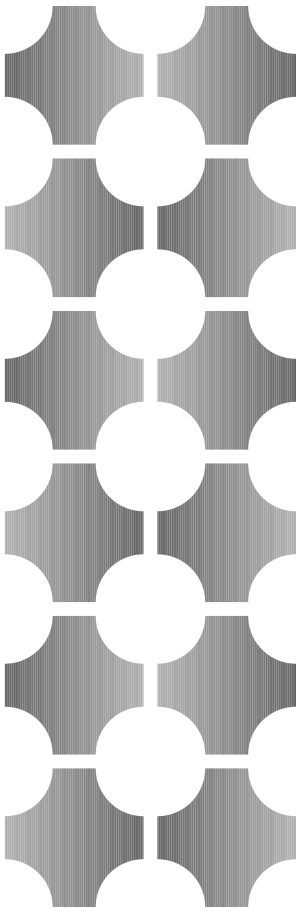
Guidelines for the **Blood Transfusion Services in the United Kingdom**

7th Edition 2005



London: TSO

Published by TSO (The Stationery Office) and available from:

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Seventh Edition 2005

ISBN 0 11 703371 5

Web Access

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http://www.official-documents.co.uk/document/other/0117033715/0117033715.asp

Printed in the United Kingdom for The Stationery Office

179955 C15 9/05

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Preface to seventh edition

This seventh edition takes account of the European Directives on blood and tissues and the resulting United Kingdom Regulations. We have tried to indicate throughout this edition which of our guidelines are now legal requirements. The book continues to include best practice guidelines and detailed technical procedures.

Blood Safety and Quality Regulation definitions and terminology are used throughout. A list of definitions is included. The four National Blood/Blood Transfusion Services in the UK are blood establishments in accordance with the EU Directives as are the individual centres associated with them. Hospital blood banks fulfil different functions but several Articles in the 'Blood Directives' and hence the Blood Safety and Quality Regulations 2005 apply to them; these are explained.

The format of the book has changed with the material presented in continuous chapters rather than in parts. All chapters have been revised and several new chapters added; therefore changes introduced in this edition are not indicated in the text.

We have included a few blank pages in the front of the publication where changes notified by the Joint UKBTS/NIBSC Professional Advisory Committee (JPAC) can be attached.

The 'Red Book' is as up to date as we can make it at the time of printing. Further EU Directives are in preparation and will in time be transposed into UK Regulations. Changes in these guidelines will therefore continue to occur. These will be notified on the JPAC website www.transfusionguidelines.org.uk and through the management of each of the UK Blood Services.

We hope the users of these guidelines and regulations find this edition clear and helpful.

We wish to thank the members of the Standing Advisory Committees who undertook the task of revising or writing the relevant chapters. In particular we wish to thank Caroline Smith for her painstaking preparation of the material for this publication.

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Change notification

For the attachment of change notifications as they are issued and notified on www.transfusionguidelines.org.uk.

Chapter 1

The regulatory environment in the United Kingdom in 2005

Introduction

Development of the 'Red Book'

The *Guidelines for the Blood Transfusion Services* in the United Kingdom were first published in 1990 by HMSO. They were compiled by experts from the then Regional Transfusion Centres and the National Institute of Biological Standards and Control (NIBSC), and aimed to define guidelines for all materials produced by the United Kingdom Blood Transfusion Services for both therapeutic and diagnostic use. The driving force for this joint initiative, which started in 1987, was the imminent EU Directive which would bind member states to introduce product liability by July 1988. It was understood that human blood and substances derived from it would be defined as 'products' in terms of this Directive, and guidelines against which manufacturers could be inspected would be required.

Since then six editions of the 'Red Book' (as the guidelines became known) have appeared. They are compiled by a group of experts involving many from outside the blood transfusion services, now called the Joint UKBTS/NIBSC Professional Advisory Committee (JPAC).

There are four National Blood/Blood Transfusion Services in the United Kingdom:

- the National Blood Service in England (NBS) is managed by the National Blood Authority (NBA). From 1 October 2005 it will be managed by the NHS Blood and Transplant Authority (NHSBT)
- the Scottish National Blood Transfusion Service (SNBTS) is managed by NHS National Services Scotland
- the Northern Ireland Blood Transfusion Service (NIBTS) is managed by the Northern Ireland Blood Transfusion Special Agency
- the Welsh Blood Service (WBS) is provided and managed by Velindre NHS Trust.

These are blood establishments. Between them they deliver services through approximately 20 centres (sites).

Following devolution of governments in the United Kingdom, the UK Blood Services Forum was established in 1999 comprising the chief executives and medical directors of

the four Services and JPAC became accountable to the medical directors who themselves are accountable to their chief executives. The close working relationship with NIBSC has been maintained through the director of NIBSC.

JPAC, with its standing advisory committees, undertakes regular review of the Guidelines in the light of developments in the field, both scientific and regulatory. The overall aim is to ensure as far as possible the safety of blood transfusion in the UK.

The current Standing Advisory Committees (SACs) of JPAC are shown in Figure 1.1.

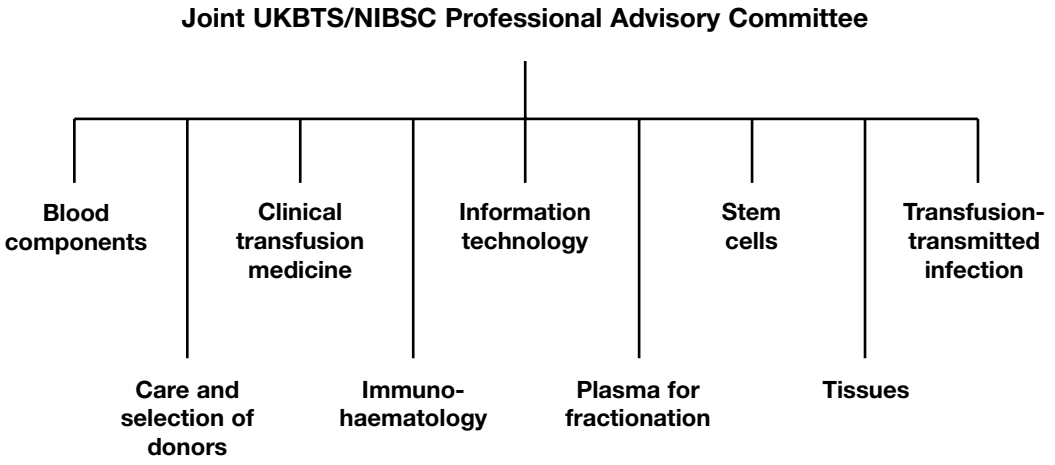


Figure 1.1 Standing Advisory Committees (SACs) of JPAC

EU Blood Directives and UK Blood Regulations

Directives 2002/98/EC[1] and 2004/33/EC[2] constitute the 'Blood Directives' in 2005. Two further Directives, one on haemovigilance/traceability and one on quality systems, are expected in 2006.

The Blood Safety and Quality Regulations 2005 transpose these Directives into UK law.[3] The Regulations apply throughout the UK, including the private sector and come into force on 8 November 2005.

The Regulations use the term 'blood establishment' for organizations that collect, process and distribute blood and blood products. In the UK the blood establishments are the four national blood services (NBS, SNBTS, NIBTS and WBS). The regulations retain the term blood bank for the laboratories within hospitals that hold, match and supply blood components to individual patients.

Several Articles in the Directives apply to hospital blood banks and these have been transposed into the Regulations. The Directives on quality systems and haemovigilance/ traceability will also impact on hospital blood banks. These will lead to amendments of the Regulations. The EU Directive on Tissues (2004/23/EC) will become legally binding in the UK from April 2006.

The EU Directives and UK Regulations are available on www.transfusionguidelines.org.uk.

Role of JPAC in developing guidelines, standards and regulations for the UK blood transfusion and transplantation services

The 'Red Book' contains guidelines reflecting best practice, sets standards to be met by the products, describes technical details of the processes involved and states the legally binding requirements introduced in 2005 under the Blood Quality and Safety Regulations, Statutory Instrument 2005 No. 50.[3]

Guidelines reflect best practice and are developed by professionals in the field. JPAC consists of such professionals in blood transfusion and tissue transplantation, appointed for their expertise from throughout the UK. The 'Red Book' reflects their work as it is implemented in the UK. The book concentrates on the products rather than their use; to do so effectively it provides technical details for all the processes involved.

Clinical use of blood and blood components is outlined in the *Handbook of Transfusion Medicine*,[4] produced by collaboration between JPAC and the British Committee for Standards in Haematology (BCSH).

Professional guidelines are not legally binding but, as they reflect consensus best practice, may be taken into account by the UK judiciary. Such national guidelines have to be taken into account when EU Directives are formulated. EU Directives have to be transposed – that is, written into UK laws – and these are legally binding.

Professionals throughout the EU member states are involved in deciding which guidelines should have the force of law. The wording of the law is the remit of lawyers and department of health officials, but the essence of what becomes law rests with the judgement of the professionals in the field. JPAC is represented on the Council of Europe and European Union expert working groups.

1.1 The key institutions involved in developing guidelines and regulations relevant to the UK

A brief description of some international organizations and their interrelationships is required for an understanding of the regulatory environment in the UK in 2005.

World Health Organization

www.who.int

Established in 1948 as the United Nations' specialized agency for health, the World Health Organization (WHO) is governed by 192 member states through the World Health Assembly. Its aim is the attainment of the highest possible levels of health by all people and clearly the availability of safe blood contributes to this aim.

The WHO produces recommendations, programmes and educational materials. The Global Collaboration in Blood Safety programme started in 1995. It was recognized that with the increased movement of populations and plasma and plasma-derived medicinal products blood safety could only be improved through global collaboration. Consensus proposals and recommendations are addressed to the participant countries.

WHO guidelines and recommendations are not legally binding in any of the 192 countries; however EU legislation in this field states that the advice emanating from the WHO has to be taken into account by the member states when formulating their own legislation.

Council of Europe and the European Union

The Council of Europe (CoE) and the EU are two totally distinct organizations. They are easily and often confused as much of the same terminology is used. In 2004 the CoE has 45 member states with approximately 770 million inhabitants and the EU 25, with a population of 470 million. All member states of the EU are member states of the CoE.

The Council of Europe Recommendations are not legally binding in the EU although they have to be taken into account by the EU member states. European Union Directives, however, are legally binding in the member states and have to be transposed into the laws of the member states, either existing laws or new ones, within clearly defined time frames.

Council of Europe

www.coe.int

Founded in 1949, one of its founding principles was the promotion of increased cooperation between member states to improve the quality of life for the population of Europe. In the field of health the CoE has consistently addressed ethical issues; the most important of these is the non-commercialization of human substances: blood, tissues and organs. In the 1950s member states started to cooperate in blood transfusion activities. Through its committees and working parties composed of national experts the CoE has produced recommendations to ensure the quality of blood components and tissues and publishes guidelines as annexes to the Recommendations. These annexes are updated regularly to take account of advances in worldwide knowledge and technology.[5, 6]

Neither the recommendations nor the guidelines are legally binding but they are generally regarded as constituting basic best practice and many form the basis of EU Directives.

European Union

www.europa.eu.int

The EU was first proposed by the French Foreign Minister Robert Schuman in 1950, following the devastation of the Second World War. It was conceived to prevent further such wars. Initially it consisted of six countries and the European Coal and Steel Community (ECSC) was created in 1951: coal and steel had played a major role in the Second World War and cooperation over these assets was seen as a means of preventing further such cataclysms. The European Atomic Energy Community (EAEC or Euratom) came into force in 1958, as did The European Economic Community (EEC) created by the Treaty of Rome in 1957. The Treaty of Maastricht 1992 (the Treaty on the European Union) amended the three existing treaties giving the three Communities (ECSC, EAEC and EEC) increased powers. The EEC was renamed European Community (EC). A 'competence' in EU terminology is a subject over which it has legislative powers. These competences are agreed by the treaties and outlined in the *acquis communautaire*. Competence in the field of blood and blood components was conferred on the EU by the Treaty of Amsterdam 1999 Article 152. It is important to note that this Article stipulates that 'member states cannot be prevented from maintaining or introducing more stringent protective measures as regards standards of quality and safety of blood and blood components'.

In 2005 the EU does not have competence over member states' healthcare services nor clinical practice. This means that the laws governing blood, blood components and tissues extend only to cover the safety of the products and not their clinical use.

The five key EU institutions are:

- Parliament: elected by the peoples of the member states
- Council of the European Union: representing the governments of the member states
- European Commission: the driving force and executive body
- European Court of Justice: ensuring compliance with EU law
- European Court of Auditors: controlling sound and lawful management of the European budget.

Amendments to the institutions and their roles to take account of increasing membership of the EU are suggested in the proposed EU constitution in 2004.

The European Commission is the only body that can initiate legislation; the processes whereby legislation is proposed and finally adopted are complex.

Issues regarding health come under a Co-decision Procedure, which means that both the European Parliament and Council (European Council *not* Council of Europe) have to agree the Commission proposals.

There is open consultation on any proposed legislation. The texts of the Directives and stages in the consultation process can be found on www.europarl.eu.int

Directives come into force on the day they are published in the *Official Journal of the European Union* (*OJ*) but defined time for transposition and implementation is allowed.

European Pharmacopoeia

www.pheur.org

This CoE initiative was ratified by the EU and 30 participating member states. The Pharmacopoeias are collections of standardised specifications that define the quality of pharmaceutical preparations, their constituents and even their containers. The European Pharmacopoeia (*Ph Eur*) monographs are binding on the EU and the participating member states.

The success of the biological standardization programme for medicines for human use of the European Pharmacopoeia Secretariat led to further collaboration between the Commission of the EU and the CoE. The European Pharmacopoeia Secretariat changed its name to the European Directorate for the Quality of Medicines (EDQM): it is part of the administrative structure of the CoE.

The EDQM is organised in four divisions including the network of laboratories involved in the quality control of medicines for human and veterinary use (Official Medicines Control Laboratories (OMCL) network).

The European Medicines Agency (EMEA) www.emea.eu.int is a decentralized body of the EU based in London. Its main responsibility is the protection and promotion of public health through the evaluation and supervision of medicines for human and veterinary use. The EMEA coordinates the evaluation and supervision of medicinal products throughout the EU.

The Committee for Medicinal Products for Human Use is involved in evaluating industrially prepared plasma derivatives.

The CoE and EU work together in this field. Industrially prepared, fractionated plasma products are medicinal products and the *Ph Eur* monographs are mandatory.

Blood components are not 'medicinal products' and are now regulated by the EU 'Blood Directives'.

United Kingdom

www.cabinetoffice.gov.uk

The United Kingdom is one member state in the EU, although since 1997 there has been devolved government in Wales and Scotland and sporadically in Northern Ireland. EU legislation must be transposed into member states' own legislation within a defined time frame.

An account of European decision-making and transposition is given in the *Transposition Guide: How to Implement European Directives Effectively*. This is available on www.cabinetoffice.gov.uk/regulation/publications.

The EU Directives regarding blood and blood components are transposed into The Blood Safety and Quality Regulations 2005[3] under Section 2[2] of the European Community Act (Maastricht 1992) and are binding across the UK.

1.2 EU legislation relevant to blood transfusion and tissue transplantation

- Directives governing medicinal products (plasma derivatives)
- Directives governing blood, blood components and tissues
- other Directives directly impacting on transfusion and transplantation.

Directives governing medicinal products

Directive 2001/83/EC 'on the Community code relating to medicinal products for human use'.[7] This Directive assembles all previous Directives governing medicinal products.

A medicinal product is defined as any substance or combination of substances presented for treating or preventing disease in human beings. A substance is defined as any matter, irrespective of origin, which may be human, animal, vegetable or chemical.

The Directive states that member states 'need to take measures to prevent the transmission of infectious diseases, apply the monographs of the European Pharmacopoeia and recommendations of the Council of Europe and WHO as regards, in particular, the selection and testing of blood and plasma donors. The member states should also promote community self-sufficiency and encourage voluntary unpaid donations'.

The Directive specifically excludes blood and blood components.

Directive 2003/94/EC[8] lays down the principles and guidelines of good manufacturing practice in respect of medicinal products for human use and investigational medicinal products for human use. These are clearly outlined in a Medicine Control Agency publication: *Rules and Guidance for Pharmaceutical Manufacturers and Distributors 2002*, commonly referred to as the 'Orange Book'.[9]

Directives governing blood, blood components and tissues

Directive 2002/98/EC 'setting standards of quality and safety for the collection, testing, processing and storage and distribution of human blood and blood components'[1] was adopted and came into force in 2003 with an implementation date of February 2005, extended in the UK to November 2005.

Recognising that knowledge and technology advance rapidly in this field and that laws are difficult to change, the Directive sets up a regulatory committee of the Commission whose task it is to ensure that detailed technical requirements are adapted to technical and scientific progress. The regulatory committee, consisting of representatives nominated by the member states has to take account of certain recommendations of the CoE, the monographs of the European Pharmacopoeia and recommendations of the WHO. It also takes account of member states' interests.

Commission Directive 2004/33/EC[2] implementing Directive 2002/98/EC as regards certain technical requirements for blood and blood components becomes legally binding at the same time as Directive 2002/98/EC. Further Commission Directives dealing with quality systems and traceability are expected later in 2005.

Legislation in tissue transplantation follows a similar pattern. Directive 2004/23/EC[10] on setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells, came into force in April 2004 and is expected to be implemented in member states by April 2006. Just as for blood and blood components, a Commission Directive dealing with more detailed technical aspects and taking account of the work of the CoE and WHO is expected in 2005.

Testing and processing of donations has to take account of two complex Directives:

Directive 98/79/EC in vitro diagnostic medical devices

An *in vitro* diagnostic medical device is defined as 'any medical device which is a reagent, reagent product, calibrator, control material, kit, instrument, apparatus equipment or

system whether used alone or in combination intended by the manufacturer to be used in vitro for the examination of specimens including blood and tissue donations derived from the human body solely or principally for the purpose of providing information: concerning a physiological or pathological state or concerning a congenital abnormality or, – to determine the safety and compatibility with potential recipients or to monitor therapeutic measures'.[11]

Directive 2000/70/EC amending Directive 93/42/EEC on medical devices to include devices incorporating stable derivatives of human blood or human plasma

A 'medical device' is defined (*inter alia*) as 'any instrument, apparatus, appliance, material or other article, whether used alone or in combination, including the software necessary for its proper application, intended by the manufacturer to be used for human beings for the purpose of: – diagnosis, prevention, monitoring, treatment or alleviation of disease'.[12]

These two Directives appear to cover all equipment and reagents, including software used in the transfusion services.

Other Directives directly impacting on transfusion and transplantation: consumer protection; confidentiality

Directive 85/374/EEC on 'the approximation of laws, regulations and administrative provision of the member states concerning liability for defective products'.[13] Article 6 of the Directive states that a product is defective if it does not provide the safety that persons generally are entitled to expect. Blood/blood components and plasma derivatives are products under this Directive. In the UK this Directive was transposed into the Consumer Protection Act 1987.

Directive 95/46/EC: Protection of individuals with regard to processing of personal data and free movement of such data [14] also applies to the transfusion and transplantation services. In the UK this was transposed into the Data Protection Act 1998.

1.3 The Blood Safety and Quality Regulations 2005

As stated in the introduction, these Regulations[3] transpose into UK law Directive 2002/98/EC and Directive 2004/33/EC and are binding across the UK.

At present blood establishments are licensed as manufacturers of medicinal products under the Medicines Act 1968; the regulations will amend the Medicines Act so that human blood and blood components will fall outside the scope of the Medicines Act and be regulated solely by the Regulations.

Competent authority

The Secretary of State is designated the competent authority for the purposes of these Directives. The Medicines and Healthcare Products Regulatory Agency will discharge the enforcement obligations of the Secretary of State. It will monitor compliance with the Regulations and carry out inspections of blood establishments. This responsibility is expected to transfer to the Regulatory Authority for Tissue and Embryos when this new Regulatory Authority is set up.

Definitions

The definitions used in this edition of the 'Red Book' are those given in the Blood Safety and Quality Regulations 2005 unless otherwise indicated (see Definitions).

References

All these Directives and the Blood Safety and Quality Regulations 2005 can be accessed directly from the JPAC website www.transfusionguidelines.org.uk.

OJ = Official Journal of the European Community

EU member states in 2005: Germany, United Kingdom, France, Italy, Spain, Netherlands, Greece, Belgium, Portugal, Sweden, Austria, Denmark, Finland, Ireland, Luxembourg, Poland, Czech Republic, Hungary, Slovakia, Lithuania, Latvia, Slovenia, Estonia, Cyprus, Malta.

Candidate Countries in 2005: Bulgaria, Romania, Croatia, and Turkey.

1. EU Directive 2002/98/EC 'setting standards of quality and safety for the collection, testing, processing, storage and distribution of human blood and blood components and amending Directive 2001/83/EC'. *OJ*, L 33, 08.02.2003, p30.

2. Commission Directive 2004/33/EC 'implementing Directive 2002/98/EC of the European Parliament and of the Council as regards certain technical requirements for blood and blood components'. *OJ*, L 91, 30.03.2004, p25.

3. *Statutory Instrument 2005 No. 50. The Blood Safety and Quality Regulations 2005*. ISBN 0 11 051622 2 available at www.opsi.gov.uk.

4. *The Handbook of Transfusion Medicine, Third Edition*. ISBN 0 11 322427 3 available at www.transfusionguidelines.org.uk.

5. Council of Europe (2004) *Guide to the Preparation Use and Quality Assurance of Blood Components, Eleventh Edition*. ISBN 92 871 5667 0 Council of Europe Publishing.

6. Council of Europe (2004) *Guide to the Safety and Quality Assurance for Organs, Tissues and Cells, Second Edition*. ISBN 92 871 4891 0 Council of Europe Publishing.

7. Directive 2001/83/EC of the European Parliament and of the Council – on the community code relating to medicinal products for human use. *OJ*, L 311, 28.11.2001, p67.

8. Commission Directive 2003/94/EC 'laying down the principles and guidelines of good manufacturing practice in respect of medicinal products for human use and investigational medicinal products for human use'. *OJ*, L 262, 14.10.2003, p22.

9. Medicines Control Agency (2002). *Rules and Guidance for Pharmaceutical Manufacturers and Distributors 2002, Sixth Edition*. Norwich: The Stationery Office, ISBN 0 11 322559 8.

10. Directive 2004/23/EC 'on setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells'. *OJ*, L 102, 07.04.2004, p48.

11. Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on 'in vitro diagnostic medical devices'. *OJ*, L 331, 07.12.1998, p1.

12. Directive 2000/70/EC of the European Parliament and of the Council of 16 November 2000 amending Council Directive 93/42/EEC 'as regards medical devices incorporating stable derivatives of human blood or human plasma. *OJ*, L 313, 13.12.2000, p22.

13. Council Directive 85/374/EEC of 25 July 1985 on 'the approximation of the laws, regulations and administrative provisions of the member states concerning liability for defective products' *OJ*, L210, 7.8.1985, pp0029–30.

14. Directive 95/46/EC of the European Parliament and of the Council of 24 October 1995 on 'the protection of individuals with regard to the processing of personal data and on the free movement of such data'. *OJ*, L281, 23.11.1995, pp0031–50.

Chapter 2

Quality in blood and tissue establishments and hospital blood banks

Introduction

The quality environment

The previous format of this chapter was designed in the late 1980s to support the United Kingdom Blood/Blood Transfusion Services in meeting the challenge of complying with UK Medicines Licensing requirements for the first time. It meant that Blood Services had to interpret the requirements of the European Community Good Pharmaceutical Manufacturing Practice (ECGPMP), and formally apply them to their collection, testing, processing and distribution services for the first time. The chapter was written to support Blood Services by demonstrating through an adaptation of BS 5750 'Quality Systems' how they could create a working quality management system that would support that licensing requirement. At that time, whilst the UK Guidelines covered other aspects of a blood service's activity, there was little requirement for those activities to be specifically regulated or accredited.

Whilst the UK Guidelines for Blood Transfusion Services in the United Kingdom continue to provide support for the majority of work of the blood establishments, these are complemented by a number of other legislative and/or accreditation requirements.

Taking all this into account the authors of this chapter feel it inappropriate to continue using the original format that was extended to cover the development of ISO 9001, which replaced BS 5750 Part 1. It was still dedicated to describing a quality management system for the collection of blood together with the subsequent testing, processing and distribution of blood and blood components. We also feel that trying to rewrite ISO 9000: 2000 and try to make this fit every aspect of a blood establishment's work would be inappropriate. For example, a blood establishment may wish to manufacture and distribute reagents. This requires the organization to be registered with a Notified Body and be awarded, following audit, the ability to apply the CE Mark to its products. To achieve this there are a number of routes from so called 'Full Quality Assurance' to independent batch testing of reagents before release by the Notified Body. The decision as to which route to take is individual to each organization and will be based on considerations such as: the categorization of the reagent; the scope of their operation; how much they wish to do to comply with the legislation; and how much they would wish to award to a third party.

To try and write a 'Quality' chapter that described a 'one size fits all' quality system would be inappropriate for this reason. It also seems inappropriate because of the huge leap in quality management skills within the blood establishments; providing a detailed description of how to install a quality system now seems redundant.

What the authors did want to do, was provide readers with the bigger picture. To describe how thinking on quality has developed within the Health Services of the United Kingdom into a holistic approach to assure stakeholders that donors, patients and staff meet their statutory duty of quality.

To that end, the following are described:

- how, through the Health Services Governance initiatives, the *Guidelines for the Blood Transfusion Services of the United Kingdom* help meet our statutory duty of quality
- key initiatives that are designed to improve the use of blood and blood components
- key European initiatives that have an impact on the work of the blood establishments and blood banks
- key principles that need to be considered when implementing a quality system
- key standards and guidelines that need to be considered during the development and implementation of a quality management system.

This chapter is considered to be a primer in developing an understanding of how quality principles can be used to support the work of 21st century blood establishments and blood banks. We hope they will encourage an open and informed dialogue with 'quality' professionals, which will help ensure that the blood and tissue establishments and hospital blood banks implement, and more importantly use their quality management systems to measure and improve on the quality of donor and patient care.

Governance

The fundamental purpose of governance/quality is to provide 'assurance' to stakeholders. Blood establishments and blood banks deliver care to donors and patients through a range of products and services. The convergence of clinical and corporate governance has emphasized an increasing understanding that assurance is imperative, in respect of these products and services.

NHS bodies are required to provide assurance particularly that risks are being managed effectively through an annual 'Statement on Internal Control' that encompasses the statutory duty of quality. Licensing and accreditation systems are an integral part of a blood and tissue establishment's and hospital blood bank's assurance framework and should be seen and utilized as such.

The Chief Medical Officers' 'Better Blood Transfusion' initiatives

The first initiative, in 1998, resulted from concerns regarding the sufficiency and increasing cost of blood, and concerns regarding safety arising from successive SHOT (Serious Hazards of Transfusion) reports and awareness of the possibility of vCJD transmission. It was recognized that the development of a blood safety strategy must involve clinical users as well as the blood services. The second initiative, in 2001, was aimed at setting the agenda for NHS transfusion services for the next three years and focused on:

- providing better information to patients
- avoiding unnecessary transfusion
- making transfusion safer.

This helped ensure that 'Better Blood Transfusion' was an integral part of NHS care. These objectives were further defined in Health Service Circulars in the four countries.

The UK blood establishments have a central part to play in the delivery of these objectives not only by ensuring optimum safety of blood components but also by working with users to develop comprehensive strategies for blood conservation.

Duty of care to patients and donors

Guidelines on the care and selection of donors are aimed at minimizing clinical risks to both the patient who receives the blood or tissue and the donor who gives it. This requires that the principles of clinical governance be in place as follows:

- clear lines of accountability for the overall quality of clinical care
- a comprehensive programme of quality improvement activities
- clear policies aimed at managing risks
- procedures for all professional groups to identify and remedy poor performance.

There is therefore a requirement for donation to be conducted in an appropriate environment, with adequate staffing levels, including suitably trained and competent health care professionals working to agreed clinical standards.

Appropriate safeguards must be in place to govern access to and storage of confidential patient and donor information as recommended in the Caldicott Report on the Review of Patient Identifiable Information. The quality of clinical care, including record keeping, should be monitored by clinical audit and adverse incidents affecting patients and donors must be reported and reviewed in order that lessons may be learned from them.

2.1 Key European initiatives

Directive 2004/23/EC of the European Parliament and the Council on setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells. Expected implementation is by April 2006.

Directive 2002/98/EC of the European Parliament and of the Council of 27 January 2003 setting standards of quality and safety for the collection, testing, processing, storage and distribution of human blood and blood components and amending Directive 2001/83/EC.

This Directive came into force in UK law on the 8 February 2005, although the requirements become effective in November 2005. It provides a framework to assure common standards for the collection of donated units, and their subsequent testing, processing and issue. Associated with Article 29 of this Directive are a number of requirements, for example, a quality management system for blood establishments, and a haemovigilance system. Commission Directive 2004/33/EC implements Directive 2002/98 as regards certain technical requirements for blood and blood components. At the time of writing a Commission Directive on quality is in preparation.

In Vitro Diagnostic Medical Devices Directive – 98/79/EC

This is currently being implemented in UK Law. There are two key milestones for users of *in vitro* diagnostic devices IVDs. The first was 5 December 2003. Users of IVDs must ensure that any stock produced and introduced into the supply chain after that date is CE marked. The second key milestone is 5 December 2005. After this date only CE marked stock can be purchased and used. There are a number of other obligations placed upon users, for example they can be held criminally liable if they knowingly encourage the supply of non-CE marked IVDs produced after 5th December 2003. The main implication for the Blood Services surrounds the provision of reagents to third parties for their use, where CE marking is required, even if there is no payment for the reagent supplied. This is a complicated piece of legislation, blood and tissue establishments and hospital blood banks are significant users and producers of IVDs, and they should ensure they are compliant with the legislation and should take appropriate advice to ensure they work within the legislation.

Medical Devices Directive (MDD) – 93/42/EEC

This Directive has been brought into UK law. It was however amended by the Directive 98/79/EC to recognize the definition of an *in vitro* diagnostic device, which was not originally defined, and to ensure there were common definitions between the two directives, such as the precise meaning of 'putting into service'. It is anticipated that whilst blood and tissue establishments and hospital blood banks may not manufacture medical devices, as they are key users of such devices, from blood bags to donation beds, knowledge of the legislation may be beneficial.

2.2 Other standards

There are a number of other standards that help define how a quality management system should be designed to meet the needs of a particular aspect of the Service's work. Table 2.1 provides information on some key inspection/licensing/accreditation/certification standards. They are all applicable within England. Some apply directly to the whole of the United Kingdom, for example the International Standards, others to England and Wales, for example the NHS Litigation Authority Risk management assessment programme. Some have been developed in England and then adapted to other parts of the United Kingdom, for example the Controls Assurance Standards. Where there is not a direct cross-reference the reader should investigate further to determine how the standards might apply. However, all the primary sources cited here are places where sound advice on management systems to address the various requirements of a modern blood service can be found. These will support the design and establishment of a system that can be confidently subjected to an external inspection process. The list is not intended to be exhaustive and by the nature of change is current at the time of publication. It is for this reason version numbering has not been applied to the available standards; they will be constantly updated. It will provide the sound basis for an excellent reference guide to assuring that a blood establishment can demonstrate it is delivering a safe and efficacious service to patients.

