre shall ensure by suitable identification, and preferably by storage in a separate location, that products which have not been released for issue can be identified from those which conform to specification and have received their final inspection.

2.16 Protection and preservation of product quality

2.161 Each RTC and each National Fractionation Centre shall establish and maintain a system to control the packing and preservation of the products during their shelf-life to the extent that is necessary to ensure that during this period they conform to specified requirements. This includes any transportation which may be required.

2.162 The requirements under the following headings in the current Guide to Good Pharmaceutical Manufacturing Practice shall be followed.

Labelling

Storage

Transportation

2.17 Training

2.171 Each RTC and each National Fractionation Centre shall establish a system for identifying the training needs and certification requirements for all staff.

2.172 Specific requirement

Personnel working in controlled environments shall be given training related to maintaining the integrity of that controlled environment.

2.18 Product recall and notification of defects

Each RTC and each National Fractionation Centre shall establish a procedure for the recall of a product suspected or known to be defective or hazardous in accordance with the specific requirements in the current Guide to Good Pharmaceutical manufacturing Practice (HMSO).

Chapter 3

Specification for plasma intended for fractionation

3.1 Features common to all plasma types

3.11 **Donor qualifications**

3.112 Each donor must meet all donor health criteria as defined in the relevant document (see Volume 1, Chapter 5).

3.113 Each donation must be tested and found negative for HBsAg using an ELISA or RIA test which detects at least 1 iu per ml of HBs antigen.

3.114 Each donation must be tested and found to be non-reactive for antibody to HIV-1.

3.12 **Donation handling**

3.121 Handling techniques shall comply with Good Manufacturing Practice at each stage of plasma preparation.

3.122 Periodic sampling and monitoring should be undertaken to ensure that microbial contamination does not exceed 10 colony forming units per ml.

3.123 Plasma is either collected by plasmapheresis or is obtained from anticoagulated whole blood. In either case, the manufacturer of the anticoagulant used shall hold a current UK product licence.

3.124 The plasma separation technique should ensure minimal cellular content. There shall be no visible red cells or haemoglobin and the plasma platelet count should be kept to a minimum.

3.125 The plasma donation shall be frozen in a plastic pack of a type agreed with the Fractionation Centre.

3.126 Each plasma donation should be frozen as soon as possible after collection.

3.127 The preferred storage temperature for plasma is -40°C, both within the RTCs and during transit. If this is not possible, then minimal acceptable storage conditions should ensure that the plasma temperature does not exceed -30°C during storage or transportation. Transient temperature perturbations in the plasma should be kept to a minimum and shall not exceed 5°C during storage or transportation.

3.13 **Documentation**

3.131 Each plasma donation shall be labelled clearly with a unique donation number unless the plasma from a single donor by apheresis is collected into more than one pack.

Under these circumstances the same donation number may be present on more than one plasma pack. RTCs should build in a security procedure to ensure that when more than one pack bears the same number that the Fractionation Centre shall always be advised of the total number of packs to trace or exclude in the event of a plasma incident or plasma recall.

3.132 Frozen plasma packs of an identical plasma type are packaged for transportation in containers approved by the Fractionation Centre. Each

container shall clearly carry a unique identification code defining container number, Transfusion Centre of origin and plasma type.

3.133 Adequate documentation shall exist which permits the RTC to trace from the container number each individual plasma donation to the donor. Comprehensive records providing donor traceability and test results appropriate to the specification shall be maintained at the RTC for a minimum of 15 years.

3.134 Each plasma container shall be accompanied by a fully completed despatch document approved by the Fractionation Centre. It should be noted that this is also a release document and should be signed by the appropriate Q.A. manager to state that the plasma despatched conforms to specifications.

3.14 **Plasma notifications**

Once plasma has been sent, the Fractionation Centre must be notified by the Transfusion Centre in the following circumstances.

3.141 If the donor did not meet the current donor health criteria.

3.142 If it is discovered that HBsAg testing or HIV antibody testing has not been carried out according to agreed procedures.

3.143 If the donor subsequently develops an infectious disease, within a period consistent with disease incubation at the time of donation.

13.144 If the donor may be implicated in an episode of post-transfusion infection.

3.145 If the plasma is considered to be unsatisfactory in any other manner.

3.2 Features specific to defined plasma types

3.21 Plasma, fresh frozen (FFP) destined for FVIII production may be obtained from the following sources.

3.211 Recovered plasma from single whole donations.

3.212 Plasma collected by plasmapheresis using manual methods.

3.213 Plasma collected using automated plasmapheresis techniques.

3.22 The requirements for FFP are as follows.

3.221 Recovered plasma, frozen fresh should be prepared only from donations obtained from a clean venepuncture and an uninterrupted procedure (see Volume I, Annex I).

3.222 The donation and the anticoagulant should be mixed during collection.

3.223 Separated plasma should be monitored regularly to demonstrate that the platelet count is consistently low. The target platelet count will depend on the separation technique used as follows.

a) Manual methods :<30 x 10^9/Litre

b) Membrane apheresis :<10 x 10^9/Litre

c) centrifugal apheresis:<40 x 10^9/Litre

3.224 Each RTC should establish appropriate quality control procedures to monitor the FVIII content of the frozen plasma (Volume I, Chapter 8).

3.225 Recovered fresh plasma should be frozen to a solid state as soon as possible.

Two categories of plasma are recognised:

a) the period from collection to freezing is less than 8 hours.

b) the period from collection to freezing is less than 18 hours.

3.226 The rate of cooling must be as rapid as possible and ideally should bring the core temperature of the plasma down to -40°C or below within 60 minutes. If this is not possible, the minimum acceptable rate of freezing must bring the core temperature down to -30°C within 4 hours, as demonstrated by regular performance tests.

3.23 **Cryosupernatant plasma** shall be derived from plasma which meets the requirements for FFP, except that the cryoprecipitate has been removed by an approved technique.

3.24 **Outdated plasma**

3.241 Plasma in this category may have been held at +4°C for more than 35 days prior to separation.

3.242 Time-expired plasma recovered from whole-blood returned after issue to hospitals is acceptable if the RTC is satisfied that the blood donation has been stored in a manner which has maintained its fitness for clinical use.

3.243 Separated plasma donations with visible red cell contamination or which are grossly lipaemic should not be sent for fractionation.

3.25 **Immune plasma**

3.251 Immune plasma is obtained from donors who meet the normal health criteria, but who are found to have circulating plasma antibody concentrations of sufficient potency to warrant inclusion of their plasma in pools destined for the manufacture of hyperimmune immunoglobulin.

3.252 Suitable donors may be selected in one of three ways.(see also Volume I, Chapter 7)

3.2521 Known natural exposure to an infective agent or to red cell stimulation.

3.2522 Known immunisation against infective agent or deliberate red cell stimulation.

3.2523 Testing of donations chosen at random.

3.253 Acceptable minimum antibody potency must be demonstrated using an assay system agreed with the Fractionation Centre. The requirements for each plasma type are specified below.

3.2531 *Anti-D*

Antibody potency should be estimated in international units using an approved AutoAnalyser-based assay system. Minimum potency should be agreed with the Fractionation Centre.

3.2532 *Cytomegalovirus*

Antibody potency should be assayed using a quantitative assay agreed with the Fractionation Centre (e.g. ELISA, immunofluorescence, complement fixation).

The donation must be shown to be of a potency equal to or greater than that of a control sample provided by the Fractionation Centre.

3.2533 *Anti-endotoxin*

Antibody potency should be assayed using a quantitative assay (e.g. ELISA) agreed with the Fractionation Centre.The donation must be shown to be of a potency equal to or greater than that of a control sample provided by the Fractionation Centre.

3.2534 *Hepatitis B*

Antibody potency should be calibrated in international units using an approved

assay system (e.g. RIA, ELISA) which detects antibody to hepatitis B surface antigen. The minimum acceptable potency is 10 iu/ml.

3.2535 *Measles*

Antibody potency should be assayed using a quantitative assay (e.g. ELISA, immunofluorescence, complement fixation) agreed with the Fractionation Centre.The donation must be shown to be of a potency equal to or greater than that of a control sample provided by the Fractionation Centre.

3.2536 *Pseudomonas*

Antibody potency should be assayed using a quantitative assay (e.g. ELISA) agreed with the Fractionation Centre.The donation must be shown to be of a potency equal to or greater than that of a control sample provided by the Fractionation Centre.

3.2537 *Rabies*

It is not practicable to assay plasma donations for rabies antibody. However, a donor should be considered to have acceptable antibody titres between 1 and 3 months after a second dose of vaccine, or between 1 and 3 months after a booster dose of vaccine. Plasma should not be collected from persons immunised after exposure to infection by rabies virus.

3.2538 *Tetanus*

Antibody potency should be calibrated in international units using an approved assay system which correlates well with the mouse neutralisation assay. The minimum acceptable potency is 10 iu/ml.

3.2539 *Zoster (varicella)*

Antibody potency should be assayed using a quantitative assay (e.g. ELISA, immunofluorescence, complement fixation) agreed with the Fractionation Centre.The donation must be shown to be of a potency equal to or greater than that of a control sample provided by the Fractionation Centre.

Chapter 4

Product characteristics

4.1 Factor VIII concentrates

In addition to meeting relevant pharmacopoeial requirements, the product has the following characteristics.

4.11 Each manufacturer is expected to provide a single type of concentrate suitable, in terms of potency and freedom from adverse effects at high doses, for the treatment of all patients with inherited Factor VIII deficiency and those with acquired inhibitors of Factor VIII. The concentrate should be suitable for self-administration.

4.12 The concentrate may not necessarily be designed to be effective in von Willebrand's disease and any claims to be effective should be made only after extensive clinical trial of the product.

4.13 The method of preparing the concentrate should ensure maximum Factor VIII:C yield. Factor VIII C yield should, therefore, not be sacrificed in the interest of specific activity except insofar as increased purity has been shown to affect substantially the product's safety or efficacy.

4.14 The concentrate should have been treated during processing, in the final container, or both, with the intention of inactivating bloodborne viruses such as human immunodeficiency virus (HIV), hepatitis B virus (HBV) , and non-A, non-B viruses (NANB). Safety from transmission of viruses must not rely solely on donor screening.

4.15 The concentrate should dissolve readily within 10 minutes of addition of water, without warming above room temperature, to a potency >10 iu/ml. Vial contents should be in the range 200-1000 iu.

4.16 Specific activity should exceed 0.3 iu/mg protein and 0.5 iu/mg fibrinogen. Other major contaminating plasma proteins should be characterised.

4.17 The concentrate should not contain more than the equivalent of 0.5 iu endotoxin/iu Factor VIII determined by the European Pharmacopoeial method.

4.18 After injection into adult patients with no concurrent bleeding, the recovery of Factor VIII should be approximately 1.8-2.4% rise.iu/kg (75-100% of theoretical values).

4.19 After injection into adult patients with no concurrent bleeding, at a dose sufficient to raise the patient's Factor VIII activity to 0.5 iu/ml, the average half-disappearance time as determined in several patients should be comparable to that found after injection of concentrates from other manufacturers.

4.2 Factor IX concentrates

4.21 A single type of concentrate should be suitable, in terms of potency and

freedom from adverse effects at high doses, for the treatment of all patients with inherited Factor IX deficiency and those with acquired inhibitors of Factor IX. The concentrate should be suitable for self-administration.

4.22 The concentrate may not necessarily be designed to be effective in the treatment of liver disease or other acquired deficiencies of the vitamin K-dependent proteins. Claims to be effective should be made only after extensive clinical trials.

4.23 The concentrate may contain amounts of Factor II and Factor X which makes it suitable for replacement therapy in inherited deficiencies of these factors. The content of these factors should be stated on the vial label.

4.24 The concentrate should have been treated during processing, in the final container, or both, with the intention of inactivating bloodborne viruses such as HIV, HBV and NANB. Safety from transmission of viruses must not rely solely on donor screening.

4.25 The concentrate should dissolve readily within 5 minutes of addition of water, without warming above room temperature, to give a potency of >20 iu/ml. Vial contents of Factor IX should be in the range 250-1000 iu.

4.26 Specific activity should exceed 1.0 iu Factor IX/mg protein. No specific activity limit is set for the other vitamin K-dependent proteins which may be present. The major contaminating plasma proteins should be characterised.

4.27 When first developed, and after any substantial modification to processing methods, typical batches of concentrate should be subjected to tests of thrombogenicity by injecting at least the equivalent of a maximum human dose into experimental animals and monitoring indices of intravascular coagulation.

This is intended to ensure that, for each product or modified product, currently used in vitro tests for activated coagulation factors retain the assumed correlation with the thrombogenicity of the concentrate.

4.28 After injection into adult patients with no concurrent bleeding, the recovery of Factor IX should be approximately 1.0-1.6% rise.iu/kg (40-66% of theoretical values).

4.29 After injection into adult patients with no concurrent bleeding, at a dose sufficient to raise the patients' Factor IX activity to 0.5 iu/ml, the average half disappearance time as determined in several patients should be comparable to that found after injection of concentrates from other manufacturers.

4.3 Albumin products

4.31 This section applies to albumin products which are formulated between 43 g/l to 250 g/l.

4.32 Albumin may be prepared from any plasma which meets the criteria detailed in the specification for plasma intended for fractionation.

4.33 The process yield should be maximised, consistent with the Pharmacopoeial description. The expected yield should be greater than 20 g/l.

4.34 The endotoxin content of the final product should be controlled and would be expected to be less than 0.5 iu/ml for 4-5% albumin preparations.

4.35 The metal ion content should be controlled. In particular, the aluminium content should be kept below 200 ug/L.

4.36 The level of pre-kallikrein activator should be less than 35 iu/ml.

4.37 The product must be pasteurised in the final container at 60°C for 10 hours. Appropriate stabilisers are added to protect the product during pasteurisation.

4.4 Immunoglobulin preparations

4.41 The products should comply with the relevant Pharmacopoeial monographs and any additional requirements of the Licensing Authority.

4.42 Immunoglobulin preparations should be concentrated and purified from human plasma, collected in accordance with the guidelines in 3.1 and 3.2, in a manner which does not affect the structural and functional integrity of the immunoglobulins. The process should maximise yield without unduly reducing product quality.

4.43 Normal immunoglobulin products should be prepared from the pooled plasma from at least 1000 normal donors; the range of antibody activities should be representative of the donor population. The concentration of antibody to at least one viral and one bacterial antigen in a 16% solution of normal immunoglobulin should be greater than 10 times that in the source plasma pool.

4.44 Specific immunoglobulin products should be prepared from the pooled plasma from donors who have been selected for elevated antibody levels to the required specific antigen, e.g. Rh(D), tetanus toxin, rabies virus or hepatitis B virus. Specific immunoglobulin products may be prepared from the pooled plasma from fewer donors than used for normal immunoglobulin. Specific immunoglobulin products should meet the potency requirements of the relevant pharmacopoeial monographs or the specifications of the Licensing Authority. When such requirements do not exist, the concentration of the required antibody in the specific immunoglobulin preparations should be at least 5-fold higher than in a normal immunoglobulin preparation with an equivalent protein concentration.

4.45 The products should be safe for administration by the chosen route and should not cause undue discomfort or inconvenience to the recipient when administered at the required dose by the chosen route.

4.46 The product should not transmit viral infections and evidence should be available for the removal and/or inactivation of viral contaminants by the process used for the preparation of the immunoglobulins. Departures from conventional cold ethanol fractionation processes, which have a long-established safety record on epidemiological grounds, should be fully validated in vitro by methods similar to those described in Chapter 6.

4.47 Each batch of product must be tested for the presence of HBsAg and antibodies to HIV-1, and shown to be negative.

4.48 The concentration of IgG in the plasma of recipients of the products should rise substantially; an increase in antibody activity should be demonstrable in recipients.

4.49 The half-life of the preparations should be studied in suitable patients; for example, after injection into hypogammaglobulinaemic patients the plasma half-life of the product should be not less than 15 days.

4.410 The products should contain the four IgG subclasses in ratios similar to those in the plasma pools from which they were derived.

4.411 Antigen binding and Fc mediated functions of the immunglobulin should be retained.

4.412 The concentration of immunoglobulins, including IgA and contaminating

proteins should be specified. The IgG should not be less than 90% of the total protein.

4.413 There should be no detectable increase in the concentration of immunoglobulin fragments in preparations after storage at 37°C for four weeks.

4.414 Characteristics specific to intramuscular immunoglobulin preparations:

The content of monomeric/dimeric IgG in the product should not be less than 85%, the content of aggregates should not be greater than 10% and the content of fragments should not exceed 5%.

4.415 Characteristics specific to intravenous immunoglobulin preparations:

The content of monomeric/dimeric IgG in the product should not be less than 90%, the content of aggregates should not exceed 5% and the content of fragments should not exceed 5%.

The kallikrein activity should not exceed 0.1 units per mt; the pre-kallikrein activator activity should not exceed 35 iu/ml

The anti-complementary activity should be controlled.

Chapter 5

Assignment of potency to batches of Factor viii and ix concentrates

5.1 Standards

Factor VIII concentrates should be assayed against the current British Working Standard (BWS) for Factor VIII concentrate. Factor IX concentrates should be assayed for Factors II, IX and X against the current BWS for Factors II, IX, X concentrates.

5.2 Assay methods

The method shall be either that described in the European Pharmacopoeia or another method that has been shown to give comparable results on at least 12 batches of product.

5.3 Number of assays

At least two bottles from each batch should be tested, preferably with two independent assays per bottle. Each assay should consist of replicate sets of at least three dilutions of standard and test preparation.

5.4 Analysis of results

5.41 Each individual assay should be analysed by the parallel line bioassay method, such as described by Kirkwood and Snape [1]. Results of individual assays should be combined to give a weighted geometric mean if they are homogeneous or an unweighted geometric mean if they are heterogeneous

.5.42 The content of each bottle is calculated by multiplying the potency of the solution by the volume of liquid, which should be measured accurately after reconstitution.

5.43 The 95% confidence limits on the overall mean potency should not be greater than plus or minus 10% of the mean.

Reference

1. Kirkwood, T. B L., Snape, T. J. Biometric principles in clotting and clot lysis assays.

Clin.Sci.Haematol., 1980, 2: 155-167.

Chapter 6

Viral inactivation

6.1 Specification for the validation of virus inactivation procedures used during the manufacture of clotting factor concentrates

6.11 The demonstration that HIV can be transmitted by Factor VIII and Factor IX products has led to the requirement that fully validated virus inactivation procedures must be included in the processes used to manufacture each type of coagulation factor concentrate. This specification outlines the minimum requirements for the virus validation of a particular product.

6.12 It should be emphasised that the inactivation of viruses in blood products involves a complex interaction of a number of factors, including product type, product concentration, product formulation, presence of stabilisers and type and conditions of inactivation process. For this reason, each manufacturer must validate each product and cannot rely on data from other products or other manufacturers.

6.2 Source plasma

In order to minimise the virus challenge to the manufacturing process, each individual plasma donation used for the manufacture of coagulation factor concentrates must be tested for the presence of hepatitis B surface antigen and for antibody to the human immunodeficiency virus, type 1 (HIV-1). In future, it may become necessary to test for other viral contaminants. The assay methods used must comply with the minimum requirements defined in the appropriate specification for each product.

6.3 Process validation

6.31 Validation of formal virus inactivation step

6.311 Data should be generated which demonstrates that at least one single stage in the manufacturing process is capable of inactivating at least 10^5 infectious particles of HIV per ml of solution (i. e. a 5 log reduction in the concentration of viable virus).

6.312 It is widely recognised that the transmission of NANBH is a major potential problem. The agent(s) of NANBH have not yet been cultured and it is, therefore, recommended that data are generated on the ability of the process to inactivate a range of other 'model' viruses to include RNA and DNA viruses, both enveloped and non-enveloped.