Table 2.1 List of some key inspection/licensing/accreditation/certification standards

Key Standards[1]	Applicable to	Responsible Body	Website
A Code of Practice for Tissue Banks	Public Sector Tissue banks in the United Kingdom	Department of Health, Richmond House, 79 Whitehall, London, SW1A 2NL, United Kingdom +44 207 210 4850	www.dh.gov.uk
BS 15000 IT Service Management Standard	Service Management	BSI British Standards HQ, 389 Chiswick High Road, London, W4 4AL, United Kingdom +44 208 996 9000 BSI Online, Technical Indexes Limited, Willoughby Road, Bracknell, Berkshire, RG12 8DW, United Kingdom +44 1344 404429	www.bsi-global.com
Caldicott Report 1997, implementation 1998	Confidentiality of patient data	Department of Health, Richmond House, 79 Whitehall, London, SW1A 2NL, United Kingdom +44 207 210 4850	www.dh.gov.uk

Table 2.1 List of some key inspection/licensing/accreditation/certification standards – *continued*

Key Standards(1)	Applicable to	Responsible Body	Website
CHAI assessment	Independent inspection of NHS and private and voluntary healthcare	Healthcare Commission (for England and Wales), Finsbury Tower, 103–105 Bunhill Row, London, EC1Y 8TG, United Kingdom +44 207 448 9200	www.chai.org.uk
Controls Assurance Self Assessment Standards	Key supporting management systems such as Medical Device Management/Infection Control/Medicines Management.	Department of Health, Richmond House, 79 Whitehall, London, SW1A 2NL, United Kingdom. +44 207 210 4850	www.info.dh.gov.uk
	Standards are updated, added to or refined on an annual basis	Supported by Health Care Standards Unit, Innovation Centre, Keele University, Keele, Staffordshire, ST5 5NB, United Kingdom +44 1782 583503	www.hcsu.org.uk
or			
The Welsh Risk Management Standards		Welsh Risk Pool, HM Stanley, Denbighshire, LL17 0RS, Wales +44 1754 589799	
or			
CNORIS		Scottish Executive Health Department, St Andrew's House, Edinburgh, EH1 3PG, Scotland +44 131 244 2663	
or			
Northern Ireland Department of Health, Social Services and Public Safety, Controls Assurance Standards		Planning and Performance Unit, Department of Health, Social Services and Public Safety, Castle Buildings, Stormont Estate, Belfast, BT4 3SQ, Northern Ireland +44 28 9052 2902 Fax +44 28 9052 3206	
EFQM Self-Assessment	Measurement of the effectiveness and, over time, the improvement in a Blood Service's management system. Helping understand where they are on the path to excellence	British Quality Foundation, 32–34 Great Peter Street, London, SW1P 2QX, United Kingdom +44 207 654 5000	www.quality-foundation.co.uk www.efqm.org

Table 2.1 List of some key inspection/licensing/accreditation/certification standards – *continued*

Key Standards(1)	Applicable to	Responsible Body	Website
European Federation for Immunogenetics (EFI)	H&I - Reference and Tissue typing	European Federation for Immunogenetics (EFI), EFI Central Office, c/o Dept. of Immunohematology and Blood Transfusion, Leiden University Medical Centre, Building 1 E3-Q, PO Box 9600, 2300 RC Leiden, The Netherlands	www.efiweb.org/contact.html
General Standards for Tissue Banking A series of Technical Guides	Tissue Banks who are members of the British Association of Tissue Banks	British Association of Tissue Banks, c/o Institute of Biology, 20–22 Queensbury Place, London, SW7 2DZ, United Kingdom	www.batb.org.uk
Good Automated Manufacturing Practice (GAMP) Guide for Validation of Automated Systems in Pharmaceutical Manufacture	Validation of Computer System	International Society for Pharmaceutical Engineering, European Office, 7 Ave des Gaulois, B-1040, Brussels, Belgium +32 2 743 44 22	http://www.ispe.org
International standards for unrelated hematopoietic stem cell donor registries WMDA Accreditation Program	Stem Cell and Donor Registries	World Marrow Donor Association, WMDA Office, Europdonor Foundation, Plesmanlaan 1b 2333 BZ Leiden, The Netherlands Fax: +31 71 5210457	www.worldmarrow.org/
ISO 17799 Information Security Management	Information Security	BSI British Standards HQ, 389 Chiswick High Road, London, W4 4AL, United Kingdom +44 208 996 9000 BSI Online, Technical Indexes Limited, Willoughby Road, Bracknell, Berkshire, RG12 8DW, United Kingdom +44 1344 404429	www.bsi-global.com

Table 2.1 List of some key inspection/licensing/accreditation/certification standards – *continued*

Key Standards(1)	Applicable to	Responsible Body	Website
ISO 9000 2000 Quality Management System Requirements	Quality Management System	BSI British Standards HQ, 389 Chiswick High Road, London, W4 4AL, United Kingdom +44 208 996 9000 BSI Online, Technical Indexes Limited, Willoughby Road, Bracknell, Berkshire, RG12 8DW, United Kingdom + 44 1344 404429	www.bsi-global.com
Joint Accreditation ICT Europe and EBMT (JACIE) assessment standard	SCI – HPC Collection, Processing, and Storage	The Joint Accreditation Committee EBMT-EuroISHAGE (JACIE) Alvaro Urbano-Ispizua, JACIE Office, Hospital Clínic, Villarroel 170, 08036 Barcelona, Spain +34 93 454 9543 Fax: +34 93 453 1263	www.celltherapy.org/jacie/tguide1.htm
MHRA publications: Rules and guidance for pharmaceutical manufacturers and distributors 2002 ISBN 0-11-322559 8	Pharmaceutical Environments	MHRA (Medicines) Market Towers, 1 Nine Elms Lane, London, SW8 5NQ, United Kingdom +44 207 084 2000	www.mhra.gov.uk Can be purchased from: www.tsoshop.co.uk or from TSO, PO Box 09, Norwich, NR3 1GN. 0870 600 5522
NHSLA risk management assessment programme for NHS trusts	Management of claims and litigation	National Health Service Litigation Authority, Napier House, 24 High Holborn, London, WC1V 6AZ, United Kingdom +44 207 430 8700	www.nhsla.com

Table 2.1 List of some key inspection/licensing/accreditation/certification standards – *continued*

Key Standards(1)	Applicable to	Responsible Body	Website
PRINCE2	Project control	Office of Government Commerce, Service Desk, Rosebery Court, St Andrew's Business Park, Norwich, Norfolk, NR7 0HS, United Kingdom +44 845 000 4999	www.ogc.gov.uk
Standards for the Medical Laboratory	Medical Laboratories	Clinical Pathology Accreditation (UK) Ltd, 45 Rutland Park, Botanical Gardens, Sheffield, S10 2PB, United Kingdom +44 114 251 5800	www.cpa-uk.co.uk

1 Information correct at 24/03/2004.

2.3 Systems

Quality management system

Within a blood/tissue establishment an effective quality management system (QMS) is a well designed, structured and organized method of quality assuring the provision of consistent, safe and efficacious products and services. It provides both a means to confirm to regulatory bodies, management and customers that the establishment service is in compliance with relevant standards, and also a basis whereby improvement in quality may be demonstrated.

In practice, an efficient QMS comprises a series of inter-related elements.

Good manufacturing practice

The application of good manufacturing practice (GMP) is the cornerstone of an effective QMS and provides the structure upon which the elements of the quality system can be built. The objective of GMP is formally stated as being 'to assure the quality of the product "manufactured" for the safety, well-being and protection of the patient'. The 'Orange Book', published by the MHRA under the title 'Rules and Guidance for Pharmaceutical Manufacturers and Distributors 2002', gives guidelines for GMP. They are presented under separate headings, and in practical terms all of these must be considered for each and every procedure or process to conform to the principles of good manufacturing practice.

The topics included are Quality Management, Personnel, Premises and Equipment, Documentation, Production, Quality Control, Contract Manufacture and Analysis, Complaints and Product Recall and Self-Inspection. To provide more practical relevance within the Blood Transfusion Services, many of these elements essential in maintaining GMP are expanded later in this section.

Documentation

Effective documentation, whether in written or electronic format, must be accurate, authorized, controlled at issue, and reviewed on a regular basis to ensure that it remains relevant. It provides clear instructions on what to do and prevents errors that may result from spoken communication. It ensures consistency of manufacture and service provision, provides objective evidence that tasks have been correctly performed, permits investigation if problems arise, and facilitates traceability from donor to patient and vice versa.

Within a Blood Transfusion Service comprehensive documentation includes a hierarchy of documentation starting with:

- a quality manual
- policies
- specifications
- standard operating procedures (SOPs)
- forms and worksheets, batch processing records, labels, equipment logbooks and investigation/validation records.

Effective document control must be practised to ensure that documents being used are current and an archive of superseded documents should be established to provide an historical record.

Change control

There should be a system of change control in process. Its aims should be to ensure that changes are evaluated and made only if they provide tangible benefits to the organization as judged by, for example, benefit to patients through risk reduction. It may also be driven by efficiency savings to ensure that maximum resource is devoted to patient care.

The system should then ensure that the change is planned and implemented in a controlled way, incorporating training for staff in new procedures, and demonstration that the expected outcome has been delivered. Supporting documentation, including for example SOPs, should ensure there is a record of the processes operated before and after the change, that the date of the change is known, and material processes through the changed system can be identified.

There should also be a system to ensure that the effectiveness of the newly implemented process is monitored and opportunities for further improvement investigated and where relevant implemented. It should support the organization in trying to learn from incidents, complaints and other event information, as analysis of this will help identify potential beneficial changes.

Validation

Validation is a pre-defined exercise to ensure that equipment or a procedure (either current or proposed) is fit for its intended purpose and meets its pre-defined specification. The benefits of validation include assurance that critical aspects of a process are in control, increased probability of uniform product quality, reduced product waste, and reduced customer complaints. New equipment, blood packs, and manufacturing processes are examples where validation is essential before they are introduced into routine application.

Manufacture

Manufacturing processes must follow clearly defined procedures in order to obtain products or services of the requisite quality. The inputs to any process must be controlled, for example the use of approved suppliers to agreed specifications. Goods requiring incoming inspection must be held in quarantine until the inspection has been performed. During manufacture any in-process controls should be carried out and recorded. Statistical techniques may be used to provide confidence that processes remain in control.

Calibration

Calibration is a procedure that confirms, under defined conditions, the relationship between values obtained from an instrument or system with those obtained using an appropriate certified standard. Examples include any equipment from which physical measurements are obtained, for example, weights, scales, temperature loggers, thermometers, light sources etc.

Quality control and quality monitoring

These provide confirmation either during or at completion of a process that manufacturing materials, processes and products meet their pre-defined specification. They may be release requirements (Quality Control Tests) such as a non-reactive microbiological test result, and demonstration of the effectiveness of a new batch of reagents. They may provide evidence that systems are operating as expected (Quality Monitoring), such as meeting a stated leucodepletion requirement by random sampling of finished product, testing white cell content and then subjecting the result to statistical analysis perhaps by the use of control charts. These latter tests would not normally prevent the issue of material.

Proficiency testing

Proficiency testing monitors the capability to perform procedures within defined limits of accuracy by analysis of unknown samples. Successful outcomes are dependent on the combined outputs of operators, equipment and process. Proficiency testing exercises are applied to a wide spectrum of laboratory procedures and may be managed on a local or national basis. National External Quality Assurance Schemes (NEQAS) are widely used in the UK.

Traceability

There must be a system to ensure that material can be traced through the procurement, testing, and production and issue systems to a patient. If the material is donated then traceability must be maintained to the donor. Any products must be uniquely identified to help support traceability. For example, for reagents this can be to batch level. Where appropriate this should be to individual units, for example apheresis donations split into multiple doses. Any material obtained from outside the EU must maintain a standard of traceability to its origin equivalent to that expected within a blood establishment.

Resources and staff

The service must ensure that adequate resources are provided to implement and operate the quality management system, to continually improve its effectiveness and to satisfy customer requirements. Staff must be competent; to ensure the work required is of the necessary standard. The physical resources to undertake the work must be suitable to attain the required standards, this will include equipment, consumables, work areas, utilities etc.

Recall

A system (usually, but not necessarily computer software) must be in place to allow full traceability of products. This will ensure that efficient recall of products can be effected and that look-back studies can be undertaken. Recall operation must be capable of being initiated promptly and at any time. It is essential that all recalled products are stored separately and securely until a decision is made on the fate of the product. Records of recall must be maintained. A review of the recall procedures for effectiveness needs to be carried out periodically.

2.4 Planned improvement

Incidents and error reporting

Incidents, errors and complaints identified at any stage in a collection, testing, manufacture or distribution process should be reported to a designated individual or department; these should be corrected on an individual basis.

Such reports are a valuable source of information from which to learn and improve. They must be reviewed and analysed on a continuous basis with a view to identifying the root cause of system failures, so that error can be minimized or eliminated, and to identify improvements that can be introduced to the system. The review should be designed to identify trends either adverse or beneficial.

It is important to take a holistic view using all available information and use information derived from analysis of incidents, errors, near misses and complaints as well as from audit processes, litigation and peer organizations. This approach will help prioritize those improvements that will be most beneficial to patients, donors and staff. As root-cause analysis places a significant drain on expert resources it should be targeted on activities that on the balance of risk are most critical to the organization. This process should be linked to the blood establishment's planning process so that improvements that require significant resources can be given sufficient consideration and support in their implementation.

Audit

Quality audit is a planned process of inspection conducted in an independent and detailed way by competent, trained individuals to ensure that procedures and associated quality assurance comply with the principles of GMP. The results of such inspections should be recorded and non-compliances reported in writing to a designated individual whose responsibility it is to ensure corrective and preventive actions are applied in an effective and timely manner.

There will also be an opportunity to learn from the problems identified through audit, to identify underlying root cause and possibly to support conclusions on areas to improve, identified through Incidents and Error reporting. As noted above this process should also be linked to the Blood Service's planning process so that improvements that require significant resources can be given sufficient consideration and support in their implementation.

2.5 Reporting of incidents to external bodies

Serious adverse events and serious adverse reactions (as defined in the EU Directives) must be reported to the Competent Authority. The MHRA has been identified as the Competent Authority for the United Kingdom for the EU 'Blood Safety' directive for five years from April 2005.

There may be additional local requirements which also must be met, for example, in Northern Ireland there has been a recent directive that all critical adverse incidents be reported directly to the NI Department of Health, Personal and Social Services and Public Safety.

Serious Hazards of Transfusion

www.shotuk.org

The Serious Hazards of Transfusion (SHOT) scheme collects data on serious sequelae of transfusion of blood components. Through the participating bodies, the information obtained contributes to: improving the safety of the transfusion process; informing policy within the Transfusion Services; improving standards of hospital transfusion practice; aiding production of clinical guidelines for the use of blood components.

Participation in the scheme is voluntary, and covers both NHS and private hospitals in the United Kingdom and Ireland.

SHOT is affiliated to the Royal College of Pathologists. Ownership of the scheme and data generated from it resides with the Steering Group, which has representation from the following Royal Colleges and professional bodies:

- Royal College of Pathologists
- British Blood Transfusion Society
- British Society for Haematology
- Faculty of Public Health Medicine

- Institute of Biomedical Science
- Institute of Health Care Managers
- NHS Confederation
- Public Health Laboratory Service/Communicable Disease Surveillance Centre
- Royal College of Anaesthetists
- Royal College of Nursing
- Royal College of Obstetricians and Gynaecologists
- Royal College of Paediatrics and Child Health
- Royal College of Physicians
- Royal College of Surgeons
- UK Transfusion Services.

UK Transfusion Services should report 'near misses' to SHOT. These are incidents where an action has placed a patient at risk. This could include, for example, the placing in stock of incorrectly labelled blood components where the discrepancy in blood group, genotype or test status would have placed a patient at risk of an adverse outcome if the component had been transfused.

It is assumed that if transfusion of products in this 'near miss' category occurs resulting in adverse outcome the incident would be reported back to the supplying service, so they can investigate, identify root cause and prevent further occurrence. In this case it is important that it is understood that in these situations capturing data about events is *not* about assigning blame or liability, but *is* about improving systems and reducing risk. Such incidents should also be reported to SHOT.

National Patient Safety Agency

www.npsa.nhs.uk

The National Patient Safety Agency (NPSA) is a Special Health Authority created in July 2001 to coordinate the efforts of the entire country to report, and more importantly to learn from mistakes and problems that affect patient safety. As well as making sure errors are reported in the first place, the NPSA is trying to promote an open and fair culture in the NHS, encouraging all healthcare staff to report incidents without undue fear of personal reprimand. It will then collect reports from throughout the country and initiate preventative measures, so that the whole country can learn from each case, and patient safety throughout the NHS can be improved. Blood Services that operate in the UK outside the NHS should utilize similar reporting mechanisms where they exist.

At the time of writing mechanisms for reporting incidents from Blood Services direct to the NPSA are being developed.

Medicines and Healthcare Products Regulatory Agency (MHRA)

www.mhra.gov.uk

The Executive Agency of the Department of Health protecting and promoting public health and patient safety by ensuring that medicines, healthcare products and medical equipment meet appropriate standards of safety, quality, performance and effectiveness, and are used safely.

Blood Services should have a mechanism to report problems with medicines, medical devices or *in vitro* diagnostic devices to the MHRA. This will provide an opportunity for problems with medicines and devices to be viewed on a UK or European-wide level.

Chapter 3

Care and selection of blood donors (including donors of pre-deposit autologous blood)

Introduction

All blood donors in the United Kingdom are non-remunerated volunteer donors. These selection guidelines have two purposes: firstly, to protect donors from any potential harm which may occur as a direct result of the donation process; secondly, to protect recipients of blood transfusions from adverse effects, such as transmission of infectious diseases or other medical conditions and unwanted effects caused by any medications taken by the donor.

The criteria for selecting blood donors apply to donors of whole blood and of components (cells and/or plasma) collected by apheresis. Additional criteria for component donors are detailed in Chapter 6. Guidelines for donors of pre-deposit autologous donations are outlined in Section 3.9. The criteria for donors of stem cells and tissues are found in Chapters 24 and 22.

More detailed and frequently updated criteria are found in the Joint UKBTS/NIBSC Professional Advisory Committee's (JPAC) *Donor Selection Guidelines*[1] which form a constituent part of this chapter and *must* be consulted.

3.1 General principles

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

- A prospective donor's medical history must be evaluated on the day of donation by a suitably qualified person who has been trained to utilize the JPAC *Donor Selection Guidelines*.[1]
- If there is any doubt about the suitability of a prospective donor, a donation should not be taken and the details referred to a designated medical officer.
- Each blood establishment responsible for the collection of blood should include a medical consultant (with responsibility for donors) who will take professional responsibility for the care and selection of donors. The immediate responsibility is that of the medical practitioner or registered nurse in attendance at the session.

Patients referred for therapeutic venesection shall not be accepted at donation sessions (but see Section 3.4 on donors with genetic haemochromatosis).

Donors with hazardous occupations or hobbies

Occupations where a delayed faint may present a hazard either to the donor or to others can be accepted only when the individual is going off duty. This would apply, for example, to train, HGV or bus drivers; heavy machine or crane operators; work involving climbing ladders or scaffolding; miners working underground.

'Hazardous' hobbies should not be followed on the day of donation, e.g. gliding, powered flying, car or motor cycle racing, climbing, diving, etc.

3.2 Assessment of fitness to donate

The combination of assessing each donor clinically (at every attendance) and testing each donation for markers of infection is essential to maximize donor and recipient safety.

Each donor must undergo an assessment based on the JPAC *Donor Selection Guidelines*[1] to determine their eligibility to donate. This requires each donor to complete a questionnaire and answer a series of standard questions relating to their general health, lifestyle, past medical history and medication.

It is the responsibility of session staff to ensure that donors clearly understand the nature of the donation process and the associated risks involved as explained in the available literature. The donors must also understand the health check and other medical information presented to them. Donors are asked about confidential and sensitive aspects of their medical history and lifestyle. It is therefore important that blood collection sessions have facilities that offer privacy for donor interviews and that donors are assured of the confidentiality of any information they provide.

Third-party interpreters should not be used as there is no guarantee of understanding or of truth telling to the interpreter; particularly if they are a friend, family member or are otherwise known to the donor.

Potential donors who are unable to read the literature should be informed of its contents by a suitably trained member of staff.

Donor age: donors shall be between the ages of 17 and 65 years; i.e. from their seventeenth to sixty fifth birthday. Regular donors may be allowed to donate beyond their 65th birthday with permission of the physician in the blood establishment, given annually.

It is normal practice to set an upper age limit of 60 years (sixtieth birthday) for first-time donors. However, older donors may be accepted at the discretion of the physician in the blood establishment.

Frequency of donation: an interval of 16 weeks between donations of whole blood is reasonable. The minimum interval is 12 weeks. Normally, no more than three donations should be collected from any donor during any 12-month period.

Volume of donation: a donation of 450 mL±10% is required to ensure the final red cell component meets specification. No more than 13% of the estimated blood volume should be taken during any one donation. In general 470–475 mL of blood, excluding samples, is collected into the main pack. A policy of withdrawing a smaller volume from first-time donors is NOT recommended, as there is no evidence that the frequency of vasovagal reactions is reduced.

Donors following their normal meal pattern may be accepted.

A cup of fluid and biscuits should be offered at the session to donors who have missed their normal meal prior to collection of the donation.

3.3 Medical history of donors

General considerations

All donors should clearly understand any information and questionnaire presented to them and must sign an appropriate document which also attests to their consent for the blood to be taken, tested and used for the benefit of patients. Any condition declared shall be discussed with the medical practitioner or registered 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.

For the details of information to be supplied to and obtained from donors see Chapter 5.

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

Individuals currently undergoing medical investigations or who have been referred for a specialist opinion or are on a hospital waiting list should normally be deferred. If, however, the condition or potential condition concerned would not of itself be a contraindication to donation they may be able to donate.

Donors taking part in clinical trials cannot be accepted until their involvement in the trial has finished, or the consultant with responsibility for donors has examined the trial protocol and agreed that donors participating in that trial can be accepted. A 'clinical trial' normally implies that the donor is participating in an intervention programme – usually taking a drug or a potential drug which may be either active or a placebo. Participating in questionnaires does not constitute a clinical trial.

All donors should be made aware that recipients are at risk from transfusion, and shall be asked to report any illness that develops within 14 days of donation.

Information about either the donor or the donation which becomes available after the blood or any derivative has been issued or transfused, and which is, or may be, relevant to the safety of that blood for transfusion, should be reported to the appropriate individual e.g. consultant in charge of the hospital blood transfusion laboratory. Donor confidentiality must be respected.

The member of staff carrying out the donor assessment must confirm they have done so by signing the donation record. Any reason for deferral, whether temporary or permanent, must be explained to the donor and recorded.

Conditions necessitating permanent deferral

More detailed and specific criteria for the acceptance should be obtained by referring to the current version of the JPAC *Donor Selection Guidelines*.[1] In cases of doubt, the donor should be asked for written permission to contact his/her general practitioner or specialist and donation should be postponed until further information is available.

- Cardiovascular diseases: individuals with circulatory disorders are especially subject to cardiovascular and cerebrovascular disturbances resulting from sudden haemodynamic changes. Thus prospective donors with active or past serious cardiovascular disease, except congenital abnormalities with complete cure, must be permanently deferred.

- Central nervous system diseases: in general, these conditions are contraindications to donation. Such individuals may be unduly susceptible to sudden haemodynamic changes. Conditions of infectious or unknown aetiology or where there is evidence of impaired cognition are also reason to exclude a donor. Prospective donors with a history of serious CNS disease must be deferred.

- History of convulsions: prospective donors with epilepsy must be permanently deferred (not childhood convulsions) unless at least three years have elapsed since the date the donor last took anticonvulsant medication and there has been no recurrence of symptoms. Donors who report repeated episodes of syncope should also be permanently deferred.
- Gastrointestinal diseases: diseases which render the individual liable to iron deficiency through impaired iron absorption or blood loss should normally be a reason for exclusion. Individuals with coeliac disease may be accepted.
- Genitourinary, haematological, immunological, metabolic, renal or respiratory diseases: most prospective donors with a history of serious disease will be permanently deferred.
- Diabetes: donors on insulin treatment must be permanently deferred.
- Malignant neoplasms, including leukaemias and myeloproliferative disorders: these are causes for permanent deferral, although exceptions may be made for certain conditions at the conclusion of successful therapy, as listed in the JPAC *Donor Selection Guidelines*.[1]
- Infectious diseases: donors with the following infectious diseases must be permanently deferred: hepatitis B; hepatitis C; HIV 1 and 2; HTLV I /II; babesiosis; Kala-azar (visceral leishmaniasis); *trypanosomiasis cruzi* (Chagas' disease)
- Transmissible spongiform encephalopathies (TSEs) eg CJD vCJD: see Section 3.7.
- Parenteral drug use: donors with any history of non-prescribed intravenous (IV), intramuscular (IM) or subcutaneous (SC) drug use, including bodybuilding steroids or hormones, must be permanently deferred.
- Sexual behaviour: persons whose sexual behaviour puts them at high risk of acquiring severe infectious diseases that can be transmitted by blood must be permanently deferred.
- Xenotransplant recipients: donors who have received a xenotransplant, and their sexual partners, must be permanently deferred.

Conditions necessitating temporary deferral or qualified acceptance

Reference must be made to the current JPAC *Donor Selection Guidelines*[1] because these conditions are very varied and subject to rapid changes.

3.4 Genetically determined conditions

An increasing number of genetically determined conditions that potentially affect donor health are being identified, and some donors have had specific tests which confirm that they possess variant genes. These include not only the haemoglobinopathies and thalassaemias, but also more recently discovered conditions such as the 'thrombophilias' (including factor V Leiden) and haemochromatosis genes. Mere possession of such genetic variants does not debar from donation if the donor is otherwise healthy and fulfils all other selection criteria.

Genetic haemochromatosis

This is a special case. Blood from individuals with genetic haemochromatosis (GH) who have no symptoms arising from their GH is intrinsically safe for transfusion. However, before patients with GH who require continued venesection for the maintenance of their health are accepted as blood donors, the consultant with responsibility for donors must ensure that the following criteria are met:

- the selection criteria/methods for all donors with GH preserve the principles of altruism

- blood donated for therapeutic use by any donor known to have GH meets all other criteria (except donation frequency) in the JPAC *Donor Selection Guidelines*.[1] If it is clinically appropriate for individuals to donate more frequently than the minimum donation interval, specific permission must be obtained from the designated medical officer
- the donor is under the continuing care of a physician who is able to offer alternative venesection facilities whenever, for any reason, the donor does not meet all other criteria in the JPAC *Donor Selection Guidelines*.[1]

3.5 Donors on treatment with medications (drugs)

Donor deferral for most drugs is based on the underlying illness suffered by the donor rather than for the properties of the drug itself, e.g. cardiovascular disease, diabetes, anaemia and malignancies. Since, in general, traces of drugs in blood and blood components are believed to be harmless to patients, many people taking medications – even when prescribed – are acceptable as blood donors so long as the reason for which the medication is taken is acceptable.

A pragmatic view should be taken of treatment of infections with antimicrobials. Providing the donor is in good health, deferral is limited to two weeks from full recovery and one week after cessation of antimicrobial therapy, whichever is the longer. This is based on what may be regarded as a reasonable recovery period for the infection and is not related to the antimicrobial therapy itself.

Donors taking drugs which are proven or potential teratogens (e.g. vitamin A derivatives) or who are taking drugs that accumulate in tissues over long periods, should not be accepted for blood donation. Some such drugs may be taken to prevent diseases to which the donor – though currently healthy – is prone. An example is Tamoxifen taken by women with a strong family history of breast cancer. A decision to accept should be taken after considering the pharmacodynamics of the specific drug, and its mode of action. The period of deferral after finishing a course of treatment is set out in the JPAC *Donor Selection Guidelines*.[1]

The current JPAC *Donor Selection Guidelines* [1] must be referred to for all donors who have had immunizations.

Sporadic self-medication with some drugs (e.g. vitamins, aspirin, sleeping tablets) need not prevent a donation being accepted, providing the donor is in good health.

If the donor has taken drugs affecting platelet function (e.g. aspirin) within the last five days the donation shall not be used for preparing platelets. A list of such drugs is in the JPAC *Donor Selection Guidelines*.[1] Other drugs or tablets may be acceptable. However the taking of some drugs may indicate a disease which would automatically make a donor ineligible.

3.6 Transfusion transmissible infectious diseases

Every effort is made to prevent transmission of disease by careful and appropriate selection of donors. This includes ensuring that the donor is provided with clear, understandable and up to date information and also ensuring that the donor has understood this information (see Chapter 5).

Donors must be assessed for their exposure to any risk of acquiring a transfusion transmissible infection. These risks include a history of

- endoscopic examination using flexible instruments
- mucosal splash with blood or needle stick injury
- transfusion of blood components
- tissue or cell transplant

- major surgery
- tattoo or body piercing
- acupuncture
- close household contact with persons with certain infectious diseases.

Travel history

Increased and rapid travel of the population may lead to asymptomatic people donating infectious blood. A clear and detailed travel history must be obtained from all donors to minimize the risk of transmission of malaria, T. cruzi and emerging diseases such as West Nile Virus.

The latest JPAC *Donor Selection Guidelines*[1] should be consulted for any donor with a relevant travel history.

The Blood Services and JPAC maintain close links with the WHO and Health Protection Agency (HPA) and base the donor deferral criteria on the advice obtained. Any changes to current selection guidelines need to be rapidly communicated and this will happen through Change Notifications and the website www.transfusionguidelines.org.uk

3.7 Prion-associated diseases including sporadic Creutzfeldt-Jakob Disease (CJD) and variant CJD (vCJD)

Individuals who are identified as having an increased risk of developing a prion-associated disease must be permanently excluded from donation. This includes

- individuals who have received human pituitary-derived hormones
- patients who have received human dura mater grafts or corneal grafts or scleral grafts
- persons identified as being members of a family at risk of inherited prion diseases
- persons who are known to have received a blood transfusion since 1980. For these purposes, a transfusion is defined as any product containing red cells, platelets, granulocytes, fresh frozen plasma, cryoprecipitate and intravenous human normal immunoglobulin.

The current edition of the JPAC *Donor Selection Guidelines*[1] provides detailed advice and should be consulted.

3.8 Physical examination of donors

General considerations

A detailed medical assessment procedure must be conducted on all donors, as referred to above, i.e. based on the JPAC *Donor Selection Guidelines*.[1] Particular attention is required for the assessment of first time or 'returning' donors. Returning donors are defined as those who – although formerly registered as a blood donor to one of the four National Blood Transfusion Services – have not been assessed for donation for two years or more.

Assessment of blood pressure is not recommended because the circumstances at blood collection sessions are not conducive to obtaining meaningful measurements. Routine measurement of blood pressure could also give the impression that blood establishments offer a general health screening service which might be construed as an inducement to donate.

Inspection of the donor: the donor should be in good health. Note should be taken of poor physique, debilitation, under-nutrition, plethora, jaundice, cyanosis, dyspnoea,

intoxication and mental instability. When in doubt the donor should be deferred until further advice has been obtained from a designated medical officer.

Weight: the minimum weight for donation is 50 kg (7 stone 12 lb). Those who weigh less than 50 kg are more likely to suffer adverse reactions, in particular dizziness and fainting, after a standard donation. This is because the volume taken represents a greater proportion of their blood volume. It should be noted that donors who are obese but are towards the lower weight limit may not have a sufficient blood volume to ensure a safe donation.

Estimation of the concentration of haemoglobin (Hb) in donor blood

The Hb concentration should be determined each time a potential donor presents. The acceptable lower limits for venous blood are 125 g/L for female donors, and 135 g/L for male donors.

The precise method of screening donors for their blood Hb concentration may be determined by the consultant with responsibility for donors. An acceptable strategy is to apply the gravimetric method using solutions of copper sulphate on blood samples obtained by fingerprick.

A donor whose fingerprick sample fails the gravimetric screen should be offered a test on a sample of venous blood for accurate determination of their Hb concentration. This is to enable the donor to receive appropriate advice either from the consultant with responsibility for donors or the donor's general practitioner. The Hb concentration in the venous sample may be determined immediately at the session if a suitably validated haemoglobinometric device capable of rapid and accurate analysis is available. If the concentration so determined is at or exceeds those quoted above the donor may be invited to give a full donation. Haemoglobinometry is not recommended for fingerprick samples.

Donors whose Hb concentration is below the minimum values should not be bled. The reason for deferral should be explained and the donors advised to see their own general practitioner if this is considered to be appropriate.

If a quantitative method of Hb determination is employed, before or after the donation, individuals found to have a concentration of Hb above the normal upper limit should be referred for further investigations.

3.9 Donors of pre-deposit autologous donations

Autologous pre-deposit donations must be collected according to the same requirements as allogeneic donations but the deferral criteria vary. These donations must be clearly identified as such and kept separate from allogeneic donations.

Deferral criteria

The deferral criteria for donors of autologous pre-deposit donations in the UK, originally agreed by the British Committee for Standards in Haematology Blood Transfusion Task Force and published in 1993, are currently under revision.[2]

The two main deferral criteria are serious cardiac disease (where the clinical setting of the blood collection must be taken into account) and active bacterial infection.

3.10 Donors of immune plasma

Recruitment of donors for specific immune globulins has been suspended in the UK until such time as the UK government decision to use only non-UK source plasma has been rescinded.

References

1. Joint UKBTS/NIBSC Professional Advisory Committee's (JPAC) *Donor Selection Guidelines* available at www.transfusionguidelines.org.uk

2. British Committee for Standards in Haematology Blood Transfusion Task Force (1993) Drafted by the Autologous Transfusion Working Party of the NBTS. Guidelines for autologous transfusion. I. Pre-operative autologous donation. *Transfusion Medicine*, **3**, pp307–16.

Chapter 4

Premises and quality assurance at blood donor sessions

This section applies to the collection of donations of whole blood at permanent sites or by mobile blood collection teams. Further information on premises and quality assurance for component collections is found in Chapter 6.

4.1 Premises

Premises used for the preparation of components from blood and plasma will be subjected to scrutiny by the Competent Authority, the Medicines and Healthcare Products Regulatory Agency in 2005. Such facilities must comply with the principles embodied in the *Rules and Guidance for Pharmaceutical Manufacturers and Distributors 2002*.[1]

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 *WHO Expert Committee on Biological Standardisation 43rd Report, Technical Report Series No. 840 1994*.[2]

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.

Selecting a venue

Account must be taken of the following activities/requirements when selecting a venue:

- registration of donors and all other necessary data processing. There should be immediate access to a working telephone
- appropriate facilities to assess the fitness of individuals to donate
- withdrawal of blood from donors without risk of contamination or errors
- flooring should be non-slip
- social and medical care of donors, including those who suffer reactions. Sufficient seating should be provided for donors and staff, with allowance made for possible queues during busy periods

- storage of equipment, reagents and disposables
- storage during the session of blood and components, if they are not to be transferred immediately to the blood processing centre or to appropriate storage in the team vehicle
- access to an adequate electrical supply to support all electrical equipment used for the session
- the space required for these activities will depend on the anticipated workload.

Health and safety factors

The requirements of the Health and Safety at Work Act must be taken into account when selecting sessional venues. Premises should be safe, clean and comfortable for donors and staff. In particular, the following points should be borne in mind:

- 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 shall be even and well lit. The space to be used should preferably not entail carriage of equipment on stairs. A similar safe approach should be ensured for donors, with as much provision as possible for car parking. Notices should be displayed, directing donors to the appropriate entry point of the building, and to the room being used
- furniture and equipment within the available space should be arranged to minimize crowding (with the increased risk of mistake or accident), enabling adequate supervision and ensuring a smooth and logical workflow
- fire exits must be unobstructed and operational. All sessional staff must be aware of the location of the fire extinguishers and exits
- lighting should be adequate for all the required activities. Provision should be made for the use of emergency lighting in the event of interruption of the electricity supply
- environmental control may not be within the power of a mobile team, but every effort should be made to ensure that the space does not become too hot, cold or stuffy. 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
- facilities for the provision of refreshments for donors and staff should be separated from the other activities of a donor session whenever possible. Every effort should be made to ensure that equipment used in this area poses the minimum threat of danger to all persons
- toilet facilities for male and female donors and staff should be provided. Separate washing facilities are desirable for those staff involved in 'clean' procedures
- adequate facilities must be available for the disposal of waste. On mobile sessions, all waste should be collected and contained in a suitable manner for subsequent disposal in accordance with relevant regulations.

4.2 Collection of the donation

The ultimate responsibility for the correct safe procedure for the collection of blood is that of the medical consultant with responsibility for donors; the immediate responsibility for the operation of the blood collection session is that of the medical practitioner or senior nurse in attendance.

Each Blood Transfusion Service 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.

Guidance for blood donation procedures is given in Chapter 5. Guidance for laboratory testing procedures is given in Chapters 10 and 13.

Donor identification

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

Labelling

Session staff must ensure that a set of labels with a unique number is assigned to each donation and that the same unique number appears on the donor session record, the primary and secondary collection packs and all the sample tubes used. Great caution is necessary to avoid crossover or duplication of numbers.

Arrangements should be such as to avoid the possibility of errors in the labelling of blood containers and blood samples. The blood bag and corresponding samples must not be removed from the donor's couch until a satisfactory check on correct labelling has been carried out. It is recommended that each donor couch has its own individual facilities for the handling of samples during donation and labelling.

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

4.3 Records

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

Donor session records

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.

The records of blood donation sessions should allow identification of each important step associated with the donation. All donations must be recorded; the reason for any unsuccessful donations must be recorded. All adverse reactions must also be recorded together with the action taken. Full details of any other incidents, including those only involving staff must be recorded.

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

Control of purchased material and services

Specification and inspection of blood bags

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 non-sterile air entering the system.

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

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

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

Batch numbers of the blood packs used shall be recorded.

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

Prior to release from the blood collection session the pack and its associated tubing should be reinspected for defects and its integrity should be checked by applying pressure to the pack to detect any leaks. Any defective pack should be marked for disposal and held separately from intact packs. Details of the defect(s) should be recorded for future analysis and action (see Section 5.10).

Inspection of labels for printing errors

All donors' 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 or equivalent on machine-readable labels.

Protection and preservation of product quality

Requirements for labelling, storage and transportation are given in Chapters 7 and 8.

References

1. Medicines Control Agency (2002). *Rules and Guidance for Pharmaceutical Manufacturers and Distributors 2002, Sixth Edition*. Norwich: The Stationery Office, ISBN 0 11 322559 8.

2. *World Health Organization (1994) WHO Expert Committee on Biological Standardisation. World Health Organ. Tech. Rep. Ser.*, **840**, pp1–218.

Chapter 5

Collection of a blood donation

Introduction

This chapter describes the steps involved in the collection of a blood donation from the information to be provided to a donor to the information required from the donor post-donation.

Sections 5.1 and 5.2 are taken from the Blood Safety and Quality Regulations 2005.[1]

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

- Accurate educational materials, which are written in terms which can be understood by members of the general public, about the essential nature of blood, the blood donation procedure, blood components, and the important benefits to patients
- For both allogeneic and autologous donations, the reasons for requiring a medical history, the testing of donations and the significance of informed consent
- For allogeneic donations, the criteria for self-deferral, temporary and permanent deferral, and the reasons why individuals are not to donate blood or blood components if there could be a substantive risk for them or the recipient
- For autologous donations, the possibility of deferral and the reasons why the donation procedure would not take place in the presence of a health risk to the individual whether as donor or recipient of the autologous blood or blood components
- Information on the protection of personal data, including confirmation that there will be no disclosure of the identity of the donor, of information concerning the donor's health and of the results of the tests performed, other than in accordance with the requirements of these Regulations
- The reasons why individuals are not to make donations which may be detrimental to their health
- Specific information on the nature of the procedures involved either in the allogeneic or autologous donation process and their respective associated risks. For autologous donations, the possibility that the autologous blood and blood components may not suffice for the intended transfusion requirements
- Information on the option for donors to change their mind about donating prior to proceeding further, or the possibility of withdrawing or self-deferring at any time during or after the donation process, without any undue embarrassment or discomfort

- The reasons why it is important that donors inform the blood establishment of any subsequent event that may render any prior donation unsuitable for transfusion
- Information on the responsibility of the blood establishment to inform the donor, through an appropriate mechanism, if test results show any abnormality of significance to the donor's health
- Information explaining why unused autologous blood and blood components will be discarded and not transfused to other patients
- Information that test results detecting markers for viruses, such as HIV, HBV, HCV or other relevant blood transmissible microbiologic agents, will result in donor deferral and destruction of the collected unit
- Information on the opportunity for donors to ask questions at any time
- If the donated blood is to be used for purposes other than clinical transfusion specific information *must* be provided.

5.2 Information to be obtained from donors by blood establishments at every donation

Donor identification

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

Health and medical history of the donor

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

Signature of the donor

The donor must sign the donor questionnaire, countersigned by the qualified health professional responsible for obtaining the health history confirming that the donor has

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

5.3 Haemoglobin or haematocrit screening

The objective is to ensure that prior to each donation the donor has a normal haemoglobin concentration (at least 125 g/L in, at least 135 g/L in males) In the UK, testing using the gravimetric method is widely used for blood donor screening, usually backed up by a second level (spectrophotometric) test. Several non-invasive techniques are currently being evaluated.

Copper sulphate haemoglobin screen

Aqueous copper sulphate, coloured blue, with a specific gravity of 1.053, equivalent to 125 g/L haemoglobin is normally used to test female donors. Copper sulphate, coloured green, with a specific gravity of 1.055, equivalent to 135 g/L is normally used to test male donors. These stock solutions should be colour-coded and labelled accordingly.

Copper sulphate storage

Stock solutions shall 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. Alternatively, copper sulphate solutions of required standards may be obtained in individually labelled containers, predispensed in 25 to 30 mL aliquots direct from manufacturers.

Copper sulphate for routine use

Designated staff shall 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 in accordance with relevant regulations. The calibration temperature of the copper sulphate must 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 °C. If kept chilled, the copper sulphate solutions must be given time to warm to ambient temperatures prior to use. When dispensed or kept in plastic containers, care must be taken to avoid accumulation of electrostatic charge, as this can interfere with penetration by blood drops.

Procedure for estimating Hb concentration on a fingerprick blood sample using copper sulphate

1. 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

2. 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

3. Blood from earlobe puncture should not be used as it has a higher haemoglobin and haematocrit than blood from a fingerprick sample and may allow donors with unsuitably low levels to give blood

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

5. One drop of blood is allowed to fall by unassisted gravity from the tube from a height of 1 cm 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

6. Results are recorded as pass or fail.

Spectrophotometric method for Hb concentration screening

1. If a haemoglobin photometer is used to provide a quantitative measurement of Hb at the donor session, standard operating procedures for the use of the instrument must be available in the session procedure manual
2. They should include a technique whereby the performance of the photometer is validated by the regular use of appropriate calibration working standards
3. In addition, a system of regular and frequent assessment of the accuracy of performance of any photometric equipment must be established.

The microhaematocrit method applied to a fingerprick sample of blood

1. The microhaematocrit centrifuge must be calibrated when first placed in service, after repairs, and annually thereafter
2. The time and speed should be checked at a minimum of six months and preferably every three months by an appropriate qualified person using a precision RPM meter and a stopwatch to check speed, acceleration and retardation
3. A calibration method that provides quality control and allows selection of optimal centrifugation time is examination of replicate specimens or red cell suspensions within, below and above the acceptable haematocrit range
4. The time selected for routine use should be the minimum time at which maximum packing occurs. A deviation of 2% between replicates is acceptable
5. If a microhaematocrit method is employed for Hb screening, standard operating procedures for the use of this instrument must be available in the session procedure manual
6. The blood samples should be obtained as described in 'Procedure for estimating Hb concentration', above, Points 1 and 2
7. The minimum acceptable microhaematocrit values are 0.38 for women and 0.40 for men.

5.4 Preparation of the venepuncture site

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

Although it is not possible to guarantee sterility of the skin surface for venepuncture, a strict standardized and validated procedure for the preparation of the venepuncture site should be in operation to achieve surgical cleanliness and thus to provide maximum possible assurance of a sterile product.

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

5.5 Preparation of the blood pack

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

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

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

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

5.6 Performance of the venepuncture

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

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

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

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

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

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

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

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

Sample collection

At the start of the donation up to 30 ml of blood should be diverted into a pouch. It is recommended that this pouch has a means of access opposite the entry line which allows blood to be sampled for haematological and serological testing without compromising the environmental integrity of the blood in the main pack.

5.7 Blood donation

If necessary, the donor should be asked to open and close his/her hand slowly every 10-12 seconds to encourage a free flow of blood.

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

Blood anticoagulation

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

Blood flow

Blood flow should be constantly observed to ensure that the flow is uninterrupted. Blood should be mixed regularly during the period of donation which should not exceed 15 minutes.

Blood volume monitoring

- The volume of blood withdrawn must be controlled to protect the donor from excessive loss of blood and to maintain the correct proportion of anticoagulant to blood
- The most efficient way of measuring the blood volume in plastic bags is by weight. The mean weight of 1 mL of blood is 1.06 g; e.g., a unit containing 470 mL of blood should therefore weigh 470 × 1.06 g plus the weight of the pack(s) and the anticoagulant
- If it is not possible to adjust the weighing device in use for the tare weight of the container and anticoagulant solution it is advisable to record the minimum and maximum weight for the brand of pack in use as products from different manufacturers may vary considerably
- Several kinds of weighing equipment are available and such devices should be used according to the manufacturer's instructions for weighing blood into its plastic pack and periodically calibrated by appropriate techniques.

Completion of the donation

- The pressure cuff must be deflated and the needle then removed from the arm. Immediate pressure must then be applied to the venepuncture site through a suitable clean dressing
- The needle must be discarded into a special container designed to minimize risk to personnel
- The pack must be inverted gently several times to ensure the contents are thoroughly mixed
- Provision of the line for compatibility testing:
  - if a pack system is used which is not designed for in-line leucodepletion, the sealed tubing from the donor line should be retained for cross-matching purposes. The blood contained in the donor line should be expressed into the pack containing the blood donation and allowed to flow back into the tube to ensure anticoagulation. The line number must be completely and clearly readable, and it must be made possible for the tubing to be separated from the pack without compromising the sterility of the pack
  - for pack systems designed for in-line leucodepletion in which the donor line becomes detached from the final red cell pack, and hence unavailable for compatibility testing, the line should be sealed close to the collection pack, according to clearly defined procedures. This sealing may be done without expressing the contents of the line into the main pack if the contents of the line are deemed to be of no further use
- The arm and general well-being of the donor should be checked before the donor leaves the session venue.

5.8 Information to be provided to the donor post-donation

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

5.9 Adverse reactions in donors

All adverse reactions in donors should be documented and reported according to standard protocols.

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

5.10 Adverse events

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

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

Any safety-related defects in equipment, including single use items, must be reported via the head of department to the Department of Health in accordance with the requirements of the Competent Authority, currently the Medicines and Healthcare Products Regulatory Agency.[3]

Serious adverse events must be reported to the Competent Authority according to the blood establishment protocol.[3]

References

1. *Statutory Instrument 2005 No. 50. The Blood Safety and Quality Regulations 2005.* ISBN 0 11 051622 2 available at www.opsi.gov.uk

2. *Joint UKBTS/NIBSC Professional Advisory Committee's (JPAC) Donor Selection Guidelines* available at www.transfusionguidelines.org.uk

3. Online reporting is available at www.mhra.gov.uk.

Chapter 6

Component donation: apheresis

Introduction

These guidelines relate to the collection of blood components by automated apheresis. Their purpose is to ensure the safety of volunteer donors undergoing apheresis procedures and to ensure the quality of collected apheresis components. They relate only to the apheresis of healthy volunteer donors and not to the clinical use of cell separators for plasma exchange and other therapeutic procedures. The latter is covered by the *Guidelines for the Clinical Use of Blood Cell Separators*, prepared by the Clinical Haematologists Task Force of the British Committee for Standards in Haematology.[1]

A medically qualified consultant experienced in apheresis must be ultimately responsible for the selection, health and welfare of the apheresis donors. He or she should ensure that all staff are appropriately trained and that clinical standards are maintained. Extreme care should be taken to ensure that undue pressure is not put on persons to donate.

6.1 Criteria for acceptance of donors

Other than in exceptional circumstances (to be decided by a designated medical officer), donors for apheresis procedures shall meet the usual criteria for ordinary whole blood donations. These are described in Chapter 3 and comply with criteria laid down in the current Joint UKBTS/NIBSC Professional Advisory Committee's (JPAC) *Donor Selection Guidelines*.[2] First-time apheresis donors should have given at least one routine blood donation without untoward effect within the last two years (as this may give an indication of their ability to tolerate an apheresis procedure).

In addition the following criteria should be observed for apheresis donors:

- first-time donors should be aged 18-60 years. Donors may continue to donate by this method up to the day before their 65th birthday
- donors must not be less than 50 kg in weight. For donors between 50 and 60 kg in weight, the extra-corporeal volume (ECV) must be calculated and never exceed 20% (see Appendix III)
- the minimum pre-donation platelet count must be 150×10^9/L
- the predicted post procedure platelet count must not be less than 100×10^9/L
- persons with sickle cell trait should not be accepted as apheresis donors

- deferral periods for platelet donors following ingestion of drugs affecting platelet function (e.g. aspirin or non-steroidal anti-inflammatory drugs) should be in accordance with the JPAC *Donor Selection Guidelines*.[2]

Red cell donation by apheresis technology

- Where a single unit of red cells is collected by this method, selection criteria and interdonation intervals as for whole blood apply (see Chapter 3) including those applicable to apheresis donors as above.

- For the collection of double units of red cells by apheresis, special considerations apply. Male and female donors must be greater than 70 kg in weight.

- The haemoglobin level to donate double units of red cells must be 140 g/L for both males and females.

- The interdonation interval for regular donation of double red cells by apheresis should not be less than 26 weeks (6 months) in the absence of iron supplementation. A shorter interval may be acceptable only if confirmation of iron-replete body stores can be accurately demonstrated and monitored.

Informed consent

Informed consent must be obtained by a doctor or registered nurse, fully conversant with the procedure. The consultant with responsibility for the apheresis programme must be personally responsible for delegating this task. A consent form, an example of which can be found in Appendix I, must be signed by each donor before the first donation. Separate informed signed consent for leucapheresis should be obtained by a medical officer on each occasion (see Appendix II).

Leaflets (nationally, locally or commercially produced) about donor apheresis should be available at the session and studied by prospective donors to assist in the process of obtaining fully informed consent.

In obtaining donor consent, the doctor or registered nurse must satisfy themselves that the donor has read the leaflet and has understood the following information:

- the purpose of the donation

- a description of the proposed apheresis procedure and its likely duration

- an explanation that a voluntary donor can withdraw consent at any stage of the procedure or of the apheresis programme

- a description of the common risks and discomfort involved in apheresis procedures. These include:

  - dizziness and fainting

  - haematoma formation

  - citrate toxicity

  - red cell loss if the procedure has to be aborted and it is considered unsafe to return the red cells

  - chilling on reinfusion.

If the donor asks further questions relating to more remote hazards, they must be answered truthfully, however unlikely these hazards may be.

At each attendance the donor must complete and sign the appropriate blood donor questionnaires, fitness to donate forms and consent to microbiological testing.

Medical assessment of donors

On entry to the apheresis programme the donor's health and general suitability should be assessed (see Chapter 3 and the JPAC *Donor Selection Guidelines*[2] or equivalent local document containing at least the same minimum standards). In addition as a minimum requirement for all donors the blood pressure, pulse and weight must be assessed and recorded. The donor's medical history, general health and age may mean that, at the discretion of the consultant with responsibility for apheresis, a more extended medical examination is required.

At each subsequent attendance the donor's health and suitability to continue on an apheresis programme should be reassessed.

If necessary, with the donor's consent, his/her General Practitioner may be contacted for further information.

Blood tests

At the initial visit, the following blood tests should be performed:

- full blood count for all donors
- serum albumin and total serum protein levels for plasma donors (total serum protein has no relevance to platelet donors).

The lower limit of acceptability for haemoglobin level should be as for normal whole blood donation. Special considerations as above apply to red cell donation by apheresis.

For all types of donor apheresis procedures, mandatory screening tests (see Chapter 10) must be performed at each donor attendance. In addition the platelet count should be performed at each visit for plateletpheresis donors.

The full blood count must be carried out at least annually for all donors and serum albumin and total serum proteins must be measured at least annually for plasma donors. A system must be in operation for regular review of these results, together with a documented protocol of the action to be taken in the light of any abnormal findings.

6.2 General specifications for apheresis sessions

Donor apheresis procedures for the collection of plasma, platelets, red cells or combinations of these may be carried out at fixed or mobile collection sites.

Leucapheresis procedures to collect, e.g. granulocytes, lymphocytes, peripheral blood progenitor cells, should only be performed at fixed apheresis units.

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

The consultant with responsibility for apheresis must ensure that, *as a minimum requirement*, all healthcare professionals involved with apheresis procedures receive basic life support training annually.

Resuscitation equipment as required by local and National Guidelines for blood donor sessions must be available at all sessions undertaking routine apheresis procedures.

6.3 Frequency of apheresis

A donor should not undergo a total of more than 24 plasma/plateletpheresis procedures per annum including not more than 12 leucapheresis procedures per annum. There should normally be a minimum of 48 hours between procedures and a donor should not

normally undergo more than two procedures within a seven-day period. Not more than 15 litres of plasma should be donated by one donor in a year.

Not more than 2.4 litres of plasma should be donated by one donor in any one-month period.

After a whole blood donation, or the loss of an equivalent number of red cells during an apheresis procedure, a donor should not normally donate plasma, platelets or leucocytes for a period of eight weeks. Intervals between successive red cell donations, by whatever technique should comply with existing Guidelines (see Chapter 3).

6.4 Volume collected

Attention must be paid during apheresis to the extra-corporeal volume (ECV) in order to avoid rendering the donor significantly hypovolaemic. Consideration must be given to the following factors:

- donor weight and estimated blood volume
- type of apheresis procedure: intermittent flow or continuous flow
- donor's haematocrit: this influences volume of plasma collected during any one cycle of an intermittent flow procedure (see Appendix V).

For any single apheresis procedure, the final collection volume should not exceed 15% of the total blood volume (TBV) excluding anticoagulant.

During apheresis procedures the ECV should not exceed 20% TBV (excluding anticoagulant). Some procedures may result in a total ECV of as much as 1 litre. In donors under 70 kg in weight this may represent more than 20% of their total blood volume and procedure may need to be adjusted to suit each individual donor's safety tolerance limits. Special considerations should be given during intermittent flow apheresis procedures (see Appendices III, IV and V). In non-obese donors TBV can be estimated as 70 mL/kg (see Appendix III).

ECV is the total volume of blood and plasma removed from the donor at any time. It includes all blood and plasma in collection packs and contained within the machine harness (volumes contained within collection harness can be obtained by reference to manufacturers' manuals).

Anticoagulant ratio during collection influences the volume of anticoagulant in collected plasma, e.g. anticoagulant in 1:12 ratio forms 14% of the final volume collected in a donor with haematocrit of 45% (see Appendix IV).

6.5 Staffing and training principles for apheresis sessions

- The medical consultant with responsibility for the apheresis programme should ensure that there are adequate staffing levels and ensure that staff are properly trained. This consultant may delegate day-to-day clinical responsibility to appropriately trained clinical staff. When donors are undergoing leucapheresis procedures (e.g. granulocyte, lymphocyte, peripheral blood progenitor cell collections) a suitably trained doctor must be immediately available to attend to the donor.
- One or more suitably trained doctors or registered nurses must be responsible for supervising the performance of venepunctures and for the supervision of machine procedures. The administration of drugs, e.g. local anaesthetic and citrate, must be supervised by a registered professional in accordance with *Guidelines for Administration of Medicines* (October 1992). During donation, donors should never be left in a room without the presence of an appropriately trained doctor or registered nurse.
- Training and certification of registered nurses undertaking apheresis procedures including training and monitoring of staff, performing venepunctures and obtaining

informed consent, must be in accordance with the current Nursing and Midwifery Council (NMC) Code of Professional Conduct.[3]

- The consultant in charge of apheresis in consultation with the nurse manager must ensure that there is an appropriate staffing level and skill mix to ensure donor safety and adequate monitoring of the equipment in use. Planned staffing levels should ensure that normally there is at least one member of suitably trained staff present for every two machines in use. For leucapheresis procedures, higher staffing ratios are required. A programme should be established for initial and continued training to ensure an appropriate level of proficiency.

  Basic life-support training, as defined by the UK Resuscitation Council, must be arranged for all donor facing healthcare professionals (see Section 6.2).

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

6.6 Collection, testing and storage of apheresis components

Specification and inspection of apheresis sets

Blood components must be collected by apheresis using sterile, single use, disposable items that are licensed by the Medicines and Healthcare Products Regulatory Agency (MHRA) and must be CE marked. The apheresis set for collection of components for direct clinical use must have a preconnected access needle to ensure a sterile pathway, and incorporate a bacterial filter in all non-preconnected fluid lines (e.g. anticoagulant saline, SAG-M) the anticoagulant line (not required if anticoagulant bag is preconnected). For dual needle procedures a preconnected needle is only essential for the access venepuncture.

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

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

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

Any faults detected before or during a procedure must be recorded in accordance with local quality systems. Any defects must be reported (see Section 6.8).

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

Specifications for automated donor apheresis machines (see also Section 9.5)

Machines must be correctly installed and commissioned according to each manufacturer's instructions. They must be CE marked.

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

Machines must comply with the relevant aspects of the Health and Safety at Work Act 1974,[4] Good Automated Manufacturing Practice (GAMP) *Guide for Validation of Automated Systems in Pharmaceutical Manufacture*[5].

Automated apheresis machines must have the following features:

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

Apheresis machines must be serviced in accordance with the manufacturer's instructions. A planned maintenance scheme should be followed. Machine maintenance and servicing must be documented and be in accordance with the procedures outlined in the appropriate Medicines and Healthcare Products Regulatory Agency publications: DB 9801, DB 9801 Supplement 1 and DB 2000(02).[6]

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

Anticoagulant

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

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

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

Guidance for collection procedures is identical to that for normal whole blood donations except for the following points:

- Labelling: apheresis packs and donor sample tubes must be labelled in accordance with local SOPs.

- Performance of the venepuncture: once the venepuncture is performed subsequent procedures such as releasing clamps on the bleed line should follow the protocol for the particular type of apheresis procedure being undertaken.
- Anticoagulation: occurs automatically in apheresis, but instructions are needed to ensure apheresis machine operators monitor flow of anticoagulant.
- Blood flow and monitoring: blood flow occurs automatically in apheresis, unless a satisfactory flow rate cannot be maintained.
- Instructions are needed for the apheresis operator in the event of a low flow or no flow situation. Particular care is needed when monitoring the return flow rate since most apheresis procedures operate with a pumped red cell return such that haematomas can rapidly form unless appropriate action is taken to prevent this from occurring.
- Sample collection: in apheresis sampling may take place at the beginning of a donation. The methods employed shall ensure an aseptic technique with no risk of contamination and be clearly defined in the sessional procedures SOP manual.
- Completion of the donation and quality control samples: a length of tubing should be left attached to the collection pack(s) as required for laboratory testing purposes. All used disposable equipment must be discarded in such a way as to prevent any risk to personnel, according to Health and Safety regulations.
- Final donation inspection: the collected apheresis components must be inspected routinely for the presence of haemolysis, unwanted red cell contamination, other abnormal appearance or evidence of clotting. Such changes may require a review of the apheresis procedure and/or equipment. Any suspected apheresis component abnormality must be recorded , the donation identified and reported in accordance with local quality systems.

Apheresis component testing and storage

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

6.7 Adverse events and adverse reactions

Serious adverse events and serious adverse reactions as defined in the EU Directives and Blood Safety and Quality Regulations 2005 (see Chapter 1 for full list of references) must be reported to the designated Competent Authority and Heads of Quality according to the protocols of the relevant blood establishment.

6.8 Notification of hazards

Any safety-related defects in the apheresis equipment, including the single use items, must be reported promptly via the established UK Blood Services quality mechanisms.

6.9 Donor compensation

The UK Transfusion Services have established procedures to ensure that any claim by a donor for compensation for any injury or loss allegedly attributable to having donated by apheresis will be considered sympathetically and decided promptly. A system of ex gratia payments and compensation operates throughout the UK Blood Services.

References

1. British Committee for Standards in Haematology (1998) Joint Working Party of the Transfusion and Clinical Haematology Task Forces. 'Guidelines for the clinical use of blood cell separators'. *Clinical and Laboratory Haematology*, **20**, pp265–78.

2. Joint UKBTS/NIBSC Professional Advisory Committee's (JPAC) *Donor Selection Guidelines* available at www.transfusionguidelines.org.uk.

3. Nursing and Midwifery Council (NMC) Code of Professional Conduct available at www.nmc-uk.org.

4. *Health and Safety at Work Act 1974*. Her Majesty's Stationery Office www.opsi.gov.uk.

5. Good Automated Manufacturing Practice (GAMP) *Guide for Validation of Automated Systems in Pharmaceutical Manufacture* available at www.ispe.org.

6. Medicines and Healthcare Products Regulatory Agency publications available at www.mhra.gov.uk:
DB 9801 *Medical Device and Equipment Management for Hospital and Community-based Organisations*.
DB 9801 Supplement 1 *Checks and Tests for Newly Delivered Medical Devices*.
DB 2000(02) *Medical Device and Equipment Management: Repair and Maintenance Provision*.