It is suggested that these might include Vaccinia and Semliki Forest Virus as these have both proved fairly resistant to heat inactivation.

Model virus data may not provide information of direct clinical relevance, but are very useful for comparing the overall inactivation potential of different processes.

6.313 Virus inactivation experiments must simulate the full scale process steps as closely as is possible. For example, if dry-heating of a freeze-dried product is used, every effort must be made to simulate the exact freeze-drying conditions used in large-scale manufacture.

6.32 Validation of rest of process

6.321 In addition to the inactivation data generated from the evaluation of the formal virus inactivation step, further data should be generated to indicate the extent to which other stages in the overall manufacturing process are capable of inactivating/eliminating infectious HIV particles present in starting plasma.

6.322 It is, however, recognised that small-scale laboratory simulations of certain processing stages such as cryoprecipitation, centrifugation, precipitation, adsorption, chromatography and diafiltration, will riot precisely replicate the full-scale manufacturing process. Therefore, undue weight must not be attached to the data from this part of the validation, which should be considered to be indicative only.

6.4 Virus assay methods

6.41 The assessment of the efficiency of virus inactivation can only be made using a validated assay for infectious virus. For example, in work with HIV-1, it is not acceptable to rely solely on data obtained from measurements of virus-associated reverse transcriptase.

6.42 Appropriate infectivity controls must be run in each assay.

6.43 The virus culture technique used must be fully validated to demonstrate that the coagulation factor concentrate does not inhibit the detection of virus.

6.5 Validation of process modifications

6.51 Once the process is established and virus validation has been completed, then all aspects of the process must be controlled within tightly defined limits representative of the conditions employed during validation.

Significant changes from these conditions will lead to a requirement for revalidation.

6.52 Certain process modifications may be expected to influence the extent of virus inactivation. Such modifications must be validated.

6.53 Process modifications agreed with the Licensing Authority as minor may be validated using model virus systems.

6.54 Process modifications agreed with the LicensingAuthority as major will necessitate a full revalidation as defined in section 6.3.

ANNEX 1

Glossary of terms

1. **Quality Assurance** is a total scheme to ensure that the product meets specification.

2 **Quality Control** is a part of a Quality Assurance programme and consists of end product tests which must be completed with satisfactory results before either the results of a set of tests are accepted or a product is released for issue.

3 **Quality Audit** is a review of the quality system.

4 **Quarantine** is the status of material or products set apart from others whilst awaiting a decision on their suitability for processing or issue.

5. **Sensitivity** is at term defining the limit of detectable specific reactions using reagents or test systems. The document specifies levels of sensitivity which must be achieved.

6. **Specificity** is a term defining the ability of a reagent or test system to react selectively. In practical terms, it represents the absence of false positive reactions.

7. **Validation of a test procedure** is a part of a Quality Assurance programme and consists of those steps which are taken in advance to ensure that the test results provide the required information.

8. **Validation of a manufacturing method** is a part of a Quality Assurance programme and consists of those steps which are taken in advance 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.

9. **A Working Standard** is a preparation prepared nationally or locally containing a known or agreed concentration of the activity being measured and it should be assayed with each group of tests to establish the sensitivity or calibration of the unknown tests in the group.

ANNEX 2

Present and past members of UKBTS/NIBSC Liaison Group

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Dr. D.R. Bangham	Secretary	NIBSC
Professor J.D. Cash		National Headquarters SNBTS
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Mr.P. Harrison		BPL, Elstree
Mr.R. Knight		RTC, N. London
Dr.P. Minor		NIBSC
Dr.T. Wallington		RTC, Bristol

ANNEX 3

British (B.S.) and International (I.S.) Standards available at NIBSC

3rd	I.S.	Factor VIII Concentrate (80/556)
8th	B.S.	Factor VIII Concentrate (88/590)
2nd	I.S.	Factor VIII - related activities in plasma (87/718)
16th	B.S.	Factor VIII:C Plasma (88/584)
3rd	B.S.	Blood Coagulation Factors, Plasma (87/658)
1st	I.S.	Factors II, IX, X Concentrate (84/681)
1st	I.S.	Factors II, VII, IX, X Plasma (84/665)
2nd	B.S.	Factor IX Concentrate (87/532)
1st	I.S.	Antithrombin III, Plasma (72/1)
1st	I.S.	Protein C, Plasma (86/622)
1st	I.S.	Anti-D Immunoglobulin, Human (68/419)
1st	B.S.	Anti-D Antibodies, Human (72/229)

Standards required

British Working Standards

Anti-CMV
Anti-endotoxinn
Anti-HBsAg (plasma)
Anti-measles
Anti-pseudomonas
Anti-zoster (varicella)

VOLUME III

Guidelines for reagents for blood group serology and HLA typing

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Chapter 1

Introduction

1.1 The United Kingdom Blood Transfusion Service (UKBTS) comprises twenty Regional Transfusion Centres (RTCs). Those in England are managed by Regional Health Authorities (RHAs), in Wales and Northern Ireland by the respective Health Offices and in Scotland by the Common Services Agency.

1.2 The National Directors in England and Scotland are responsible for the implementation of national policies and co-ordination of the work of the RTCs. The Headquarters Unit for Scotland was established in 1974 and the National Directorate for England and Wales in 1988.

1.3 During 1987 representatives of the UKBTS formed a liaison with those of the National Institute for Biological Standards and Control (NIBSC) to identify and define guidelines for all materials produced by UKBTS both for therapeutic and diagnostic use.

1.4 Working Groups were formed to consider:

(i) RTC derived blood components

(ii) Fractionated plasma products

(iii) Reagents for blood group serology and HLA typing

(iv) Microbiological aspects, providing advice to the other Working Groups

1.5 The resulting Guidelines give advice, guidance and, where appropriate, general specifications. Details of methods have been included when relevant. Standards have been identified within the Guidelines. Not all are available at the present time.

1.6 The Guidelines have been published in three volumes with topics corresponding to the first three Working Groups stated in paragraph 1.4

1.7 The Guidelines relate to blood and blood products from voluntary, non-remunerated donors and to reagents produced within the NHS, i.e. both within the UKBTS and the Central Blood Laboratories Authority (CBLA).

Separate guidance for hospital departments is being prepared by the Blood Transfusion Task Force of the British Committee for Standardisation in Haematology.

However, both hospitals and the Pharmaceutical Industry may find parts of the Guidelines helpful.

1.8 It is not intended that the Guidelines should replace detailed specifications and standard operating procedures (SOPs), but they should be used in the preparation of specifications and SOPs. In this context most of the recommendations within the Guidelines state that they should be observed. However, there are certain recommendations, which by common consent, must be observed and in these circumstances the word 'shall' or 'must' has been used.

1.9 The Guidelines, in general, should be used in conjunction with the current Guide to Good Pharmaceutical Manufacturing Practice (HMSO) and the Requirements for the Collection, Processing and Quality Control of Blood, Blood Components and Plasma Derivatives; (WHO: Requirements for Biological substances, No. 27, revised 1988). Where other texts are relevant, reference will be made to them.

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1.10 There are many reasons why UKBTS should achieve and maintain the highest standard of operations. That some uniformity should be engendered in the determination of those procedures which will ensure maximum safety of blood and its products has been highlighted by two events, viz;

(i) It is intended that Crown Privilege, under which the UKBTS and its associated Fractionation Centres have operated is to be withdrawn. Thus, fractionated plasma products will require to be licensed and whilst it is unlikely that licensing procedures will be required for cellular products, licences could not be approved for fractionated products if the RTC producing the source plasma was not operating within agreed standards

(ii) A Directive of the Council of European Committees (85/374/EEC) bound member states to introduce product liability by July 1988. In the UK this became a legal requirement on 1st March 1988. (Consumer Protection Act 1987, Chapter 43, Part I)

Human blood and substances prepared from it are products within the terms of the Act..

The contents of the Guidelines are not mandatory, but the recommendations contained in them do reflect the current state of technology and therapy in transfusion medicine and as such are advisory. They will be revised as technology advances and new products become available.

1.11 Volume III contains recommendations for the production and use of reagents used in blood group serology and HLA typing.

In Chapters 1 and 2 there are general guidelines for the preparation and use, respectively, of reagents relating to red cell serology.

Subsequent chapters contain guidelines for specific reagents, reagent red cells, low ionic strength solutions, enzyme treated red cells, anti-human globulin reagents, bovine serum albumin, the use of reagents in manual, microplate and automated systems and HLA typing reagents.

Chapter 2

General guidelines

2.1 Introduction

General guidelines applicable to all reagents used for the typing of human red cells, and the detection of red cell antibodies are presented in this section. In other sections additional guidelines are given for particular reagents.

In Chapter 12, guidelines are given for the tests that should be performed before accepting for use ABO and Rh D blood grouping reagents and polyspecific anti-human globulin reagents intended for manual and microplate use. Those users unable to perform these tests should satisfy themselves that the tests have been carried out and the results are satisfactory before using the reagents.

This document uses Fisher's notation to describe the presumed Rh genotype of red cell samples to be used. where $R_z r$ or $r^y r$ red cells samples are to be used, the probable genotype should be confirmed by appropriate testing.

2.2 Standards

It is intended that the following working standards will be available:
anti-A
anti-B
anti-D (IgM)
anti-D (IgG)
anti-human IgG
anti-human C3c (polyclonal anti-C3c + C3d)
anti-human C3d
(monoclonal IgM)
bovine serum albumin
anti-HLA-A2
rabbit complement for use in HLA serology

2.3 Definitions

2.31 **Saline** is a solution of 8.5 to 9.0 g/l NaC1 that is 0. 14·5 to 0. 154M buffered with phosphate salts to pH 7.0 ± 0.2 at 20°C. The molarity of the buffer should be such to maintain the pH in the presence of likely contaminants. Phosphate buffered saline generally has a final buffer molarity of 10 to 15 mM.

2.32 **A low ionic strength solution** (LISS) is one comprising 0.03M NaC1, 0.003M $Na_2 HPO_4$: $NaH_2 PO_4$ buffer pH6.7 and 0.24M glycine.

2.33 **Water** should be either distilled or deionised for use in the preparation of reagents or buffers.

2.34 **Fresh plasma or serum for complement activity** should be less than 12 hours from donation if used either in the liquid state or after being stored frozen at -70°C or below. Unless validated, the maximum period of storage shall be six months.

2.35 **Inert blood group compatible serum** should be from one or more individuals, as appropriate, of an ABO group compatible with the test red cells. The serum should give negative reactions when tested against a panel of red cells for antibody identification (see 5.13) by appropriate methods.

2.36 **A batch** of reagent or red cells 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.

Each batch or sub-batch should be identified by a distinctive combination of numbers and/or letters (batch reference) which permits its history to be traced.

2.37 In the testing of reagents, the term **'undiluted'** in these guidelines means the reagent as intended by the manufacturer for use. This includes diluted solutions of the reagent if supplied to the user in a form requiring dilution prior to use, as specified in the manufacturer's package insert.

2.38 A reagent recommended by the manufacturer for the determination of a blood group of an individual should be designated **'blood grouping reagent'**.

2.39 A reagent recommended by the manufacturer for the detection of A (that is sub groups 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 (that is sub groups A_1 and A_2) and B but not of A_x, should be named anti-**A+B 'blood grouping reagent.'**

2.310 **Polyspecific anti-human globulin reagent** should be the name for a reagent which contains anti-human IgG and anti-human complement activity, and is recommended by the manufacturer for use in both the direct and indirect anti-human globulin technique, that is for the detection of red cell bound human IgG, C3c and C3d, irrespective of the presence of other anti-human immunoglobulin or anti-human complement specificities.

2.311 The name for a blood grouping reagent derived from material produced by somatic cell hybridization should include the word **'monoclonal'.**

2.312 The name of reagents produced by mixing preparations of different specificity or different monoclonal preparations from different sources and of the same apparent specificity, should include the word **'blended'**. Details of the blend, together with the reason, should appear in the package insert, for example, 'the reagent comprises a blend of two IgG monoclonal anti-D preparations, formulated to give the required reactivity against the widest range of Rh D Positive cell samples'.

Reagents formulated by mixing Polyclonal preparations obtained from the same individual, or by mixing different culture supernatants of the same monoclonal preparation should not be termed blended.

2.313 The name of reagent red cells intended for use in ABO grouping should include the words **'for use in ABO grouping'**.

The name of reagent red cells intended for use in Rh D grouping should include the words for **'use in Rh D groupings'**.

The name of reagent red cells intended for use in the control of the anti-human globulin technique should include the words **'for use in the control of the**

anti-human globulin technique'.

The name of enzyme-treated reagent red cells should include the words **'enzyme-treated'**.

The name of reagent red cells intended for detection of red cell antibodies should include the words **'for antibody screening'** or **'for antibody detection'**. The name of reagent red cells intended for the identification of irregular antibodies should include the words **'for antibody identification'**.

2.314 For reagent red cells, the antigens represented by the lower case letters c, k and s should be referred to on the final labels, package inserts and the antigenic profiles of reagent red cells, with an overscored bar, that is as c, k and s.

Similarly, for antisera, the antibody specificities represented by the lower case letters c, k and s should be referred to on the final labels and package inserts, with an overscored bar, that is as c, k and s.

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

2.316. **IgM anti-D** refers to anti-D blood grouping reagentsof IgM class, recommended by the manufacturer for use with red cells suspended in saline or LISS.

2.317 **Anti-D for albumin displacement** refers to an anti-D reagent, the use of which by the recommended method of the manufacturer requires the user to add albumin to the tube in order to displace the cell suspending medium.

2.318 **Chemically modified anti-D** refers to anti-D blood grouping reagents that have been chemically modified to effect agglutination of red cells suspended in saline or LISS.

2.319 **Other anti-D** refers to anti-D blood grouping reagents that are for use with an enzyme, or include in the formulation a potentiating substance or agent such as high molecular weight dextrans or modified albumin to effect agglutination of red cells suspended in saline or LISS.

2.320 **Prezone** is the term used to denote the absence or weakening of agglutination with excess of antibody.

2.321 **A monospecific** reagent is one containing an antibody or blend of antibodies specific for one antigen; e.g. anti-A.

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

2.323 **Irregular blood group antibodies** are those of specificity other than anti-A or anti-B.

2.324 **Antibody screening** is a test or combination of tests designed to determine irregular antibodies.

2.325 **Antibody identification** is a test or combination of tests designed to determine the specificity of irregular antibodies.

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

2.327 **Shelf-life** is the period for which a reagent, if stored under recommended conditions, is certified by the manufacturer to perform as expected.

2.328 **Expiry date** is the date beyond which it is not recommended by the manufacturer to use a reagent.

2 .4 Good manufacturing practice

2.41 Reagents for blood group serology shall be prepared in accordance with Good Manufacturing Practice (see current Guide to Good Pharmaceutical Manufacturing Practice', HMSO, and 'The Guide to Good Manufacturing Practice for in vitro Diagnostic Reagents', Association of British Pharmaceutical Industry, London).

2.42 The method of manufacture should result in a product in the final container that is homogeneous and free of properties which adversely affect its intended use throughout its recommended shelf-life.

2.43 The manufacturer should test, as described in these Guidelines, each batch or sub-batch of a reagent obtained from the final container to be supplied for use. The final container at the point of test need not be labelled with the final label if it is the manufacturer's practice to apply the final label after testing has assured the satisfactory performance of the reagent

2.5 Guidelines for human source material

2.51 Each individual donation of human material in a reagent for blood group serology shall be tested and found negative for at least hepatitis B surface antigen and HIV antibody by a reliable, sensitive and specific test (see Vol. I, Annex 5). A statement is required in the package insert to this effect

2.52 Samples from patients used for the evaluation of reagents for blood group serology should be used, whenever practical, with the patients' consent and tested individually and found negative for hepatitis B3 surface antigen and HIV antibody by a reliable, sensitive and specific test.

2.53 A sample of plasma, or preferably serum, of at least 3ml, collected at the same time as the donation of plasma, serum, red cells or lymphocytes used in the formulation of a particular reagent, should be stored at -20°C or lower until at least six months after the expiry date of the reagent. This recommendation applies also to human blood used in the preparation of monoclonal antibody reagents.

If necessary these samples should be used for retrospective serological testing or to test for hepatitis B surface antigen or HIV antibody by tests which are more sensitive or specific than routine techniques or for any other viral determinants.

2.6 Final containers

2.61 The final container 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.

2.62 The label fixed to the final 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.

2.7 Final container label

2.71 The label of the final container for at reagent for blood group serology should conform to the requirements of the British or European Pharmacopoeia as applicable.

The information printed on the label should be in black ink. The specificity and name of the blood grouping reagent should be of a print size which is not less than 12 point for containers of less than 5 ml volume and not less than 18 point for containers of greater than or equal to 5 ml volume. The print size of other information given on the label should not exceed the minimum stipulated print size for specificity and name of the reagent.

The following information should be stated on the label.

2.72 The name or specificity of the batch or sub-batch of reagent.

2.73 If not of human origin, the source of the material, for example, 'mouse monoclonal', or 'Dolichos biflorus'.

2.74 The name of the manufacturer or supplier.

2.75 A reference number or code by which the batch or sub batch can be identified.

2.76 The expiry date after which the reagent is not to be used when stored within the final container, at the highest storage temperature recommended by the manufacturer.

2.77 A space should be indicated for the user to write the expiry date of a freeze-dried product after it has been reconstituted and stored as recommended.

2.78 The minimum net weight, or net volume, of the reagent within the final container of each batch or sub-batch, or the average net weight, (or net volume of a final container together with an 'e,' as defined by the Weights and Measures Act 1979.

2.79 A statement that the reagent contains or does not contain a preservative. If the preservative is an azide its identity and concentration should be stated.

2.710 Where reagent red cells are to be washed before use, a statement to that effect.

2.711 The recommended temperature and conditions of storage. If the reagent is to be stored only in the liquid state, a statement that the reagent is not to be frozen.

2.712 Any colour appearing on the main panel of the label should comply with the permitted colour coding of reagents, **see 2.12**, except that:-

the main panel of labels of enzyme treated reagent red cells should be coloured mauve in order to be distinguishable from non-enzyme treated reagent red cells.

the company logo or name if coloured, should be located away from the main panel of the label where details of the specificity are given, and should not cause confusion with the permitted colour coding of reagents.

2.713 A statement that the reagent is for in vitro use only.

2.714 A statement that the user should refer to the package insert for details on the use of the reagent, e.g. read package insert before use.

2.715 Further labelling guidelines specific to a particular reagent, may be described under the appropriate paragraphs.

2.8 Package insert

2.81 Each reagent for blood group serology should be supplied with an accompanying document (package insert). If two or more final containers requiring identical package inserts are placed in a single package, only one package insert is necessary.

2.82 Information in the package insert should include that required for the label of the final container together with the following.

2.821 The batch reference on the final label to which the package insert refers.

2.822 The detailed methods of use recommended by the manufacturer for the stated batch or sub-batch of reagent, including any limitations or precautions and other information relevant to the safe use, storage and disposal of the reagent.

2.83 For **blood grouping reagents**, a list of those antibodies to antigens having a prevalence of greater than 99 per cent in the general population of the U.K. and of the specificity stated below, whose presence has not been excluded.

A; A_1; B; Le^a; Le^b; K; Kp^a; Js^a; P_1; D; C; E; c; e ; C^w; M; N; S; s; Lu^a; Jk^a; Jk^b; Fy^a; Fy^b; Xg^a; Do^a; Do^b; Yt^b; Co^b; Wr^a; Bg^a and Vw

2.84 If the reagent contains material of human origin, a statement that the human material has been tested at source and found negative for Hepatitis B surface antigen and HIV antibody.