Appendix 1
Donor consent form: apheresis

A recommended format for a donor consent form where a Medical Officer is obtaining informed consent or a registered nurse – this duty having been delegated by the consultant in charge.

I..... (full name)
of..... (full address)
.....
.....Postcode.....
confirm that I have read and understood **and had an opportunity to ask questions about** the explanatory literature to **plasmapheresis/plateletpheresis/red cell apheresis. (* Delete as appropriate)**

I hereby acknowledge that I have volunteered to donate **plasma/platelets/red cells** by apheresis, using a cell separator. The nature and purpose of the apheresis procedure and the potential risks to the donor have been explained to me by:

Medical Officer/Registered Nurse..... (full name)

I consent to donate **plasma/platelets/red cells** by apheresis and I agree to undergo medical assessment which will also involve giving a sample of my blood. I consent to such further or alternative operative measures or treatment as may be found necessary during the course of the donation.

Signature of volunteer donor.....
Date.....

I confirm that I have explained the nature of the apheresis procedure and the significant risks involved, to the above donor and that he/she has read and understood the relevant literature.

Signature of Medical Officer/Registered Nurse.....
Date.....

Appendix II
Donor consent form: leucapheresis

A recommended format for a donor consent form where a Medical Officer is obtaining informed consent.

I..... (full name)
of..... (full address)
.....
.....Postcode.....
confirm that I have read and understood **and had an opportunity to ask questions about** the explanatory literature to **leucapheresis**.

I hereby acknowledge that I have volunteered to donate **white cells** by apheresis, using a cell separator. The nature and purpose of the apheresis procedure and the potential risks to the donor have been explained to me by:

Medical Officer..... (full name)

I consent to donate **white cells** by apheresis and I agree to undergo medical assessment which will also involve giving a sample of my blood. I consent to such further or alternative operative measures or treatment as may be found necessary during the course of the donation.

Signature of volunteer donor.....
Date.....

I confirm that I have explained the nature of the apheresis procedure and the significant risks involved, to the above donor and that he/she has read and understood the relevant literature.

Signature of Medical Officer.....
Date.....

Appendix III
Total blood volume and extra-corporeal volume tables

To avoid symptomatic donor hypovolaemia, final collection volume should not exceed 15% TBV (excluding anticoagulant).

Total ECV at any point should not exceed 20% TBV (excluding anticoagulant).

Table III.1 Total blood volume (TBV)* and extra-corporeal blood volume (ECV)

Weight: kg	50	55	60	65	70	75	80	85	90	95	100
Weight: st.	7.9	8.6	9.4	10.2	11.0	11.8	12.6	13.4	14.1	14.9	15.7
TBV – mL	3,500	3,850	4,200	4,550	4,900	5,250	5,600	5,950	6,300	6,650	7,000
15% TBV	525	577.5	630	682.5	735	787.5	840	892.5	945	997.5	1,050
20% TBV	700	770	840	910	980	1,050	1,120	1,190	1,260	1,330	1,400

* Based on the assumption that in the normal healthy adult TBV = 70 mL/kg, i.e. TBV of a 70 kg adult 4.9 litres

Appendix IV
Citrate anticoagulants and the avoidance of citrate toxicity

Based on studies undertaken in 1989-90 the following recommendations can be made to avoid citrate toxicity during apheresis procedures.

Intermittent flow cell separator machines

The reinfusion rate of citrated blood or plasma should not exceed 0.015 mmol citrate/kg/min.

Continuous flow cell separator machines

The continuous reinfusion rate of citrated blood or plasma should not exceed 0.01 mmol citrate/kg/min.

Maximum acceptable reinfusion rates (mL/min for a 70 kg donor)

For the four citrate anticoagulants listed that are commonly used in the UK, the above recommendations are represented in Table IV.1.

Table IV.1 Citrate anticoagulants

				Plasma		Whole blood	
AC	**AC:blood ratio**	**Average plasma citrate mmol/L**	**AC volume in collected plasma %**	**Int.**	**Cont.**	**Int.**	**Cont.**
CPD-50	1+15(1:16)	17	11	60	33	100	60
Acid CPD	1+11(1:12)	19	14	55	29	90	50
ACD-A	1+11(1:12)	16	14	60	33	100	55
ACD-A	1+7(1:8)	23	20	45	24	75	40

AC = Anticoagulant
Int. = Intermittent flow cell separator
Cont. = Continuous flow cell separator

Packed cells may be reinfused as quickly as the characteristics of the return system and the viscosity will allow, but not normally faster than 130 mL/min.

NB – For donors weighing less than 70 kg, these reinfusion rates need to be suitably adjusted downwards to avoid citrate toxicity occurring. They may also be adjusted upwards for donors above 70 kg in weight.

If different anticoagulant formulations or ratios are used other than those represented in Table IV.I, the procedure should be validated

- to ensure plasma citrate levels are within the required range for fractionation purposes, i.e. 15–25 mmol/L
- to ensure the citrate molar reinfusion rate does not exceed these recommended maximum acceptable limits.

Final collection volume must not exceed 15% TBV (excluding anticoagulant).

Appendix V
Volume of blood processed per pass

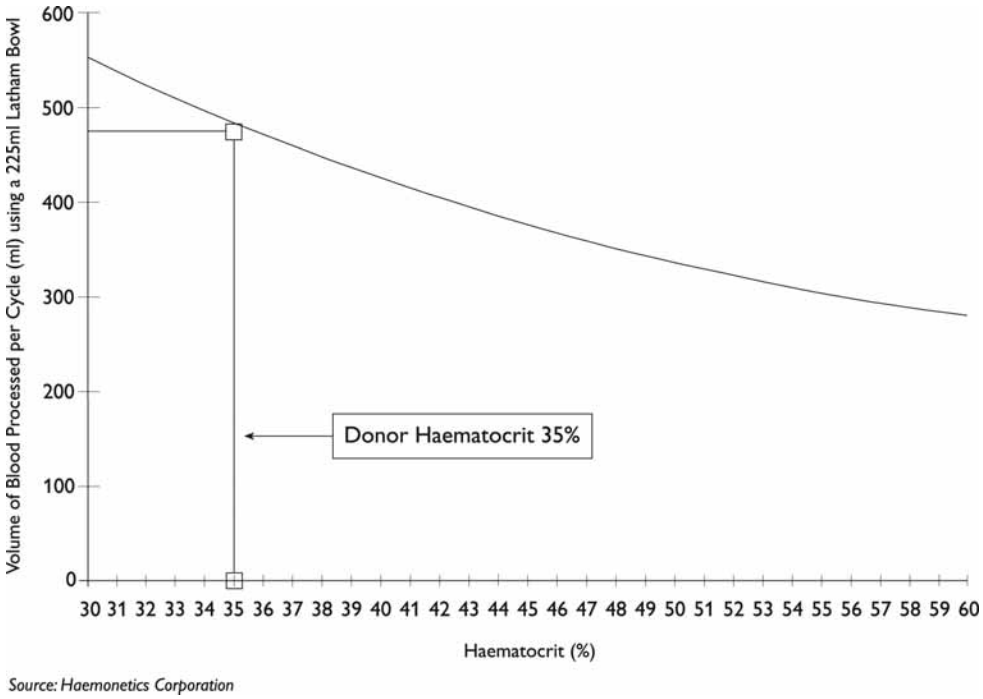


Figure V.1 Volume of blood processed per cycle vs donor haematocrit

Based on haematocrit of 0.80 in bowl and a flow rate of 60–80 mL/min. Includes harness volume of 35 mL.

These figures draw attention to the fact that for donors with a low haematocrit an increased volume of blood is processed at each pass. This will influence the ECV accumulating throughout the procedure and this particular group of donors may become symptomatically hypovolaemic.

Chapter 7

Evaluation and manufacture of blood components

Scope of the Guidelines

These Guidelines provide a framework on which blood establishments should assemble standard operating procedures (SOPs) for the manufacture of blood components.

These guidelines apply to single-donor and small-pool components (<12 donors) prepared from units of whole blood or by apheresis.

Blood establishments should ensure that the hospital blood banks, which they supply, are informed of these component production guidelines.

7.1 Setting and maintaining specifications

The wide variability of the source material from which blood components are prepared makes it difficult to set stringent limits. Nevertheless, realistic minimum specifications should be set and complied with.

Component and process quality monitoring results should be subjected to statistical analysis so that trends can be identified.

If the results of analyses show a consistent trend towards the minimum requirements specified in Chapter 8, the cause should be investigated. The criteria to be investigated must be detailed in the relevant SOP together with the corrective action to be taken. The steps to be considered should include the following:

- an investigation of the collection, testing, production and distribution procedures as appropriate
- checking that procedures are up-to-date and are not being deviated from
- checking the operation of equipment and storage conditions (this may include reviewing validation documentation and/or revalidation).

The person responsible for quality assurance and/or production may initiate investigations beyond the scope of written procedures.

7.2 Component and process monitoring tests

These Guidelines also indicate the minimum level of other process monitoring tests necessary to ensure components are prepared to specification.

Any assay used for blood component quality monitoring should be validated and documented before introduction and before any changes to methodology or manufacture are brought into use. Blood establishments should ensure that they participate in the National External Quality Assessment Scheme (NEQAS) or other available external quality assurance schemes for the assays used to assess component quality.

Each component should be visually inspected at each stage of processing and immediately prior to issue. The component must be withdrawn if there is evidence of leakage, damage to or fault in the container, excessive air, suspicion of microbial contamination or any other contraindications such as platelet clumping, unusual turbidity, haemolysis or other abnormal colour change.

Sampling procedures

Sampling procedures should be designed and validated, prior to acceptance as standard practice, to ensure the sample truly reflects the contents of the component pack. The Chair of SAC on Blood Components Technical Sub-Committee maintains a database of sampling procedures that have been validated to meet this requirement, against whole component units mixed to homogeneity.

Validation of sampling procedures should be repeated before application to new components or different quality parameters, or before the introduction of new sampling equipment. Also there should be a procedure for continuous assessment of staff competence/sampling techniques.

Where test samples are removed from a component to be issued for transfusion, the sampling procedure should be designed and validated to ensure that the sterility and essential properties of the component are not adversely affected.

Samples for leucocyte counting must be taken and tested within 48 hours of donation, unless the sampling and testing times used have been validated to yield equivalent results.

Frequency of tests

The regularity with which components are made and the extent of their compliance with specification influences the frequency with which component and process monitoring tests are required.

If there is a trend towards the minimum requirements specified in Chapter 8, the frequency of quality monitoring tests should be increased according to defined procedures until the relevant component attributes have been brought into control.

The testing protocol should take into account all major production variables and ensure samples are representative of these.

Component weight: volume

To provide information, which is useful for clinicians, the component specifications given in Chapter 8 generally require the component label to indicate a volume. This may be either the calculated volume or nominal volume and the nominal volume may be based on a national or locally established volume specification.

Since volume generally is calculated by dividing the component weight by its specific gravity, to ensure some element of standardization the following conventions should apply:

- whole blood volume is most appropriately calculated by deducting the weight of the pack assembly and dividing the resulting weight by the nominal specific gravity of 1.06
- to provide quality monitoring data that demonstrates the capability of the blood collection process, deduct the weight of the anticoagulant before converting to volume
- to provide quality monitoring data that reflects the provision of whole blood as a component, the volume given on the component label should include whole blood and anticoagulant

- for red cell components, volume is calculated by weighing the pack, deducting the weight of the pack assembly only and dividing the resultant weight by the nominal specific gravity 1.06. The weight of anticoagulant and, if relevant, additive solution are not deducted when calculating the volume of red cell components
- for platelets and plasma components, volume is calculated by weighing the pack, deducting the weight of the pack assembly and dividing the resulting weight by the nominal specific gravity of 1.03.

7.3 Component processing

Premises

Component Production areas should satisfy the requirements defined in the current *Rules and Guidance for Pharmaceutical Manufacturers and Distributors, 2002.*[1] In addition:

- the ambient temperature of blood component processing areas should be maintained within a range that would not be expected to adversely affect component viability/shelf life
- where appropriate, steps should be taken to ensure that air quality in the blood component processing environment does not increase the bioburden to which blood components are exposed.

The starting material

The starting material for component preparation is whole blood or the products of apheresis collected from donors who satisfy current donor selection criteria. Components must be collected into blood packs/apheresis harness assemblies that are CE-marked.

Before use, packs/apheresis harness assemblies that have not previously been validated, or contain component parts that have not previously been validated should be subject to validation or process qualification as appropriate according to the protocols set out in Chapter 9.

Starting material for component preparation should be transported as described in Section 7.10.

Prevention of microbial contamination

Infections associated with the microbial contamination of blood and blood components still occur. Whilst there is no evidence to suggest that routine, retrospective sterility testing of blood components diminishes or eliminates such instances of infection, the following measures will minimize the risks:

- creating and maintaining the highest level of awareness among all personnel of the constant care and attention to detail needed to minimize microbial contamination e.g. validation and periodic monitoring of the effectiveness of venepuncture site preparation
- using validated procedures designed to minimize microbial contamination of the environment and prevent microbial contamination of components
- diverting the first part of the donation into a sample pouch, to avoid entry into the primary donation. This may be used for mandatory screening tests
- monitoring the microbial load in equipment and in the environment of component preparation areas.

It is important that data derived from such monitoring exercises are accumulated and regularly examined with a view to taking appropriate action.

Screening of platelet components for bacterial contamination is being evaluated or implemented by some blood establishments to address concerns over bacterial contamination.

Closed system

The term 'closed system' refers to a system in which the blood pack assembly is manufactured under clean conditions, sealed to the external environment and sterilised by an approved method.

Venting

With the exception of the venepuncture procedure and strict requirements for open processing (see Section 7.3), the blood pack system and its contents must not be vented to the external environment at any stage during blood collection or processing.

Sealing

Blood pack and apheresis harness fluid pathways must at all times be protected from the external environment by

- hermetic seal(s) incorporated during manufacture or Blood Service use
- other validated devices for effecting a permanent seal
- break seal closure(s)*
- port(s) incorporating a tamper proof closure and pierceable membrane*
- microbial filter(s)*

* These devices must comply with the requirements of relevant standards for medical devices, including ISO 3826 Parts 1 and 3, must be validated by the manufacturer and provided with clear instructions for use.

Before severing any sub-component of the pack assembly, the pack contents must first be protected from the external environment by a minimum of one permanent seal made using a validated hermetic sealer cleaned and maintained according to SOP.

Temporary sealing clamps/clips must be used only to control the flow of fluid within a closed system. They must not be used as the sole means of protection from the external environment.

When a device for making a sterile connection is used the system can be regarded as closed providing that the process of joining and sealing has been validated and shown not to lead to an increased risk of microbial contamination of the component. The procedure for use should ensure that the operator carefully checks the suitability of every weld and also pays particular attention to effective cleaning of the working parts of the equipment.

Cleaning should be by validated procedure with regular checks to ensure conformance to procedures.

Pressure or tensile testing the strength of welds should be performed during the validation or Process Qualification of equipment. Guidance on methods for this are available from the chair of SAC on Blood Components Technical Sub-Committee.

Where a sterile connecting device has been used to add satellite packs, the components must not be issued with the weld in place.

Pre-donation sampling

Pre-donation sampling must only be carried out using blood pack assemblies that incorporate a device to prevent the return of blood and or air from the sample pouch towards the donor and donation. The procedure must be validated by the Blood establishment and documented in blood collection SOP.

After filling, the sample pouch must be permanently sealed from the donation before collecting blood samples.

In the event of inadvertent contamination of the donation by blood or air from the sample pouch, the donation must be discarded.

Open system

The term 'open system' refers to a system in which the integrity of the closed system must be breached but where every effort is made to prevent microbial contamination by operating in a clean environment, using sterilised materials and aseptic handling techniques. In such circumstances, positive pressure should be exerted on the original container and maintained until the container is sealed. Open system processing should be undertaken in a designated clean environment as defined in the current *Rules and Guidance for Pharmaceutical Manufacturers and Distributors 2002*.[1]

The sterility of components prepared in an open system should be monitored using validated methods.

Blood components prepared by an open system should be used as soon as possible. If storage is unavoidable, components with a recommended storage temperature of 22°C ±2°C should be used within six hours. Components with a recommended storage temperature of 4°C ±2°C should be used within 24 hours.

Components are rendered unsuitable for clinical use when breached and the requirements defined for an open system have not been observed, unless issued under medical concession.

Any new development in component preparation by an open procedure must be validated to ensure the maintenance of sterility before the procedure can be used to produce components for therapeutic use.

Procedures for collecting samples for sterility testing must not adversely affect the sterility of components intended for subsequent transfusion.

7.4 Component shelf life

Component storage specifications are given in Chapter 8.

Where components are pooled or undergo procedures that influence the shelf life, the maximum shelf life of the component must not exceed the expiry date of the oldest constituent component or the expiry date of the new component produced by the procedure, whichever is the shorter.

For all other components the date of collection will be assigned Day 0 of the shelf life. Day 1 of storage will commence at one minute past midnight on the day of collection.

7.5 Labelling

Component labelling

Barcoded labels and on-demand printing must be used whenever possible.

The design, content and use of labels for blood components should conform to specifications set out in Chapters 25 and 27.

Procedures should be established to ensure labels are satisfactory for their intended use.

Pre-printed labels to be attached to blood donations, documentation and components should be stored under secure conditions.

Donation/donor identification

The use of a unique bar coded/eye-readable donation number links the donation to its donor. Donation numbers must be attached to all integral packs, sample tubes and corresponding documents at the time of donation.

When component production requires the use of subsidiary packs which are not an integral part of the pack assembly, e.g. filtration, pooling, freezing, a secure system must

be in place to ensure that the correct eye-readable and barcoded donation number is placed on each additional pack used.

When components are pooled there should be a system that ensures that the pool carries a unique barcoded and eye-readable identification number(s). This barcode must be able to be read by component manufacturers and blood banks.

When a component is divided a secure system must be in place to ensure that all sub-batches can be traced.

7.6 Component storage

Specifications for component storage areas

Storage areas for blood components must operate within a specified temperature range and should provide adequate space, suitable lighting and be arranged and equipped to allow dry, clean and orderly storage.

Good manufacturing practice requires that components of different status are appropriately identified and effectively separated.

Recognised status categories include:

Quarantine

Procedures should ensure that untested components are not quarantined with components which have produced, or are likely to produce, repeatably reactive results in mandatory microbiological screening tests.

Secure and exclusive quarantine storage should be available for known biohazard material awaiting disposal (see Section 7.7).

Non-conforming

Components which do not comply with the specification for mandatory tests or are otherwise unsuitable for transfusion should be categorized as non-conforming. Normally, such components would be discarded. However, if they are to be issued for therapeutic, reagent or research use, a concessionary release procedure must be used (see Section 7.9).

Returned

Components which have been returned from blood transfusion laboratories outside the direct control of the Blood Centre normally should not be returned to stock.

Components which have been returned to the Blood Centre but which have been maintained within specification should be held securely pending possible reinstatement to stock by a designated person.

Stock

Only those components which have been deemed satisfactory for issue by a designated person should be held in stock (see Section 7.8).

Appropriate security and status labelling of component storage areas are essential.

A current inventory should be maintained of components in each storage category/area.

Areas/equipment in which components are to be stored should be validated before their introduction into routine use and checked for calibration to a documented schedule thereafter.

A permanent, continuous record of storage temperatures should be made, reviewed and stored. There should be a log of alarm events that describes the actions taken.

Procedures for component storage

Written procedures must be established for the storage of blood components. These should include the following:

- a procedure to ensure components are not released to stock unless authorized by a designated person (see Section 7.8)
- definitions of the designated storage areas including the storage specification, the status of components to be stored in each area and the persons who are authorised to access each specific area
- procedures for validating and monitoring the conditions of storage
- procedures for ensuring the good order and cleanliness of storage areas
- procedures to ensure the storage of blood components does not jeopardize their identity, integrity or quality
- a procedure which ensures appropriate stock rotation.

7.7 Non-conforming components and biohazards

Discard of non-conforming components

Procedures for the discard of non-conforming components should ensure that an appropriate record of discard is maintained. This includes:

- the donation number
- the component identity
- the reason for discard
- the date of discard
- the identity of the person effecting the discard.

If the discard process involves recording as a discard on computer software and physically discarding, then adequate records are required for both steps.

Biohazards

Components from donations that are repeatably reactive in mandatory microbiological screening tests or from donors whose records indicate their components should be destroyed because they are on a high risk deferral registry or because of previous mandatory test results are classified as biohazards

Secure and effective procedures are required to ensure that all components and samples from biohazard donations are retrieved for safe disposal in accordance with Blood Service Policies and with *Safe Disposal of Clinical Waste*, Second Edition, 1999, Health Services Advisory Committee, Health and Safety Commission.[2] Procedures should include

- a system which ensures all components prepared from any donation can be traced
- maintaining a record of the person whom retrieves each biohazard component, including laboratory samples.

When biohazard material, e.g. plasma, is retained for laboratory use, it must be appropriately labelled to prevent it ever being used for therapeutic purposes and must be stored in a secure freezer or other storage unit that is clearly labelled to prohibit the storage of material for therapeutic use. An inventory of freezer (or other storage unit) contents of such samples, record of 'sample' retention, reason for retention, and fate should be maintained.

7.8 Component release

All components must be appropriately labelled in accordance with these guideline specifications including those general guidelines outlined in Section 7.5 and Chapters 25 and 27.

Standard procedures must ensure that blood and blood components cannot be released to stock until all the required laboratory tests, mandatory and additional, have been completed, documented and approved within a validated system of work and it has been ascertained that conditions of production and storage have been satisfactory. Compliance with these requirements may be achieved by the use of a computer program, or suite of programs, which requires the input of valid and acceptable test results for all the mandatory and required laboratory tests before permitting, or withholding, the release of each individual unit.

Where a computer-based system is not used or is temporarily unavailable, documented approval for the release of each individual unit should be by a designated person.

All biohazard donations and components otherwise unsuitable for issue should be reconciled and accounted for, preferably prior to releasing blood components to stock.

7.9 Release of components which do not conform to specified requirements

Blood and/or blood components may be issued for research, reagent and, in exceptional cases, for therapeutic use when they do not conform to specified requirements. Each blood establishment must have written instructions on the procedure which detail the circumstances under which such concessionary issues can be made and the procedures to be followed.

For major non-conformances in components intended for therapeutic use (e.g. an HLA-matched platelet that is significantly below specified cell counts, extension of shelf life for an autologous donation or, in extreme circumstances, donor sample not tested for mandatory microbiological marker, etc.) the instructions should, as a minimum, include the following:

- that such component issues are authorised by a blood establishment consultant to the relevant registered medical practitioner
- that the reason for the issue is fully documented
- that a verbal and written warning indicating an increased level of risk is given by a blood establishment consultant to the receiving registered medical practitioner who should sign a statement indicating that he/she is willing to accept these risks
- that the name of the recipient is entered on the issue documentation
- that the component is clearly identified with a label indicating that it does not conform to specification, the details of the non-conformance, the name of the recipient and that it must not be used for any other patient.

Issues of non-conforming components should be subjected to a formal review process.

Minor non-conformances in components intended for therapeutic use (e.g. non-critical blood pack faults, minor label issues) should be referred for assessment by the quality manager.

7.10 Transportation of blood components

General considerations

Donated blood and blood components should be transported by a secure system using transit containers, packing materials and procedures which have been validated for the

purpose to ensure the component surface temperature can be maintained within the correct ranges during transportation (Chapter 8).

Monitoring of routine transport temperatures should be performed periodically.

Revalidation should be performed if changes are made to the transport containers, packing materials or procedures.

As far as is practicable, transit containers should be equilibrated to a component's storage temperature prior to filling.

Transport containers should be appropriately labelled and should be secure and protect components and samples from damage during transit.

Documentation should accompany components in transit to permit their identification.

Transport containers should not be exposed to temperatures beyond the range and time for which they have been validated.

Where melting ice is used to achieve an appropriate storage temperature, it should not come into direct contact with the components.

Dead air space in packaging containers should be minimised.

Written procedures for the transportation of components should be established and should ensure that the guidance given above is complied with. In addition, written procedures should include the following:

- definition of approved systems of packaging, transportation and transport conditions required for each component
- a requirement for monitoring approved systems of packaging and transportation.

Transportation from collection site to processing centre

Blood and samples from donor sessions must be transported to the receiving Blood Centre under appropriate conditions of temperature, security and hygiene.

Donations from which it is intended to prepare platelets should be transported in conditions that ensure the surface temperature of the blood packs does not drop below 18°C.

Blood and samples being transported from donor sessions must be accompanied by documentation, which ensures that all donations in the consignment can be accounted for. (NB 'documentation' includes information in writing or in electronic format).

Transport of components from blood establishments to hospital blood banks/users

Blood components should be transported under conditions which are as close as possible to their specific storage requirements and comply with the requirements of Chapter 8. Transport time should be kept to a minimum.

It should be noted that, occasionally, red cell components are issued before they have been cooled to their storage temperature (4°C ±2°C). In such circumstances, it may be neither possible nor necessary to maintain the transport temperature within the range 2°C to 10°C and local judgement should be exercised.

Components despatched from a blood establishment should be accompanied by a despatch note detailing as a minimum:

- the donation number of each component
- if relevant, the component's ABO and Rh D blood group

- the signature(s) and designation of the person(s) responsible for the issue
- space for the signature(s) and designation of the person(s) of the person receiving the consignment.

A copy of the signed and annotated despatch note should be returned to the Blood Centre for storage.

7.11 Component recall

There must be a documented system available in each blood establishment whereby adverse effects caused by the administration of any component, or the identification of a component quality problem, can enable the recall, if appropriate, of all unused components derived from that donation or all donations which are a constituent of a component pool. Similarly, there must be a documented system in each Blood Centre for the recall of any component or constituent of a component pool where reasonable grounds exist for believing it could cause adverse effects.

Any recall of a component should lead to a thorough investigation with a view to preventing a recurrence.

References

1. Medicines Control Agency (2002). *Rules and Guidance for Pharmaceutical Manufacturers and Distributors 2002, Sixth Edition*. Norwich: The Stationery Office. ISBN 0 11 322559 8.

2. Health and Safety Executive (HSE). *Safe Disposal of Clinical Waste, Second Edition*. Sudbury: HSE Books. ISBN 0 7176 2492 7.

Chapter 8

Specifications for blood components

This chapter details process, product, labelling, discard, storage and transport specifications, which are given below and tabulated in Section 8.29.

8.1 Leucocyte depletion

With very few stated exceptions (e.g. granulocytes), from November 1999 all allogeneic blood components produced in the UK have been subjected to a leucocyte depletion process. The term 'LD' may be used where necessary instead of 'Leucocyte Depleted' or 'leucocyte depletion' although component names will state 'Leucocyte Depleted' where appropriate. The UK specification for leucodepletion is that a minimum of 99% of leucocyte depleted components from relevant processes should have less than 5×10^6 leucocytes with 95% confidence and more than 90% of components should contain less than 1×10^6 leucocytes.

Leucocyte depletion can be achieved by a number of methods, which must be validated before use. If filtration is used the recommended capacity of the filter must not be exceeded.

Currently, it is not feasible to assess all components for the effectiveness of the leucodepletion process. Therefore, the UK Blood Transfusion Services should apply recognized statistical process monitoring (SPM) methodology such as those proposed by the ISBT BEST Expert Working Party, Transfusion 1996[1] to ensure the following:

- conformance of the process to the LD process specification
- identification of LD component specified limit failures
- stability of the process over time.

The residual leucocyte testing schedule should be defined in process monitoring and conformance checking procedures.

It is advisable to identify results to a production run or 'batch' and to ensure conformance of components to relevant specifications before release of components to stock or to ensure that a monitored filter batch is producing components that conform to specification.

A leucocyte depletion process is controlled if a control chart or equivalent is in use and does not currently display control limit or trend warnings.

A leucocyte depletion process is uncontrolled if a control chart or equivalent is not in operation for the process or if a current control chart or equivalent displays control limit or trend warnings.

Where SPM methodology is not judged appropriate due to an inability to control the process or the production of small numbers of components, all components must have been shown to contain less than 5×10^6 leucocytes or less than 2.5×10^6 leucocytes (Platelets for IUT only) prior to being released to stock.

Issue (to stock) of components, which do not meet the leucocyte depletion specified limit, must follow a concessionary release procedure (see Section 7.9).

Patient-designated components should not be discarded before referral to a clinician.

Secondary components or split components produced from primary components do not require a leucocyte count provided the primary process is controlled or the individual primary component is tested and found to be acceptable.

Plasma components derived from whole blood filtration do not require residual leucocytes to be monitored provided the associated red cell process is controlled.

Leucocyte or platelet counts on components produced from frozen and thawed material should be made, where necessary, prior to the initial freezing process unless otherwise validated.

If the leucodepletion process transfers the final component into a pack that was not part of the original pack assembly, a secure system must be in place to ensure the correct identification number is put on the final component pack.

Leucocyte depletion of components should take place before the end of Day 2 (Day 0 is the day of collection).

Once a red cell component has been cooled to its storage temperature (i.e. 4°C ± 2°C) prior to leucodepletion, and when leucodepletion by filtration is to take place at ambient temperature, the ambient temperature of the room in which filtration takes place should not exceed 26°C (see also Section 7.3).

If components are removed from their designated storage temperature to undergo a leucodepletion process, they must be returned to their storage temperature as soon as possible and in any event within three hours (see also Section 7.3).

8.2 Other component specifications

Other component and process monitoring specifications are detailed later in this chapter. As far as possible, all parameters tested should be derived from a single component. Because of biological variability, it is acceptable if a minimum of 75% of the results from component and process monitoring tests (other than leucocyte depletion specifications and Platelets for IUT) achieve the specifications.

Yield specifications (e.g. Platelet yield/unit, total Hb/unit) for components produced by splitting primary components should be the indicated specification for the primary component divided by the number of split components produced.

Haemolysis measurements on red cell components are performed at the end of the component shelf life. Due to intermittent availability of outdated red cell components, each primary process should be validated to give haemolysis of <0.8% of the red cell mass at the end of component shelf life in >75% of components with a minimum of 20 components tested. Re-validation of the red cell preparation processes for red cell haemolysis must be performed at least annually and after any alteration to the production methodology.

For mandatory microbiology screening and blood grouping tests, all components must conform to the requirements specified in Chapter 10. Concessionary procedures for release of components that do not conform to these requirements are given in Section 7.9.

8.3 Production advice

The timing and method of separation depends on the components to be prepared from a given donation.

If the production process, washing or splitting process transfers the final component into a pack that was not part of the original pack assembly, a secure system must be in place to ensure the correct identification number is put on the final component pack.

Where a production process amends the expiry date of the component, there are different consequences, dependent on the process.

- Further processing or irradiation may reduce the expiry date of the component. Here the expiry date of the new component must not exceed that of the primary component or the expiry date limitations conferred by the process.
- Components produced by pooling primary components must have an expiry date of the shortest dated component used.
- Remanufacturing of neonatal or paediatric red cell components into adult components, to avoid unnecessary wastage, where the expiry date may be extended.
- Processing of a red cell component to allow frozen storage will result in a lengthened expiry date.

The method of preparation should ensure that plasma components have the maximum level of labile coagulation factors with minimum cellular contamination.

Donations from donors with clinically significant HPA antibodies should not be used for the production of plasma-rich blood products (e.g. fresh frozen plasma, platelet concentrate, whole blood, cryoprecipitate). Red cells suspended in additive solution can be produced from such donations.

Platelet and plasma components should not be produced from lipaemic, icteric or red cell contaminated donations. Procedures should exist for assessing these findings.

An upper platelet concentration should be assigned for each platelet component type based on pack validation data or the pack manufacturer recommendations.

pH measurements on platelet components should be made between 20–24°C or the measurements corrected to 22°C.

Blood components for use in IUT, neonates and infants (see also Section 8.18), and plasma components for direct clinical use must be derived from selected donors who fulfil the following criteria:

- have given at least one donation in the last two years
- have not produced a repeat reactive result in microbiology tests that were designated as mandatory at that time
- negative results were obtained for mandatory microbiology markers with the current donation
- the current donation and donor status otherwise meet component release criteria.

Each component should be visually inspected at each stage of processing and immediately prior to issue. The component must be withdrawn if there is evidence of leakage, damage to or fault in the container, excessive air, suspicion of microbial contamination or any other contra-indications such as platelet clumping, unusual turbidity, haemolysis or other abnormal colour change.

8.4 Whole Blood, Leucocyte Depleted

A unit of blood collected into an anticoagulant, containing less than 5×10^6 leucocytes.

Technical information

- A unit of whole blood consists of 450 mL ± 10% of blood from a suitable donor (see Chapter 3), plus 63 mL of anticoagulant, which is then leucocyte depleted, and stored in an approved container.
- Whole Blood, Leucocyte Depleted should be transfused through a 170–200μm filter.

Labelling (for general guidelines see Section 7.5)

The following shall be included on the label:

(* = in eye-readable and UKBTS approved barcode format.)

- whole blood, Leucocyte Depleted* and volume
- the blood component producer's name*
- the donation number*
- the ABO group*
- the RhD group stated as positive or negative*
- the name, composition and volume of the anticoagulant solution*
- the date of collection
- the expiry date*
- the temperature of storage
- the blood pack lot number.*

In addition, the following statements should be made:

INSTRUCTION
Always check patient/component compatibility/identity
Inspect pack and contents for signs of deterioration or damage
Risk of adverse reaction/infection

Storage (for general guidelines see Section 7.6)

- The component may be stored for a maximum of 35 days at a core temperature of 4°C ± 2°C if an adenine supplemented anticoagulant is used, otherwise the maximum period of storage is 28 days at a core temperature of 4°C ± 2°C.
- Variation from the core temperature of 4°C ± 2°C must be kept to a minimum during storage and restricted to any short period necessary for examining, labelling or issuing the component.
- Exceptionally, i.e. due to equipment failure at a Blood Centre, red cell components which have been exposed to a core temperature not exceeding 10°C and not less than 1°C may be released for transfusion provided:
  - that the component has been exposed to such a temperature change on one occasion only
  - that the duration of the temperature change has not exceeded five hours
  - that a documented system is available in each Blood Centre to cover such eventualities
  - that adequate records of the incident are compiled and retained.

Testing

In addition to the mandatory and other tests required for blood donations described in Chapter 10, and leucocyte counting (see Sections 7.2. and 8.1.), a minimum of 75% of those components tested for the parameters shown in Table 8.1 shall meet the specified values.

Table 8.1 Whole blood, leucocyte depleted – additional tests

Parameter	Frequency of test	Specification
Volume	1%	470 mL±50 mL**
Haemolysis	As per Section 8.2	<0.8% of red cell mass
Haemoglobin content	1%	>40 g/unit
Leucocyte count*	As per Sections 7.2 and 8.1	$<5 \times 10^6$/unit

* Methods validated for counting low numbers of leucocytes must be used
** After volume losses resulting from leucodepletion

Transportation (for general guidelines see Section 7.10)

For red cell components, transit containers, packing materials and procedures should have been validated to ensure the component surface temperature can be maintained between 2°C and 10°C during transportation. Additionally:

- the validation exercise should be repeated periodically
- if melting ice is used, it should not come into direct contact with the components
- dead air space in packaging containers should be minimized
- as far as is practicable, transit containers should be equilibrated to their storage temperature prior to filling with components
- transport time normally should not exceed 12 hours
- in some instances it is necessary to issue red cell components that have not been cooled to their storage temperature prior to placing in the transit container. The transport temperature specified above is not applicable for such consignments.

8.5 Red Cells, Leucocyte Depleted

A red cell component containing less than 5×10^6 leucocytes.

Technical information

- A red cell component prepared by removing a proportion of the plasma from leucocyte depleted whole blood or by leucodepleting plasma reduced red cells.
- Red Cells, Leucocyte Depleted should be transfused through a 170–200μm filter.

Labelling (for general guidelines see Section 7.5)

The following shall be included on the label:

(⋆ = in eye-readable and UKBTS approved barcode format.)

- red cells, Leucocyte Depleted⋆ and volume
- the blood component producer's name⋆
- the donation number⋆
- the ABO group⋆
- the RhD group stated as positive or negative⋆
- the name, composition and volume of the anticoagulant solution⋆

- the date of collection
- the expiry date*
- the temperature of storage
- the blood pack lot number.*

In addition, the following statements should be made:

INSTRUCTION
Always check patient/component compatibility/identity
Inspect pack and contents for signs of deterioration or damage
Risk of adverse reaction/infection

Storage (for general guidelines see Section 7.6)

- The component may be stored for a maximum of 35 days at a core temperature of 4°C ± 2°C if an adenine supplemented anticoagulant is used, otherwise the maximum period of storage is 28 days at a core temperature of 4°C ± 2°C.
- Variation from the core temperature of 4°C ± 2°C must be kept to a minimum during storage and restricted to any short period necessary for examining, labelling or issuing the component.
- Exceptionally, i.e. due to equipment failure at a Blood Centre, red cell components which have been prepared in a closed system and exposed to a core temperature not exceeding 10°C and not less than 1°C may be released for transfusion provided:
  - that the component has been exposed to such a temperature change on one occasion only
  - that the duration of the temperature change has not exceeded five hours
  - that a documented system is available in each Blood Centre to cover such eventualities
  - that adequate records of the incident are compiled and retained.

Testing

In addition to the mandatory and other tests required for blood donations described in Chapter 10, and leucocyte counting (see Sections 7.2 and 8.1), a minimum of 75% of those components tested for the parameters shown in Table 8.2 shall meet the specified values.

Table 8.2 Red cells, leucocyte depleted – additional tests

Parameter	Frequency of test	Specification
Volume	1%	280 ± 60 mL
Haemoglobin content	1%	>40 g/unit
Haemolysis	As per Section 8.2	<0.8% of red cell mass
Leucocyte Count*	As per Sections 7.2 and 8.1	$<5\times10^6$/unit

* Methods validated for counting low levels of leucocytes must be used.

Transportation (for general guidelines see Section 7.10)

For red cell components, transit containers, packing materials and procedures should have been validated to ensure the component surface temperature can be maintained between 2°C and 10°C during transportation. Additionally:

- the validation exercise should be repeated periodically
- if melting ice is used, it should not come into direct contact with the components

- dead air space in packaging containers should be minimized
- as far as is practicable, transit containers should be equilibrated to their storage temperature prior to filling with components
- transport time normally should not exceed 12 hours
- in some instances it is necessary to issue red cell components that have not been cooled to their storage temperature prior to placing in the transit container. The transport temperature specified above is not applicable for such consignments.

8.6 Red Cells in Additive Solution, Leucocyte Depleted

A red cell component containing less than 5×10^6 leucocytes and suspended in an approved additive solution.

Technical information

- A red cell component prepared by removing a proportion of the plasma from leucocyte depleted whole blood and suspending in an approved additive solution. Leucodepletion may be carried out on either the whole blood starting material or on the final component.
- Red Cells in Additive Solution, Leucocyte Depleted should be transfused through a 170–200μm filter.

Labelling (for general guidelines see Section 7.5)

The following shall be included on the label:

(⋆ = in eye-readable and UKBTS approved barcode format.)

- red cells in additive solution, Leucocyte Depleted⋆ and volume
- the blood component producer's name⋆
- the donation number⋆
- the ABO group⋆
- the RhD group stated as positive or negative⋆
- the name, composition and volume of the additive solution⋆
- the date of collection
- the expiry date⋆
- the temperature of storage
- the blood pack lot number.⋆

In addition, the following statements should be made:

INSTRUCTION
Always check patient/component compatibility/identity
Inspect pack and contents for signs of deterioration or damage
Risk of adverse reaction/infection

Storage (for general guidelines see Section 7.6)

- The component may be stored for a maximum of 42 days at a core temperature of 4°C ± 2°C.
- Variation from the core temperature of 4°C ± 2°C must be kept to a minimum during storage and restricted to any short period necessary for examining, labelling or issuing the component.

- Exceptionally, i.e. due to equipment failure at a Blood Centre, red cell components which have been prepared in a closed system and exposed to a core temperature not exceeding 10°C and not less than 1°C may be released for transfusion provided:
  - that the component has been exposed to such a temperature change on one occasion only
  - that the duration of the temperature change has not exceeded five hours
  - that a documented system is available in each Blood Centre to cover such eventualities
  - that adequate records of the incident are compiled and retained.

Testing

In addition to the mandatory and other tests required for blood donations described in Chapter 10, and leucocyte counting (see Sections 7.2. and 8.1.), a minimum of 75% of those components tested for the parameters shown in Table 8.3 shall meet the specified values.

Table 8.3 Red cells in additive solution, leucocyte depleted – additional tests

Parameter	Frequency of test	Specification
Volume	1%	280 ± 60 mL
Haemoglobin content	1%	>40 g/unit
Haemolysis	As per Section 8.2	<0.8% of red cell mass
Leucocyte Count*	As per Sections 7.2 and 8.1	$<5 \times 10^6$/unit

* Methods validated for counting low numbers of leucocytes must be used.

Transportation (for general guidelines see Section 7.10)

For red cell components, transit containers, packing materials and procedures should have been validated to ensure the component surface temperature can be maintained between 2°C and 10°C during transportation. Additionally:

- the validation exercise should be repeated periodically
- if melting ice is used, it should not come into direct contact with the components
- dead air space in packaging containers should be minimized
- as far as is practicable, transit containers should be equilibrated to their storage temperature prior to filling with components
- transport time normally should not exceed 12 hours
- in some instances it is necessary to issue red cell components that have not been cooled to their storage temperature prior to placing in the transit container. The transport temperature specified above is not applicable for such consignments.

8.7 Red Cells, Washed, Leucocyte Depleted

A red cell component, containing less than 5×10^6 leucocytes, which has been washed with 0.9% w/v sodium chloride for injection (BP). The Red Cells, Washed, Leucocyte Depleted may then be suspended in an approved additive solution.

Technical information

- The amount of residual protein will depend on the washing protocol. Washing can be performed by interrupted or continuous flow centrifugation.
- The use of validated washing procedures that incorporate chilled saline, at least for the final wash and chilled approved additive solution for suspension, is recommended.

This will minimize the risk of bacterial contamination and helps to produce a component that meets the transit temperature requirements.

- If the washing process results in the transfer of the final component into a pack that was not part of the original pack assembly, a secure system must be in place to ensure the correct donation identification number is put on the component pack of Red Cells, Washed, Leucocyte Depleted.
- Red Cells, Washed, Leucocyte Depleted should be transfused through a 170–200μm filter.

Labelling (for general guidelines see Section 7.5)

The following shall be included on the label:

(* = in eye-readable and UKBTS approved barcode format.)

- red cells, washed, Leucocyte Depleted* and volume
- the blood component producer's name*
- the donation number*
- the ABO group*
- the RhD group stated as positive or negative*
- the name, composition and volume of the suspending solution*
- the date and time of preparation
- the expiry date and time*
- the temperature of storage
- the blood pack lot number.*

In addition, the following statements should be made:

INSTRUCTION
Always check patient/component compatibility/identity
Inspect pack and contents for signs of deterioration or damage
Risk of adverse reaction/infection

Storage (for general guidelines see Section 7.6)

The component should be used as soon as possible. Where the component has been produced from a closed system and storage is required the component should be stored at a core temperature of 4°C ± 2°C and used within 24 hours of production if suspended in saline or a defined validated period if suspended in an approved additive solution.

Testing

In addition to the mandatory and other tests required for blood donations described in Chapter 10, and leucocyte counting (see Sections 7.2 and 8.1), a minimum of 75% of those components tested for the parameters shown in Table 8.4 shall meet the specified values. Provided the component is prepared from a process that is validated for leucocyte removal, testing of washed red cells for residual leucocytes is not required.

Transportation (for general guidelines see Section 7.10)

For red cell components, transit containers, packing materials and procedures should have been validated to ensure the component surface temperature can be maintained between 2°C and 10°C during transportation. Additionally:

- the validation exercise should be repeated periodically
- if melting ice is used, it should not come into direct contact with the components
- dead air space in packaging containers should be minimized
- as far as is practicable, transit containers should be equilibrated to their storage temperature prior to filling with components
- transport time normally should not exceed 12 hours
- in some instances it is necessary to issue red cell components that have not been cooled to their storage temperature prior to placing in the transit container. The transport temperature specified above is not applicable for such consignments.

Table 8.4 Red cells, washed, leucocyte depleted – additional tests

Parameter	Frequency of test	Specification
Volume	10 per month or, if made less frequently, every component	Within locally specified volume range
Haemoglobin content		>40 g/unit
Residual protein		<0.5 g/unit
Leucocyte Count* (pre-wash)	As per Sections 7.2 and 8.1	$<5 \times 10^6$/unit

* Methods validated for counting low numbers of leucocytes must be used.

8.8 Red Cells, Thawed and Washed, Leucocyte Depleted

A red cell component, that contains less than 5×10^6 leucocytes, frozen in the presence of a cryoprotectant (preferably within five days of collection), and washed before use. Red Cells, Thawed and Washed, Leucocyte Depleted may then be suspended in an approved additive solution.

Technical information

- The concentration and nature of the cryoprotectant must provide appropriate protection of the red cells at the intended storage temperature. The entire process of freezing, thawing and washing must be validated and documented.
- The use of validated washing procedures that incorporate chilled saline, at least for the final wash and chilled approved additive solution for suspension, is recommended. This will minimize the risk of bacterial contamination and helps to produce a component that meets the transit temperature requirements.
- The target minimum haemoglobin content is 36g.
- If the washing process results in the transfer of the final component into a pack that was not part of the original pack assembly, a secure system must be in place to ensure the correct donation identification number is put on the pack in which the component is frozen and the pack in which the final component is presented.
- Red Cells, Thawed and Washed, Leucocyte Depleted should be transfused through a 170–200 μm filter.

Labelling (for general guidelines see Section 7.5)

The following shall be included on the label:

(* = in eye-readable and UKBTS approved barcode format.)

- red cells, thawed and washed, Leucocyte Depleted* and volume
- the blood component producer's name*

- the donation number*
- the ABO group*
- the RhD group stated as positive or negative*
- the name, composition and volume of the suspending solution*
- the date and time of preparation
- the expiry date and time*
- the temperature of storage
- the blood pack lot number.*

In addition, the following statements should be made:

INSTRUCTION
Always check patient/component compatibility/identity
Inspect pack and contents for signs of deterioration or damage
Risk of adverse reaction/infection

Storage (for general guidelines see Section 7.6)

The storage period for red cells in the frozen state will be influenced by the nature and concentration of the cryoprotectant but normally should not exceed 10 years. Storage is normally at –80°C or colder. Maintenance of a constant storage temperature is important, particularly if a low glycerol cryoprotectant system is used.

The component should be used as soon as possible. Where the component has been produced from a closed system and storage is required the component should be stored at a core temperature of 4°C ± 2°C and used within 24 hours of production if suspended in saline or a defined validated period if suspended in an approved additive solution.

Testing

In addition to the mandatory and other tests required for blood donations described in Chapter 10, and leucocyte counting (see Sections 7.2 and 8.1), a minimum of 75% of those components tested for the parameters shown in Table 8.5 shall meet the specified values. Provided the component is prepared from a process that is validated for leucocyte removal, testing of washed red cells for residual leucocytes is not required.

Table 8.5 Red cells, thawed and washed, leucocyte depleted – additional tests

Parameter	Frequency of test	Specification
Volume	All	Within locally defined nominal volume range
Supernatant Hb	10 per month or, if made less frequently, every component	≤2 g/unit
Red cell Hb		≥36 g/component
Leucocyte count*	As per Sections 7.2 and 8.1	$<5 \times 10^6$/unit**

*Methods validated for counting low numbers of leucocytes must be used.
**Prefreeze.

Transportation (for general guidelines see Section 7.10)

- The transport requirements for red cells in the frozen state will be influenced by the nature and concentration of cryoprotectant used: e.g. a component containing <20% glycerol requires a refrigerant colder than dry ice, such as the vapour phase of liquid nitrogen.

- For red cell components, transit containers, packing materials and procedures should have been validated to ensure the component surface temperature can be maintained between 2°C and 10°C during transportation. Additionally:
  - the validation exercise should be repeated periodically
  - if melting ice is used, it should not come into direct contact with the components
  - dead air space in packaging containers should be minimized
  - as far as is practicable, transit containers should be equilibrated to their storage temperature prior to filling with components
  - transport time normally should not exceed 12 hours
  - in some instances it is necessary to issue red cell components that have not been cooled to their storage temperature prior to placing in the transit container. The transport temperature specified above is not applicable for such consignments.

8.9 Platelets, Pooled, Buffy Coat Derived, Leucocyte Depleted

A pool of platelets, derived from buffy coats, which contains less than 5×10^6 leucocytes.

Technical information

- Donations of whole blood where the bleed time exceeded 15 minutes are not suitable for platelet production.
- The component must be prepared at ambient temperature before the red cell component is cooled to its storage temperature.
- Initial separation of buffy coat normally occurs within 12 hours of venepuncture, with secondary pooling and processing of buffy coats to produce the final component generally completed before the end of day 1.
- The volume of suspension medium must be sufficient to maintain the pH within the range 6.4–7.4 at the end of the shelf life of the component.
- The production process transfers the final component into a pack that was not part of the original pack assembly, therefore a secure system must be in place to ensure the correct identification number is put on the final component pack.
- Where the production method requires the use of a single unit of plasma for resuspension, the plasma from group O donors should be tested for high titre anti-A and anti-B and 'high titre negative' units labelled. The testing method and acceptable limits should be defined (see also Chapter 10).
- Platelets, Pooled, Buffy Coat Derived, Leucocyte Depleted, should be transfused through a 170–200 μm filter.

Labelling (for general guidelines see Section 7.5)

The following shall be included on the label:

(* = in eye-readable and UKBTS approved barcode format.)

- platelets, pooled, buffy coat derived, Leucocyte Depleted* and volume
- the blood component producer's name*
- a unique pool or batch number or the donation number of all contributing platelet units*
- the ABO group*
- the RhD group stated as positive or negative*
- the expiry date*

- the temperature of storage and a comment that continuous gentle agitation throughout storage is recommended
- the blood pack lot number*
- the name, composition and volume of the anticoagulant or additive solution.

In addition, the following statements should be made:

INSTRUCTION
Always check patient/component compatibility/identity
Inspect pack and contents for signs of deterioration or damage
Risk of adverse reaction/infection

Storage (for general guidelines see Section 7.6)

- The storage period depends on a number of factors including the nature of the container, the concentration of platelets and on whether an open or closed system is used.
- Packs currently in use for this purpose allow for storage at a core temperature of 22°C ± 2°C with continuous gentle agitation for up to five days in a closed system. Appropriate pack and platelet concentration combinations may allow storage up to seven days, but due to concerns over bacterial contamination would require either an assay to exclude bacterial contamination prior to transfusion or application of a licensed pathogen inactivation procedure.
- If any production stage involves an open system, after preparation the component should be used as soon as possible. If storage is unavoidable, the component should be stored at a core temperature of 22°C ± 2°C with continuous agitation and used within six hours.
- Platelets should be agitated during storage. If agitation is interrupted, for example due to equipment failure or prolonged transportation, the components are suitable for use, retaining the same shelf life, provided the interruption is for no longer than a single episode of 24 hours.

Testing

In addition to the mandatory and other tests required for blood donations described in Chapter 10, and leucocyte counting (see Sections 7.2 and 8.1), a minimum of 75% of those components tested for the parameters shown in Table 8.6 shall meet the specified values.

Table 8.6 Platelets, pooled, buffy coat derived, leucocyte depleted – additional tests

Parameter	Frequency of test	Specification
Volume	1% or 10 per month, whichever is greater	Within locally defined nominal volume range
Platelet count		$\geq 240 \times 10^9$/pool
pH at end of shelf life	If less than 10 per month, every available component	6.4–7.4
Leucocyte Count*	As per Sections 7.2 and 8.1	$< 5 \times 10^6$/pool

* Methods validated for counting low numbers of leucocytes must be used.

NOTE: Visual inspection of platelet components for the swirling phenomenon, clumping, excessive red cell contamination and abnormal volume is a useful pre-issue check.

Transportation (for general guidelines see Section 7.10)

Containers for transporting platelets should be equilibrated at room temperature 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, the component should be transferred to storage at a core temperature of 22°C ± 2°C with continuous gentle agitation.

Plastic overwraps should be removed prior to storage.

8.10 Platelets, Apheresis, Leucocyte Depleted

A single donor platelet component containing less than 5×10^6 leucocytes.

Technical information

- Platelets, Apheresis may be collected by a variety of apheresis systems using different protocols. Since platelet yields may vary, each procedural protocol must be fully validated, documented and specifications set accordingly.
- If filtration is used the recommended capacity of the filter should not be exceeded.
- The volume of suspension medium must be sufficient to maintain the pH within the range 6.4–7.4 at the end of the shelf life of the component.
- If the leucodepletion process transfers the final component into a pack that was not part of the original pack assembly, a secure system must be in place to ensure the correct identification number is put on the final component pack.
- The plasma from group O donors should be tested for high titre anti-A and anti-B and 'high titre negative' units labelled. The testing method and acceptable limits should be defined (see also Chapter 10).
- Platelets, Apheresis, Leucocyte Depleted should be transfused through a 170–200μm filter.

Labelling (for general guidelines see Section 7.5)

The following shall be included on the label:

(* = in eye-readable and UKBTS approved barcode format.)

- platelets, apheresis, Leucocyte Depleted* and volume
- the blood component producer's name*
- the donation number and, if divided, sub-batch number*
- the ABO group*
- the RhD group stated as positive or negative*
- the expiry date*
- the temperature of storage and a comment that continuous gentle agitation throughout storage is recommended
- the blood pack lot number*
- the name, composition and volume of the anticoagulant or additive solution.

In addition, the following statements should be made:

INSTRUCTION
Always check patient/component compatibility/identity
Inspect pack and contents for signs of deterioration or damage
Risk of adverse reaction/infection

Storage (for general guidelines see Section 7.6)

- The storage period depends on a number of factors including the nature of the container, the concentration of platelets and whether an open or closed system is used.

- Packs currently in use for this purpose allow for storage at a core temperature of 22°C ± 2°C with continuous gentle agitation for up to five days in a closed system. Appropriate pack and platelet concentration combinations may allow storage up to seven days, but due to concerns over bacterial contamination would require either an assay to exclude bacterial contamination prior to transfusion or application of a licensed pathogen inactivation procedure.

- Where any manufacturing step involves an open system the platelets should be used as soon as possible after collection. If storage is unavoidable, the component should be stored at a core temperature of 22°C ± 2°C with continuous agitation and used within six hours.

- Platelets should be agitated during storage. If agitation is interrupted, for example due to equipment failure or prolonged transportation, the components are suitable for use, retaining the same shelf life, provided the interruption is for no longer than a single episode of 24 hours.

Testing

In addition to the mandatory and other tests required for blood donations described in Chapter 10, and leucocyte counting (see Sections 7.2 and 8.1), a minimum of 75% of those components tested for the parameters shown in Table 8.7 shall meet the specified values.

Table 8.7 Platelets, apheresis, leucocyte depleted – additional tests

Parameter	Frequency of test	Specification
Volume	1% or 10 per month, whichever is greater	Within locally defined nominal volume range
Platelet count		$\geq 240 \times 10^9$ per unit
pH at end of shelf life	If less than 10 per month, every available component	6.4–7.4
Leucocyte Count*	As per Sections 7.2 and 8.1	$< 5 \times 10^6$ per unit

* Methods validated for counting low numbers of leucocytes must be used.

NOTE: Visual inspection of platelet components for the swirling phenomenon, clumping, excessive red cell contamination and abnormal volume is a useful pre-issue check.

Transportation (for general guidelines see Section 7.10)

Containers for transporting platelets should be equilibrated at room temperature 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, the component should be transferred to storage at a core temperature of 22°C ± 2°C with continuous gentle agitation.

Plastic overwraps should be removed prior to storage.

8.11 Platelets, Suspended in Additive Solution, Leucocyte Depleted

A platelet concentrate derived from buffy coats or apheresis, which contain less than 5×10^6 leucocytes and where the suspending medium is additive solution. This component is indicated for patients with reactions to plasma containing components.

Technical information

- Donations of whole blood where the bleed time exceeded 15 minutes are not suitable for platelet production.
- The platelet component must be prepared at ambient temperature before the red cell component is cooled to below 20°C.
- Where prepared from buffy coats, initial separation of buffy coat normally occurs within 12 hours of venepuncture, with secondary pooling and processing of buffy coats to produce the final component generally completed before the end of day 1.
- The volume of suspension medium must be sufficient to maintain the pH within the range 6.4–7.4 at the end of the shelf life of the component.
- Where the production process transfers the final component into a pack that was not part of the original pack assembly, a secure system must be in place to ensure the correct identification number is put on the final component pack.
- Platelets, Suspended in Additive Solution, Leucocyte Depleted, should be transfused through a 170–200 μm filter.

Labelling (for general guidelines see Section 7.5)

The following shall be included on the label:

(* = in eye-readable and UKBTS approved barcode format.)

- Platelets, Suspended in Additive Solution, Leucocyte Depleted* and volume
- the blood component producer's name*
- a unique pool or batch number or the donation number of all contributing platelet units*
- the ABO group*
- the RhD group stated as positive or negative*
- the expiry date*
- the temperature of storage and a comment that continuous gentle agitation throughout storage is recommended
- the blood pack lot number*
- the name, composition and volume of the additive solution.

In addition, the following statements should be made:

INSTRUCTION
Always check patient/component compatibility/identity
Inspect pack and contents for signs of deterioration or damage
Risk of adverse reaction/infection

Storage (for general guidelines see Section 7.6)

- The storage period depends on a number of factors including the nature of the container, the concentration of platelets and on whether an open or closed system is used.
- Platelets, Suspended in Platelet Additive Solution, Leucocyte Depleted, should be used within 24 hours of production.

- If any production stage involves an open system, after preparation the component should be used as soon as possible. If storage is unavoidable, the component should be stored at a core temperature of 22°C ± 2°C with continuous agitation and used within six hours.

Testing

In addition to the mandatory and other tests required for blood donations described in Chapter 10, and leucocyte counting (see Sections 7.2 and 8.1), a minimum of 75% of those components tested for the parameters shown in Table 8.8 shall meet the specified values.

Table 8.8 Platelets, suspended in platelet additive solution, leucocyte depleted – additional tests

Parameter	Frequency of test	Specification
Volume	1% or 10 per month whichever is greater	Within locally defined nominal volume range
Platelet Count		$\geq 240 \times 10^9$/pool
pH at end of shelf life	If less than 10 per month, every available component	6.4–7.4
Leucocyte Count*	As per Sections 7.2 and 8.1	$< 5 \times 10^6$/pool

*Methods validated for counting low levels of leucocytes must be used.

NOTE: Visual inspection of platelet components for the swirling phenomenon, clumping, excessive red cell contamination and abnormal volume is a useful pre-issue check.

Transportation (for general guidelines see Section 7.10)

Containers for transporting platelets should be equilibrated at room temperature 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, the component should be transferred to storage at a core temperature of 22°C ± 2°C with continuous gentle agitation.

Plastic overwraps should be removed prior to storage.

8.12 Granulocytes, Apheresis

A component prepared from anticoagulated blood, which is separated into components by a suitable apheresis machine with retention of granulocytes as the major cellular product, suspended in a portion of the plasma. The remaining elements may be returned to the donor.

Technical information

- Granulocytes may be collected by a variety of apheresis systems using different protocols. Since yields may vary, each procedural protocol must be fully validated, documented and specifications set accordingly.
- The component must not be agitated during storage.
- The component must be gamma irradiated before use.
- Granulocytes, Apheresis should be transfused through a 170–200 μm filter.

Labelling (for general guidelines see Section 7.5)

The following shall be included on the label:

(* = in eye-readable and UKBTS approved barcode format.)

- granulocytes, apheresis* and volume

- the blood component producer's name*
- the donation number*
- the ABO group*
- the RhD group stated as positive or negative*
- the date of collection
- the expiry date and time*
- the temperature of storage
- the statement 'Do not agitate'
- the blood pack lot number*
- the name, composition and volume of the anticoagulant solution.

In addition, the following statements should be made:

INSTRUCTION
Always check patient/component compatibility/identity
Inspect pack and contents for signs of deterioration or damage
Risk of adverse reaction/infection

Storage (for general guidelines see Section 7.6)

Granulocytes, apheresis should be used as soon as possible after their preparation. Whether prepared in an open or closed system, if storage is unavoidable, the component should be stored, without agitation, at a core temperature of 22°C ± 2°C and used within 24 hours of collection.

Plastic overwraps should be removed prior to storage.

Testing

In addition to the mandatory and other tests required for blood donations described in Chapter 10, all components tested for the parameters shown in Table 8.9 shall meet the specified values.

Table 8.9 Granulocytes, apheresis – additional tests

Parameter	Frequency of test	Specification
Volume	10 per month or, if made less frequently, every component	Within locally defined nominal volume range
Total Granulocyte Count		$>5 \times 10^9$/unit

Transportation (for general guidelines see Section 7.10)

Containers for transporting granulocytes, apheresis should be equilibrated at room temperature before use. During transportation the temperature of the component must be kept as close as possible to the recommended storage temperature and on receipt, unless intended for immediate therapeutic use, the component should be transferred to storage at a core temperature of 22°C ± 2°C.

Plastic overwraps should be removed prior to storage.

8.13 Fresh Frozen Plasma, Leucocyte Depleted

Plasma that has been obtained from whole blood or by apheresis from a previously tested donor (as defined in Section 8.3). The plasma contains less than 5×10^6 leucocytes per component and has been rapidly frozen to a temperature that will maintain the activity of labile coagulation factors.

Technical information

- Donations of whole blood where the bleed time exceeded 15 minutes are not suitable for the production of plasma components for direct clinical use.
- Ideally the plasma should be separated before the red cell component is cooled to its storage temperature.
- The method of preparation should ensure the component has the maximum level of labile coagulation factors with minimum cellular contamination.
- The production process should be validated to ensure that components meet the specified limits for FVIII:C concentration.
- Greater FVIII:C yields will be obtained when the plasma is separated as soon as possible after venepuncture and rapidly frozen to –30°C or below.
- Component samples collected for the Quality Monitoring assessment of FVIII:C should have approximately the same ABO group distribution as issued components.
- Fresh Frozen Plasma, Leucocyte Depleted should be transfused through a 170–200μm filter.