2.85 If the reagent contains material of animal or human origin, a statement that the reagent cannot be assumed to be free from infectious agents and care is to be taken in the use and disposal of the container and its contents.

2.86 A statement that the reagent is for in vitro use only.

2.87 If the reagent is supplied at the optimal dilution for use, a statement that the reagent is to be used as supplied without dilution or addition.

2.88 If the reagent is supplied to the user in a form requiring dilution for use, the extent of which is determined by the user, full details of the diluent and dilution procedure, together with a statement that the user is to perform the tests to assure the correct performance of the diluted reagent. That is, at the dilution and for the techniques selected for use, tests for potency, specificity, stability and, for a reagent to be used by a slide technique, for avidity.

2.89 If the reagent is supplied in a freeze-dried form, full details of the reconstitution and reconstitution medium, together with the period during which it may be used following reconstitution, when stored as recommended by the manufacturer. The manufacturer should include a statement to the effect that after reconstituting the dried reagent the user should record the recommended expiry date on the space provided on the label.

2.810 A statement that the reagent has been characterised by the procedures recommended in the package insert and that its suitability for use in other techniques must be determined by the user.

2.811 For reagents other than reagent red cells, a statement that the reagent is not to be used if a precipitate, gel, particles or turbidity are present.

2.812 A statement that storage of the reagent at temperatures outside the recommended range may result in an acceleration in the rate of loss of reactivity.

2.813 The nature of any colourant added to the reagent.

2.814 If a blood grouping reagent is supplied for use with a reagent control, a statement that each test red cell sample is to be tested in parallel with the blood

grouping reagent and control, and that no determination of the blood group is possible if the reagent control effects agglutination of the test cell sample.

In addition, a statement that caution should be exercised in the interpretation of results of tests performed with such reagents at temperatures lower than 37°C

2.815 For blood grouping reagents containing monoclonal antibody(ies), the identity of the cell line(s) from which the monoclonal antibody(ies) has been derived.

2.816 For blended reagents, other than anti-human globulin, polyclonal preparations obtained from the same individual and different culture supernatants of the same monoclonal preparation, details of the blend (see 2.312)

2.817 For reagent red cells a statement that the reagent is not to be used if it is obviously discoloured or if the suspension medium indicates an obvious haemolysis.

2.818 For reagent red cells, one or more of the following statements, as appropriate:

'for use in ABO grouping'

'for use in Rh D grouping'

'for use in antibody identification'

'for use in antibody detection' 'for use in the control of the anti-human globulin technique'

2.819 For reagent red cells that are to be washed prior to use, instructions on the washing and resuspension of the red cells.

2.820 Loss of reactivity may occur during the stated shelf life of the red cells.

A list of the antigens most likely to deteriorate should be stated.

(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).

2.821 When red cells are preserved in LISS, especially in the presence of aminoglycoside antibiotics, e.g. neomycin sulphate, they should be discarded within 24 hours of resuspension. (There is accelerated deterioration in the reactivity of Fy^a, Fy^b and s and to a lesser extent S antigens under these conditions).

2.822 Reagent red cells which have been washed and resuspended in saline or low ionic strength solution are to be discarded not more than 24 hours after their preparation.

2.823 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. In addition, a statement that the efficacy of enzyme-treated reagent red cells should be confirmed daily or each time the reagent is used, whichever is shorter.

2.824 A statement that the reagent does or does not comply with the recommendations contained in Volume III of the UKBTS/NIBSC Guidelines.

2.825 Further package insert guidelines specific to a particular reagent, may be described under the appropriate paragraph.

2.9 Date of manufacture

2.91 For reagents other than reagent red cells, the date of manufacture is the date of commencement of the last potency test that indicates attainment of the required

specification.

2.92 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 first donation should be recorded as the date of manufacture.

2.93 Where a freezing process is used to preserve red cells before their preparation for issue as reagent red cells, the freezing process should be undertaken not more than 5 days after the donation is collected and should be capable of maintaining a temperature of -65°C or lower. At such temperatures, red cells may be stored for 10 years before their preparation for use as a reagent.

Other systems for the frozen storage of red cells may be appropriate. All systems used to maintain red cells in the frozen state prior to reagent red cell manufacture must be validated to confirm their suitability.

2.10 Stability data for reagents other than reagent red cells

2.101 The manufacturer should have data to validate the expiry date beyond which the reagent for blood group serology is not to be used when stored at the highest temperature recommended by the manufacturer.

2.102 If the reagent is supplied as a freeze-dried product then in addition to **2.10**, data should be available to validate the expiry date assigned to the reconstituted material as intended for use, stored at the highest temperature recommended by the manufacturer.

2.103 Before the release of a batch of a new formulation of a reagent, the manufacturer should have data to indicate the compliance with the specifications for a non-released batch of that reagent within the final container stored at the highest temperature recommended by the manufacturer for a period of at least six months.

2.104 A provisional expiry date of not more than one year from the date of manufacture may be assigned to the first released batch of reagent made to the same formulation and by the same processes as the non-released batch for which at least six months data are available.

2.105 Data should be collected and reviewed at least three-monthly from both the non-released and released batches of reagent, in order to determine the expiry date ultimately assigned to batches subsequently made to that formulation.

2.106 If the data indicate an expiry date for that formulation earlier than the provisional expiry date assigned to the released batch, the manufacturer should notify immediately all primary consignees of that batch or sub-batch.

2.11 Stability data for reagent red cells

2.111 The maximum expiry date of reagent red cells supplied in a preservative medium should be 40 days from the date of manufacture, excluding any period of storage in the frozen state at a temperature of -65°C or lower (see 2.93).

2.112 The manufacturer should validate the expiry date assigned to reagent red cells by establishing that significant deterioration does not occur, by their processing methods, in the expression of those antigens stated in the antigenic profile

2.113 The maximum expiry date of reagent red cells stored as recommended by the

manufacturer and supplied in a medium not specifically formulated to preserve the reactivity of antigens, for example CPD-A, should be 21 days from the date of manufacture in unopened final containers and not more than 5 days after opening.

2.12 Colour coding

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

polyspecific anti-human globulin reagent may be coloured green, anti-A may be coloured blue, anti-B may be coloured yellow

tests for specificity, potency, avidity and stability should be performed on the reagent as intended to be supplied for use, that is including the presence of any colourant. The colourant should not interfere with the observation of the test result.

2.13 Sterility of reagents

Reagents should be processed in a manner known to produce a final product free from microbial contaminants that adversely affect the product either during storage at the recommended temperature or in use.

2.131 A preservative may be included in the reagent to minimise the effects of contamination during use if the preservative has been assured not to adversely affect the product during storage or use. The efficacy of the preservative should be assessed during the development of the reagent and should reflect the expected usable period of the reagent. Organisms which have been shown from experience to cause deterioration of the reagent should be included in the test procedure in addition to those listed in the monograph 'Efficacy of Antimicrobial Preservatives in Pharmaceutical Products' (Appendix: XVIC, British Pharmacopoeia 1988, Volume II) .

2.132 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 0.2 µm filter. All reagents should be dispensed into the final container under aseptic condition

2.133 The production process including dispensing into the final containers, should be validated to produce a sterile product.

2.134 For reagent red cells, the manufacturer should perform viable count estimations on the final product as part of process and product monitoring, but these need not form part of product release requirements. In the event of such monitoring disclosing contamination likely to affect adversely the performance of the reagent during the expiry period, the manufacturer should notify immediately all primary consignees of that batch or sub-batch to withdraw existing stocks from distribution and, if necessary re-validate the manufacturing process.

2.135 Tests for sterility alone do not give absolute assurance of freedom from microbial contamination. In addition, the opening of a container may compromise the sterility of the reagent. Bactericidal agents in common use for blood grouping reagents do not guarantee the absence of microbial agents after opening of the container.

Adequate controls should be used to detect any aberrant serological reactivity

caused by contamination of the reagent.

2.14 Retained samples

2.141 A minimum of 5 final containers of **each batch of reagents** other than reagent red cells should be retained by the manufacturer and stored at the highest recommended temperature, to enable analysis of reported defects. Such samples should be retained for at least 6 months beyond the expiry date.

2.142 A minimum of 2 final containers of **each batch of reagent red cells** should be retained by the manufacturer and stored at the highest recommended temperature, to enable analysis of reported defects. Such samples should be retained for at least 28 days beyond the expiry date.

2.15 Packaging and despatch of reagents

2.151 The manufacturer should ensure that the method of despatch does not cause deterioration in the performance of the reagent.

2. 152 If distributed by UK postal services, the packaging shall conform to the UK postal requirements and be sufficient to protect the reagent from foreseeable mechanical damage.

2.153 Packaging containing solid carbon dioxide should be vented and precautions taken to prevent inactivation of the reagent by entry of carbon dioxide gas.

2.154 Reagents despatched frozen and intended to remain so during transit should contain an indicator to demonstrate that the recommended temperature for the reagent has not been exceeded.

2.155 The label on the packaging should include all the information required on the label of the final container and be sufficient to ensure the appropriate handling and correct storage on receipt by the addressee.

CHAPTER 3

GUIDELINES FOR SEROLOGICAL TESTS

3.1 Grading system for agglutination

The following grading system should be used for manual serological testing. If a cumulative (titration) score is required to assess the characteristics of a blood grouping reagent in a titration, then the score as indicated should be used.

Grade 5: Cell button remains in one clump, macroscopically visible. (Titration score of 12).

Grade 4: Cell button dislodges into several clumps, macroscopically visible. (Titration score of 10).

Grade 3: Cell button dislodges into many small clumps, macroscopically visible. (Titration score of 8).

Grade 2: Cell button dislodges into finely granular but definite, small clumps, macroscopically visible. (Titration score of 5).

Grade 1: Cell button dislodges into fine granules, macroscopically visible. (Titration score of 3).

Grade 0: Negative result. (Titration score of 0).

3.2 Test red cells

3.21 Records should be kept of all red cells used in the assessment of a reagent during manufacture.

3.22 For specificity testing, red cells stored in the liquid state in a medium not specifically formulated to preserve the reactivity of antigens, should be used within 7 days of collection. Red cells stored in a medium proven to preserve the antigens for which the red cells are being used should be used within the shelf-life assigned to red cells stored in that solution.

3.23 Red cells of any age may be used to test the potency and avidity of antibodies.

3.24 Red cells may be stored frozen and thawed for use in tests for potency, avidity and specificity. For use in tests for specificity the method of freezing, storage and thawing should be known to preserve the antigens for which testing will be performed. Frozen red cells should be used on the day they are thawed and resuspended unless they are suspended in a medium proven to preserve the antigens for which the red cells are being used.

3.25 Red cells for testing reagents for blood group serology which require an anti-human globulin technique, should be tested on the day they are used and found negative by the direct anti-human globulin technique.

3.26 Unless red cells are stored in a preservative medium and recommended for use without washing, red cells should be washed at least twice in saline before use. The supernatant after the last wash should be clear. Red cells to be suspended in LISS should be given at least one additional wash in LISS. Red cells suspended in saline or LISS should be discarded after 24 hours.

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3.27 Red blood cells from different individuals used for specificity or potency tests should not be pooled. Cord red cells of a given ABO and Rh D group may be pooled.

3.28 Unless otherwise stated, the concentration of test red cell suspensions should be two to three percent by volume.

3.3 Reactivity against Gm and Km immunoglobulin determinants and non-specific reactions against sensitised red cells

3.31 Blood grouping reagents should not contain antibodies reactive against the immunoglobulin determinants Gm and Km when used by any method recommended by the manufacturer.

3.32 The reagent should be tested against red cells coated with IgG blood group antibody known to express the common Gm allotypes, to effect a grade 5 reaction in the anti-human globulin technique. Blood group antibodies from at least six individuals should be tested separately and include both IgG1 and IgG3 subclasses.

3.4 Number of final containers to be tested

3.41 For reagents other than red cells, a minimum of 1 percent of the batch or 5 final containers, whichever is less, should be tested for compliance with the specification for serological reagents.

3.42 For reagent red cells, since the number of final containers is limited by the volume of the donation of red cells, a minimum of 1 percent of the batch or 2 final containers, whichever is less, should be tested for compliance with the specifications for serological reagents.

3.43 If the volume of material required for the serological testing exceeds the volume available from a single container, then the contents from two or more containers should be pooled, mixed and used for testing.

3.5 Guidelines for blood grouping reagents

3.51 The manufacturer should test the blood grouping reagent as intended for use for the specificity of the reactivity claimed for the reagent by all methods recommended for the use of the reagent by that manufacturer.

3.52 Specificity should be determined by testing the reagent with red cells from a minimum of 4 different donors known to express the antigen corresponding to the specificity of the reagent and 4 different individuals known to lack that antigen. The reagents should be tested for specificity by all methods recommended by the manufacturer for its use.

3.53 Rouleaux formation, prezone (see Definitions, 2.320) or haemolysis should not occur in any of the methods recommended by the manufacturer.

3.54 Manufacturers should ensure that those properties of the reagent which may affect the performance of reagents in blood group serology, for example, EDTA

concentration, NaCl concentration, pH and total protein content, are not detrimental to the reactivity of the reagent.

3.55 Contaminating antibodies to antigens having a prevalence of greater than 99 per cent 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 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 antigen specificities should be stated in the package insert as not having been excluded in specificity testing:

A ; A_1 ; B ; Le^a ; Le^b ; K ; Kp^a ; Js^a ; P_1; D ; C; E ; c ; e ; C^w ; M ; N ; S ; s ; Lu^a ; Jk^a ; Jk^b ; Fy^a ; Fy^b ; Xg^a ; Do^a ; Do^b ; Yt^b ; Co^b ; Wr^a ; Bg^a ; and Vw.

Where practicable, red cells used for this testing should have homozygous expression of these antigens.

3.56 Tests for the presence of contaminating ABO antibodies should be performed with red cells from a minimum of 2 individuals of group A_1; 2 of group B and 2 of group 0 who lack the antigen corresponding to the antibody specificity under test.

3.57 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

3.6 Potency tests for manual and microplate blood grouping reagents

3.61 UK reference preparations for use in manual, automated or microplate techniques will be made available for the following reagents:

Anti-A ; anti-B; anti-D (IgM); anti-D (IgG); anti-human IgG anti-human C3 (polyclonal anti-C3c+C3d); anti-human C3d (monoclonal IgM) ; bovine serum albumin ; anti-HLA A2 and rabbit complement for HLA serology.

3.62 Where the appropriate UK reference preparation exists, manufacturers should compare the potency of the reference preparation and test blood grouping reagent in parallel, as detailed in these guidelines. For anti-A+B and anti A,B blood grouping reagents, the anti-A and anti-B UK reference preparations should be used separately. Blood grouping reagents should at all dilutions attain or exceed that reaction grade of the corresponding UK reference preparation, tested in parallel.

3.63 The manufacturer should assay the potency of each batch or sub-batch of blood grouping reagent recommended for use by an immediate spin tube method using the following procedure.

Add one volume of each dilution of the reagent to a separate tube.

Add one volume of the test cell suspension to each tube.

After dispensing each of the required cell suspension to the series of tubes mix thoroughly and centrifuge the tubes immediately.

Determine the reaction grade.

3.64 The manufacturer should test each batch or sub-batch of blood grouping

reagent recommended for use by an albumin displacement method using the following procedure.

Add one volume of each dilution the reagent to a separate tube.

Add one volume of the test cell suspension to each tube. Mix thoroughly and incubate for 45 minutes at 37°C.

Add one volume of 200g/L albumin (not deliberately polymerised or otherwise potentiated) to run down the inner wall of the 50-60 x 7mm tube to displace the saline and tie over the sedimented red cells. Do not mix.

Incubate for 15 minutes at 37°C.

Determine the reaction grade.

3.65 The manufacturer should test each batch or sub-batch of blood grouping reagent recommended for use by a 5 minute agglutination method using the following procedure.

Add one volume of each dilution of the reagent to a separate tube.

Add one volume of the test red cell suspension to each tube.

Mix thoroughly and incubate for five minutes at 37°C in a water bath or heated dry block.

Centrifuge the tubes.

Determine the reaction grade.

3.66 The manufacturer should test each batch or sub-batch of blood grouping reagent recommended for use by a microplate method using the following procedure.

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.

BROMELIN

To 1g of bromelin powder add 5ml saline. Mix to form a thin homogenous paste. Add 100 ml saline and mix thoroughly for 5 minutes. Centrifuge the suspension. Discard any insoluble material. Add saline to the supernatant to a final volume of 1000 ml.

TEST RED CELLS

Test red cells, washed at least once in saline, are suspended in the bromelin solution to a suspension of 3 percent.

Incubate at 20°C for 2 minutes before use. Cell suspensions not used within one hour are washed, resuspended to a 3 percent suspension in saline and used within 24 hours of

preparation.

CONTROL OF BROMELIN TREATMENT

A pool of R_1r red cells from 4 individuals are treated with bromelin as above and tested using the microplate method described below, with an inert group-compatible serum and anti-D (0.25 IU/ml).

If a grade 4-5 reaction is not observed with the anti-D (0.25 IU/ml), the use of a more potent bromelin preparation is investigated.

The group-compatible, inert serum must effect a negative reaction with the bromelin-treated R_1r red cells.

MICROPLATE METHOD

Using a microplate, add 30,µl of serum to 30 µl of bromelin - treated test cells.

Mix the contents of the wells using a microplate shaker. Incubate at 20°C for 15 minutes.

Centrifuge the microplate at 100g for 40 seconds.

Gently dislodge the red cells using a microplate shaker.

Determine the reaction grade using a concave microplate reading mirror.

3.67 The manufacturer should test each batch or sub-batch of blood grouping reagent recommended for use by a non automated method if the method differs from that referred to in 3.63 to 3.66 using the following procedures.

Using each dilution of the reagent perform the tests, with each test cell suspension.

If a range of incubation times is given, the shortest time should be used, except for enzyme-addition techniques where the longest recommended incubation time should also be used.

If a range of incubation temperatures is given for blood grouping reagents where the antibody/antigen reaction is favoured by a colder temperature, the highest temperature should be used; for other blood grouping reagents, the lowest temperature should be used.

Centrifuge using the recommended rcf and time.

Determine the reaction grade.

3.68 Avidity tests

The manufacturer should test the avidity of each batch or sub-batch of blood grouping reagent recommended for use by a slide method using the following procedure.

Mix over an oval area of approximately 20mm x 40mm on a glass slide, one volume of the undiluted reagent with one volume of a 30 to 45 percent red cell suspension in homologous serum or ABO group-compatible plasma.

Maintain the slide at the recommended temperature for the slide test. If a range of incubation temperatures is given, for blood grouping reagents where the antibody/antigen reaction is favoured by a colder temperature, the highest temperature should be used; for other blood grouping reagents, the lowest temperature should be used.

Determine the time from mixing at which macroscopic agglutination first appears and the reaction grade at 1 minute.

3.7 Quality control of blood grouping reagents

3.71 A blood grouping reagent is unsatisfactory if it fails, with red cells from more than one person which express the corresponding antigen, to effect the required grade of reaction by the above recommended methods of tests for potency, avidity, or by any method recommended for use by the manufacturer.

3.72 When the blood grouping reagent is shown to be unsatisfactory by failing to meet the criteria in 3.71 above, red cells from a minimum of 3 further persons with the same antigen phenotype as the red cells which failed to attain the reaction grade, should be tested.

3.73 If a blood grouping reagent produces agglutination, by any method recommended for use by the manufacturer, of red cells lacking the antigen corresponding to the antibody specificity of the reagent but sensitised with IgG antibody to effect a grade 5 reaction in the anti-human globulin technique, that blood grouping reagent should be supplied with a reagent control which has been shown to be suitable for use with that batch or sub-batch of blood grouping reagent.