Labelling (for general guidelines see Section 7.5)

The following shall be included on the label:

(* = in eye-readable and UKBTS approved barcode format.)

- fresh frozen plasma, Leucocyte Depleted* and volume
- the blood component producer's name*
- the donation number and, if divided, sub-batch number*
- the ABO group*
- the RhD group stated as positive or negative*
- the date of collection
- the expiry date of the frozen component*
- the temperature of storage
- the blood pack lot number*
- a warning that the component must be used within four hours of thawing if maintained at 22°C ± 2°C, or 24 hours of thawing if stored at 4°C ± 2°C
- the name, composition and volume of the anticoagulant.

In addition, the following statements should be made:

INSTRUCTION
Always check patient/component compatibility/identity
Inspect pack and contents for signs of deterioration or damage
Risk of adverse reaction/infection

Storage (for general guidelines see Section 7.6)

- The component should be stored at a core temperature of –30°C or below for a maximum of 24 months.
- Although a storage temperature below –30°C improves the preservation of labile coagulation factors, lower temperatures increase the fragility of plastic. Particular care must be taken when handling such packs.

- The component should be thawed at 37°C in a waterbath or other equipment designed for the purpose, within a vacuum sealed overwrap bag. Protocols must be in place to ensure that the equipment is cleaned daily and maintained to minimize the risk of bacterial contamination.
- Once thawed, the component must not be refrozen and should be transfused as soon as possible. If delay is unavoidable, the component may be stored and should be used within 4 hours if maintained at 22°C ± 2°C or 24 hours if stored at 4°C ± 2°C, but it should be borne in mind that extended post-thaw storage will result in a decline in the content of labile coagulation factors.

Testing

In addition to the mandatory and other tests required for blood donations described in Chapter 10, and leucocyte counting (see Sections 7.2 and 8.1), a minimum of 75% of those components tested for the parameters shown in Table 8.10 shall meet the specified values.

Table 8.10 Fresh frozen plasma, leucocyte depleted – additional tests

Parameter	Frequency of test	Specification
Volume	1%	Within locally defined nominal volume range
Total Protein	1%	>50g/L
Platelets	1%	$<30 \times 10^9$/L**
Red Cells	1%	$<6 \times 10^9$/L**
FVIII:C	1%	>0.70 IU/mL
Leucocyte Count*	As per Sections 7.2 and 8.1	$<5 \times 10^6$/unit**

* Methods validated for counting low numbers of leucocytes must be used.
** Prefreeze in starting component.

Transportation (for general guidelines see Section 7.10)

Every effort should be made to maintain the core storage temperature during transportation. Unless the component is to be thawed and used straightaway it should be transferred immediately to storage at the recommended temperature.

8.14 Fresh Frozen Plasma, Methylene Blue Treated and Removed, Leucocyte Depleted

Fresh Frozen Plasma Methylene Blue Treated (MBT) and Removed, Leucocyte Depleted, is plasma that has been obtained from whole blood or by apheresis from a previously tested donor (as defined in Section 8.3), contains less than 5×10^6 leucocytes and has been treated with methylene blue and exposure to visible light to inactivate pathogens.

Following methylene blue treatment and removal, the plasma is rapidly frozen to a temperature that will maintain the activity of labile coagulation factors.

Technical information

Where the starting component is sourced outwith the UK, a detailed and agreed specification must be available.

- Donations of whole blood where the bleed time exceeded 15 minutes are not suitable for the production of plasma components for direct clinical use.
- Ideally the plasma should be separated before the red cell component is cooled to its storage temperature.
- The method of preparation should ensure the component has the maximum level of labile coagulation factors with minimum cellular contamination.

- Intact white blood cells in the plasma should be reduced to less than 5×10^6 per unit prior to exposure to methylene blue and visible light.
- The production process should be validated to ensure that components meet the specified limits for FVIII:C content.
- Greater FVIII:C yields will be obtained when the plasma is separated as soon as possible after venepuncture, MB treated and rapidly frozen to –30°C or below.
- Component samples collected for the Quality Monitoring assessment of FVIII:C should have approximately the same ABO group distribution as issued components.
- The MBT process reduces the FVIII:C content by approximately 30% when compared to standard FFP.
- The process for methylene blue removal should be validated to give components with a methylene blue concentration ≤0.30 μM (< approximately 30 μg per unit).
- Component samples collected for the Quality Monitoring assessment of FVIII:C should have approximately the same ABO group distribution as issued components.
- Fresh Frozen Plasma, Methylene Blue Treated and Removed should be transfused through a 170–200 μm filter.

Labelling (for general guidelines see Section 7.5)

The following shall be included on the label:

(* = in eye-readable and UKBTS approved barcode format.)

- fresh frozen plasma, methylene blue treated and removed, Leucocyte Depleted* and volume
- the blood component producer's name*
- the donation number*
- the ABO group*
- the RhD group stated as positive or negative*
- the date of collection
- the expiry date of the frozen component*
- the temperature of storage
- the blood pack lot number*
- a warning that the component should be used within four hours of thawing
- the name, composition and volume of the anticoagulant.

In addition the following statements should be made:

INSTRUCTION
Always check patient/component compatibility/identity
Inspect pack and contents for signs of deterioration or damage
Risk of adverse reaction/infection

Storage (for general guidelines see Section 7.6)

- The component should be stored at a core temperature of –30°C or below for a maximum of 24 months.

- Although a storage temperature below –30°C improves the preservation of labile coagulation factors, lower temperatures increase the fragility of plastic. Particular care must be taken when handling such packs.

- The component should be thawed at 37°C in a waterbath or other equipment designed for the purpose, within a vacuum sealed overwrap bag. Protocols must be in place to ensure that the equipment is cleaned daily and maintained to minimize the risk of bacterial contamination.

- Once thawed, the component must not be refrozen and should be used immediately. If delay is unavoidable, the component should be stored at ambient temperature and used within four hours.

Testing

In addition to the mandatory and other tests required for blood donations described in Chapter 10, and leucocyte counting (see Sections 7.2 and 8.1), a minimum of 75% of those components tested for the parameters shown in Table 8.11 shall meet the specified values.

Table 8.11 Fresh frozen plasma, methylene blue-treated and removed, leucocyte depleted – additional tests

Parameter	Frequency of test	Specification
Volume	1% or 10 per month, whichever is greater. If less than 10 per month, every component.	Within locally defined nominal volume range and within any limits specified for the MBT process used.
Platelets		$<30 \times 10^9$/L**
FVIII:C		>0.50 IU/mL
Leucocyte Count*	As per Sections 7.2 and 8.1	$<5 \times 10^6$/unit**

*Methods validated for counting low numbers of leucocytes must be used.
**Prefreeze in starting component.

8.15 Cryoprecipitate, Leucocyte Depleted

The component represents a source of concentrated FVIII:C, and von Willebrand factor, fibrinogen, Factor XIII and fibronectin from a unit of fresh frozen plasma. The plasma from which the cryoprecipitate was produced contains less than 5×10^6 leucocytes per component and derived from a previously tested donor (as defined in Section 7.3).

Technical information

- Donations of whole blood where the bleed time exceeded 15 minutes are not suitable for the production of plasma components for direct clinical use.

- Cryoprecipitate, Leucocyte Depleted is the cryoglobulin fraction of plasma obtained by thawing a single donation of fresh frozen plasma, Leucocyte Depleted at 4°C ± 2°C.

- For storage, Cryoprecipitate, Leucocyte Depleted should be rapidly frozen to a core temperature of –30°C or below within two hours of preparation.

- Component samples collected for the Quality Monitoring assessment of FVIII:C should have approximately the same ABO group distribution as issued components.

- Cryoprecipitate, Leucocyte Depleted should be transfused through a 170–200 μm filter.

Labelling (for general guidelines see Section 7.5)

The following shall be included on the component label:

(* = in eye-readable and UKBTS approved barcode format.)

- Cryoprecipitate, Leucocyte Depleted* and volume
- the blood component producer's name*
- the donation number*
- the ABO group*
- the RhD group stated as positive or negative*
- the date of collection
- the expiry date of the frozen component*
- the temperature of storage
- the blood pack lot number*
- a warning that the component must be used within four hours of thawing
- the name, composition and volume of the anticoagulant.

In addition, the following statements should be made:

INSTRUCTION
Always check patient/component compatibility/identity
Inspect pack and contents for signs of deterioration or damage
Risk of adverse reaction/infection

Storage (for general guidelines see Section 7.6)

- The component should be stored at a core temperature of –30°C or below for a maximum of 24 months.
- Although a storage temperature below –30°C improves the preservation of labile coagulation factors, lower temperatures increase the fragility of plastic. Particular care must be taken when handling such packs.
- The component should be thawed at 37°C in a waterbath or other equipment designed for the purpose, within a vacuum sealed overwrap bag. Protocols must be in place to ensure that the equipment is cleaned daily and maintained to minimize the risk of bacterial contamination.
- Once thawed, the component must not be refrozen and should be used immediately. If delay is unavoidable, the component should be stored at ambient temperature and used within four hours.

Testing

In addition to the mandatory and other tests required for blood donations described in Chapter 10, and leucocyte counting (see Sections 7.2 and 8.1), a minimum of 75% of those components tested for the parameters shown in Table 8.12 shall meet the specified values.

Transportation (for general guidelines see Section 7.10)

Every effort should be made to maintain the core storage temperature during transportation. Unless the component is to be thawed and used straight away it should be transferred immediately to storage at the recommended temperature.

Table 8.12 Cryoprecipitate, leucocyte depleted – additional tests

Parameter	Frequency of test	Specification
Volume	1%	Within locally defined nominal volume range
Fibrinogen	1%	>140 mg/unit
FVIII:C	1%	>70 iu/unit
Leucocyte Count*	As per Sections 7.2 and 8.1	$<5 \times 10^6$/unit**

*Methods validated for counting low numbers of leucocytes must be used.
**Prefreeze in starting component.

8.16 Cryoprecipitate, Methylene Blue Treated and Removed, Leucocyte Depleted

Refer to Section 8.25.

8.17 Plasma, Cryoprecipitate Depleted, Leucocyte Depleted

The supernatant plasma removed during the preparation of cryoprecipitate, Leucocyte Depleted. The plasma from which the plasma, cryoprecipitate depleted, Leucocyte Depleted was made contains less than 5×10^6 leucocytes per component and derived from a previously tested donor (as defined in Section 7.3).

Technical information

- Donations of whole blood where the bleed time exceeded 15 minutes are not suitable for the production of plasma components for direct clinical use.
- Plasma, Cryoprecipitate Depleted, Leucocyte Depleted should be frozen to a core temperature of –30°C or below within two hours of separation from its cryoprecipitate, Leucocyte Depleted.
- Plasma, Cryoprecipitate Depleted, Leucocyte Depleted should be transfused through a 170–200μm filter.

Labelling (for general guidelines see Section 7.5)

The following shall be included on the component label:

(* = in eye-readable and UKBTS approved barcode format.)

- plasma, cryoprecipitate depleted, Leucocyte Depleted* and volume
- the blood component producer's name*
- the donation number*
- the ABO group*
- the RhD group stated as positive or negative*
- the date of collection
- the expiry date of the frozen component*
- the temperature of storage
- the blood pack lot number*
- a warning that the component must be used within four hours of thawing if maintained at 22°C ± 2°C, or 24 hours of thawing if stored at 4°C ± 2°C.
- the name, composition and volume of the anticoagulant.

In addition, the following statements should be made:

INSTRUCTION
Always check patient/component compatibility/identity
Inspect pack and contents for signs of deterioration or damage
Risk of adverse reaction/infection

Storage (for general guidelines see Section 7.6)

- The component should be stored at a core temperature of –30°C or below for a maximum of 24 months.
- Although a storage temperature below –30°C improves the preservation of labile coagulation factors, lower temperatures increase the fragility of plastic. Particular care must be taken when handling such packs.
- The component should be thawed at 37°C in a waterbath or other equipment designed for the purpose, within a vacuum sealed overwrap bag. Protocols must be in place to ensure that the equipment is cleaned daily and maintained to minimize the risk of bacterial contamination.
- Once thawed, the component must not be refrozen and should be transfused as soon as possible. If delay is unavoidable, the component may be stored and should be used within four hours if maintained at 22°C ± 2°C or 24 hours if stored at 4°C ± 2°C, but it should be borne in mind that extended post-thaw storage will result in a decline in the content of labile coagulation factors.

Testing

In addition to the mandatory and other tests required for blood donations described in Chapter 10, and leucocyte counting (see Sections 7.2 and 8.1), a minimum of 75% of those components tested for the parameters shown in Table 8.13 shall meet the specified values.

Table 8.13 Plasma, cryoprecipitate depleted, leucocyte depleted – additional tests

Parameter	Frequency of test	Specification
Volume	1%	Within locally defined nominal volume range
Platelets	1%	$<30 \times 10^9$/L**
Red Cells	1%	$<6 \times 10^9$/L**
Leucocyte Count*	As per Sections 7.2 and 8.1	$<5 \times 10^6$/unit**

*Methods validated for counting low numbers of leucocytes must be used.
**Prefreeze in starting component (FFP).

Transportation (for general guidelines see Section 7.10)

Every effort should be made to maintain the core storage temperature during transportation. Unless the component is to be thawed and used straight away it should be transferred immediately to storage at the recommended temperature.

8.18 Components Suitable for Use in Intrauterine Transfusion, Neonates and Infants Under One Year

General requirements

- Components for use in intrauterine transfusion, neonates and infants under one year must be prepared from previously tested donors who fulfil the following criteria:
  - have given at least one donation in the last two years

  - have not produced a repeat reactive result in microbiology tests that were designated as mandatory at that time
  - negative results were obtained for mandatory microbiology markers with the current donation
  - the current donation and donor status otherwise meet component release criteria.
- Red cell and platelet components should be negative for CMV antibodies although leucodepleted components may be used if CMV antibody negative components are not available.
- Components should be tested and shown to be free of clinically significant, irregular blood group antibodies including high titre anti-A and anti-B.
- It is good practice to provide neonates, who are likely to be repeatedly transfused, with components in which the original donation has been split, thereby providing the potential to reduce donor exposures in this vulnerable group of recipients.
- When a component is to be split for neonatal use, the original pack must first be mixed thoroughly by a validated procedure to ensure that the contents are homogeneous.
- When a component is split for neonatal use, it is sufficient to undertake leucocyte counting on the parent pack or process.
- When a component is split for neonatal use, each 'split' must be identified by a unique number to ensure all splits can be accounted for.

8.19 Red Cells for Intrauterine Transfusion (IUT), Leucocyte Depleted

A component for intrauterine transfusion, prepared by removing a proportion of the plasma from fresh whole blood. The component should be leucocyte depleted to less than 5×10^6 leucocytes/unit.

Technical information

- The component must be prepared and used for IUT by the end of day 5, should be free from clinically significant irregular blood group antibodies including high titre anti-A and anti-B (see Chapter 10), and should be negative for antibodies to CMV.
- The component must be gamma irradiated and should be transfused within 24 hours of irradiation. See BCSH Transfusion guidelines for neonates and older children.[2]
- Unless the Blood Centre recommends screening is unnecessary, the component should be Haemoglobin S screen negative.
- Red Cells for IUT, Leucocyte Depleted should be transfused through a 170–200 μm filter.

Labelling (for general guidelines see Section 7.5)

The following shall be included on the label:

(* = in eye-readable and UKBTS approved barcode format.)

- red cells for IUT, Leucocyte Depleted* and volume
- the blood component producer's name*
- the donation number*
- the ABO group*
- the RhD group stated as positive or negative*

- the name, composition and volume of the anticoagulant solution*
- the date of collection
- the expiry date*
- the temperature of storage
- the blood pack lot number.*

In addition the following statements should be made:

INSTRUCTION
Always check patient/component compatibility/identity
Inspect pack and contents for signs of deterioration or damage
Risk of adverse reaction/infection

Storage (for general guidelines see Section 7.6)

- The component may be stored for a maximum of five days at a core temperature of 4°C ± 2°C.
- The component must be used within 24 hours of irradiation and within the overall maximum five-day shelf life.
- Variation from the core temperature of 4°C ± 2°C must be kept to a minimum during storage and restricted to any short period necessary for examining, labelling or issuing the component.
- Exceptionally, i.e. due to equipment failure at a Blood Centre, red cell components which have been prepared by a closed system and exposed to a core temperature not exceeding 10°C and not less than 1°C may be released for transfusion provided:
  - that the component has been exposed to such a temperature change on one occasion only
  - that the duration of the temperature change has not exceeded five hours
  - that a documented system is available in each Blood Centre to cover such eventualities
  - that adequate records of the incident are compiled and retained.

Testing

In addition to the mandatory and other tests required for blood donations described in Chapter 10, and leucocyte counting (see Sections 7.2 and 8.1), the component shall be free from clinically significant irregular blood group antibodies and high titre anti-A and/or anti-B, and antibodies to CMV. Furthermore, a minimum of 75% of those components tested for the other parameters shown in Table 8.14 shall meet the specified values.

Table 8.14 Red cells for intrauterine transfusion (IUT), leucocyte depleted – additional tests

Parameter	Frequency of test	Specification
Volume	1% or 10 per month, whichever is greater	Within locally defined nominal volume range
Haematocrit	If less than 10 per month, every component	Within local specification but not less than 0.70
Haemoglobin content		Locally defined
Leucocyte Count*	As per Sections 7.2 and 8.1	$<5 \times 10^6$/unit

*Methods validated for counting low levels of leucocytes must be used.

Transportation (for general guidelines see Section 7.10)

For red cell components, transit containers, packing materials and procedures should have been validated to ensure the component surface temperature can be maintained between 2°C and 10°C during transportation. Additionally:

- The validation exercise should be repeated periodically
- if melting ice is used, it should not come into direct contact with the components
- dead air space in packaging containers should be minimized
- as far as is practicable, transit containers should be equilibrated to their storage temperature prior to filling with components
- transport time normally should not exceed 12 hours
- in some instances it is necessary to issue red cell components that have not been cooled to their storage temperature prior to placing in the transit container. The transport temperature specified above is not applicable for such consignments.

8.20 Whole Blood for Exchange Transfusion, Leucocyte Depleted

A component for exchange or large volume transfusion of neonates, containing less than 5×10^6 leucocytes/unit.

Technical information

- The component must be prepared and used for exchange transfusion by the end of day 5, should be free from clinically significant irregular blood group antibodies including high titre anti-A and anti-B (see Chapter 10) and should be negative for antibodies to CMV.
- The component should be gamma irradiated and transfused within 24 hours of irradiation. See BCSH Transfusion guidelines for neonates and older children.[2]
- Unless the Blood Centre recommends screening is unnecessary, the component should be Haemoglobin S screen negative.
- Whole blood for exchange transfusion, Leucocyte Depleted should be transfused through a 170–200μm filter.

Labelling (for general guidelines see Section 7.5)

The following shall be included on the label:

(* = in eye-readable and UKBTS approved barcode format.)

- whole blood for exchange transfusion, Leucocyte Depleted* and volume
- the blood component producer's name*
- the donation number*
- the ABO group*
- the RhD group stated as positive or negative*
- the name, composition and volume of the anticoagulant solution*
- the date of collection
- the expiry date*
- the temperature of storage
- the blood pack lot number.*

In addition the following statements should be made:

INSTRUCTION
Always check patient/component compatibility/identity
Inspect pack and contents for signs of deterioration or damage
Risk of adverse reaction/infection

Storage (for general guidelines see Section 7.6)

- The component may be stored for a maximum of five days at a core temperature of 4°C ± 2°C.
- The component should be used within 24 hours of irradiation and within the overall maximum five-day shelf life.
- Variation from the core temperature of 4°C ± 2°C must be kept to a minimum during storage and restricted to any short period necessary for examining, labelling or issuing the component.
- Exceptionally, i.e. due to equipment failure at a Blood Centre, red cell components which have been prepared in a closed system and exposed to a core temperature not exceeding 10°C and not less than 1°C may be released for transfusion provided:
  - that the component has been exposed to such a temperature change on one occasion only
  - that the duration of the temperature change has not exceeded five hours
  - that a documented system is available in each Blood Centre to cover such eventualities
  - that adequate records of the incident are compiled and retained.
- If whole blood for exchange transfusion, Leucocyte Depleted is unused within its specified shelf life, the Blood Centre may return the component to stock provided:
  - the component was stored within specification
  - the component is appropriately relabelled as whole blood leucocyte depleted and, if necessary, 'irradiated'
  - that the storage restrictions of irradiated red cells are observed i.e. use within 14 days of irradiation.

Testing

In addition to the mandatory and other tests required for blood donations described in Chapter 10, and leucocyte counting (see Sections 7.2 and 8.1), the component shall be free from clinically significant irregular blood group antibodies and high titre anti-A and/or anti-B, and antibodies to CMV. Furthermore, a minimum of 75% of those components tested for the other parameters shown in Table 8.15 shall meet the specified values.

Table 8.15 Whole blood for exchange transfusion, leucocyte depleted – additional tests

Parameter	Frequency of test	Specification
Volume	1% or 10 per month, whichever is greater	Within locally defined nominal volume range
Haemoglobin Content	If less than 10 per month, every component	>40 g/unit
Leucocyte Count*	As per Sections 7.2 and 8.1	$<5 \times 10^6$/unit

*Methods validated for counting low levels of leucocytes must be used.

Transportation (for general guidelines see Section 7.10)

For red cell components, transit containers, packing materials and procedures should have been validated to ensure the component surface temperature can be maintained between 2°C and 10°C during transportation. Additionally:

- the validation exercise should be repeated periodically
- if melting ice is used, it should not come into direct contact with the components
- dead air space in packaging containers should be minimized
- as far as is practicable, transit containers should be equilibrated to their storage temperature prior to filling with components
- transport time normally should not exceed 12 hours
- in some instances it is necessary to issue red cell components that have not been cooled to their storage temperature prior to placing in the transit container. The transport temperature specified above is not applicable for such consignments.

8.21 Red Cells for Exchange Transfusion, Leucocyte Depleted

A component for exchange or large volume transfusion of neonates prepared by leucodepleting fresh whole blood to less than 5×10^6 leucocytes per component and removing a proportion of the plasma.

Technical information

- The component must be prepared and used by the end of day 5, should be free from clinically significant irregular blood group antibodies including high titre anti-A and anti-B (see Chapter 10), and should be negative for antibodies to CMV.
- The component should be gamma irradiated and transfused within 24 hours of irradiation. See BCSH Transfusion guidelines for neonates and older children.[2]
- Unless the Blood Centre recommends screening is unnecessary, the unit should be Haemoglobin S screen negative.
- Red cells for exchange transfusion, Leucocyte Depleted should be transfused through a 170–200 μm filter.

Labelling (for general guidelines see Section 7.5)

The following shall be included on the label:

(* = in eye-readable and UKBTS approved barcode format.)

- red cells for exchange transfusion, Leucocyte Depleted* and volume
- the blood component producer's name*
- the donation number*
- the ABO group*
- the RhD group stated as positive or negative*
- the name, composition and volume of the anticoagulant solution*
- the date of collection
- the expiry date*
- the temperature of storage
- the blood pack lot number.*

In addition the following statements should be made:

INSTRUCTION
Always check patient/component compatibility/identity
Inspect pack and contents for signs of deterioration or damage
Risk of adverse reaction/infection

Storage (for general guidelines see Section 7.6)

- The component may be stored for a maximum of five days at a core temperature of 4°C ± 2°C.
- The component should be used within 24 hours of irradiation and within the overall maximum five-day shelf life.
- Variation from the core temperature of 4°C ± 2°C must be kept to a minimum during storage and restricted to any short period necessary for examining, labelling or issuing the component.
- Exceptionally, i.e. due to equipment failure at a Blood Centre, red cell components which have been prepared in a closed system and exposed to a core temperature not exceeding 10°C and not less than 1°C may be released for transfusion provided:
  - that the component has been exposed to such a temperature change on one occasion only
  - that the duration of the temperature change has not exceeded 5 hours
  - that a documented system is available in each Blood Centre to cover such eventualities
  - that adequate records of the incident are compiled and retained.
- If red cells for exchange transfusion, Leucocyte Depleted are unused within their specified shelf life, the Blood Centre may return them to stock provided:
  - the component was stored within specification
  - the component is appropriately relabelled as red cells, leucocyte depleted and, if necessary, 'irradiated'
  - that the storage restrictions of irradiated red cells are observed i.e. use within 14 days of irradiation.

Testing

In addition to the mandatory and other tests required for blood donations described in Chapter 10, and leucocyte counting (see Sections 7.2 and 8.1), the component shall be free from clinically significant irregular blood group antibodies and high titre anti-A and/or anti-B, and antibodies to CMV. Furthermore, a minimum of 75% of those components tested for the other parameters shown in Table 8.16 shall meet the specified values.

Table 8.16 Red cells for exchange transfusion, leucocyte depleted – additional tests

Parameter	Frequency of test	Specification
Volume	1% or 10 per month, whichever is greater If less than 10 per month,	Within locally defined nominal volume range
Haematocrit	every component	0.50–0.60
Haemoglobin content		>40 g/unit
Leucocyte Count*	As per Sections 7.2 and 8.1	$<5 \times 10^6$/unit

*Methods validated for counting low levels of leucocytes must be used.

Transportation (for general guidelines see Section 7.10)

For red cell components, transit containers, packing materials and procedures should have been validated to ensure the component surface temperature can be maintained between 2°C and 10°C during transportation. Additionally:

- the validation exercise should be repeated periodically
- if melting ice is used, it should not come into direct contact with the components
- dead air space in packaging containers should be minimized
- as far as is practicable, transit containers should be equilibrated to their storage temperature prior to filling with components
- transport time normally should not exceed 12 hours
- in some instances it is necessary to issue red cell components that have not been cooled to their storage temperature prior to placing in the transit container. The transport temperature specified above is not applicable for such consignments.

8.22 Red Cells for Neonates and Infants, Leucocyte Depleted

A red cell component suitable for neonates and infants under one year that contains less than 5×10^6 leucocytes (per starting component). The red cells for neonates and infants, Leucocyte Depleted may be divided into approximately equal volumes using a closed system.

Technical information

The component should be free from clinically significant irregular blood group antibodies including high titre anti-A and anti-B and should be negative for antibodies to CMV.

Red cells for neonates and infants, Leucocyte Depleted should be transfused through a 170-200 μm filter.

Labelling (for general guidelines see Section 7.5)

The following shall be included on the label:

(* = in eye-readable and UKBTS approved barcode format.)

- red cells for neonates and infants* and volume
- the blood component producer's, Leucocyte Depleted name*
- the donation number and, if divided, sub-batch number*
- the ABO group*
- the RhD group stated as positive or negative*
- the name, composition and volume of the anticoagulant solution*
- the date of collection
- the expiry date*
- the temperature of storage
- the blood pack lot number.*

In addition the following statements should be made:

INSTRUCTION
Always check patient/component compatibility/identity
Inspect pack and contents for signs of deterioration or damage
Risk of adverse reaction/infection

Storage (for general guidelines see Section 7.6)

- For top-up transfusions of neonates and infants under one year, this component may be stored for a maximum of 35 days at a core temperature of 4°C ± 2°C if an adenine supplemented anticoagulant is used, otherwise the maximum period of storage is 28 days at a core temperature of 4°C ± 2°C.
- For large volume transfusion of neonates, this component should be used within five days of venepuncture.
- Variation from the core temperature of 4°C ± 2°C must be kept to a minimum during storage and restricted to any short period necessary for examining, labelling or issuing the component.
- Exceptionally i.e. due to equipment failure at a Blood Centre red cell components which have been prepared in a closed system and exposed to a core temperature not exceeding 10°C and not less than 1°C may be released for transfusion provided:
  - that the component has been exposed to such a temperature change on one occasion only
  - that the duration of the temperature change has not exceeded 5 hours
  - that a documented system is available in each Blood Centre to cover such eventualities
  - that adequate records of the incident are compiled and retained.

Testing

In addition to the mandatory and other tests required for blood donations described in Chapter 10, and leucocyte counting (see Sections 7.2 and 8.1), the component shall be free from clinically significant irregular blood group antibodies and high titre anti-A and/or anti-B, and antibodies to CMV. Furthermore, a minimum of 75% of those components tested for the other parameters shown in Table 8.17 shall meet the specified values.

Table 8.17 Red cells for neonates and infants, leucocyte depleted – additional tests

Parameter	Frequency of test	Specification
Volume	1% or 10 per month, whichever is greater If less than 10 per month,	Within locally defined nominal volume range
Haemoglobin content	every component	Locally defined
Haemolysis (only required if produced as a primary component)	As per Section 8.2	<0.8% of red cell mass
Leucocyte Count*	As per Sections 7.2 and 8.1	$<5 \times 10^6$/starting component

*Methods validated for counting low levels of leucocytes must be used.

Transportation (for general guidelines see Section 7.10)

For red cell components, transit containers, packing materials and procedures should have been validated to ensure the component surface temperature can be maintained between 2°C and 10°C during transportation. Additionally:

- the validation exercise should be repeated periodically
- if melting ice is used, it should not come into direct contact with the components
- dead air space in packaging containers should be minimized

- as far as is practicable, transit containers should be equilibrated to their storage temperature prior to filling with components
- transport time normally should not exceed 12 hours
- in some instances it is necessary to issue red cell components that have not been cooled to their storage temperature prior to placing in the transit container. The transport temperature specified above is not applicable for such consignments.

8.23 Red Cells in Additive Solution for Neonates and Infants, Leucocyte Depleted

A red cell component suitable for neonates and infants under one year containing less than 5×10^6 leucocytes (per starting component). The red cells are suspended in an additive solution and may be divided into approximately equal volumes using a closed system.

Technical information

- The component should be free from clinically significant irregular blood group antibodies including high titre anti-A and anti-B and should be negative for antibodies to CMV.
- Red cells in additive solution for neonates and infants, Leucocyte Depleted should be transfused through a 170–200μm filter.

Labelling (for general guidelines see Section 7.5)

The following shall be included on the label:

(* = in eye-readable and UKBTS approved barcode format.)

- red cells in additive solution for neonates and infants, Leucocyte Depleted* and volume
- the blood component producer's name*
- the donation number and, if divided, sub-batch number*
- the ABO group*
- the RhD group stated as positive or negative*
- the name, composition and volume of the additive solution*
- the date of collection
- the expiry date*
- the temperature of storage
- the blood pack lot number.*

In addition the following statements should be made:

INSTRUCTION
Always check patient/component compatibility/identity
Inspect pack and contents for signs of deterioration or damage
Risk of adverse reaction/infection

Storage (for general guidelines see Section 7.6)

- Red cells in additive solution, Leucocyte Depleted for top up transfusion of neonates and infants under 1 year may be stored for a maximum of 35 days at a core temperature of 4°C ± 2°C.