3.74 The reagent control should effect a similar degree of non-specific reaction with sensitised red cells as the corresponding blood grouping reagent.

3.75 Manufacturers who provide blood grouping reagents which require the user to add a potentiator, such as albumin or an enzyme, should provide the potentiator which has been shown to be suitable for use with that batch of reagent.

3.8 Potency titrations

METHOD

The use of a semi-automatic pipette is recommended; one volume being in the order of 40 µl.

A separate pipette tip or Pasteur pipette should be used for each reagent.

If the reagent is formulated with a medium to enhance its reactivity then the same formulation, but with no blood group activity is used as the diluent for the determination of the potency titre. Otherwise, dilutions should be prepared in saline

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containing a final concentration of 20g/l bovine serum albumin which has not been deliberately polymerised or otherwise potentiated.

Beginning with the undiluted blood grouping reagent, separate doubling dilutions (1 in 2, 1 in 4, 1 in 8, etc.) of the reagent should be prepared.

When preparing doubling dilutions, after the addition of a volume of reagent or diluted reagent to an equal volume of the diluent, the tip of the Pasteur pipette is emptied and blotted before the dilution is mixed and a volume transferred to prepare the subsequent dilution.

The potency titre should be the reciprocal of the highest dilution of the reagent which effects a grade 2 reaction using the required technique. The dilution caused by the addition of the cell suspension should not be considered in determining the potency titre.

3.9 Test tubes

Unless otherwise stated 10-12 x 75mm glass tubes should be used.

3.10 Centrifugation following the addition of anti-human globulin reagent

In the anti-human globulin technique, after the addition of the anti-human globulin reagent, the reactants should be centrifuged within 15-30 seconds of mixing, unless otherwise stated.

3.11 Centrifugation and reading of serological results

The centrifugal force should be sufficient to create a button of cells with clearly defined edges but not such to make the button difficult to dislodge. Many combinations of relative centrifugal. force (rcf) and time give similar results e.g. 110 rcf for 1 minute, 200 rcf for 30 seconds, 500 rcf for 15 seconds or 1000 rcf for 10 seconds.

3.111 ANY OF THE FOLLOWING READING TECHNIQUES SHOULD BE USED

Pipette transfer of cell button to microscope slides.

The tube is not agitated at any stage. The transfer pipette is clean and has a 1.5-2.0mm internal diameter bore and the tip is free from any irregularities. The cell button is drawn, by the minimum suction possible, into the stem of the

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clean pipette and then gently ejected onto a slide and drawn out over an area of some 2 cm^2. The angle of the pipette above the horizontal controls the width of the spread. The test is observed macroscopically and microscopically if required.

Tip and roll

The tube is held almost horizontally (70-80° to the vertical) between the thumb and first two fingers and slowly rotated without any shaking or agitation, until the cell button is dislodged from the tube. The free cell button/agglutinates are only allowed to move a maximum of lcm down the tube. The test is read macroscopically, with the tube head horizontally over an illuminated light source A x5 or x6 magnifying mirror, or an x6 hand lens can be used.

Readings can then be obtained by examination of the tube placed horizontally on the stage of an inverted microscope, or by transferring the tube contents to a microscope slide, either by pipette or by touching the lip of the tube on the slide, pouring the contents and moving the tube slowly along it.

Gentle agitation

The tube is held almost vertically between the thumb and first two fingers and gently agitated using a trembling or vibrating movement. The test is read as described above.

A 'Shake' technique should not be used. This type of procedure has been developed by some workers from the gentle agitation procedure that is in wide usage. Its over-vigorous action disrupts agglutination and is responsible for many false negative tests in blood group serology.

3.112 Examination for haemolysis

Haemolysis can be determined by the visual detection of haemoglobin in the supernatant fluid.

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Chapter 4

Serological guidelines for ABO and Rh D blood grouping reagents

4.1 Introduction

4.11 The determination of the ABO and Rh D group is of prime importance in ensuring the safe transfusion of blood. It. is essential that reagents for ABO and Rh D grouping are prepared using reliable manufacturing procedures that are consistently capable of producing safe and efficacious products.

4.12 Monoclonal preparations, particularly anti-D, may have a restricted reactivity compared with polyclonal preparations of the same specificity.

4.13 The term D^u is used in these recommendations to indicate a weakened expression of a normal D antigen. There is a gradation of the expression of the D^u, antigen; in general such cells are negative or weakly positive with IgM blood grouping reagents, but are positive with IgG Rh D blood grouping reagents used in an anti-human globulin technique.

4.14 The term D variant is used in these recommendations to indicate the expression of only a part of the normal D antigen. The reactivity of Rh D blood grouping reagents against D variant red cells is determined by the nature of the D variant, the anti-D reagent and the technique used.

4.2 Quality control of the specificity of ABO blood grouping reagents for use in manual or microplate tests

4.21 As a minimum the following red cells should be tested using all methods recommended for use by the manufacturer.

Blood Grouping	Number of red cell samples to be tested						
Reagent	A_1	A_2	A_1B	A_2B	B	0	A_x
anti-A	2			2	2	2	3*
anti-B	2		2		2	2	
anti-A,B	1	2			2	4	3
anti-A+B	1	2			2	4	

* only if the anti-A is recommended for the detection of A_x cells.

4.22 The blood grouping reagent is satisfactory if not less than a grade 4 reaction is effected 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.

4.23 Anti-A blood grouping reagent is satisfactory if not less than a grade 4 reaction is effected with all the A_2B samples tested by all the methods recommended for use by the manufacturer.

4.24 Anti-A, B blood grouping reagents and anti-A blood grouping reagents recommended for the detection of Ax should effect at least a grade 3

agglutination with each of the Ax red cell samples by those techniques recommended by the manufacturer for the detection of A_x.

4.25 If further reactivity is claimed by the manufacturer against subgroups of A other than A_1 and A_2. red cells from three individuals of each claimed subgroup should be tested.

Such blood grouping reagents should effect at least a grade 3 agglutination with each of the red cell subgroup samples by those techniques recommended by the manufacturer for the detection of those subgroups.

4.3 Quality control of the potency of ABO reagents for use in manual or microplate tests.

4.31 As a minimum, the following red cell samples should be used.

Blood Grouping	Number of red cell samples to be tested				
Reagent	A_1	A_2	A_1B	A_2B	B
anti-A	1			3	
anti-B			3		1
anti-A,B	1	2			2
anti-A+B	1	2			2

4.32 For blood grouping reagents recommended for use by a tube, slide or microplate technique:

anti-A blood grouping reagents should effect a potency titre at least equal to the UK reference anti-A preparation with the red cell samples tested.

anti-B blood grouping reagents should effect a potency titre at least equal to the UK reference anti-B preparation with the red cell samples tested.

anti-A,B blood grouping reagents should effect a potency titre at least equal to the UK reference anti-A preparation with the A_1 and A_2 red cell samples tested and a potency titre at least equal to the UK reference anti-B preparation with the group B red cell samples tested.

anti-A+B blood grouping reagents should effect a potency titre at least equal to the UK reference anti-A preparation with the A_1 and A_2 red cell samples tested and the potency titre at least equal to the UK reference anti-B preparation with the group B red cell samples tested.

4.4 Quality control of the avidity of ABO blood grouping reagents for use in manual slide tests.

4.41 As a minimum, the following red cell samples should be used.

Blood Grouping Reagents	Number of red, cell samples to be tested.				
	A_1	A_2	A_1B	A_2B	B
anti-A	1			3	
anti-B			3		1
anti-A,B	1	2			2
anti-A+B	1	2			2

4.42 The grade of agglutination with the blood grouping reagent should at least equal that of the corresponding UK reference preparation(s) with the red cell samples tested within one minute of mixing.

4.5 Quality control of specificity of anti-D blood grouping reagents for use in manual or microplate tests.

4.51 As a minimum the following red cells should be tested using all methods recommended for use by the manufacturer:

R_1r from two individuals

R_or or R_oR_o f rom two individuals

r'r from one individual

r"r" from one individual

rr from one individual

and, if the anti-D blood grouping reagent is recommended by the manufacturer for use by the anti-human globulin technique,

rr cells with strong expression of Bg^a from three individuals.

The blood grouping reagent should ,effect a grade 5 reaction with all D positive red cell samples by all methods recommended for use by the manufacturer and negative reactions with D negative red cell samples. The blood grouping reagent, if recommended for use by the anti-globulin technique should effect negative reactions with the rr cells with strong Bg^a antigens.

4.52 Anti-D blood grouping reagents recommended by the manufacturer for the detection of D^u should be tested with a minimum of three D^u red cell samples using those techniques recommended by the manufacturer for the detection of D^u.

The blood grouping reagent should effect at least a grade 3 reaction with the three D^u red cell samples using those techniques recommended by the manufacturer for the detection of D^u.

4.53 Anti-D blood grouping reagents recommended by the manufacturer for the detection of D variant should be tested with a minimum of 2 examples of the particular D variant against which reactivity is claimed, using those techniques recommended by the manufacturer for the detection of that D variant.

The blood grouping reagent should effect at least a grade 3 reaction with the 2 examples of the particular D variant against which reactivity is claimed, using those techniques recommended by the manufacturer for the detection of that D variant.

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4.54 Anti-D blood grouping reagents, supplied by the manufacturer for use without a reagent control should be tested with a minimum of 3 different group 0 rr red cells, sensitised with anti-e to effect grade 5 agglutination in the anti-human globulin technique.

> The blood grouping reagent should not agglutinate by any method of use recommended by the manufacturer, the 3 different group 0 rr red cells, sensitised with anti-e

4.6 Quality control of the potency of anti-D grouping reagents for use in manual or microplate tests

As a minimum, R_1r red cells from 2 individuals should be used.

4.61 For anti-D (for albumin displacement) blood grouping reagents recommended for use by a tube technique

> There should be grade 5 agglutination with the undiluted reagent and at least a grade 2 agglutination with the reagent diluted 1 in 8, with all the R_1r test red cell samples

4.62 For anti-D blood grouping reagents recommended for use by a microplate technique.

> There should be grade 5 agglutination with the undiluted reagent and at least a grade 2 agglutination with the reagent diluted 1 in 8, with all the R_1r test red cell samples

4.63 For IgM anti-D blood grouping reagents recommended for use by a tube technique.

> There should be a grade 5 agglutination with the undiluted reagent and at least a grade 2 agglutination with the reagent diluted 1 in 8, with all the R_1r test red cell samples.

4.64 For anti-D blood grouping reagents recommended for use by a slide technique.

> There should be a grade 5 agglutination with the undiluted reagent and at least a grade 2 agglutination with the reagent diluted 1 in 8, with all the R_1r test red cell samples.

4.65 For chemically modified anti-D blood grouping reagents recommended for use by a tube technique.

> The potency titre should be grade 5 with the undiluted reagent and at least a grade 2 agglutination with reagent diluted 1 in 4, with all the R_1r test red cell samples.

4.66 For other anti-D blood grouping reagents recommended for use by a tube technique.

> The potency titre should be grade 5 with the undiluted reagent and at least a grade 2 agglutination with the reagent diluted 1 in 8, with all the R_1r test red cell samples.

4.67 For other anti-D blood grouping reagents recommended for use by a slide

technique.

The potency titre should be grade 5 with the undiluted reagent and at least a grade 2 agglutination with the reagent diluted 1 in 8, with all the R_1r test red cell samples.

4 7 Quality control of the avidity of anti-D blood grouping reagents for use in manual slide tests

As a minimum, R_1r red cells from 2 individuals should be used.

Agglutination should be grade 3 within one minute of mixing.

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Chapter 5

Guidelines for reagent red cells

5.1 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 irregular (allo) red cell antibodies.

5.2 Guidelines for testing reagent red cells

5.21 When testing reagent red cells, in order to confirm the presence or absence of antigens listed in the protocol of antigenic profile, a sample from each individual (especially from those not used previously for such reagent red cells) should be tested whenever possible, with a minimum of two different examples of blood grouping reagents corresponding to each antigen specifically listed.

5.22 Where such testing produces conflicting results, further testing with at least one additional example of the relevant antibody/ies should be undertaken before the antigenic status of that cell is committed to the antigen profile included within the package insert.

5.23 Where such testing has been performed with only one example of any blood grouping reagent, this information should be included on the antigen profile included within the package insert.

5.24 Reagent red cells should be shown not to produce unwanted positive reactions by the methods recommended for use by the manufacturer.

5.25 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 using the techniques recommended for use by the reagent manufacturer.

5.3 Preparation of reagent red cells

5.31 Red cells for use in reverse ABO grouping, in the control of blood grouping reagents or in the detection of irregular antibodies in blood donations, may be pooled. In such instances, equal volumes of red cells from a maximum of two individuals should be used and the reagent red cells prepared by a procedure which ensures adequate mixing of the constituent red cells.

5.32 With the exception of umbilical cord blood, red cells used to test patient's

samples for irregular antibodies should not be pooled.

5.33 Reagent red cells should be processed by a method consistently shown to yield a product capable of detecting, throughout its shelf-life, all antibodies directed against the antigens specified in the antigen profile included within the package insert.

5.34 Unless instructions are given to wash the reagent red cells before use, all reagent red cells should be free of ABH specific blood group substances and blood group antibodies, including anti-A, anti-B, demonstrable by the manufacturer's recommended methods of use.

5.4 Final container label and package insert

(In addition to the guidelines in 2.6 and 2.7).

5.41 The final container label and package insert of reagent red cells prepared from pooled material should include the statement 'POOLED CELLS'. In addition, the package insert should include the statement 'DO NOT USE WHEN TESTING PATIENT'S SERUM FOR IRREGULAR ANTIBODIES'.

5.42 The antigen profile of reagent red cells should accompany or be included with the package insert. Where such reagent red cells are intended for use in ABO grouping or control of ABO or Rh D blood grouping reagents, or comprise pooled umbilical cord blood, only the ABO and Rh D group need be stated.

5.43 When the reagent red cells are a multi-container product such as a red cell panel, the label on the 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 antigen profile

5.44 The date of expiry of reagent red cells should be stated in the antigen profile.

5.45 Where reagent red cells are provided suspended in preservative medium, the components of the medium should be stated in the package insert.

5.46 The concentration of the red cell suspension should be stated in the package insert and antigenic profile.

5.5 Other reagent red cells

Manufacturers who wish to provide additional reagent red cells other than those described in paragraphs 5.6 to 5.14 should comply with the relevant guidelines listed herein.

5.6 Enzyme-treated reagent red cells

5.61 Reagent red cells pretreated with proteolytic enzymes may be provided for antibody screening and identification.

5.62 Enzymes used to treat red cells for manual tests should comply with the guidelines listed in Chapter 8 of this document.

5.63 Enzymes used to prepare red cells for ABO or Rh D grouping using microplates should be prepared as described in 3.66.

5.64 The method of enzyme treatment used by the manufacturer should be shown consistently to yield a product capable of detecting, throughout its shelf-life, those antibodies which the reagent is intended to detect.

5.7 Reagent red cells for use in ABO grouping

5.71 Introduction

Determination of the ABO group of donor and patient is vital in ensuring safe transfusion practice. This is primarily achieved by testing unknown red cells with standard blood grouping reagents. Confirmation of the observed ABO group can be achieved by demonstrating ABO specific antibodies in serum or plasma - a procedure known as 'reverse' grouping. Appropriate reagent red cells are required for this purpose and for controlling ABO blood grouping reagents.

Reagent red cells for use in ABO grouping need only be tested for A ; A_1, ; B and Rh D.

5.72 Reagent red cells for reverse groupingReagent red cells of groups A_1 and B should be used, although A_2 may be included..

Reagent red cells for reverse grouping should be Rh D negative.

5.73 Controls for ABO blood grouping reagents

Reagent red cells of groups A_1; A_2; B and O should be used for the control of each batch of tests with anti-A ; anti-B ; anti-A,B ; anti-A+B or anti-A_1.

Reagent red cells of group A_2B should be used for the daily control of anti-A reagents. Reagent red cells of group A_1B should be used for the daily control of anti-B reagents.

5.8 Reagent red cells for use in Rh grouping

5.81 Introduction

Determination of the Rh D group of donor and patient is required to ensure safe transfusion practice. Reagent red cells which confirm the efficacy of anti-D blood grouping reagents are essential.

5.82 Reagent red cells for the control of Rh D blood grouping need only be ABO grouped and Rh phenotyped with anti-C. anti-D. anti-E. anti-e and anti-e.

5.83 Reagent red cells of group O, R1r and r'r should be used for the control of each batch Rh D blood grouping tests.

5.9 Additional control cells

Additional red cells intended for the control of blood group reagents, whenever possible should be from individuals whose zygosity for the appropriate antigen/s is known.

5.10 Reagent red cells for use in antibody screening

5.101 Introduction

The detection of irregular antibodies in the serum of a patient is of greater clinical significance than if such antibodies are detected in blood donors. Consequently it is permissible to use reagent red cells of a lesser specification when performing antibody screening tests on blood donors.

5.102 General guidelines

5.1021 Reagent red cells for use in antibody screening should be confirmed as group O by an ABO blood grouping procedure which is capable of demonstrating the A_x phenotype.

5.1022 Where practicable, reagent red cells known to express low frequency antigens (that is, those antigens having a frequency of less than 1 percent in the general population of the UK) should not be included in reagent red cells for antibody screening.

5.1023 Where practicable, red cells from individuals known consistently to effect troublesome reactions with HLA specific antibodies should not be used as reagent red cells for antibody screening.

5.11 Reagent red cells for use in antibody screening of patient samples

5.111 Introduction

Antibody screening tests on patients' serum samples provide an opportunity for the detection and subsequent identification of irregular antibodies, thereby facilitating the selection of blood for transfusion and permitting early prediction of possible haemolytic disease of the foetus and newborn.

5.112 General guidelines

5.1121 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; P_1; Le^a and Le^b

5.1122 As a minimum, reagent red cells from two individuals should be provided. These red cells should not be pooled.

5.1123 At least one of the red cell samples should express the probable Rh haplotype R_2.

5.1124 Apparent homozygous expression of the following antigens in the stated order of priority, is desirable;

D; c; Fy^a; Jk^a; Jk^b; S; s and Fy^b

5.12 Reagent red cells for use in antibody screening of donor samples

5.121 Introduction

Antibody screening of donor samples permits identification of those donors

whose plasma contains relatively potent irregular antibodies and the exclusion of those donations which have the potential to cause adverse reactions on transfusion.

5.122 General guidelines

5.1221 It is preferable that reagent red cells are provided, unpooled, from a minimum of two individuals but the reagent may be supplied as a pool of red cells from no more than two donors.

5.1222 Pooled reagent red cells for antibody screening should 'be used only for testing samples from blood donors.

5.1223 As a minimum the following antigens should beexpressed

C; c; D; E; e; K; k; Fy^a; Fy^b; Jk^a; Jk^b; S and s.

5.1224 At least one of the red cell samples should expressthe probable Rh haplotype R_2.

5.13 Reagent red cells for use in antibody identification

5.131 Introduction

Identification of the specificity of irregular antibodies detected during antibody screening is important, particularly in pretransfusion and pre-natal testing programmes.

5.132 General guidelines

5.1321 Reagent red cells for use in the identification of irregular antibodies should be confirmed as group 0 by an ABO blood grouping procedure which is capable of demonstrating the A_x phenotype.1

5.1322 In some instances, identification of the specificityof an irregular antibody requires the use of reagent red cells other than group 0, for example, red cells of group A, and A_2 are particularly useful in assigning the specificity of anti-HI.