- Variation from the core temperature of 4°C ± 2°C must be kept to a minimum during storage and restricted to any short period necessary for examining, labelling or issuing the component.
- Exceptionally i.e. due to equipment failure at a Blood Centre, red cell components which have been prepared in a closed system and exposed to a core temperature not exceeding 10°C and not less than 1°C may be released for transfusion provided:
  - that the component has been exposed to such a temperature change on one occasion only
  - that the duration of the temperature change has not exceeded 5 hours
  - that a documented system is available in each Blood Centre to cover such eventualities
  - that adequate records of the incident are compiled and retained.

Testing

In addition to the mandatory and other tests required for blood donations described in Chapter 10, and leucocyte counting (see Sections 7.2 and 8.1), the component shall be free from clinically significant irregular blood group antibodies and high titre anti-A and/or anti-B, and antibodies to CMV. Furthermore, a minimum of 75% of those components tested for the other parameters shown in Table 8.18 shall meet the specified values.

Table 8.18 Red cells in additive solution for neonates and infants, leucocyte depleted – additional tests

Parameter	Frequency of test	Specification
Volume	1% or 10 per month, whichever is greater	Within locally defined nominal volume range
Haemoglobin content	If less than 10 per month, every component	Locally defined
Haemolysis (only required if produced as a primary component)	As per Section 8.2	<0.8% of red cell mass
Leucocyte Count*	As per Sections 7.2 and 8.1	$<5 \times 10^6$/starting component

*Methods validated for counting low numbers of leucocytes must be used.

Transportation (for general guidelines see Section 7.10)

For red cell components, transit containers, packing materials and procedures should have been validated to ensure the component surface temperature can be maintained between 2°C and 10°C during transportation. Additionally:

- the validation exercise should be repeated periodically
- if melting ice is used, it should not come into direct contact with the components
- dead air space in packaging containers should be minimized
- as far as is practicable, transit containers should be equilibrated to their storage temperature prior to filling with components
- transport time normally should not exceed 12 hours
- in some instances it is necessary to issue red cell components that have not been cooled to their storage temperature prior to placing in the transit container. The transport temperature specified above is not applicable for such consignments.

8.24 Fresh Frozen Plasma, Neonatal Use, Methylene Blue Treated and Removed, Leucocyte Depleted

Fresh Frozen Plasma Methylene Blue Treated (MBT) and Removed, Leucocyte Depleted, is plasma that has been obtained from whole blood or by apheresis from a previously tested donor (as defined in Section 8.18), contains less than 5×10^6 leucocytes and has been treated with methylene blue and exposure to visible light to inactivate pathogens.

Using a closed system the component may be sub divided into approximately equal volumes and rapidly frozen to a temperature that will maintain the activity of labile coagulation factors.

Technical information

- Where the starting component is sourced outwith the UK, a detailed and agreed specification must be available.
- Donations of whole blood where the bleed time exceeded 15 minutes are not suitable for the production of plasma components for direct clinical use.
- The component should be free from clinically significant irregular blood group antibodies including high titre anti-A and anti-B. Testing for CMV antibodies is not required.
- Ideally the plasma should be separated before the red cell component is cooled to its storage temperature.
- The method of preparation should ensure the component has the maximum level of labile coagulation factors with minimum cellular contamination.
- Intact white blood cells in the plasma should be reduced to less than 5×10^6 per unit prior to exposure to methylene blue and visible light.
- The production process should be validated to ensure that components meet the specified limits for FVIII:C content.
- Greater FVIII:C yields will be obtained when the plasma is separated as soon as possible after venepuncture, MB treated and rapidly frozen to –30°C or below.
- The MBT process reduces the FVIII:C content by approximately 30% when compared to standard FFP.
- Component samples collected for the Quality Monitoring assessment of FVIII:C should have approximately the same ABO group distribution as issued components.
- The process for methylene blue removal should be validated to give components with a methylene blue concentration ≤0.30 μM. The methylene blue content of the final component is the initial content of the unsplit starting component (< approximately 30 μg per unit) divided by the number of split components produced.
- Fresh Frozen Plasma, Methylene Blue Treated and Removed, Leucocyte Depleted should be transfused through a 170–200 μm filter.

Labelling (for general guidelines see Section 7.5)

The following shall be included on the label:

(* = in eye-readable and UKBTS approved barcode format.)

- fresh frozen plasma, neonatal use, methylene blue treated and removed, Leucocyte Depleted* and volume
- the blood component producer's name*
- the donation number*

- the ABO group*
- the RhD group stated as positive or negative*
- the date of collection
- the expiry date of the frozen component*
- the temperature of storage
- the blood pack lot number*
- a warning that the component should be used within four hours of thawing
- the name, composition and volume of the anticoagulant.

In addition the following statements should be made:

INSTRUCTION
Always check patient/component compatibility/identity
Inspect pack and contents for signs of deterioration or damage
Risk of adverse reaction/infection

Storage (for general guidelines see Section 7.6)

- The component should be stored at a core temperature of –30°C or below for a maximum of 24 months.
- Although a storage temperature below –30°C improves the preservation of labile coagulation factors, lower temperatures increase the fragility of plastic. Particular care must be taken when handling such packs.
- The component should be thawed at 37°C in a waterbath or other equipment designed for the purpose, within a vacuum sealed overwrap bag. Protocols must be in place to ensure that the equipment is cleaned daily and maintained to minimize the risk of bacterial contamination.
- Once thawed, the component must not be refrozen and should be used immediately. If delay is unavoidable, the component should be stored at ambient temperature and used within four hours.

Testing

In addition to the mandatory and other tests required for blood donations described in Chapter 10, and leucocyte counting (see Sections 7.2 and 8.1), the component shall be free from clinically significant irregular blood group antibodies and high titre anti-A and/or anti-B. Furthermore, a minimum of 75% of those components tested for the other parameters shown in Table 8.19 shall meet the specified values.

Table 8.19 Fresh frozen plasma, neonatal use, methylene blue treated and removed, leucocyte depleted – additional tests

Parameter	Frequency of test	Specification
Volume	1% or 10 per month, whichever is greater If less than 10 per month, every component	Within locally defined nominal volume range and within any limits specified for the MBT process used
Platelets		$<30 \times 10^9$/L**
FVIII:C		>0.50 IU/mL
Leucocyte Count*	As per Sections 7.2 and 8.1	$<5 \times 10^6$/unit**

*Methods validated for counting low numbers of leucocytes must be used.
**Prefreeze in starting component.

Transportation (for general guidelines see Section 7.10)

Every effort should be made to maintain the core storage temperature during transportation. Unless the component is to be thawed and used straight away it should be transferred immediately to storage at the recommended temperature.

8.25 Cryoprecipitate, Methylene Blue Treated and Removed, Leucocyte Depleted

The component represents a source of concentrated FVIII:C, and von Willebrand factor, fibrinogen, Factor XIII and fibronectin from a unit of Fresh Frozen Plasma, Methylene Blue Treated and Removed. The plasma from which the cryoprecipitate, methylene treated and removed, Leucocyte Depleted was produced contains less than 5×10^6 leucocytes per component and derived from a previously tested donor (as defined in Section 8.18).

Technical information

- Where the starting component is sourced outwith the UK, a detailed and agreed specification must be available.
- Donations of whole blood where the bleed time exceeded 15 minutes are not suitable for the production of plasma components for direct clinical use.
- Cryoprecipitate, Methylene Blue Treated and Removed, Leucocyte Depleted is the cryoglobulin fraction of plasma obtained by thawing a single donation of Fresh Frozen Plasma, Methylene Blue Treated and Removed at 4°C ± 2°C.
- The process for methylene blue removal should be validated to give components with a methylene blue concentration ≤0.30 μM (< approximately 30 μg per unit) in the starting component.
- For storage, Cryoprecipitate, Methylene Blue Treated and Removed should be rapidly frozen to a core temperature of –30°C or below within two hours of preparation.
- Component samples collected for the Quality Monitoring assessment of FVIII:C should have approximately the same ABO group distribution as issued components.
- Cryoprecipitate, Methylene Blue Treated and Removed, Leucocyte Depleted, should be transfused through a 170–200 μm filter.

Labelling (for general guidelines see Section 7.5)

The following shall be included on the component label:

(* = in eye-readable and UKBTS approved barcode format.)

- Cryoprecipitate, Methylene Blue Treated and Removed, Leucocyte Depleted* and volume
- the blood component producer's name*
- the donation number*
- the ABO group*
- the RhD group stated as positive or negative*
- the date of collection
- the expiry date of the frozen component*
- the temperature of storage
- the blood pack lot number*
- a warning that the component must be used within four hours of thawing

- the name, composition and volume of the anticoagulant or additive solution.

In addition, the following statements should be made:

INSTRUCTION
Always check patient/component compatibility/identity
Inspect pack and contents for signs of deterioration or damage
Risk of adverse reaction/infection

Storage (for general guidelines see Section 7.6)

- The component should be stored at a core temperature of –30°C or below for a maximum of 24 months.
- Although a storage temperature below –30°C improves the preservation of labile coagulation factors, lower temperatures increase the fragility of plastic. Particular care must be taken when handling such packs.
- The component should be thawed at 37°C in a waterbath or other equipment designed for the purpose, within a vacuum sealed overwrap bag. Protocols must be in place to ensure that the equipment is cleaned daily and maintained to minimize the risk of bacterial contamination.
- Once thawed, the component must not be refrozen and should be used immediately. If delay is unavoidable, the component should be stored at ambient temperature and used within four hours.

Testing

In addition to the mandatory and other tests required for blood donations described in Chapter 10, and leucocyte counting (see Sections 7.2 and 8.1), a minimum of 75% of those components tested for the parameters shown in Table 8.20 shall meet the specified values.

Table 8.20 Cryoprecipitate, methylene blue treated and removed, leucocyte depleted – additional tests

Parameter	Frequency of test	Specification
Volume	1%	Within locally defined nominal volume range
Fibrinogen	1%	>140 mg/unit
FVIII:C		>70 IU/unit
Leucocyte Count*	As per Sections 7.2 and 8.1	$<5 \times 10^6$/unit**

*Methods validated for counting low numbers of leucocytes must be used.
**Prefreeze in starting component.

Transportation (for general guidelines see Section 7.10)

Every effort should be made to maintain the core storage temperature during transportation. Unless the component is to be thawed and used straight away it should be transferred immediately to storage at the recommended temperature.

8.26 Platelets for IUT, Leucocyte Depleted

A hyperconcentrated platelet component for intrauterine transfusion prepared by apheresis that contains less than 2.5×10^6 leucocytes per donation.

Technical information

- The component should be free from clinically significant irregular blood group antibodies including high titre anti-A and anti-B and should be negative for antibodies to CMV.

- The component must be used by the end of day 1.
- The component must be gamma irradiated. See BCSH Transfusion guidelines for neonates and older children.[2]
- The component should contain a concentration of platelets between 2–4 × 10^{12}/litre in a collected volume generally in the range of 50–100 mL.
- All components should be quality monitored and achieve the specified requirements. The testing need not necessarily be performed before component release.
- If platelets are to be issued as HPA-matched (e.g. HPA-1a or HPA-5b negative) then donors should be screened and found negative for all clinically significant HPA antibodies and HLA antibodies. This screening can be done on an initial sample and does not need repeating at each donation unless the donor has been transfused or pregnant since the last antibody screen.
- A record which demonstrates that the donor has not been transfused since the initial negative screen for antibodies and in case of female donors that the donor has not been pregnant since the initial negative screen for antibodies needs to be maintained.
- Platelets for IUT, Leucocyte Depleted should be transfused through a 170–200 μm filter.

Labelling (for general guidelines see Section 7.5)

The following shall be included on the label:

(* = in eye-readable and UKBTS approved barcode format.)

- platelets for IUT, Leucocyte Depleted* and volume
- the blood component producer's name*
- the donation number*
- the ABO group*
- the RhD group stated as positive or negative*
- the date of collection
- the expiry date and time*
- the temperature of storage and a comment that continuous gentle agitation during storage is recommended
- the blood pack lot number*
- the name, composition and volume of the anticoagulant.

In addition, the following statements should be made:

INSTRUCTION
Always check patient/component compatibility/identity
Inspect pack and contents for signs of deterioration or damage
Risk of adverse reaction/infection

Storage (for general guidelines see Section 7.6)

- The component should be stored at a core temperature of 22°C ± 2°C for use up to the end of Day 1.
- The component should be gently and continuously agitated during storage.

Testing

In addition to the mandatory and other tests required for blood donations described in Chapter 10, and leucocyte counting (see Sections 7.2 and 8.1), the component shall be free from clinically significant irregular blood group antibodies and high titre anti-A and/or anti-B and antibodies to CMV. Furthermore, all components tested for the other parameters shown in Table 8.21 shall meet the specified values.

Table 8.21 Platelets for IUT, leucocyte depleted – additional tests

Parameter	Frequency of test	Specification
Volume	Every component	Within locally defined range
Platelet concentration		2–4 × 10^{12}/L
pH at end of shelf life**		
Leucocyte Count*	As per Sections 7.2 and 8.1	<2.5 × 10^{6}/unit

*Methods validated for counting low numbers of leucocytes must be used.
**The shelf life of this hyperconcentrated platelet component has been set to reflect validation data. Therefore once this has been validated locally, there is no need to measure pH at expiry on a routine basis.

NOTE: Visual inspection of platelet components for the swirling phenomenon, clumping, excessive red cell contamination and abnormal volume is a useful pre-issue check.

Transportation (for general guidelines see Section 7.10)

Containers for transporting platelets should be equilibrated at room temperature 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, the component should be transferred to storage at a core temperature of 22°C with continuous gentle agitation.

Plastic overwraps should be removed prior to storage.

8.27 Platelets for Neonatal Use, Leucocyte Depleted

A single donor platelet component for neonatal use that contains less than 5 × 10^{6} leucocytes per starting component.

Technical information

- The component should be free from clinically significant irregular blood group antibodies including high titre anti-A and anti-B and should be negative for antibodies to CMV.
- The component may be prepared by splitting an apheresis platelet, Leucocyte Depleted using a closed system.
- The component should contain >40 × 10^{9} platelets in sufficient plasma to maintain the pH between 6.4 and 7.4 at the end of the shelf life of the component.
- The component may be leucodepleted as part of an apheresis process or by subsequent filtration of the platelet component.
- If platelets are to be issued as HPA-matched (e.g. HPA-1a or HPA-5b negative) then donors should be screened and found negative for all clinically significant HPA antibodies (as defined in Chapters 15 and 17) and HLA antibodies. This screening can be done on an initial sample and does not need repeating at each donation unless the donor has been transfused or pregnant since the last antibody screen.
- A record which demonstrates that the donor has not been transfused since the initial negative screen for antibodies and in case of female donors that the donor has not

been pregnant since the initial negative screen for antibodies needs to be maintained. Donations from donors with clinically significant HPA antibodies should not be used for the production of plasma-rich blood products (e.g. plasma, platelet concentrate, whole blood, cryoprecipitate). Red cells suspended in additive solution can be produced from such donations.

- Platelets for neonatal use, Leucocyte Depleted should be transfused through a 170–200 μm filter.

Labelling (for general guidelines see Section 7.5)

The following shall be included on the label:

(* = in eye-readable and UKBTS approved barcode format.)

- platelets for Neonatal Use, Leucocyte Depleted* and volume
- the blood component producer's name*
- the donation number and, if divided, sub-batch number*
- the ABO group*
- the RhD group stated as positive or negative*
- the date of collection
- the expiry date*
- the temperature of storage and a comment that continuous gentle agitation throughout storage is recommended
- the blood pack lot number*
- the name, composition and volume of the anticoagulant or additive solution.

In addition, the following statements should be made:

INSTRUCTION
Always check patient/component compatibility/identity
Inspect pack and contents for signs of deterioration or damage
Risk of adverse reaction/infection

Storage (for general guidelines see Section 7.6)

- The component should be stored at a core temperature of 22°C ± 2°C for up to five days. Appropriate pack and platelet concentration combinations may allow storage up to seven days, but due to concerns over bacterial contamination would require either an assay to exclude bacterial contamination prior to transfusion or application of a licensed pathogen inactivation procedure.
- Platelets should be agitated during storage. If agitation is interrupted, for example due to equipment failure or prolonged transportation, the components are suitable for use, retaining the same shelf life, provided the interruption is for no longer than a single episode of 24 hours.

Testing

In addition to the mandatory and other tests required for blood donations described in Chapter 10, and leucocyte counting (see Sections 7.2 and 8.1), the component shall be free from clinically significant irregular blood group antibodies and high titre anti-A and/or anti-B and antibodies to CMV. Furthermore, a minimum of 75% of those components tested for the other parameters shown in Table 8.22 shall meet the specified values.

Table 8.22 Platelets for Neonatal Use, Leucocyte Depleted – additional tests

Parameter	Frequency of test	Specification
Volume	1% or 10 per month, whichever is greater	Within locally defined range
Platelet count	If less than 10 per month,** every component if available	>40 × 10^9/unit
pH at end of shelf life		6.4–7.4
Leucocyte Count*	As per Sections 7.2 and 8.1	<5 ×10^6/starting component

*Methods validated for counting low levels of leucocytes must be used.
**If producing low numbers, use of most units is likely to make testing of outdated units impossible. In this situation periodic checks to ensure end-of-shelf-life quality should be undertaken with the combination of blood pack platelet concentration and storage conditions in routine use.

NOTE: Visual inspection of platelet components for the swirling phenomenon, clumping, excessive red cell contamination and abnormal volume is a useful pre-issue check.

Transportation (for general guidelines see Section 7.10)

Containers for transporting platelets should be equilibrated at room temperature 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, the component should be transferred to storage at a core temperature of 22°C with continuous gentle agitation.

Plastic overwraps should be removed prior to storage.

8.28 Irradiated components

It is not necessary to irradiate the following components:

- cryopreserved red cells after washing
- plasma components.

Irradiated components not used for the intended recipient can safely be used for recipients who do not require irradiated components provided the other requirements of Chapters 7 and 8 have been satisfied. However, any reduction in shelf life resulting from the irradiation process must be observed.

Irradiated components should conform to their appropriate specification previously given in this chapter. In addition, the guidelines shown below should be observed.

Description

Irradiated components are components that have been irradiated by a validated procedure.

Technical information

- Other than for use in intrauterine or exchange transfusion, red cells can be irradiated at any time up to 14 days after collection.
- Platelets can be irradiated at any stage in their storage.
- Granulocytes should be irradiated as soon as possible after production.
- For red cells, platelets and granulocytes the recommended minimum dose achieved in the irradiation field is 25Gy, with no part receiving >50Gy.
- Platelets to be transfused *in utero* to treat alloimmune thrombocytopenia must be irradiated.

- Laboratories performing irradiation of blood components must work to a clearly defined specification and are strongly recommended to work closely with a medical physicist. The defined irradiation procedure must be validated and there must be regular monitoring of the blood component dosimetry and the laboratory equipment.

- It is recommended that irradiation of blood components is carried out using dedicated blood irradiation machines. If radiotherapy machines are used, equivalent protocols should be developed.

- Gamma ray sensitive labels should be used as an aid to differentiating irradiated from non-irradiated components. However, it may not be necessary to attach a gamma ray sensitive label to every component pack provided that the irradiation procedure follows a validated, documented and well controlled system of work that is integrated to the component labelling and release mechanism and permits retrospective audit of each stage of the irradiation process.

- There should be a permanent record of all units irradiated. This should include details of irradiation batch and donation numbers, component type, the site of irradiation, when irradiation was performed and by whom.

Labelling

- Irradiated components must be identified by the applied labelling and include the date of irradiation and any reduction in shelf life.

- Labels which are sensitive to gamma rays and change from 'NOT IRRADIATED' to 'IRRADIATED' are available and are considered a useful indicator of exposure to gamma rays. The dose at which the label changes to 'IRRADIATED' must be marked on the label. It must be remembered that such labels simply reflect that the unit has been exposed to gamma rays and their use does not replace the need for regular and precise dosimetry nor carefully controlled working procedures.

Storage (for general guidelines see Section 7.6)

- Red cell components, other than for intrauterine or exchange transfusion can be irradiated at any time up to 14 days after collection and stored for up to 14 days thereafter provided the other requirements of this section are adhered to.

- Where irradiated red cells are intended for intrauterine or exchange transfusion or where the patient is at particular risk from hyperkalaemia, red cells should be transfused within 24 hours of irradiation. Furthermore, red cells intended for intrauterine or exchange transfusion should be transfused within five days of collection.

- Irradiated platelets can be stored up to their normal shelf life of five days after collection.

- Granulocytes must be irradiated and should be used with minimum delay after irradiation but within the shelf life specified earlier in this chapter.

8.29 Tabulated information

The tables following the references summarize technical information, transport, storage and component specifications and should be read in conjunction with advice given elsewhere within Chapters 7 and 8. (Key for tables: LSL = Lower specified limit; USL = Upper specified limit.)

References

1. Dumont, L., Dzik, W., Rebulla, P., Brandwein, H. and members of the BEST Expert Working Party of the ISBT (1996) 'Practical guidelines for process validation and process control of white cell-reduced blood components: report of the Biomedical Excellence for Safer Transfusion (BEST) Working Party of the International Society of Blood Transfusions (ISBT)'. *Transfusion*, **36**, pp11–20.

2. British Committee for Standards in Haematology (2004) Blood Transfusion Task Force. 'Transfusion guidelines for neonates and older children'. *British Journal of Haematology*, **124**, pp433–53.

Table 8.23 Red cell components

Component name	Technical information	Storage temp. (°C)	Max. storage period (days)	Transport temp. (°C)	Volume (mL)		Hb. content (g/unit)	Other
					LSL	USL	LSL	
Whole Blood, Leucocyte Depleted	A unit of blood collected into an anticoagulant. A unit of whole blood, Leucocyte Depleted consists of 450 mL ± 10% of blood from a suitable donor (see Chapter 3), plus 63 mL of anticoagulant, which is then leucocyte depleted, and stored in an approved container.	4 ± 2	35 (solution containing adenine) 28 (other)	2–10	420	520	40	Haemolysis see Section 8.2
Red Cells, Leucocyte Depleted	A red cell component prepared by removing a proportion of the plasma from leucocyte depleted whole blood or by leucodepleting plasma reduced red cells.	4 ± 2	35 (solution containing adenine) 28 (other)	2–10	220	340	40	Haemolysis see Section 8.2
Red cells in Additive Solution, Leucocyte Depleted	A red cell component prepared by removing a proportion of the plasma from leucocyte depleted whole blood and suspending in an approved additive solution or by removing a proportion of the plasma from whole blood, suspending in an approved additive solution followed by leucodepletion of the red cells.	4 ± 2	42	2–10	220	340	40	Haemolysis see Section 8.2
Red cells, Washed, Leucocyte Depleted	A red cell component, which has been washed with 0.9% w/v sodium chloride for injection (BP). The Red Cells, Washed, Leucocyte Depleted may then be suspended in an approved additive solution. The amount of residual protein will depend on the washing protocol. Washing can be performed by interrupted or continuous flow centrifugation. The use of validated washing procedures that incorporate chilled saline, at least for the final wash and chilled approved additive solution for suspension, is recommended. This will minimize the risk of bacterial contamination and helps to produce a component that meets the transit temperature requirements. After preparation, the component should be used as soon as possible.	4 ± 2	1 (not suspended in additive solution) An increased storage period may result from validation work.	2–10	Local	Local	40	Protein <0.5g/unit
Red Cells, Thawed and Washed, Leucocyte Depleted	A red cell component frozen in the presence of a cryoprotectant (preferably within 5 days of collection), and washed before use. The Red Cells, Thawed and Washed, Leucocyte Depleted may then be suspended in an additive solution. The concentration and nature of the cryoprotectant must provide appropriate protection of the red cells at the intended storage temperature. The entire process of freezing, thawing and washing must be validated and documented. The use of validated washing procedures that incorporate chilled saline, at least for the final wash and chilled approved additive solution for suspension, is recommended. This will minimize the risk of bacterial contamination and helps to produce a component that meets the transit temperature requirements. After preparation, the component should be used as soon as possible.	4 ± 2	1 (not suspended in additive solution) An increased storage period may result from validation work.	2–10	Local	Local	36	Supernatant Hb <2g/unit

Table 8.24 Platelet and granulocyte components

Component name	Technical information	Storage temp. (°C)	Max. storage period (days)	Transport temp. (°C)	Volume (mL)		Platelet yield ($\times 10^9$/ unit)	pH at out date		Other
					LSL	USL	LSL	LSL	USL	
Platelets, Pooled, Buffy Coat Derived, Leucocyte Depleted	A pool of platelets derived from buffy coats. The component must be prepared at ambient temperature before the red cell component is cooled to its storage temperature. Initial separation of buffy coat occurs within 12 hours of venepuncture, with secondary pooling and processing of buffy coats to produce the final component completed before the end of day 1. Packs currently in use for this purpose allow for storage at a core temperature of 22°C ± 2°C with continuous gentle agitation for up to 5 days in a closed system. Appropriate pack and platelet concentration combinations may allow storage up to 7 days, but due to concerns over bacterial contamination would require either an assay to exclude bacterial contamination prior to transfusion or application of a licensed pathogen inactivation procedure.	22 ± 2	5	20–24	Local	Local	240	6.4	7.4	
Platelets, Apheresis, Leucocyte Depleted	A single donor platelet component. Platelets, Apheresis, Leucocyte Depleted may be collected by a variety of apheresis systems using different protocols. Packs currently in use for this purpose allow for storage at a core temperature of 22°C ± 2°C with continuous gentle agitation for up to 5 days in a closed system. Appropriate pack and platelet concentration combinations may allow storage up to 7 days, but due to concerns over bacterial contamination would require either an assay to exclude bacterial contamination prior to transfusion or application of a licensed pathogen inactivation procedure.	22 ± 2	5	20–24	Local	Local	240	6.4	7.4	

Table 8.24 Continued

Component name	Technical information	Storage temp. (°C)	Max. storage period (days)	Transport temp. (°C)	Volume (mL)		Platelet yield (× 10^9/unit)	pH at out date		Other
					LSL	USL	LSL	LSL	USL	
Platelets, Suspended in Additive Solution, Leucocyte Depleted	A platelet concentrate, derived from buffy coats or apheresis, which contain less than 5×10^6 leucocytes and where the suspending medium is platelet additive solution. Where prepared from buffy coats, initial separation of buffy coat normally occurs within 12 hours of venepuncture, with secondary pooling and processing of buffy coats to produce the final component generally completed before the end of day 1.	22 ± 2	1	20–24	Local	Local	240	6.4	7.4	
Granulocytes, Apheresis	A component prepared from anticoagulated blood, which is separated into components by a suitable apheresis machine with retention of granulocytes as the major cellular product, suspended in a portion of the plasma. The remaining elements may be returned to the donor. Granulocytes may be collected by a variety of apheresis systems using different protocols. The component must be stored at ambient temperature without agitation. The component must be gamma irradiated before use. Granulocytes apheresis should be used as soon as possible after their preparation, whether prepared in an open or closed system. If storage is unavoidable, the component should be stored, without agitation.	22 ± 2 No agitation	1	20–24	Local	Local				Granulocyte count >5 × 10^9/unit

Table 8.25 Plasma components

Component name	Technical information	Storage temp. (°C)	Max. storage period (months)	Transport temp. (°C)	Volume (mL)		Platelet yield ($\times 10^9$/L)	FVIIIc (IU/mL)	FVIIIc (IU/unit)	Fibrinogen (mg/unit)
					LSL	USL	USL	LSL	LSL	LSL
Fresh Frozen Plasma, Leucocyte Depleted	Plasma that has been obtained from whole blood or by apheresis from a previously tested donor (as defined in Section 8.3). The plasma has been rapidly frozen to a temperature that will maintain the activity of labile coagulation factors. The Total Protein content should be >50g/L.	<–30	24 (Up to 24 hours post thaw at 4°C)	<–30	Local	Local	30 (Plt) 6 (Rbc)	0.70		
Fresh Frozen Plasma, Methylene Blue Treated and Removed, Leucocyte Depleted	The components are produced from plasma that has been obtained from whole blood or by apheresis from a previously tested donor (as defined in Section 8.3) and has been treated with methylene blue and exposure to visible light to reduce pathogens. The Methylene Blue is subsequently removed using an adsorption filter. The MB removal filter must have been shown to be capable of reducing methylene blue in the final component to $\leq$0.30μM (approximately 30μg per unit). The concentration pre removal is approximately 1μM (<100μg/unit). The components need not be tested for leucocyte or platelet contamination where it is derived from frozen and thawed plasma unless the counting methodology is validated for frozen/thawed plasma or where the source plasma is monitored. Ideally the plasma should be separated before the red cell component is cooled to its storage temperature. The Methylene Blue treatment process reduces the FVIII:C content by approximately 30% when compared to standard FFP.	<–30	24	<–30	Local	Local	30 (Plt)	0.50		
Cryoprecipitate, Leucocyte Depleted	The component represents a source of concentrated FVIII:C, and von Willebrand factor, fibrinogen, Factor XIII and fibronectin from a unit of Fresh Frozen Plasma, Leucocyte Depleted. The plasma from which the Cryoprecipitate, Leucocyte Depleted was derived is from a previously tested donor (as defined in Section 8.3). Cryoprecipitate, Leucocyte Depleted is the cryoglobulin fraction of plasma obtained by thawing a single donation of fresh frozen plasma at 4°C $\pm$ 2°C.	<–30	24	<–30	Local	Local			70	140
Cryoprecipitate, Methylene Blue Treated and Removed, Leucocyte Depleted. See Table 8.28.										
Plasma, Cryoprecipitate Depleted, Leucocyte Depleted	The supernatant plasma removed during the preparation of Cryoprecipitate, Leucocyte Depleted. The plasma from which the Plasma, Cryoprecipitate Depleted, Leucocyte Depleted was made was derived from a previously tested donor (as defined in Section 8.3).	<-30	24 (Up to 24 hours post thaw at 4°C)	<–30	Local	Local	30 (Plt) 6 (Rbc)			

Table 8.26 Neonatal and infant red cell components (produced from previously tested donors; see Section 7.17)

Component name	Technical information	Storage temp. (°C)	Max. storage period (days)	Transport temp. (°C)	Volume (mL)		Haematocrit (L/L)		Hb. content (g/unit)
					LSL	USL	LSL	USL	LSL
Red Cells for Intrauterine Transfusion (IUT), Leucocyte Depleted	A component for intrauterine transfusion, prepared by removing a proportion of the plasma from fresh whole blood. The component must be gamma irradiated before use.	4 ± 2	5 (24 hrs post irradiation)	2–10	Local	Local	Not <0.70		Locally defined
Whole Blood for Exchange Transfusion, Leucocyte Depleted	A component for exchange or large volume transfusion of neonates. The component must be gamma irradiated before use.	4 ± 2	5 (24 hrs post irradiation)	2–10	Local	Local			40
Red Cells for Exchange Transfusion, Leucocyte Depleted	A component for exchange or large volume transfusion of neonates prepared by removing a proportion of the plasma from fresh whole blood. The component must be gamma irradiated before use.	4 ± 2	5 (24 hrs post irradiation)	2–10	Local	Local	0.50	0.60	40
Red Cells for Neonates and Infants, Leucocyte Depleted	A red cell component suitable for neonates and infants under 1 year. The red cells may be divided into approximately equal volumes using a closed system. For haemolysis specification see Section 8.2 (only required when the component is made from a primary process).	4 ± 2	35 (solution containing adenine) 28 (other)	2–10	Local	Local			Locally defined
Red Cells in Additive Solution for Neonates and Infants, Leucocyte Depleted	A red cell component suitable for neonates and infants under 1 year. The red cells are suspended in an additive solution and may be divided into approximately equal volumes using a closed system. For haemolysis specification see Section 8.2 (only required when the component is made from a primary process).	4 ± 2	35 (solution containing adenine)	2–10	Local	Local			Locally defined