5.1323 Where practicable, red cells from individuals known consistently to effect troublesome reactions with HLA specific antibodies should not be used in reagent red cells for antibody identification.

5.1324 Reagent red cells for antibody identification should comprise red cells from eight or more group 0 individuals and permit the confident identification of those clinically significant alloantibodies which are most frequently, encountered, for example, anti-D, anti-E, anti-c, anti-K and anti-Fy^a.

5.1325 Red cells from the eight or more individuals which comprise the red cell panel for antibody identification, should be tested, as a minimum,with antibodies of the following specificities;

C; C^w; c; D; E; e; K; k; Fy^a; Fy^b; Jk^a; Jk^b; S; s; Le^a; Le^b; M; N; P_1 and Lu^a.

5.1326 A distinct pattern of reactivity should be apparent or each of the commonly encountered alloantibodies, or example, anti-D, anti-E, anti-c, anti-K and anti-Fy^a.

5.1327 The antigenic profile of reagent red cells for antibody identification, as far as possible, should permit assignment of specificity in test sera containing more than one commonly encountered alloantibody, for example, anti-D+K.

5.1328 For reagent red cells for antibody identification, the minimum characteristics are:

one individual should be $R_1{}^wR_1$ and one R_1R_1. Between them these two

individuals should express the antigens; K; k; Fy^a; Fy^b; Jk^a; Jk^b; S and s.

one individual should be R_2R_2 and one r'r.

A minimum of four individuals should lack the Rh antigens C and D. One of these individuals should be K positive and one should be E positive. Between them, this minimum of individuals should exhibit apparent homozygous expression of the antigens;

c; k; Fy^a; Fy^b; Jk^a; Jk^b; S and s.

5.14 Reagent red cells for use in the control of the anti-human globulin technique

5.141 Introduction

The anti-human globulin technique is the single most reliable procedure for the detection of alloantibodies. Consequently, careful control of the procedure is essential. In addition, reagent red cells are required to ensure active anti-human globulin reagent is present in each negative test.

5.142 Reagent red cells for controlling anti-IgG reactivity

The reactivity of anti-IgG, either as a monospecific reagent or as a component of polyspecific anti-human globulin reagent, should be controlled by testing a selected weak anti-D reagent with group 0 R_1r red cells. These reagent red cells should be coated to effect a grade of reaction not greater than 3 when subsequently used in the direct anti-human globulin technique.

5.143 Reagent red cells for controlling anti-complement reactivity.

The reactivity of anti-C3 as an anti-complement reagent or as a component of polyspecific anti-human globulin reagent, should be controlled by testing with red cells coated with complement in vitro. For preparation of such red cells see Annex 1.

5.144 Reagent red cells for assuring anti-IgG activity in tests with negative results.

Inadequate washing of red cells in the anti-human globulin technique may result in the neutralisation of anti-human globulin with consequent false negative tests. Also, following completion of the wash phase in the anti-human globulin technique, excess residual saline may dilute the anti-human globulin reagent added subsequently.

5.1441 In order to confirm that each anti-human globulin test has been conducted correctly, following completion of the test, control red cells coated with IgG antibody are added to all negative tests.

5.1442 Reagent red cells for this purpose may be provided ready for use, comprise a suspension of red cells coated with IgG antibody- Alternatively such reagents red cell may be produced by the user by incubating an IgG anti-D reagent with a concentration not greater than 0.3 iu/ml with group 0 R_1r red cells which are subsequently washed free of protein.

Using the procedures recommended by the maufacturer of the anti-human globulin reagent, the IgG sensitised reagent red cells should produce:

A grade 3-4 reaction with polyspecific anti-human globin reagent.

A reaction of not less than grade 4 with monospecific anti-IgG reagent.

A grade 0 reaction with monospecific anti-complement reagent.

Chapter 6

Guidelines for low ionic strength solution (LISS) for red cell suspensions

6.1 Introduction

By reduction of the ionic strength of the antibody/antigen reaction mixture, the use of LISS as a red cell suspending medium permits a substantial reduction in incubation time and an increase in test sensitivity with most antibody specificities. These advantages of LISS are entirely dependent on its correct preparation and use.

Test procedures which incorporate low ionic strength conditions are particularly useful in pretransfusion testing.

6.2 Provision of LISS

LISS solutes may be provided in solution ready for use by the preferred method. However, in view of transport and storage difficulties, manufacturers may provide LISS solutes as a concentrated solution to be diluted prior to use, or a dry powder. Powders should be packaged so that the total contents are dissolved in a stated volume of water.

6.3 Physical characteristics

Measurements of pH, conductivity and osmolality should be made at 23°C. The acceptable value for these measurements should be:

pH	=	6.7 ± 0.2
Conductivity	=	3.7 ± 0.3 mS/cm
Osmolality	=	295 ± 5 mosmol/Kg

6.4 Shelf-life

6.41 The shelf life of LISS is influenced by the method by which it is used; LISS which is transferred to a second container may become more quickly contaminated and unsuitable for use than if LISS is dispensed aseptically from a sterile primary container that is collapsible or is fitted with a sterile airway.

Manufacturers should advise users of the most appropriate means of dispensing LISS.

6.42 As a guide, the following usable period is recommended for the storage of LISS at 18-22 0C.

Ready for use:	unopened		1 year
	opened, used aseptically		2 weeks
	opened		3 days
Concentrate:	unopened	1 year	
	opened	2 weeks	
LISS prepared from concentrate or dry powder, not sterilised		3 days	

6.5 Quality control, serological testing

6.51 Comparative titrations

A minimum of 4 different antibodies known to be reactive by the indirect anti-human globulin technique should be selected for testing. At least 1 Rh specific antibody and 1 of specificity outside the Rh system should be used. These antibodies should give potency titres of not less than 1 in 4 and not greater than 1 in 16 in tests with red cells having an heterozygous expression of the appropriate antigens, using a polyspecific anti-human globulin reagent by its recommended LISS technique.

An acceptable batch of LISS should be tested in parallel.

Prepare doubling dilutions of the selected antibodies in saline containing 20g/L bovine serum albumin.

Using the batch of LISS, test each dilution using the indirect anti-human globulin technique using a polyspecific anti-human globulin reagent by its recommended LISS technique.

The potency titration of each antibody using the test LISS should be similar to that using the acceptable batch of LISS.

6.52 Freedom from unwanted positive reactions

LISS should be shown not to produce a significant number of unwanted positive reactions by methods for which its use is recommended.

For each recommended LISS technique, red cell samples from a minimum of 10 individuals should be tested; each with one, different, fresh, group-compatible serum sample.

All reactions should be negative by macroscopic examination. If one red cell/serum sample produces an unwanted macroscopic positive reaction, a further 10 red cell samples should be tested, each with a different, fresh, group compatible serum. There should be not more than one unwanted macroscopic positive reaction in the 20 tests should be unacceptable.

A few small clumps of red cells seen microscopically, although not desirable, are acceptable.

6.6 Final container label and package insert

In addition to the guidelines in Chapter 2.

6.61 The label of concentrates LISS should include the statement 'Dilute before use - see instructions'.

6.62 In the event that the preparation of LISS is to be completed by the user, the package insert should give clear instructions how this is achieved together with quality control tests that are required, how these are performed and the test values which are acceptable.

6.63 The package insert for LISS concentrate should include instructions for checking the container for deposit of solute which, if present, should be redissolved before dilution of that concentrate.

6.64 The package insert should include the following additional cautionary statements.

6.641 That suspension of red cells in LISS is associated with an accelerated deterioration in the expression of Fy^a, Fy^b, s and S antigens and that red cells suspended in LISS are to be discarded within 24 hours of their preparation.

6.642 That thorough mixing of equal volumes of serum and LISS suspended red cells is required for LISS procedures.

6.643 That for optimum sensitivity, the LISS indirect anti-human globulin technique requires a minimum incubation time of 15 minutes at 37°C.

6.644 That red cells should be washed at least twice in normal ionic strength saline before they are finally washed and resuspended in LISS, in order to avoid the non-specific uptake of autologous complement by the red cells.

6.645 That false positive reactions are likely to be encountered if the temperature of the red cell suspension, LISS or serum is less than 18-22°C before use.

6.646 That LISS solution is readily contaminated. Clear instructions are to be given for a reliable means of preventing bacterial and fungal contamination during the usable period of the solution.

6.647 That no single test is capable of detecting all clinically significant antibodies.

Chapter 7

Guidelines for anti-human globulin reagents

7.1 Introduction

7.11 The spin-tube anti-human globulin technique is an essential test for the detection and identification of antibodies and for compatibility ('crossmatch') tests to ensure safe transfusion practice

7.12 Monoclonal antibodies may be 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 activity to detect in-vitro bound complement and limited amounts of anti-C3d to detect in-vivo bound complement. However, some monclonal 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

7.2 Antibodies required in polyspecific anti-human globulin reagents

7.21 The majority of red cell alloantibodies are non-complement binding IgG therefore anti-IgG is the essential component.

7.22 The presence of anti-complement in the reagent is necessary.

7.23 Anti-C3 components are more important that anti-C4.

7.24 Anti-C4d should be avoided. It is accepted that very low titres of anti-C4c may occur in anti-complement reagents of animal polyclonal origin.

7.3 Specificity testing of polyspecific anti-human globulin and anti-IgG reagents for use in manual tests

7.31 **TESTS FOR IgM AND IgG RED CELL HETEROSPECIFIC ANTIBODIES**

Divide 12 test tubes into 2 sets of 6.

Into each of the first set of tubes, add 1 volume of washed 3 percent untreated red cells in saline from two group A_1 Rh D positive, two group a Rh D positive and two group 0 Rh D positive individuals.

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Into each of the second set of tubes add 1 volume of washed 3 percent enzyme-treated red cells (papain, bromelin or ficin) in saline from the same group A_1 Rh D positive, group 8 Rh D positive and group 0 Rh D positive individuals.

Add 2 volumes of the anti-human globulin reagent as intended to be supplied for use, to each test tube. Mix thoroughly.

Incubate the reactants for 5 minutes at 20-25 °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 at least a grade 4 reaction with each washed, enzyme-treated, red cells sample by the following method:

To separate tubes, add 1 volume of the weak IgG anti-D to 1 volume of each washed, 3 percent suspension of enzyme-treated, Rh D positive red cells. Mix thoroughly. Incubate for 5 minutes at 37°C. Centrifuge the tubes. Determine the reaction grade.

The anti-human globulin reagent should not agglutinate or haemolyse washed red cells from two individuals of group A_1 Rh D positive, two individuals of group B Rh D positive and two individuals of group 0 Rh D positive, that have not been treated with enzyme or that have been treated with proteolytic enzyme, that is papain, bromelin or ficin.

7.32 Tests for unwanted positive reactions.

These test for excess anti-C3d and anti-C3c and for the presence of any undesirable antibodies within the reagent.

PREPARATION OF THE RED CELL SUSPENSIONS.

Select blood collection tubes from 2 packs of group A_1, two packs of group B and two packs of group 0 blood stored at 3-7°C to within 5 days of their recommended expiry date.

Wash each of the red cell samples 4 times with saline.

Prepare suspensions of each red cell sampLe as 3 percent in saline and as 1.5 percent in LISS.

INCUBATION OF RED CELLS AND FRESH GROUP-COMPATIBLE SERUM

Each of the six red cecil samples described above is tested as a saline and a LISS suspension with one, different, fresh, group - compatible serum.

For each anti-human globulin reagent to be assessed, prepare 2 sets of 6 tubes

To the first tube of the first set of 6 tubes and the first tube of the second set of 6 tubes, add 1ml of a fresh, single donor group-compatible serum. Add 1ml of a second fresh, single donor group-

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compatible serum to the second tube of each set, and so on for the 6 different, fresh, group-compatible, sera.

To the first tube of the first set of 6 tubes, add 0.5ml of a red celt sample as a 3 percent suspension in saline. Add 1ml of the same red cell sample as a 1.5 percent suspension in LISS to the first tube of the second set of 6 tubes. Add 0.5ml of the second red cell sample as a 3 percent suspension in saline to the second tube of the first set of tubes and 1ml of the same red cell sample as a 1.5 percent 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 tests 4 times with large volumes of saline and resuspend the red cells to 3 percent suspension in saline.

TESTS WITH ANTI-HUMAN GLOBULIN REAGENTS

For each anti-human globulin reagent, prepare 2 sets of 6 tubes. To each of the first set of 6 tubes, add in sequence 1 volume of the 3 percent suspension of washed red cells from the saline test above.

To each of the second set of 6 tubes, add in sequence 1 volume of the washed 3 percent suspension of washed red cells from the LISS tests above.

Add 2 volumes of undiluted anti-human globulin that is as intended to be supplied for use, to each of the 12 tubes. mix thoroughly.

Centrifuge the tubes.

Determine the reaction grade.

All reactions should be negative on macroscopic examination. A few small clumps of red cells seen microscopically, although not desirable, are acceptable.

7.4 Anti-IgG potency: polyspecific anti-human globulin and anti-IgG reagents for use in manual tests

The UK anti-IgG reference reagent should be tested in parallel with the test reagent.

7.41 The anti-IgG potency titre with red cells heavily sensitised with IgG antibody should be tested as follows.

TEST CELLS

A 3 percent suspension in saline of washed pooled group OR_1r red cells is prepared from 4 individuals.

SELECTION OF THE STRONG IgG ANTI-D SERUM FOR USE BY THE MANUFACTURER.

A pool of potent IgG anti-D approximately 70 iu/ml, is selected. A minor anti-C or anti-E component are acceptable in the potent IgG anti-D preparation. Alternatively, a pool of potent anti-D which satisfies the requirements below may be used.

DETERMINATION OF THE ANTI-IgG POTENCY OF A TEST ANTI-HUMAN GLOBULIN PREPARATION USING RED CELLS SENSITISED WITH SERIAL DILUTIONS OF IgG ANTI-D.

TEST METHOD

From an initial 1 in 4 dilution of the potent IgG anti-D, prepare in 10 tubes twofold serial dilutions of the anti-D serum to 1 in 2048 (10 tubes).

Place 2 volumes of each dilution into each of the series of 10 tubes. Add 1 volume of the 3 percent suspension of pooled group $0R_1r$ red cells to each tube.

Mix thoroughly. Incubate the tubes at 37°C for 45 minutes.

Wash the red cells 4 times with a large volume of saline.

To each tube add 2 volumes of the undiluted anti-human globulin that is as intended to be supplied for use.

Mix thoroughly. Centrifuge the tubes.

Determine the reaction grade.

Anti-D suitable for use in this application should have a potency titre of > 1 in 512.

The same procedure should be performed in parallel using the UK anti-IgG reference reagent

DETERMINATION OF THE ANTI-IGG POTENCY TITRE OF A TEST ANTI-HUMAN GLOBULIN PREPARATION USING RED CELLS STRONGLY SENSITISED WITH IgG ANTI-D.

Preparation of the strongly sensitised red cells

To 4ml of the potent IgG anti-D add 2ml of the 3 percent suspension of pooled group 0 R_1r red cells.

Mix and incubate at 37°C for 45 minutes.

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Wash the red cells 4 times with large volumes of saline and resuspend the red cells to 3 percent in saline.

TEST METHOD

Prepare 1ml 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 (10 tubes).

Prepare a set of 10 tubes for each anti-human globulin reagent to be assessed.

Place 2 volumes of each dilution into each of the series of 10 tubes.

Add 1 volume of the 3 percent suspension of pooled sensitised R_1r red cells to each tube, mix and centrifuge the tests.

Determine the potency titre.

CONTROLS

The washed, strongly sensitised 3 percent suspension of R_1r 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).

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

7.42 The anti-IgG potency by chequerboard titration studies with red cells weakly sensitised with weak IgG antibodies (anti-D, anti-K and anti-Fy^a) should be tested as follows.

SELECTION OF THE WEAK, SINGLE-DONOR IgG ANTIBODIES

An IgG anti-D is selected to an anti-human globulin potency titre of 1 in 8 to 1 in 16 using a pool of group 0 R_1r red celts from 4 individuals.

An IgG anti-K containing a final concentration of 0.01M neutralised EDTA is selected to give an anti-human globulin potency titre of 1 in 8 to 1 in 16 using a pool of Kk red cells from 4 individuals.

IgG anti-Fy^a containing a final concentration of 0.01M neutralised EDTA is selected to give an anti-human globulin potency titre of 1 in 8 to 1 in 16 using a pool of Fy(a+b+) red cells from 4 individuals.

PREPARATION OF THE RED CELL SUSPENSIONS

Prepare 10ml of 3 percent suspensions of

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washed, pooled R_1r, Kk and Fy(a+b+) red cells in saline.

METHOD FOR SENSITIZATION OF THE RED CELLS IN BULK

Anti-D

Using a set of 5 glass containers each of 20 to 25ml volume, prepare 4ml volumes of serial twofold dilutions of the anti-D from undiluted to 1 in 16.

Add 2ml of the 3 percent suspension of pooled R,r red cells in saline to each container. Mix and incubate at 37°C for 45 minutes.

Wash the red cells 4 times with 20ml volumes of saline at each wash and remove the last supernatant.

Add 2ml of saline to the packed washed red cells to prepare the 3 percent suspensions of sensitised red cells.

Anti-K

As above, but using the anti-K with the pooled Kk red celts.

Anti-Fy^a

As above, but using the anti-Fy^a with the pooled 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 2ml volumes of twofold serial dilutions of the test anti-human globulin from undiluted, that is as intended to be supplied for use, to 1 in 16.

TEST METHOD FOR ANTI-IgG OR ANTI-HUMAN GLOBULIN POTENCY BY CHEQUERBOARD TITRATION

Anti-D sensitised red cells

Prepare 5 sets of 5 tubes for each anti-human globulin reagent under test and the anti-IgG reference reagent.

Place 2 volumes of the anti-human globulin dilution, undiluted to 1 in 16 in the appropriate tubes for each of the 5 sets of 5 tubes.

Using the 3 percent suspension of red cells sensitised with the undiluted anti-D for the first set of 5 tubes, the 3 percent suspension of red cells sensitised with the anti-D diluted 1 in 2 for the second set of 5 tubes, and so on, finishing with the 3 percent suspension of red cells sensitised using the anti-D diluted 1 in 16 for the fifth set of 5 tubes, add 1 volume of the washed

red cells to each of the sets of anti-human globulin dilutions. See below.

Anti-D used to coat red cells Set		Dil. 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	

Mix thoroughly. Centrifuge the tubes.

Determine the reaction grade

Anti-K sensitised red cells

As above, but using the anti-K sensitised pool of Kk cells.

Anti-Fy^a sensitised red cells

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

CONTROLS

The 3 percent red cell suspensions sensitised with the undiluted anti-D, anti-K and anti-Fy^a give a negative results when centrifuged and give 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).

The anti-globulin or anti-IgG reagent is satisfactory if the reaction grade at all dilutions attains or exceeds that of the UK reference reagent without significant prezone, 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.

7.5 Anti-complement potency; polyspecific anti-human globulin reagents for use in manual tests.

Although complement binding antibodies such as anti-Le^a and anti-Jk^a are scarce, manufacturers should validate the activity of the anti-complement activity by tests with complement-fixing antibodies.

7.51 Preparation of the complement sensitised red cells.

Various very low ionic strength medium techniques are used to prepare the iC3b, C3 ("C3b"), C4b, C3d and C4d sensitised red cells that are necessary for the assessment of anti-complement reagents.

C3 and C4 activation states produced on red cells by the various methods

Method	Initial State	State after trypsin treatment
Very low ionic strength medium* 37°C	iC3b/C4b	iC3d/C4d
Very low ionic strength medium* 0°C	C3 ('C3B')	C3d
Cold acquired haemolytic anaemia (alpha 2D, CHAD)	C3dg	C3d
Very low ionic strength medium* 37°C with EDTA	C4b	C4d

*These media are not to be confused with low ionic stength solution (LISS).

See Annex 1 for methods of preparation of these sensitised red cells. As a minimum, red cell samples from 2 individuals are to be prepared and tested as described below.

7.52 Anti-C4c potency

METHOD

Prepare a set of 3 tubes for each anti-human globulin reagent under test.

Prepare doubting dilutions of the anti-human globulin reagent from undiluted to 1 in 4.

Place 2 volumes of each anti-human globulin dilution in the appropriate tubes.

Add 1 volume of 3 percent 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.

The anti-human globulin reagent should have an anti-C4c titre of 1 in 2 or less.

7.53 Anti-C4d potency

METHOD

Place 2 volumes of undiluted anti-human globulin in a tube.

Add 1 volume of 3 percent EC4d red cells. Mix thoroughly. Incubate for 5 minutes at 20-30°C. Centrifuge the tubes.

Determine the reaction grade.

CONTROLS

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

The anti-human globulin reagent should not effect a macroscopic reaction with Ec 4d red cells.

7.54 Anti-C3c potency

The term anti-C3b is not used as C3b has several C3 determinants. The component on C3 sensitised red cells is not C3b but whole C3 (less the C3a component).

The presence of traces of anti-C4c in conventional anti-human globulin reagents does not obscure the reactions with EiC3b red cells which also have C4b. Therefore, EiC3b/C4b red cells may be used for these tests. They are simpler to prepare than EC3 ("EC3b") cells, but must be prepared on the day of use whereas EC3 ("EC3b") cells can be stored at 4°C for use within 3 days of preparation.

METHOD

Prepare a set of 8 tubes for each anti-human globulin reagent under test and the UK anti-C3c reference preparation which is tested in parallel.

Prepare doubting dilutions of the anti-human globulin reagent from undiluted to 1 in 28.

Place 2 volumes of each anti-human globulin dilution in the appropriate tube.

Add 1 volume of 3 percent EiC3b/C4b or EC3 ("EC3b") red cells to each tube. Mix thoroughly. Centrifuge the tubes. Determine the reaction grade.

The anti-human globulin reagent should attain or exceed the potency titre of the UK anti-C3 (anti-C3+C3d) reference reagent..

7.55 Anti-C3d potency

7.551 Conventional (polyclonal) anti-human globulin or anti-human globulin containing monoclonal IgG anti-C3d. 5 minute incubation method.

Note: Polyspecific anti-human globulin reagents with IgM monoclonal anti-C3d need need not be assessed by this technique.

METHOD

Prepare a set of 5 tubes for each anti-human globulin under test and the UK anti-C3 (C3c+C3d) reference reagent which is tested in parallel.

Place 2 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 16).

Add 1 volume of the 3 percent EiC3d/EC4d or EC3d red cells to each tube. Mix thoroughly. incubate for 5 minutes at 20-30°C. Centrifuge the tubes.

Determine the reaction grade.

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CONTROLS

The EiC3d/EC4d or EC3d 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.

The reagent should have a potency titre attaining or exceeding that of the UK anti-C3 (anti-C3c+C3d)reference reagent.

7.552 Polyspecific anti-human globulin with IgM monoclonal anti-C3d. Immediate Spin Method.

Use the method described in 7.551 above, but test in parallel with the UK anti-C3d reference preparation with no incubation prior to centrifugation.

The reagent should have a potency titre attaining or exceeding that of the UK anti-C3d reference reagent.

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Chapter 8

Serological guidelines for papain and bromelin

8.1 Introduction

8.11 Proteolytic enzymes, predominantly papain or bromelin, are used in red cell serology to modify the red cell surface to enhance or destroy the reactivity of many red cell antigens as an adjunct to grouping, antibody screening or antibody identification procedures.

8.12 Purified bromelin does not require activation to achieve a high specific activity (enzyme activity per unit weight) whereas purified papain does require the presence of an activator (such as a cysteine or activated cysteine) which itself may be unstable, to achieve a high specific activity. However, papain can be formulated without such an activator if a high concentration of enzyme is used to achieve a satisfactory activity. The activity of bromelin and particularly papain does show a dependence on the pH of the reactants.

8.13 Enzyme preparations may be formulated with EDTA to chelate heavy metal ion contaminants which can inhibit activity. Enzyme preparations autolyse unless this is prevented by frozen storage at an appropriate temperature or by the presence of a displaceable temporary inhibitor.

8.2 Test procedures

8.21 The manufacturer should ensure that the following tests give satisfactory results.

Tests for unwanted positive reactions

Select integral segment lines from 2 packs of group A_1, 2 packs of group B, two packs of group 0 blood stored at 3-7°C to within 5 days of their recommended expiry date.

Using recommended methods for use with the enzyme preparation, test each red cell sample with fresh, single donor, group-compatible serum, using a different serum for each red cell sample.

All reactions should be negative macroscopically.

8.22 Tests for potency

8.221 Papain and bromelin reagents microplate procedures.

Each batch of enzyme reagent should potentiate, by all methods recommended for use, the reaction between IgG anti-D and group 0 R_1r red cells from three individuals. IgG anti-D should

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have a maximum concentration of 0.25 IU/ml which may be achieved by diluting a more potent anti-D with inert, group-compatible, serum. An antiserum with an anti-D content in excess of 10 IU/mt for this purpose should not be used.

The enzyme reagent should effect, as a minimum and with all methods recommended for use, a grade 4 reaction with the three R_1r samples tested.

8.222 Papain and bromelin reagents for use in automated procedures

The ability of each batch of enzyme reagent to potentiate the reaction between 1gG anti-D and R_1r red cells from three individuals should be tested as follows.

Using the automatic procedure(s) recommended for use, test the three unpooled group 0 R_1r red cell samples with IgG anti-D at a concentration, when sampled by the automated system of 0.5 IU/ml.

The IgG anti-D may be obtained by diluting a more potent anti-D with inert, group compatible, serum. An antiserum with an anti-D content in excess of 10 IU/ml should not be used for this purpose.

The enzyme reagent should effect with all methods recommended for use, the unequivocal detection of the reaction between the IgG anti-D and the three unpooled group 0 R_1r samples tested.

8.3 Package insert

The package insert should comply with the requirements in Chapter 2 and, in addition, should include the following.

8.31 A statement that microscopic reading of the results of enzyme tests is not recommended.

8.32 One-stage mix techniques in which enzyme, serum and red cells are mixed and incubated together are not recommended for use in the screening of patients' sera for a typical antibodies or in the compatibility testing of patients' sera with donors' red cells.

One stage procedures where there is a defined pre-incubation of cell suspension and enzyme followed by a defined incubation of the cell/enzyme mixture with serum, or

layer techniques in which serum is overlayed with enzyme which is in turn overlayed with the red !cell sample in a 7 x 50mm glass tube or,

inhibitor techniques in which red cells are treated with enzyme which is inactivated before the subsequent addition of the serum to the mixture, may be recommended for use in the screening of patients' sera for atypical antibodies or in compatibility tests

8.33 A list of those red cell antigens that can stimulate clinically significant antibodies (see 5.1121), whose reactivity is removed or measurably diminished by the methods recommended for use of the product.

8.34 A statement that enzyme tests do not detect all antibodies of probable clinical

significance.

8.35 A statement that enzyme care should be taken to maintain sterility of preparations since they readily become contaminated with micro-organisms which can result in false negative or false positive reactions.

8.36 If stored frozen prior to use, a statement that the reagent is to be discarded within 24 hours of thawing.

8.37 If stored as a liquid prior to use in the absence of any preservative, a statement that the reagent is to be discarded within 24 hours of opening the container when stored as recommended.

8.38 If stored as a liquid prior to use with a preservative shown to be effective against common contaminants, a statement that the reagent is to be discarded within 48 hours of opening when stored as recommended.

8.39 Where the method(s) recommended for use involve the pre-treatment of red cells prior to incubation with serum, the recommended method of pre-treatment should be described in addition to the recommended method(s) of use of the treated red cells.

8.40 A statement that deviation from the recommended methods of use may result in false positive or false negative results. This includes very slight changes in buffers or in solutions which may result in a sub-optimal pH for enzyme treatment.

8.41 A statement that procedures involving enzymes should include procedures to ensure the adequate enzyme-treatment of red cells. For example, in the use of enzyme-treated rr red cells for antibody screening procedures, the use of anti-c known to react weakly with adequately treated red cells would be appropriate.

Chapter 9

Guidelines for bovine serum albumin

9.1 Introduction

Bovine serum albumin (BSA) has various applications in blood group serology. It is used to

- potentiate the reactions of blood group antibodies which do not directly agglutinate red cells suspended in saline for LISS. For this purpose it is generally added at a concentration of 200 g/l (20 percent w/v);
- potentiate the haemagglutination reaction in automated blood grouping equipment;
- potentiate the haemagglutination reaction in microplate test procedures;
- stabilise blood grouping reagents, in which its final concentration is generally less than 80g/l.

9.11 BSA is generally provided as a 300g/l solution. Some preparations may be deliberately polymerised or contain additives to enhance the potentiating properties.

9.12 Where BSA is used in the formulation of a reagent control, it is essential that the reagent control is formulated in the same way as the blood grouping reagent it is to control, but without the specific blood group reactivity of the reagent..

9.13 Albumin sources other than bovine may be used provided the guidelines of this document are satisfied.

9.2 Test procedures.

9.21 Tests for potentiating abilityThe UK reference albumin preparation should be tested in parallel.

SELECTION OF TEST ANTIBODIES

Examples should be selected from IgG antibodies with the following specificities and indirect antiglobutin potency titres when tested with red cells from a pool of 4, group 0 R_1r individuals.

Specificity	Potency titre
Anti-D	1 in 64 to 1 in 128
Anti-D	1 in 8 to 1 in 16
Anti-c	1 in 16 to 1 in 32

Unless the albumin is intended to be used :s provided, dilute the test albumin reagent to 200g/L in saline.

METHOD

Prepare doubting dilutions of the antibodies in saline (not containing BSA) to two dilutions

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beyond its potency titre.

Prepare a 3 percent suspension of washed pooled group 0 R_1r red celts in saline.

Each antibody preparation and its dilutions istested in duplicate in parallel with the test albumin, the reference albumin and saline in place of albumin, using the method described below.

Those albumin preparations recommended for use by methods other than albumin displacement should be tested using the method recommended for use.

Add one volume of each dilution of the reagent to a separate tube.

Add one volume of the test cell suspension to each tube.

Mix thoroughly and incubate for 45 minutes at 37°C.

Add one volume of 200 g/L albumin (not deliberately polymerised or otherwise potentiated) to run down the inner wall of the tube to displace the saline and tie over the sedimented red cells. Do not mix.

Incubate for 15 minutes at 37°C.

Determine the reaction grade.

The albumin reagent is satisfactory if:

(i) the potency titre is equal to or attains that with the use of the reference albumin,

(ii) with each dilution and the undiluted antisera, the reaction grades using the test albumin are at least equal to those with the use of the reference albumin,

(iii) there is no significant prozone,

(iv) for those albumin preparations recommended for use in AutoAnalyzers, these should be tested to ensure their suitability for use by that procedure. The testing should include comparative quantitation of the UK anti-D Working Standard for AutoAnalyzers with the test and reference albumin preparations.

9.22 Tests for unwanted positive reactions

Albumin preparations which contain low concentrations of fatty acids in the presence of significant amounts of polymerised albumin (for example, more than 7 percent of the albumin as trimer or more than 8 percent (of the albumin as oligomer) are likely to cause unwanted positive reactions.

METHOD

Select integral segment Lines from 3 packs of group A_1 ; 3 packs of group B and 3 packs of group 0 CPDA-1 blood stored at 3-7°C to within 5 days of their recommended expiry date. Wash the red cells 4 times in saline. Prepare a 3 percent suspension of each cell sample in saline.

Test each red cell suspension with a different

fresh, group-compatible serum by those methods for which the albumin is recommended for use by the manufacturer.

Where the albumin is intended for use as a reagent stabiliser, use the described method below.

Add one volume of each dilution of the reagent to a separate tube.

Add one volume of the test cell suspension to each tube.

Mix thoroughly and incubate for 45 minutes at 37°C.

Add one volume of 200 g/L albumin (not deliberateLy polymerised or otherwise potentiated) to run down the inner wall of the tube to displace the saline and lie over the sedimented red cells. Do not mix.

Incubate for 15 minutes at 37°C.

Determine the reaction grade.

The reagent is satisfactory for use if no positive reactions, rouleaux or haemolysis is observed.

9.23 Tests for freedom from IgG protein

The absence of IgG protein is particularly important if the albumin preparation is used to stabilise anti-human globulin reagents. Standard electrophoretic or nephelometric assays may be used in addition to the following serological assessment.

Prepare red cells sensitised within a weak IgG anti-D, see Chapter 7, 7.42. Prepare doubling dilutions of polyspecific anti-human globulin reagent from undiluted to 1 in 32 using saline containing no BSA.

Prepare 3 sets of 6 tubes. Add one volume of the undiluted anti-human globulin to the first tube in each of the 3 sets. Add 1 volume of the anti-human globulin reagent diluted 1 in 2 to the second tube of each set, and so on until adding 1 volume of the anti-human globulin reagent diluted 1 in 32 to the sixth tube of each set.

To the tubes of the first set, add 1 volume of the test albumin as intended to be supplied for use. To the second set, add 1 volume of saline. To the third set, add 1 volume of pooled normal human serum diluted 1 in 20 in saline containing no BSA.

Add 1 volume of the 3 percent suspension of red cells weakly sensitised with IgG anti-D.

Mix and centrifuge the tubes. Determine the reaction grade.

The potency titre with the first set of tubes should be at least equal to that with the second set of tubes. The potency titre of the third set of tubes should be clearly reduced to indicate inhibition of the anti-IgG by the diluted human serum.

9.24 Tests for freedom from blood group substances.

Albumin solutions may contain substances similar to human blood group substances A or B. The absence of such activity is important if the albumin is to be used to stabilise ABO blood grouping reagents. The presence of substances similar to other soluble blood group antigens should be excluded if the albumin is used to stabilise other blood grouping reagents, for example, anti-Le^a or anti-Le^b.

Prepare doubling dilutions of anti-A and anti-B blood grouping reagents, or of the blood grouping antibody to be stabilised, in saline not containing BSA.

For each blood grouping antibody, prepare three sets of tubes. Add one volume of the undiluted blood grouping reagent to the first tube in each of the 3 sets. Add 1 volume of the blood grouping reagent diluted 1 in 2 to the second tube of each set, and so on.

To the tubes of the first set, add 1 volume of the test albumin as intended to be supplied for use. To the second set of tubes, add 1 volume of saline containing no BSA. To the third set of tubes, add one volume of blood group substance at a concentration known to be inhibitory in this procedure.

Mix and add 1 volume of a 3 percent suspension of red cells in saline containing no RSA. Use red cells with a weak expression of the corresponding antigen. For example, anti-A use A_2B and for anti-B use A_1B red cells.

Mix and incubate at 20-25°C for 15 minutes.

Determine the reaction grade.

The potency titre with the first set of tubes should be at least equal to that with the second set of tubes. The potency titre of the third set of tubes should be clearly reduced to indicate inhibition of the antibody by the blood group substance.

9.25 Tests for freedom from neuraminidase-like activity

Albumin preparations may contain neuraminidase-like substance capable of exposing the red cell cryptantigen T. Anti-T is a naturally occurring antibody found in human sera.

Obtain integral segment Lines from 3 packs of group 0 CPDA-1 blood stored at 3-7°c to within 5 days of their recommended expiry date. Wash each red celt sample 4 times with saline and make a 3% suspension in saline (containing no BSA).

Prepare two sets of 3 tubes. To the tubes of one set add 1ml of test albumin preparation. To the tubes of the second set add 1ml saline.

Add 1ml of the 3 percent suspension of the first cell sample to the first tube of each set, of the second cell sample to the second tube of each set, and of the third cell sample to the third tube of each set.

Mix and incubate for 3 hours at 37°c. Wash the cells four times in saline (containing no BSA) and resuspend to a 3 percent suspension in saline.

Prepare doubling dilutions of anti-T reagent in saline containing no BSA, from undiluted to 1 in 8.

Test each red cell suspension with each dilution of anti-T reagent using the described method below. Use a 3 percent suspension of neuraminidase-treated red cells as a control of the anti-T reagent dilutions.

Add one volume of each dilution of the reagent to a separate tube.

Add one volume of the test cell suspension to each tube.

Mix thoroughly and centrifuge the tubes immediately.

Determine the reaction grade.

The albumin reagent is satisfactory if no agglutination is observed.

9.26 Tests for freedom from inhibitors of erythrocyte agglutination

Although the tests for the potentiating ability of an albumin preparation will detect the presence of an inhibitor of erythrocyte agglutination, this phenomenon is more pronounced in automated systems, particularly where bromelin is incorporated as an added reagent rather than used to pretreat the red cells.

A previously accepted preparation of albumin and the test albumin should be tested in parallel in the automated system.

Assess the British Working Standards anti-D for AutoAnatyzer use or other reference anti-D preparations.

Only the albumin preparations under test are to be present within the automated system.

The albumin preparation is satisfactory if its performance is at least as good as the acceptable preparation. Attention is paid to the performance of the automated system when no antibody is being assessed as well as when antibody is being assessed.

9.3 Final container label

In addition to the guidelines in Chapter 2 the final label should contain the following.

9.31 A statement that the albumin is for use in blood group serology.

9.32 The concentration of the albumin.

9.33 Whether the albumin has been deliberately polymerised.

9.34 Whether any other potentiators of haemagglutination have been added.

9.35 If the albumin is to be diluted prior to use, the statement 'dilute for use'. If no dilution is required, the statement 'do not dilute'.

9.4 Package insert

In addition to the guidelines in Chapter 2, the package insert should contain the following.

9.41 The concentration of trimeric and oligomeric albumin expressed as a percentage of the total albumin.

9.42 A statement that agglutinins to albumin are found in a small proportion of serum samples.

9. 43 A statement that the efficacy of albumin reagents is to be controlled throughout their use.

9.44 A statement that albumin preparations are not to be used as a negative control for potentiated IgG blood grouping reagents.

9.45 If the albumin is to be diluted prior to use, the recommended dilution, diluent, method of dilution, the concentration of albumin produced by the dilution, together with the storage conditions and usable period of the diluted albumin.

9.46 The osmolality of the albumin preparation as recommended for use.

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Chapter 10

Guidelines for blood grouping reagents of specificity other than ABO and Rh D

10.1 Anti-C ; anti-c ; anti-E ; anti-e ; anti-CDE and anti-C^w

10.11 Specificity

10.111 In addition to the guidelines in Chapter 2 as a minimum, the antisera should be tested with test red cells of the following Rh genotypes (or presumptive genotypes) by all the methods recommended for use by the manufacturer. Numbers in parentheses represent the minimum number of each presumptive genotype to be tested.

Red cells of Rh genotype R_zr or r^yr may be used in place of R_2R_z red cells in the testing of anti-C or in place of R_1R_z red cells in the testing of anti-E.

Red cells of Rh genotype R_zR_z or r^yr^y may be used in place of R_1R_2 red cells in the testing of anti-c or in place of R_2R_z red cells in the testing of anti-e.

10.112 Anti-C
positive reactors: R_1R_2 (2) ;
R_2R_Z (1) ;
r'r (1)
r'^sr (1)
$R_1{}^wR_1$ or $R_1{}^wr$ or $R_1{}^wR_2$ (1)

In addition, the reagent may be tested with $R_1{}^xr$ or $R_1{}^xR_2$ (1) when reactivity with C^x red cells is to be included in the package insert.

negative reactors: R_2R_2 (1) ;
$r''r''$ (1) ;
rr (1) .

10.113 Anti-c
positive reactors: R_1R_2 (2);
R_1r (2);
r'r (1).

negative reactors: R_1R_1 (1);
R_1R_z (2)

10.114 Anti-E
positive reactors: R_1R_2 (2);
R_1R_z (1);
$r''r$ (1).

negative reactors R_1R_2 (1);
r'r' (1) ;
rr (1) .

10.115 Anti-e
positive reactors: R_2r (2);

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	R_1R_2 (2); r"r (1).
negative reactors:	R_2R_2 (1); R_2R_2 (2).

10.116 Anti-CDE

positive reactors:	R_or or R_oR_o (2); r'r' (2); r"r" (2).
negative reactors:	rr (1).

10.117 Anti-C^w

positive reactors:	$R_1{}^w$r or $R_1{}^wR_2$ (2); r'^wr (1)
Negative reactors:	R_1R_1 (1) ; r'r' (1) ; rr (1).

10.12 Specificity

10.121 The reagent should produce a reaction grade of not less than 5 when tested with any red cell sample expressing the corresponding antigen, including those listed at 10.11 by any method recommended for use by the manufacturer.

10.122 A blended reagent comprising anti-D with anti-C and/or anti-E activity, recommended for the detection of G antigen, should produce a reaction grade of not less than 4 when tested with red cells of the presumptive Rh genotype r^Gr by any method of use recommended by the manufacturer for the detection of G antigen.

10.13 Potency testing

10.131 As a minimum, the manufacturer should determine the potency of the test reagent with red cells of the following presumptive Rh genotypes. Numbers in parentheses represent the minimum number of each presumptive genotype to be tested.

10.132 The methods described in 3.6 which correspond to those recommended by the manufacturer should be used.

Anti-C	R_1R_2 (2); r'r (1).
Anti-c	R_1R_2 (2); R_1r(2).
Anti-E	R_1R_2 (2); r"r (1).
Anti-e	R_1R_2 (2); R_2r (2).
Anti-CDE	R_or or R_oR_o (2); r'r (2); r"r (2).
Anti-C^w	$R_1{}^w$r or $R_1{}^w$ R_2 or r'^wr (2).

10.14 Blood grouping reagents recommended for use by an albumin displacement technique.

10.141 The reagent should produce with all red cell samples tested, as a minimum, grade 5 agglutination when undiluted and grade 2 agglutination when diluted 1 in 8.

10.15 Blood grouping reagents recommended for use with red cells suspended in saline or LISS by tube technique.

10.151 The reagent should produce with all red cell samples tested, as a minimum, grade 5 agglutination when undiluted and grade 2 agglutination when diluted 1 in 8.

10.152 Reagents produced by chemical modification, such as reduction and alkylation, should produce with all red cell samples tested, as a minimum, grade 5 agglutination when undiluted and grade 2 agglutination when diluted 1 in 4.

10.16 Blood grouping reagents recommended for use with red cells suspended in saline or LISS by slide technique.

10.161 The reagent should produce with all red cell samples tested, as a minimum, grade 5 agglutination when undiluted and grade 2 agglutination when diluted 1 in 8.

10.162 Reagents produced by chemical modification, such as reduction and alkylation, should produce with all red cell samples tested, as a minimum, grade 5 agglutination when undiluted and grade 2 agglutination when diluted 1 in 4.

10.17 Blood grouping reagents recommended for use with other techniques.

10.171 Reagents recommended for use by a tube technique should produce with all red cell samples tested by the methods of use recommended by the manufacturer, as a minimum, grade 5 agglutination when undiluted and grade 2 agglutination when diluted 1 in 8.

10.172 Reagents recommended for use by a slide technique should produce, with all red cell samples tested by the methods of use recommended by the manufacturer, as a minimum, grade 5 agglutination when undiluted and grade 2 agglutination when diluted 1 in 8.

10.18 Avidity testing

10.181 The avidity of reagents recommended for use by slide techniques should be determined, using the method described in 3.68.

10.182 Agglutination should be grade 3 within 1 minute of mixing.

10.2 Anti-K; anti-k; anti-Fy^a; anti-Fy^b; anti-Jk^a anti-Jk^b; anti-S and anti-s.

10.21 Specificity testing

10.211 In addition to the guidelines in 3.5, as a minimum the manufacturer should test the blood grouping reagent with red cells from a minimum of 4 individuals known to exhibit heterozygous (or weaker) expression of the antigen corresponding to the antibody under test, by all methods of use recommended by the manufacturer.

10.212 Anti-K reagents should additionally be tested by all methods of use recommended by the manufacturer with red cells from a minimum of 2 individuals known to be heterozygous for K, k and Kp^a antigens.

10.22 Specificity guidelines

10.221 Anti-K, anti-Fy^a, anti-jk^a and anti-S should produce a reaction grade of not less than 5 when tested by any method of use recommended by the manufacturer with any red cell sample expressing the corresponding antigen, including those listed at 10.311.

10.222 Anti-k should produce, when tested by any method of (use recommended by the manufacturer, a reaction grade of not less than 4 with red

cells from Kk Kp(a-) individuals and not less than 3 with red cells from Kk Kp(a+) individuals.

10.223 Anti-Fy^b, anti-Jk^b and anti-s should produce a reaction grade of not less than 4 when tested by any method of use recommended by the manufacturer with any red cell sample expressing the corresponding antigen, including those listed at 10.311.

10.23 Potency testing

The methods described iri 3.6 corresponding to the methods recommended for use by the manufacturer should be used, using red cells from a minimum of two individuals known to exhibit heterozygous expression of the antigen corresponding to the antibody under test.

10.24 Potency guidelines

10.241 Anti-K, anti-Fy^a, anti-Jk^a and anti-S should produce, as a minimum and with all red cell samples tested, grade 5 agglutination when undiluted and grade 2 agglutination when diluted 1 in 8.

10.242 Anti-k, anti-Fy^b, anti-Jk^b and anti-s should produce with all red cell samples tested, as a minimum, grade agglutination when undiluted and grade 2 agglutination when diluted 1 in 4.

10.3 Anti-A_1; anti-P_1; anti-M; anti-N; anti-Le^a and anti-Le^b.

10.31 Specificity testing

10.311 In addition to the guidelines in 3.5, as a minimum the blood grouping reagents should be tested by all methods of use recommended by the manufacturer with red cells from 4 individuals known to express the antigen under test, in heterozygous or weakened form if possible.

10.312 Anti-A_1 blood grouping reagents should be tested with red cells from a minimum of 2 individuals each of blood groups A_1; A_2 ; A_1B ; A_2B ; B and 0 by all methods of use recommended by the manufacturer.

10.313 Anti-M and anti-N blood grouping reagents should be tested from red cells from a minimum of 3 individuals of phenotypes NNSS and MMSS respectively by all methods of use recommended by the manufacturer.

10.32 Specificity guidelines

10.321 The blood grouping reagent should produce a reaction grade of not less than 5 when tested by any method of use recommended by the manufacturer with any red cell sample expressing the corresponding antigen, including those listed at 10.311 and 10.312

10.322 Anti-M and anti-N should not agglutinate red cells of phenotype NNSS and MMSS, respectively, by any method of use recommended by the manufacturer.

10.33 Potency testing

The methods described in 3.6 corresponding to the methods recommended for use by the manufacturer should be used, using red cells from a minimum of 2 individuals known, where relevant to exhibit heterozygous expression (or in the case of P_1, weak expression) of the antigen corresponding to the antibody under test.

10.34 Potency guidelines

The blood grouping reagents should produce, as a minimum and with all red

cell samples tested, grade 5 agglutination when undiluted and grade 2 agglutination when diluted 1 in 4.

10.4 Blood grouping reagents of other specificities

10.41 Blood grouping reagents of specificity not mentioned in these guidelines should be tested for potency and specificity as described in 3.5 using the methods corresponding to those recommended for use by the manufacturer.

10.42 These reagents should produce a reaction grade of not less than 3 with all red cell samples tested which express the corresponding antigen and by all methods of use recommended by the manufacturer, using red cells from a minimum of 2 individuals known, where relevant, to exhibit heterozygous or weakened expression of the antigen specific to the antibody under test.

CHAPTER 11

GUIDELINES FOR REAGENTS USED IN AUTOMATED SYSTEMS

11.1 Introduction

In automated systems, blood grouping reagents produce optimal reactions over a narrower range of dilutions than reagents characterised for manual use, e.g. anti-A reagent used undiluted may react too strongly with A_1 cells causing the instrument to misread the result. However, the same anti-A diluted 1 in 2 may fail to react reliably with A_2B cells.

11.2 Specificity

11.21 The specificity of the undiluted reagent should be assessed by preparing red cells as described in 3.66 and should test these with the candidate automated system reagents by the 5 minute incubation agglutination technique, 3.64.

11.22 For reagents to be used at room temperature, preheat the reagents to and incubate the tests at that temperature. For reagents to be used at 37°C, preheat the reagents to and incubate the tests at 37°C.

11.23 For the selection of red cells to be tested see 3.54. In addition, for ABO and Rh D blood grouping reagents see 4.2 and 4.5. For other Rh specific blood grouping reagents see 10.11. For blood grouping reagents of specificity other than ABO and Rh see Chapter 10.

11.24 Specificity requirements - blood grouping reagents intended for use in automated systems should not produce a positive reaction when tested with red cells lacking the antigen corresponding to the antibody specificity under test by the procedure described in 11.21 (see also 3.72).

In addition:

- for ABO blood grouping reagents see 4.22 to 4.24.
- for Rh D blood grouping reagents see 4.52 and 4.53.
- for other Rh specific blood grouping reagents see 10.12.
- for blood grouping reagents of specificity other than ABO and Rh see Chapter 10.

11.3 Potency

11.31 The optimum dilution of the reagent is that which maximises the correct detection whilst minimising the false reading rate due to either excessively dilute or potent reagent.

11.32 The optimum dilution for the reagent should be determined by the following procedure.

11.321 **METHOD**

Using the diluent recommended by the manufacturer of the automated system, prepare a series of dilutions of the candidate reagent, e.g. 1 in 10, 1 in 20, 1 in 30, 1 in 40.

11.322 Using the method recommended by the manufacturer of the automated system, test each of these reagent dilutions with the following numbers of red cell samples.

	A_1	A_2	A_1B	A_2B	B	A_X
Anti-A	3			3		
Anti-B			3		3	
Anti-A+B	2	2			3	
Anti-A,B	2	2			3	3
	R_1r.	R_2r	R_o	r'r	r"r	
Anti-D	3		2			
Anti-C	3			3		
Anti-c	3			2		
Anti-E		3			3	
Anti-e		3			2	
Anti-C+D	2		2	2		
Anti-D+E		2	2		2	
Anti-CDE			2	2	2	

Reagents of specificity other than ABO or Rh should be tested with red cells from a minimum of 2 individuals known, where relevant, to exhibit heterozygous or weakened expression of the antigen corresponding to the antibody under test.

11.33 That reagent dilution which provides optimal results with the test red cells described at 11.321 and 11.322 should be selected for repeat testing. This should include the preparation and testing of reagent dilutions 25 percent less and 25 percent greater than that initially selected as optimal, e.g. for a reagent which initially produces optimal results at a dilution of 1 in 20, repeat testing should be performed with the reagent diluted 1 in 15, 1 in 20 and 1 in 25.

11.331 Potency

The following test procedure should be used.

Using the method recommended by the manufacturerof the automated system, test each of the 3 reagent dilutions with the red cell samples described at 11.322.

The dilution prepared or recommended by the manufacturer of reagents for use in automated systems should produce, qualitatively, the same result at dilutions ± 25% of the optimal dilution.

11.332 Specificity

a) For ABO and Rh reagents recommended for use inautomated procedures the following numbers of red cell samples should be tested.

b) Reagents of specificity other than ABO or Rh should be tested with red cell samples from at

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least 4 unrelated individuals known to lack the antigen corresponding to the antibody under test.

Reagent	A_1	B	0
Anti-A	0	3	3
Anti-B	3	0	3
Anti-A+B	0	0	5
Anti-A,B	0	0	5
	rr	r'r	r"r
Anti -D	2	2	2
Anti-C	2		2
Anti-E	2	2	
Anti-C+D	2		2
Anti-D+E	2	2	
Anti-CDE	2		

In all instances, tests at 1.332 a) and b) should be negative.

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Chapter 12

Guidelines for the user to test blood grouping reagents intended for manual and microplate tests

12.1 General guidelines

12.11 Prior to acceptance, users may wish to confirm the performance of blood grouping reagents by those methods of use recommended by the manufacturer that are selected as suitable for their use. Users who are unable to perform such tests should satisfy themselves that the tests have been performed and are satisfactory.

12.12 Appearance. A blood grouping reagent should not be used if a precipitate, a gel, particles or turbidity is present.

12.2 ABO blood grouping reagents for use in manual or microplate tests

12.21 At least one example each of group A_1 ; A_2 ; A_1B A_2B ; B and 0 red cells should be tested by those methods recommended by the manufacturer that are selected for use.

12.22 Where the user needs to detect A_x red cells with a blood grouping reagent and a technique is recommended by the manufacturer to detect A_x cells, at least one example of A_x red cells should be tested by the methods selected for use.

12.23 Anti-A blood grouping reagent should agglutinate A_1, A_2 ; A_1 B and A_2B cells but not agglutinate group B and 0 cells.

Anti-B should agglutinate group A_1B ; A_2B and B cells but not agglutinate group A_1 ; A_2 or 0 cells.

Anti- A+B should agglutinate group A_1 ; A_2 ; A_1B ; A_2B and B cells, but not group 0 cells.

Anti-A,B should agglutinate group A_1 ; A_2 ; A_1B ; A_2B ; A_x and B cells, but not group 0 cells.

12.24 Users should be aware that the strength of reaction of group A_2B cells with anti-A, and of A_x red cells with anti-A,B blood grouping reagents may be variable. on the other hand, examples of anti-A blood grouping reagents may agglutinate A_x red cells.

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12.3 Anti-D blood grouping reagents for use in manual or microplate tests

12.31 At least one example each of group A_1 rr, B rr, 0 rr, R_1r , r"r and r'r should be tested by those methods recommended by the manufacturer that are selected for use.

12.32 Anti-D blood grouping reagent should react with R_1r cells but not with group A_1 rr, B rr, 0 rr, 0 r"r and 0 r'r cells.

12.4 Polyspecific anti-human globulin reagent for use in manual tests

12.41 The polyspecific anti-human globulin reagent should be tested for absence of false positive reactions by simulated routine 'crossmatch' tests using a minimum of 10 fresh group-compatible sera and red cells from at least 10 donor pack integral segment lines stored 28 to 35 days at 4°C. All reactions should be negative macroscopically. A few small clumps of red cells seen microscopically, although not desirable, are acceptable.

12.42 The performance of the polyspecific anti-human globulin reagent should be compared with the polyspecific anti-human globulin reagent currently in use, using a selection of weak (preferably undiluted) , warm-reacting IgG antibodies reactive in the anti-human globulin technique.

The polyspecific anti-human globulin reagent should be comparable with the reagent in use.

12.43 A weak IgG anti-D suitable for the control of anti-human globulin tests should be titrated from undiluted to 1 in 16. Prepare suspensions of washed sensitised R_1r red cells using each of the dilutions of anti-D.

The test polyspecific anti-human globulin reagent should be titrated to 1 in 8. In a chequerboard titration each of the five sensitised cells suspensions should be tested with each of the four dilutions of anti-human globulin reagents. The polyspecific anti-human globulin reagent currently in use should be tested in parallel.

The test polyspecific anti-human globulin reagent should not show prezone by immediate spin tests. The potency titre of the test polyspecific anti-human globulin reagent should be comparable with the polyspecific anti-human globulin reagent in current use.

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Chapter 13

Guidelines for HLA typing reagents

13.1 General Guidelines

13.11 Definitions

HLA serology refers to the determination of HLA antigens and antibodies by serological methods and their immunological reactions. It includes the isolation from blood, solid tissue or cell cultures of suitable cells such as lymphocytes and their typing for HLA and other related serological procedures.

HLA serology reagents covered by this specification include HLA typing reagents, rabbit complement for HLA serology and HLA reagent lymphocytes.

13.12 Nomenclature

The nomenclature for HLA antigens and corresponding antibodies should conform to that of the World Health Organisation (WHO) Committee on HLA nomenclature.

13.13 Human materials

13.131 HLA serological reagents should comply with the guidelines in 2.5 for virological testing.

13.132 Exceptionally, reagents not tested at source for hepatitis B surface antigen and HIV antibody, 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 (HN(86)25).

These reagents should be marked 'potentially infectious — not tested at source for hepatitis B surface antigen and/or HIV antibody', as appropriate, both on the final container label, or multi-well tray or reservoir, and outer packaging.

The package insert should indicate that the reagent(s) has not been tested at source for hepatitis B surface antigen or HIV antibody as appropriate, and is to be considered as potentially infectious. In addition the package insert should give information on the safe disposal of the material.

13.14 Names of reagents used in HLA serology

13.141 A reagent for the determination of an HLA antigen is designated '**HLA typing reagent**'.

13.142 HLA typing reagent sets

HLA typing reagents produced as a set of reagents in a multi-welled tray or reservoir and used to define the products of a particular HLA locus or loci is known as an '**HLA typing set**'.

13.143 Rabbit complement recommended by the manufacturer for use in HLA class 1. serology is. designated '**Rabbit complement for HLA class I serology**'.

13.144 Rabbit complement recommended by the manufacturer for use in HLA class I and class II serology is designated '**Rabbit complement for HLA class I**

and class II serology'.

13.145 Rabbit complement recommended by the manufacturer for use with monoclonal HIA, serology reagents is designated '**Rabbit complement for HLA monoclonal reagents**'.

13.146 A preparation of lymphocytes recommended by at Manufacturer for the detection or determination of the specificity of an HLA antibody is designated '**HLA reagent lymphocytes**'.

13.15 Final container label

13.151 HLA typing reagents issued separately

The minimum labelling requirement for HLA typing reagents issued separately in individual final containers is a unique combination of numbers and/or letters to identify the donation, the reagent batch number or batch reference and the name of the manufacturer or distributor.

13.152 Multi-well tray label

The minimum labelling requirement for multi-well trays or reservoirs is a unique designation on the body, not the lid, of the tray or reservoir from which it is possible to identify the HLA locus or loci for which the HLA typing batch is intended, the HLA typing batch reference and the sub batch reference (if the HLA typing batch contains more than one tray or reservoir) and the manufacturer or distributor, can be identified.

13.16 Package insert

13.161 In addition to the information required by 2.73 to 12.710, 2.712, 2.713 and 2.8, the accompanying package insert should include the following information on each individual HLA typing reagent or HLA typing batch.

13.162 The recognised HLA specificity(ies) of the reagent, the reaction score ++, +-, -+, --, (serum/antigen), the percentage of specific reactions giving a cytotoxicity score of grade 8 (%8) and the values of coefficient r obtained by the pretesting of the reagent against a well characterised cell panel.

13.163 For HLA typing sets this may take the form of a listing of the details required in 13.162 of the individual sera comprising the set.

HLA typing sets must include a map or diagram of the multi-well tray or reservoir layout indicating the position and HLA specificity(ies) of the HLA typing reagent contained in each well.

13.164 Monoclonal antibodies must be identified to alert the user to the possibility of false positive reactions caused by the carry over of traces of a potent reagent into adjacent wells.

13.165 When reagents are supplied for the detection of a single antigen (e.g. HLA B27), the detailed methods of use (Chapter 2) should include appropriate controls for cross-reactivity.

13.166 In addition to the above, to avoid delay when HLA serological reagents are freely exchanged as a gift between different countries of the European community, each consignment is to be accompanied by a customs certificate as specified in the annex of the European Agreement on the exchange of tissue typing reagents, revised Protocol no. 84.

13.17 Preservation

13.171 HLA typing reagents may be preserved in the liquid or in the dried state. Reagents made from human serum should be kept at a temperature not above -30°C and freeze-dried reagents at a temperature not above +7°C. Monoclonal reagents may be stored at not above +7°C.

13.172 Freeze-dried reagents should be kept in an atmosphere of inert gas or in vacuo in the container in which they were dried and which should be closed

or sealed to exclude moisture.

3.173 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 supplied 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.

13.174 HLA typing reagents, after being thawed or reconstituted should be transparent and should not contain any sediment, gel or particles visible on microscopy (x200).

13.18 Stability and expiry date

13.181 HLA typing reagents, when despatched and stored as recommended by the manufacturer, should retain the requisite properties for at least one year.

13.182 The expiry date of an HLA typing reagent issued in the liquid state as given in the package insert should be not more than one year from the date of the last satisfactory potency test.

13.183 The expiry date of an HLA typing reagent issued in an individual final container may be extended for further periods of one year by, repetition by the user of potency tests with results complying with these guidelines. Details of the performance of potency tests, the results of the potency test and the minimum acceptable potency should be included in the package insert.

13.184 The expiry date of an HLA typing set issued in the liquid state in a multi-well tray or reservoir may be extended by the user by one further year from the date of commencement of the last satisfactory monitoring tests of the activity of the set with a lymphocyte panel containing two examples (of each HLA antigen detected by the set.

13.185 Users must be notified if an HLA typing reagent or a component reagent of an HLA typing batch stored as recommended fails potency or monitoring tests, performed by or on behalf of the manufacturer, within 1 year of the original date of manufacture.

13.186 Potency tests

The titre of an HLA typing reagent is determined by making successive dilutions from undiluted to 2 in 3, 1 in 2, 1 in 3, 1 in 4 and then successive doubling dilutions, e.g. 1 in 8, 1 in 16 etc., of the reagent under study in TC199 or similar tissue culture medium supplemented with a 200ml/L of inactivated foetal bovine serum.

13.187 Each dilution is tested with lymphocytes known to contain the corresponding antigen(s), using the technique recommended by the producer. The titre is the reciprocal of the highest serum dilution at which a positive reaction of 40 percent or greater cell death occurs above the background incidence of cell death.

13.19 Dispensing and volume

The volume of HLA typing reagent issued in an individual final container should be such that the contents can, if necessary, The used for the performance of the appropriate tests for potency as described.

13.2 Serological guidelines

13.21 Specificity guidelines for HLA typing reagents to be used in cytotoxicity techniques with lymphocytes

13.211 HLA typing reagents should, when used by all methods recommended by the producer, 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 50 unrelated individuals.

13.212 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 500 individuals.

13.213 If a sole reagent does not satisfy these conditions,a combination of at least three HLA typing reagents encompassing an HLA specificity listed in 13.214 must be used together.

13.214 HLA typing reagents to be used in combination if no sole reagent is available:

HLA-A 1,2,3,9,10,11,29.

HLA-B 5,7,8,12,13,14,15,16,17,18,21,22,27,40

HLA-DR 1,2,3,4,5,7

13.215 If a combination of HLA typing reagents is used to detect an HLA antigen all of them should have been shown to react with at least 80 percent of samples bearing the antigen in question.

13.216 Not more than half of the HLA typing reagents used in combination to detect an antigen should have been shown to react with the same additional HLA specificity.

13.217 None of the HLA typing reagents used in combination should have been shown to react with more than 5 percent of the separate samples of a lymphocyte panel which do not express any of the antigen(s) which the reagent is claimed to detect.

13.218 Rarer specificities

Whenever possible, at least two antisera should the employed to detect antigens not listed in 13.214. The use of several multi-specific sera is permissable.

13.3 Rabbit complement for use in HLA serology

13.31 General guidelines

13.311 Rabbit complement supplied for use in HLA serology should be collected from healthy rabbits.

13.312 It should be dispensed into final containers convenient for a small batch of HLA tests (lml-5ml).

13.313 It should be stored in the liquid state at a temperature below -70°C, or if freeze-dried, at below +7°C.

13.314 Once the complement has been thawed from the frozen state or reconstituted from the freeze-dried state it should not be refrozen. It should be kept on melting ice if not used immediately; any complement unused after one hour on ice should be discarded.

13.315 Rabbit complement after being thawed or reconstituted should be transparent and free of excess haemoglobin, it should not contain any sediment, gel or particulate matter visible on microscopy (X200).

13.32 Final container label

The label of the final container of rabbit complement for use in HLA serology

should conform to the specifications in Chapter 2.

13.33 Package insert

The package insert supplied with rabbit complement for use in HLA serology should conform to the specifications in Chapter 2.

13.34 Potency tests on rabbit complement

The following UK reference preparations will be available for use in complement assessment cytotoxicity tests.

Rabbit complement

Anti HLA-A2

13.35 Manufacturers should determine the potency titre of the reference HLA typing reagent employing the reference rabbit complement in tests against a normal lymphocyte preparation known to be positive for HLA-A2, and test their own rabbit complement for use in HLA serology in parallel as detailed in these guidelines.

13.36 Each batch of rabbit complement recommended for use in HLA serology should be tested by the following procedure.

Reconstitute UK reference anti-HLA-A2 in 1.0 mL distilled water.
Prepare successive dilutions of the UK reference antibody of Undiluted(N), 3 in 2, 1 in 2, 1 in 3, 1 in 4, 1 in 8 and 1 in 16 in TC199 or similar tissue culture medium supplemented with a 200ml/L of inactivated foetal bovine serum.

	Dilution of UK reference anti-HLA A2						
	N3 in 2	1 in 2	1 in 3	1 in 4	1 in 8	1 in 16	
Row	1	2	3	4	5	6	7
Test	1 2 3						
Ref.	4 5 6						

Add 1 µl of each dilution to six wells of an oiled microwell tray as shown above.
Add 1µ of a suspension of 1-2 10^6 ml^{-1} of normal peripheral Lymphocytes, separated from an individual known to posess the HLA-A2 antigen, to all the wells taking care to avoid carry-over.
Incubate at 220 c for 30 min.
Reconstitute the UK reference complement in 0.5ml distilled water and reconstitute or thaw the complement under test, 5 minutes before they are needed and keep on melting ice.
Add 5 µl of reference complement to each well in rows 1 to 3 and 5 µl of test complement to each well in rows 4 to 6, avoiding carry-over at all times. Reincubate at 22° C for 60 minutes.
Stain the dead cells with eosin, fix tray with formalin solution 3 minutes after staining, apply coverglass, examine with ×10 eyepiece and ×20 phase contrast optics.
Alternatively, flick off the excess serum and stain with trypan blue, examine immediately with ×10 eyepiece and ×20 objective.

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Record percentage cell death in each well.
Compare the degree of cel death observed in the 3 replicate tests of each dilution of reference antibody obtained with reference complement and the complement under test.

13.37 Rabbit complement for use in HLA serology should enable all dilutions of the reference preparation of anti HLA-A2 to attain or exceed the percentage cell death attained with the UK reference rabbit complement.

13.38 Rabbit complement toxicity tests

13.381 Rabbit complement recommended for use in HLA serology must not cause cell death in the absence of HLA antibody when tested against serum collected from 5 non-transfused male volunteers using samples of unfractionated lymphocyte samples from 10 random donors in the standard one and one half hour incubation NIH cytoxocity test.

13.382 Rabbit complement recommended for use in HLA DR serology must not cause cell death in the absence of HLA antibody when tested against serum collected from 5 non-transfused male volunteers using samples of B lymphocyte from 10 random donors in the extended 3 hour incubation cytotoxicity test.

13.383 Rabbit complement for use in HLA typing lymphoblastoid B cell lines must not cause cell death in the absence of HLA antibody when tested against samples of lymphoblastoid B cell from 5 lines in the standard one and a half hour incubation cytotoxicity test.

13.384 Rabbit complement recommended for use in screening or testing monoclonal HLA typing reagents must not cause cell death in the absence of human serum when tested against TC199 or similar tissue culture medium supplemented with 100ml/L foetal bovine serum using samples of B lymphocyte from 10 random donors in the extended 3 hour incubation cytotoxicity test.

13.4 Guidelines for HLA reagent lymphocytes

13.41 Introduction

HLA reagent lymphocytes have wide use in the detection and identification of HLA antibodies in patients with febrile reactions to blood transfusion, in patients refractory to platelet transfusions, in the regular screening of patients' serum pre and post transplantation and in the screening of donors for new sources of HLA antibodies.

13.42 Testing guidelines

13.421 HLA reagent lymphocytes used for the preliminary screening of potential donors in an HLA antibody procurement programme should be adequately typed, but may not necessarily meet the standard of phenotyping required for HLA antibody identification in clinical work.

13.422 HLA reagent lymphocytes for use in clinical studies should always be tested with a batch of HLA typing reagents of a standard equivalent to that used for HLA typing transplant recipients. The lymphocytes should give a clear reaction and an unambiguous interpretation with the routine lymphocyte typing sets. Lymphocytes with doubtful antigen specificities should not be included unless their specificity has been resolved by family studies, or by reference to experienced laboratories, or by discriminating techniques such as 2D gel electrophoresis or methods of molecular genetics or oligonucleotide probing.

13.43 Lymphocyte quality

Freshly isolated reagent lymphocytes or previously frozen reagent lymphocytes recovered according to the manufacturer's instructions, for use in antibody detection and identification by a dye exclusion technique should have 80 percent viability or more as judged by dye exclusion or phase contrast microscopy and should contain not more than 1 percent of platelets or granular cells.

13.431 Reagent lymphocytes supplied previously frozen in test trays should be dispensed such that each well contains between 1000 and 2000 cells when the lymphocytes have been recovered according t:o the manufacturer's instructions.

13.432 Reagent B lymphocytes isolated from peripheral blood for use in the identification of class II antibodies should contain not more than 20 percent of T cells when assessed by rosetting with neuraminidase-treated sheep RBC.

13.433 The background incidence of spontaneous cell death of reagent lymphocytes should be assessed in the cytotoxicity test with a negative control of serum known not to contain HLA antibody. Reagent lymphocytes with 30 percent or more dead cells observed in the negative control at the completion of the test are not suitable for use in dye exclusion cytotoxicity tests.Ç

13.44 Final container label

13.441 Final container label for HLA reagent lymphocytesissued in separate containers.

Owing to the small volumes in which HLA reagent lymphocytes are supplied, the minimum labelling requirement for HLA reagent lymphocytes issued separately in individual final containers is a unique combination of numbers and/or letters to identify the donation, the reagent batch number or batch reference and the name of the manufacturer or distributor.

13.442 Multi-well tray label

The minimum labelling requirement for multi-well trays or reservoirs is a unique designation on the body, not the lid, of the tray or reservoir from which it is possible to identify the HLA reagent lymphocytes set reference and the sub-set reference (if the complete set of HLA reagent lymphocytes contains more than one tray or reservoir) and the name of the manufacturer or distributor.

13.45 Package insert

In addition to the information required in 2.8, the package insert should include the following information on each individual preparation of HIA reagent lymphocytes or set of HLA reagent lymphocytes.

13.451 The HLA phenotype of the reagent.

13.452 HLA reagent lymphocytes intended solely for use in the detection of HLA antibodies in an antibody procurement programme should bear a statement on the insert in black ink in print of 12 point or greater "Not for clinical studies".

13.453 The nature of the HIA reagent lymphocytes (e.g. normal peripheral lymphocytes, separated peripheral B lymphocytes, separated peripheral T lymphocytes, CLL cells, splenic lymphocytes, lymph node lymphocytes or lymphoblastoid cell line).

13.454 When the HLA reagent lymphocytes are supplied as a multi container product such as a lymphocyte panel, the label on the containers and packaging should be assigned the same batch reference and carry a number or symbol to distinguish one container from another, this batch reference should also be on the package insert. The phenotype information on these multi container products may take the form of the phenotypes of each of the individual donations of the batch listed against the numbers or symbols distinguishing one donation from another.

13.455 The concentration of the lymphocyte suspension should be stated on the package insert of HLA reagent lymphocytes issued in individual containers and also on the phenotype listing of batches issued as multi container products.

13.46 HLA reagent lymphocyte sets issued in multi well trays should include a map or diagram of the tray or reservoir layout indicating the location of the individual HLA reagent lymphocytes in the wells of the tray.

13.47 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.

13.48 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 on the package insert.

13. 49 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 package insert

ANNEX 1

Methods for the preparation of complement sensitised cells

1. The use of the term 'very low ionic strength medium' in this annex is to avoid any confusion with low ionic strength solutions (LISS).

Stock Solution A: Dipotassium hydrogen orthophosphate trihydrate 1.0M

$K_2HPO_4.3H_2O$	228.2g
water	to 1 litre

Store at 4°C

Stock solution	B: Potassium dihydrogen
orthophosphate	1.0M
KH_2PO_4	136.lg
water	to 1 litre

Store at 4°C

1.1 Method for EiC3b/C4b produced by a warm very low ionic strength medium technique

1.11 REAGENTS

Solution C: Buffered Sucrose, pH 6.1

Solution D	
Stock Solution A	0.5ml
water	to 100ml
Solution E:	
Stock Solution B	5.0ml
water	to l000ml

To prepare buffer, place Solution D in 1 litre beaker. Using a magnetic mixer and pH meter, add Solution E until pH is 6.1.

Dissolve 92.4g sucrose in approximately 500ml of the buffer prepared above, and make up to 1 litre with the same buffer. Dispense in l0-20ml aliquots and store at -20°C.

METHOD

Place 8.5ml of the buffered sucrose solution C into a 20-25ml container.

Add 0.5ml of fresh inert group 0 serum.

Mix and immediately add 1.0ml of 50 percent group 0 red cells, washed three times and suspended in 0.15M NaCl.

Mix thoroughly. Incubate at 37°C for 30 minutes with occasional further mixing.

Centrifuge the container.. Remove the supernatant and wash the red cells 4 times with 20ml of saline for each wash. Remove the last wash.

Add 14.5ml of saline to the packed red cells to prepare a 3 percent suspension of EiC3b/C4b red cells.

Store at 4°C. These cells should be prepared on the day of use.

1.2 Method for EC3 ('EC3b') produced by a cold very low ionic strength medium technique

REAGENTS

Stock Solution F

Stock Solution A	1.25ml
0. 2M Na_2EDTA	5.25ml
sucrose	23.lg
water	to 250ml

Stock Solution G

Stock Solution B	1.1ml
0. 2M Na_2EDTA	5.25ml
sucrose	23.lg
water	to 250ml

Solution H
0.4M $NgCl_2$

Solution J: Buffered sucrose, pH 5.1 Add Solution F to Solution G, using a magnetic mixer until the pH of the mixture is 5.1.

METHOD

Place 19.8ml of Solution J in a beaker surrounded by melting ice in a 4°C refrigerator (or cold room). Stir gently with a magnetic mixer until the buffer solution is at 0.1°C.

Add

a) 0.5ml of packed group 0 red cells, washed three times in 0.15M NaCl.

b) 0.5ml of a 1 in 50 dilution in 0.15M NaCl of fresh serum or fresh CPD-Al plasma.

c) 0.lml of Solution H.

Incubate at 0°c for 30 minutes, mixing continuously.

Transfer the mixture to a suitable 20-25ml container and centrifuge. Remove the supernatant and wash the red cells 4 times with 20ml of saline for each wash. Remove the last wash.

Add 14.5ml of saline to the packed red cells to prepare the 3 percent EC3 ('EC3b') red cells.

Store at 4°C. These cells should be used within 3 days of preparation.

1.3 Method for Ec4b produced by a warm very low ionic strength medium technique

REAGENTS

Stock Solution K

sucrose	25g
water	to 250ml

Store frozen or use within 24 hours if stored at 4°C.

Stock Solution L

Na $_2$EDTA	7.45g
water	to 100ml

Stock Solution M

Na $_4$EDTA	8.32g
water	to 100ml

Solution N

Add equal volumes of solution L and solution M to prepare 0.2 M Na_3EDTA.

METHOD

Place l0ml of sucrose solution K into a 20-25ml container.

Add

a) 0.15ml of Solution N
b) 0.5ml of packed group 0 red cells, washed three times in 0.15M NaCl
c) 0.5ml of fresh, inert, serum

Mix thoroughly and incubate at 37°C for 15 min.

Centrifuge the container. Remove the supernatant and wash the red cells 4 times with 20ml of saline at 3-7°C. Remove the last wash.

Add 15ml of saline to the packed red cells to prepare the 3 percent EC4b red cells.

Store at 4°C. These cells should be used within 3 days of preparation.

1.4 Method for EiC3d/C4d or EC3d or EC4d prepared by trypsin treatment of EiC3b/C4b or EC3("EC3b") or EC4b respectively

REAGENTS

Stock Solution P

1 N HCl	2.5ml
water	to 50ml

Stock Solution Q: 1.0 percent Trypsin

trysin*	0.lg
Solution P	to l0ml

*twice crystallised trypsin (e.g. SIGMA CHEMICAL COMPANY, Catalogue 1987 No. T8253).

Dispense in 0.lml aliquots and store frozen.

Stock Solution R

Stock Solution A	5.0ml
water	to 50ml
Stock Solution B	l..0ml
water	to l0ml

Using a magnetic stirrer, add the diluted Solution B to diluted Solution A to pH 7.7.

Dispense in convenient volumes and store at –20°C.

EiC3b/C4b or EC3 ('EC3b')or EC4b red cells are prepared, as required.

Prepare l.0ml of 0.1 percent trypsin solution by the addition of 0.9ml of Stock Solution R to 0.lml Stock Solution Q.

METHOD

Add lml of 0. 1 percent trypsin to the 0. 5ml of the washed packed EiC3b/C4b or EC3 ('EC3b') or EC4b red cells in a 20-25ml container. Mix and incubate at 37°C for 30 minutes, with occasional further mixing.

Centrifuge the container. Wash 4 times with 20ml of saline at each wash. Remove the last wash.

Add 14.5ml of saline to the packed red cells to prepare the 3 percent EiC3d/C4d or EC3d or EC4d red cells respectively.

Store at 4°C. These cells should be used within 3 days of preparation.

Annex 2

Glossary of terms

1. **Quality Assurance** is a total scheme to ensure that the product meets specification.
2. **Quality Control** is a part of a Quality Assurance programme and consists of end product tests which must be completed with satisfactory results before either the results of a set of tests are accepted or a product is released for issue.
3. **Quality Audit** is a review of the quality system.
4. **Quarantine** is the status of material or products set apart from others whilst awaiting a decision on their suitability for processing or issue.
5. **Sensitivity** is a term defining the limit of detectable specific reactions using reagents or test systems. The document specifies levels of sensitivity which must be achieved.
6. **Specificity** is a term defining the ability of a reagent or test system to react selectively. In particular terms, it represents the absence of false positive reactions.
7. **Validation of a test procedure** is a part of a Quality Assurance programme and consists of those steps which are taken in advance to ensure that the test results provide the required information.
8. **Validation of a manufacturing method** is a part of a Quality Assurance programme and consists of those steps which are taken in advance 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.
9. **A Working Standard** is a preparation prepared nationally or locally containing a known or agreed concentration of the activity being measured and it should be assayed with each group of tests to establish the sensitivity or calibration of the unknown tests in the group

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Annex 3

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