Table 8.27 Neonatal and infant platelet components (produced from previously tested donors; see Section 8.18)

Component name	Technical information	Storage Temp. (°C)	Max. Storage Period (Days)	Transport Temp. (°C)	Volume (mL)		Platelet yield ($\times 10^9$/unit)	pH (at outdate)		Other
					LSL	USL	LSL	LSL	USL	
Platelets for IUT, Leucocyte Depleted	A hyperconcentrated platelet component for intrauterine transfusion prepared by apheresis. If platelets are to be issued as HPA-matched (e.g. HPA-1a or HPA-5b negative) then donors should be screened and found negative for all clinically significant HPA antibodies and HLA antibodies. This screening can be done on an initial sample and does not need repeating at each donation unless the donor has been transfused or pregnant since the last antibody screen. A record which demonstrates that the donor has not been transfused since the initial negative screen for antibodies and in case of female donors that the donor has not been pregnant since the initial negative screen for antibodies needs to be maintained. The component must be gamma irradiated before use. All components require quality monitoring testing.	22 ± 2	1	20–24	Generally in the range 50–100 mL					Platelet concentration in the range 2–4 × 10^{12}/L Leucocyte count <2.5 ×10^6/unit
Platelets for Neonates and Infants, Leucocyte Depleted	A single donor platelet component for neonatal use. The component may be prepared by splitting an apheresis platelet using a closed system. If platelets are to be issued as HPA-matched (e.g. HPA-1a or HPA-5b negative) then donors should be screened and found negative for all clinically significant HPA and HLA antibodies. This screening can be done on an initial sample and does not need repeating at each donation unless the donor has been transfused or pregnant since the last antibody screen. A record which demonstrates that the donor has not been transfused since the initial negative screen for antibodies and in case of female donors that the donor has not been pregnant since the initial negative screen for antibodies needs to be maintained.	22 ± 2	5	20–24	Local		Local	6.4	7.4	

Table 8.28 Neonatal and infant plasma components (produced from previously tested donors; see Section 8.18)

Component name	Technical information	Storage Temp. (°C)	Max. Storage Period (Months)	Transport Temp. (°C)	Volume (mL)		Platelet count ($\times 10^9$/L)	FVIIIc (IU/unit)	Fibrinogen (mg/unit)
					LSL	USL	USL	LSL	LSL
Fresh Frozen Plasma, Neonatal use, Methylene Blue Treated and Removed, Leucocyte Depleted	The components are produced from plasma that has been obtained from whole blood or by apheresis from a previously tested donor (as defined in Section 8.18) and has been treated with methylene blue and exposure to visible light to reduce pathogens. The Methylene Blue is subsequently removed using an adsorption filter. The methylene blue content of the final component is the initial content of the unsplitstarting component (<30μg per unit) divided by the number of split components produced. The MB removal filter in use must have been shown to be capable of reducing methylene blue in the final component to $\leq$0.30μM. The concentration pre removal is approximately 1 μM (<100μg/unit). The components need not be tested for leucocyte or platelet contamination where it is derived from frozen and thawed plasma unless the counting methodology is validated for frozen/thawed plasma or where the source plasma is monitored. Ideally the plasma should be separated before the red cell component is cooled to its storage temperature. The Methylene Blue treatment process reduces the FVIII:C content by approximately 30% when compared to standard FFP.	<–30	12	<–30	Local	Local	30	0.5	0.5
Cryoprecipitate, Methylene Blue Treated and Removed, Leucocyte Depleted	The component represents a source of concentrated FVIII:C, and von Willebrand factor, fibrinogen, Factor XIII and fibronectin from a unit of Fresh Frozen Plasma, Methylene Blue Treated and Removed, Leucocyte Depleted. The plasma from which the Cryoprecipitate was derived is from a previously tested donor (as defined in Section 8.18). Cryoprecipitate, Methylene Blue Treated and Removed, Leucocyte Depleted is the cryoglobulin fraction of plasma obtained by thawing a single donation of Fresh Frozen Plasma Methylene Blue Treated and Removed, Leucocyte Depleted at 4°C $\pm$ 2°C.	<–30	24	<–30	Local	Local		70	140

Table 8.29 Blood components suitable for use in intrauterine and exchange transfusion and neonates and infants under one year, specific requirements

Requirement	**Leucocyte depleted**	**Mandatory testing required**	**Prepared from previously tested donors**	**Gamma irradiated**	**CMV negative**	**HbS screen negative**	**Free from clinically significant red cell antibodies**	**Free from clinically significant HPA, HLA antibodies**
See also	Section 8.1	Chapter 10	Section 8.18	Section 8.28	Chapter 10 (method)		Chapter 10	
Red Cells for Intrauterine Transfusion (IUT), Leucocyte Depleted	✔	✔	✔	✔	✔	✔**1**	✔	✖
Whole Blood for Exchange Transfusion, Leucocyte Depleted	✔	✔	✔	✔	✔	✔**1**	✔	✖
Red Cells for Exchange Transfusion, Leucocyte Depleted	✔	✔	✔	✔	✔	✔**1**	✔	✖
Red Cells for Neonates and Infants, Leucocyte Depleted	✔	✔	✔	✖**2**	✔	✖	✔	✖
Red Cells in Additive Solution for Neonates and Infants, Leucocyte Depleted	✔	✔	✔	✖**2**	✔	✖	✔	✖
Platelets for IUT, Leucocyte Depleted	✔	✔	✔	✔	✔	✖	✔	✖ ✔**3**
Platelets for Neonatal Use, Leucocyte Depleted	✔	✔	✔	✖ ✔**4**	✔	✖	✔	✖ ✔**3**
Fresh Frozen Plasma, Neonatal Use, MB Treated and Removed, Leucocyte Depleted	✔	✔	✔	✖	✖	✖	✔	✖
Cryoprecipitate, Methylene Blue Treated and Removed, Leucocyte Depleted	✔	✔	✔	✖	✖	✖	✔	✖

Key: ✔= Mandatory; ✖ = not mandatory

1. Unless the Blood Centre considers unnecessary.
2. Unless clinically required.
3. Where HPA matched is required.
4. If the recipient has received an IUT previously.

Chapter 9

Evaluation of blood components: generic protocols

9.1 Novel blood components, production processes and new whole blood collection bags

Aim

To describe how a proposed novel blood component or processing method is to be evaluated to

- gain sufficient data to validate the component and production method
- gain sufficient data to support the clinical use of the component
- allow the Standing Advisory Committee on Blood Components (SACBC) to recommend to the Joint UKBTS/NIBSC Professional Advisory Committee that the component should be included in the 'Red Book'
- provide sufficient information to prevent all Blood Transfusion Centres (other than those performing a full evaluation) from having to complete a full validation of the novel component before it enters routine production. They will only need to undertake process qualification.

Introduction

This document identifies the steps that a group of investigators will need to undertake to submit a novel blood component for inclusion in the 'Red Book' thereby allowing it to be produced on a routine basis throughout the UK.

It is recognised that some novel components may be developed by a group of investigators in conjunction with a commercial company undertaking speculative research. As a result the group of investigators may wish to enter the process at Step 9. In this case SACBC will expect any requirements for data collection in the preceding steps to be complied with when the protocols and reports are submitted to the SACBC Chair for consideration by the technical subcommittee. If sufficient data are not included then a request for extra data will be made (Step 11).

Guidance on how novel components should be tested is included in Sections 9.2–9.4.

Table 9.1 Testing of novel components

Step	Details	Information
1 Investigators identify requirement for a novel blood component.	The requirement must be derived from R & D work or as the result of clinical discussions. ● The blood component needs to fulfil an unmet clinical need OR ● provide production benefit and have a blood service proposer. Investigators will need preliminary data to support their application.	The new component may be derived from a commercially available product, in this case data to support the submission may be derived from the manufacturer. Investigators must critically appraise data already available. All data must be maintained on file. It will be used to demonstrate validation has been completed in support of Blood Transfusion Centre licensing activities. Data required may include clinical outcome.
2 Investigators may obtain initial advice from SACBC Chair as to whether the component should be treated as novel.	Yes:– Go to step 3. No:– Undertake local validation and produce the component locally under the general principles of GMP and the 'Red Book'.	The proposed new component may require evaluation even if it complies with existing 'Red Book' Guidelines if ● a new production technique is involved (e.g. leucocyte depleted red cells produced by apheresis) ● there are different steps in the production process (e.g. white cell filtration immediately following collection). Definitive advice about the need for full scale evaluation will be provided from the SACBC following a written submission.
Characterise the new blood component		
3 Apply to the SACIT Chair for a development barcode.	Allocated by SACIT.	Allows component production, discard and use to be tracked using the Blood Transfusion Centre's IT system. This will allow the evaluation to be integrated within the Centre's Quality System.
4 Investigators define the intended specification for the blood component.	Written specification to include: ● expected characteristics (e.g. WBC, Platelet count) ● testing characteristics (blood grouping, microbiology, etc.) ● sampling time, sampling method and sample handling conditions to confirm that the component meets specification ● reference should also be made to the research papers from which the specification is derived.	Specify all key points which will allow subsequent production of the component to be well controlled.
5 Write the protocol for component evaluation.	Investigators' group writes procedures for: ● component production ● monitoring of performance ● clinical use ● outcome measurement ● adverse incidents in production/use of the blood component or use manufacturer's documentation to produce 'in-house' protocols.	Principles of GCP and GMP should apply. Comply with generic protocols (Sections 9.2–9.5). Laboratory studies should comply with local standards. Must include in the procedures the sampling regimes, data analysis, and expected ranges which will be used to confirm that production of the component is under control. Must include detail of the data analysis methods.
6 Investigators should ensure their protocol complies with Chapter 9 and may seek advice from SACBC.		
7 Obtain ethics committee approval.		Must comply with local consenting and ethics policies for the use of donated material.

Step	Details	Information
8 Investigators apply protocol.	Document evidence of protocol being implemented. Investigation should be subject to independent quality audit.	Audit may be carried out on behalf of collaborating manufacturers even though this may be confidential regarding the data collected. A summary outlining non-compliances against GCP, GMP must be made available to the Blood Transfusion Service involved for submission as part of the supporting documentation to the SACBC.
9 Reports submitted to SACBC Chair for technical subcommittee to review outcome.	Investigators review outcomes and produce a report, which summarises findings and supports the case for a new blood component to be listed.	Investigators who have been conducting speculative research with a manufacturer may enter the process at this point.
Obtain SACBC listing of the component		
10 Investigators submit report and supporting data to the SACBC for consideration.	SACBC decide if: ● the blood component is novel ● the data support the ability to produce the blood component on a regular basis ● the blood component is efficacious and safe.	
11 SACBC decide whether the component may be recommended for inclusion in the 'Red Book' guidelines.	If SACBC decide that the blood component will be listed, submit this recommendation to the 'Red Book' Joint Professional Advisory Committee. If SACBC decide that the blood component will not be listed, inform the submitting group and provide an explanation.	SACBC may request further data in support of the submission prior to listing the blood component.
Joint Professional Advisory Committee		
12 Consider the recommendation that a new component should be listed.	Write to SACBC notifying them of the decision. If not accepted, provide SACBC with detailed reasons for the decision.	
SACBC		
13 Communicate the Joint Professional Advisory Committee decision to appropriate parties.	If accepted inform investigators; request SACIT to proceed with the provision of appropriate labels. Write to Medical Directors of the four UK Blood Transfusion Services. Provide copies of the data and report used to accept the new blood component. If not accepted inform investigators, with supporting reasons.	
SACIT		
14 Provides codes for the new blood component.	Code will be unique. ISBT 128/ABC Codabar will be supported.	
15 Provides a component label.	Label will be unique.	Label text will describe the key attributes of the component.
Blood Establishments		
16 Begin production of the new blood component.	Base procedures on those used during validation studies. Complete process qualification.	Demonstrates without redoing the above validation that the blood component produced is equivalent to that defined in the UK guidelines.
17 Produce the blood component routinely.	Confirm procedures.	Continue to monitor production to the 'Red Book' specification.

9.2 Evaluation of new red cell components for transfusion

Introduction

In establishing any novel component, the development process is expected to involve three stages:

- Investigation: initial intensive investigation of a range of parameters on a relatively small number of units (e.g. 10) to establish concepts. This should involve *in vitro* studies with serial sampling, and may also involve *in vivo* studies. Components produced during this phase should not be used for transfusion.
- Validation: operational validation on a larger number of units (e.g. 50 to 100) to establish routine operation of the technique, normally testing for those parameters listed in the current edition of the 'Red Book'. These tests may be supplemented by a limited set of assays selected from the investigational phase to allow setting of routine quality parameters. This may involve *in vivo* studies and normally would involve sampling at the times shown below for routine testing.
- Routine: ongoing routine validation using a small set of parameters selected on the basis of the above studies. This will not normally involve *in vivo* studies. For clarity the guidance on which tests need to be performed is as shown in Table 9.2.

Red cell components may be derived either from whole blood (WB) or collected by apheresis (APH) and, in either case, the standard requirements for donor selection and for mandatory donation microbiological testing should be fulfilled. When well prepared, there is no evidence that the clinical performance of any of these products is different, and the guidance provided below applies equally to the various approaches.

In vitro assays should be performed on samples representative of the pack contents taken by an aseptic technique that does not appreciably alter the gross volume of the pack contents (must be kept to a minimum but in any case no greater than 10%). Parallel testing of units prepared by a well established method is recommended, and the use of a split-pool or crossover design will increase the power of such comparisons. If required, *in vivo* studies, preferably with parallel testing of 'standard' components, should be performed on the last day of the proposed storage period.

Red cell components will be stored for the recommended storage period or longer in the case of experimental additive solutions (AS) that are designed to extend the shelf life of RBC. Samples will be taken weekly (or minimally at day 1, 21, 35 and at the end of storage if this is >35 days) for *in vitro* studies. If required, autologous *in vivo* recovery studies should be undertaken at the end of the storage period.

In vitro studies (minimum 10 units)

The measurements described below and in Table 9.2 will be made at the time of collection of WB/APH and at the relevant stages of component preparation. An equal number of appropriate control components, e.g. standard OAS RBC should be tested in parallel. Greater consistency of information may be obtained if two or more group-compatible components are pooled and divided prior to processing for *in vitro* studies only.

At time of WB/APH collection

Weight, volume, RBC $\times 10^{12}$/U, haematocrit (l/l), Hb g/U, MCV, WBC $\times 10^{6}$/ U, platelets $\times 10^{9}$/U, red cell loss* %, platelet loss*%, Leucocyte depletion (given as residual WBC $\times 10^{6}$/U) and log depletion*. These results should be obtained by validated test procedures and be within the limits defined by the preliminary component specification.

* Relevant to procedures involving integral filtration or other methods of leucocyte removal, e.g. during apheresis collection. Validated techniques using flow cytometry or cell counting chambers should be used to count leucodepleted components and would currently be expected to exhibit a sensitivity of less than or equal to 1 leucocyte per microlitre. These parameters are measured on WB components and RBC in OAS at each stage of component preparation.

During storage

ATP, 2,3-DPG, Glucose, Lactate, Na^+, K^+, Haemolysis % (soluble haemoglobin as a percentage of total haemoglobin per ml of whole product), pH, pO_2, pCO_2, Cytokines. These may include Interleukin-1α, IL-1β, IL-6, IL-8, TNF-α and TGF-β. Measurements should wherever possible be by bioassay (seek advice from SACBC Technical Committee). Cytokine measurements are complex and may be considered optional. As red cell components are leucocyte-depleted, measurement of leucocyte-derived cytokines is probably not informative. Advice should be taken from the SACBC Technical Committee on the selection of cytokine tests.

These results should be obtained by validated test procedures and be within the limits defined by the preliminary component specification. Where manipulation of components during processing might increase the risk of bacterial contamination, microbiological sterility testing should be performed.

Separated FFP should be stored for two years and representative samples assayed at suitable time intervals, e.g. 3, 6, 9, 12 and 24 months for clotting factors and other parameters (see Section 9.4).

Autologous *in vivo* studies (minimum five components)

See Table 9.2 for testing. An equal number of appropriate control components obtained from healthy volunteer donors with ethical approval, e.g. standard OAS RBC should be tested in parallel. The number of components transfused should be justified based on the study objectives and design.

Table 9.2 Evaluation of new red cell components for transfusion: recommended tests

New characteristic Parameter	New pack	Leuco-depletion	New centrifugation/ component extractor e.g. Optipress, Compomat, etc.	Novel AS/ anti-coagulant	Apheresis OAS RBC	Apheresis OAS RBC with novel OAS/anti-coagulant	Gamma irradiated	Pathogen reduction
Weight (g)	✔	✔	✔	✔	✔	✔	✔	✔
Unit vol (mL)	✔	✔	✔	✔	✔	✔	✔	✔
RBC $\times 10^{12}$/U	✔	✔	✔	✔	✔	✔	✔	✔
Hct (L/L)	✔	✔	✔	✔	✔	✔	✔	✔
Hb g/U	✔	✔	✔	✔	✔	✔	✔	✔
MCV	✔	✔	✔	✔	✔	✔	✔	✔
WBC $\times 10^6$/U (initial)	✔	✔	✔	✔	✔	✔	✔	✔
WBC $\times 10^6$/U (post-L-D)		✔						
Leucocyte subsets % (post L-D)		?						
Platelets $\times 10^9$/U	✔	✔	✔	✔	✔	✔	✔	✔
RBC cell loss % (post L-D)		✔	✔					✔
Platelets cell loss % (post L-D)		✔	✔	✔	✔	✔	✔	✔
K^+ mmol/L	✔	✔	✔	✔	✔	✔	✔	✔
Haemolysis %	✔	✔	✔	✔	✔	✔	✔	✔
pH				✔		✔	✔	✔
Lactate mmol/L				✔		✔	✔	✔
Glucose mmol/L				✔		✔	✔	✔
ATP µmol/gHb				✔		✔	✔	✔
2-3,DPG µmol/gHb				✔		✔	✔	✔
Na^+ mmol/L				✔		✔	✔	✔
pCO_2 kPa				✔		✔	✔	✔
pO_2 kPa				✔		✔	✔	✔
Pathogen reduction*								✔
PrP^c and microvesicles		?						?
24 hour recovery %				?		?	?	?

Some components may need to be tested for a combination of parameters, e.g. apheresis red cells in a novel/experimental OAS that are also leucodepleted. In this case the sampling requirement includes that of a leucodepleted red cell component and that of an experimental OAS component.

Key: ✔ = recommended. ? = optional; other tests are not excluded. * = normally undertaken by the manufacturer.

At the end of the storage period components should be checked for sterility and a representative sample labelled with ^{51}Cr (single label method) or ^{51}Cr plus ^{131}I-Albumin (dual label method), washed, reinjected and blood samples taken at 5, 7, 10, 12.5, 15 min and 24 hours to calculate the 24 hour recovery. No more than 20% of components should have recovery <75% by either method. Alternative methods to ^{51}Cr labelling may be used if shown to yield equivalent results.

9.3 Evaluation of new platelet components for transfusion

Introduction

In establishing any novel component, the development process is expected to involve three stages:

- Investigation: initial intensive investigation of a range of parameters on a relatively small number of units (e.g. 10) to establish concepts. This should involve *in vitro* studies with serial sampling, and may also involve *in vivo* studies. Components produced during this phase should not be used for transfusion

- Validation: operational validation on a larger number of units (e.g. 50 to 100) to establish routine operation of the technique, normally testing for those parameters listed in the current edition of the 'Red Book'. These tests may be supplemented by a limited set of assays selected from the investigational phase to allow setting of routine quality parameters. This may involve *in vivo* studies and normally would involve sampling at the times shown below for routine testing

- Routine: ongoing routine validation using a small set of parameters selected on the basis of the above studies. This will not normally involve *in vivo* studies. For clarity the guidance on which tests need to be performed is as shown Tables 9.3 and 9.4.

Platelet components may be derived from whole blood using platelet-rich plasma or buffy coat methods of preparation, or by plateletpheresis and, in either case, the standard requirements for donor selection and for mandatory donation microbiological testing should be fulfilled. For components prepared in a closed system, storage in specifically designed plastic bags is currently undertaken with gentle agitation for up to 5 days at 22°C ± 2°C. Platelet components may be subjected to leucodepletion, storage in platelet additive solutions in place of plasma and, in the case of whole blood derived components, pooling of 4 to 6 units to form an adult equivalent dose. When well prepared, there is no evidence that the clinical performance of any of these products is different, and the guidance provided below applies equally to the various approaches.

In vitro assays should be performed on samples representative of the pack contents taken by an aseptic technique that does not appreciably alter the gross volume of the pack contents (must be kept to a minimum but in any case no greater than 10%) on days 1, 3, 5 and 7 (and further samples if an extension of shelf life is proposed). For studies investigating an extension to shelf life, consideration should be given to testing the component one day after the proposed limit of shelf life (e.g. Day 8 for a 7-day shelf life). Parallel testing of units prepared by a well established method is recommended, and the use of a split-pool or crossover design will increase the power of such comparisons. In vivo studies, preferably with parallel testing of 'standard' components, should be performed on the last day of the proposed storage period.

Investigational phase

Guidance

Table 9.3 recommends an assessment format for different kinds of novel development that may be expected for platelet components. While these are listed against the recommended assays above, this is not intended to be restrictive and comparable alternatives may be employed. Unless otherwise noted it is expected that tests would be done on d1, d3, d5 and d7 (and longer if an extended shelf life is proposed).

For studies investigating an extension to shelf life, consideration should be given to testing the component one day after the proposed limit of shelf life (e.g. Day 8 for a 7-day shelf life). It is recommended that any protocol for the evaluation of a novel blood component or production method be discussed with the chairman of the Standing Advisory Committee on Blood Components before finalization.

For leucodepleted components, leucocyte enumeration should involve validated techniques and would currently be expected to exhibit a sensitivity of less than or equal to 1 leucocyte per microlitre.

In vitro assessment

Background

In vitro assessments essentially use surrogate assays that are hoped to be indicative of the *in vivo* performance of platelets, as measured by haemostatic effect, *in vivo* recovery and survival and corrected count increment following transfusion. While a large number of *in vitro* assays have been proposed, only a few of these have been shown to correlate with post transfusion indices. This area has been reviewed by the BEST group[1] and can be summarised in Table 9.3 (* = correlates with *in vivo* viability).

Any platelet production system that may be considered as having the potential for an increased risk of bacterial contamination or growth should include an assessment of sterility as part of the initial validation phase. It is recommended that at least 50 apheresis units or pools (each sufficient for a standard adult dose) should be assessed for sterility by a validated technique prior to *in vivo* assessment and routine introduction of the component into clinical use.

In vivo assessment

If *in vivo* assessment is required local ethical committee approval should be obtained prior to commencing the *in vivo* assessment.

Any *in vivo* assessments should be performed at the end of the proposed storage period, following generation of sufficiently reassuring data from in vitro studies. For studies investigating an extension to shelf life, consideration should be given to testing the component one day after the proposed limit of shelf life (e.g. Day 8 for a 7-day shelf life). Due to the inherent variability of patients, use of a crossover design or dual labelling technique in stable, afebrile thrombocytopenic patients without evidence of platelet consumption (or in volunteers) is strongly recommended so that each patient acts as their own control. The number of components transfused should be justified on the basis of the study objectives and design.

Two approaches are established:

- Use of radio-isotope labelled platelets in normal volunteers: this approach is not applicable to pooled products. ^{51}Cr, or preferably ^{111} In are the recommended isotopes to determine platelet recovery and survival.[2] Alternative validated techniques may be used

- Determination of recovery after transfusion: an appropriate adult dose (>240×10^9 platelets) of ABO identical platelets may be used to determine increments and therapeutic effect (bleeding time measurements are not recommended). Patients known or suspected to have lymphocytotoxic or HPA antibodies should be excluded and should have no evidence of hypersplenism, sepsis, ongoing haemorrhage or other cause of increased platelet consumption.

Platelet counts should be assessed immediately prior to infusion of an appropriate dose of ABO identical platelets and one hour post-infusion.

Additional measurements at 4-6 and/or 24 hours post-transfusion may give some indication of platelet survival.

Table 9.3 *In vitro* assessment

	Recommended	Alternatives (may be used if validated against parameters that correlate with *in vivo* viability)
(a)	*Product content* Volume Platelet content Leucocyte content Plasma content (for additive developments only)	
(b)	*Platelet morphology (proportion of discs)* Determination of swirling Morphology index (phase microscopy)* Extent of shape change by ADP*	Mean platelet volume dMPV (change in platelet volume on EDTA addition)
(c)	*Platelet metabolism* ATP Hypotonic shock response (HSR)* pO_2/pCO_2 pH Glucose consumption Lactate production	
(d)	*Extent of platelet activation* P-selectin (CD62P) on platelet surface and in supertenant Beta thromboglobulin release	Surface GPIb/IX (CD42a/42b) Surface GPIIb/IIIa (CD41/CD61) Platelet fibrinogen binding Serotonin content or release Glycocalicin or PF4 release Annexin V binding (to phospolipid)
(e)	*Extent of platelet lysis* Supernatant lactate dehydrogenase	Soluble annexin V
(f)	*Measurements reflecting in vitro function* Aggregation in response to paired antagonists (e.g. 80 μM ADP and 8 μg/ml collagen)	*In vitro* bleeding time (in development) Platelet adhesion (e.g. Baumgartner)
(g)	*Assays indicative of possible side effects* Cytokines/chemokines, particularly platelet-derived (IL-6, IL-8, RANTES, TNF-α, TGF-B): optional, (if performed bioassay is preferable to immunoassay) Complement activation (particularly for novel plastics or filters) FXIIa formation (particularly for novel plastics or filters) Bacterial contamination (at end of shelf life only) Pathogen reduction (for these processes only)	

9.4 Evaluation of new fresh frozen plasma/cryoprecipitate components for transfusion

Introduction

In establishing any novel component, the development process is expected to involve three stages:

- Investigation: initial intensive investigation of a range of parameters on a relatively small number of units (e.g. 10) to establish concepts. This should involve *in vitro* studies with serial sampling, and may also involve *in vivo* studies. Components produced during this phase should not be used for transfusion
- Validation: operational validation on a larger number of units (e.g. 50 to 100) to establish routine operation of the technique, normally testing for those parameters listed in the current edition of the 'Red Book'. These tests may be supplemented by a limited set of assays selected from the investigational phase to allow setting of routine

Table 9.4 Evaluation of new platelet components for transfusion

Parameter	PRP or BC (single donation)	Pooled PRP or BC or apheresis	Leuco-depletion	Pathogen reduction	Extended storage	Sterile connection	New bag, additive or anticoagulant
Volume (d1)	✔	✔	✔	✔	✔	✔	✔
Platelet content	✔	✔	✔	✔	✔	✔	✔
Leucocyte content (d1)	?	?	✔	?	?		?
Leucocyte subsets (%)	?	?	?	?	?		?
Morphology, e.g. Swirl test	✔	✔	✔	✔	✔	✔	✔
Activation, e.g. B thromboglobulin	✔	✔	✔	✔	✔		✔
Lysis, e.g. Lactate dehydrogenase	✔	✔	✔	✔	✔		✔
Metabolic activity, eg. ATP, pH	✔	✔	✔	✔	✔		✔
Function e.g. Aggregation	?	?	?	?	?	?	?
Side-Effects							
Cytokines/chemokines	?	?	✔	✔	✔		✔
Complement activation	?	?	?	?	?		✔
FXIIa			?	?			?
Sterility	?	✔	if dock on	✔	✔	✔	?
PrP^C and microvesicles			?				
Pathogen reduction*			?	✔			

Key: ✔ = recommended. ? = optional; other tests are not excluded. * = normally undertaken by the manufacturer. Planned studies may fall into more than one category in which case all indicated assays should be performed. d1 = day 1.

quality parameters. This may involve *in vivo* studies and normally would involve sampling at the times shown below for routine testing

- Routine: ongoing routine validation using a small set of parameters selected on the basis of the above studies. This will not normally involve *in vivo* studies. For clarity the guidance on which tests need to be performed is as shown in Table 9.4.

Currently, FFP for direct use or as start material for cryoprecipitate production is produced either from whole blood donations or by centrifugal apheresis techniques. Novel technologies under assessment include Amotosalen and Riboflavin pathogen reduction techniques. Apheresis techniques involving filtration have been approved in the past.

In vitro evaluation of novel FFP

Suggested study design

Because of the wide normal range of some clotting factors and potential inter-batch variation of assays, it is suggested that initially 20 novel units and 20 controls be produced and assayed in parallel, with the novel technology being the only variable. A less costly alternative, if logistics permit, is to do a pooled paired comparison, where two units are pooled, and one half processed by the novel technique. This provides greater statistical power for fewer units assayed, and is particularly important for storage studies. For LD or pathogen reduction systems it is recommended that assays are performed on samples collected before and after the process under investigation. Ideally provision should be made for storing and testing aliquots from each pack at every time point, as thawing out three or four different packs at each time point introduces excessive variation. However, a prevalidation should be done to ensure that the behaviour of the aliquotted component during storage is the same as that in the main pack.

Assays required

The extent of any evaluation depends in part on the degree of novelty of the component. The list of assays below need not be applied in every setting. The attached table gives a summary of which assays are recommended in different situations. All evaluations must include the routine quality control parameters such as FVIII:C.

Before freezing:

- volume, platelet count, WBC*
- prothrombin time (PT), activated partial thromboplastin time (APTT)
- factors I (fibrinogen), II, V, VII, VIII, IX, X, XI, XIII von Willebrand factor (vWf):Ag, vWf:RiCof, which measures the functional activity or an assay validated as yielding equivalent results, vWf multimeric analysis, vWF cleaving protease
- inhibitors of coagulation – antithrombin, protein C, protein S, α_2-antiplasmin
- markers of unwanted activation of coagulation* – prothrombin fragment 1.2, fibrinopeptide A, factor XIIa, thrombin-antithrombin (TAT) complexes
- markers of unwanted activation of kinins/complement* – C3a, C5a, bradykinin.

*Particularly relevant to plasma which has been collected by any filtration technique, in which case the assays should be performed before and after filtration or to packs made of novel materials.

During storage:

- Consideration should be given to performing storage studies at >–20°C in addition to those at <–30°C to reflect hospital storage conditions. Samples should be taken at 6, 12 and 24 months. Ideally, all clotting factors should be assayed at each time point, if only in a few packs. FVIII should be assayed at each time point as a minimum in addition to the proteins most severely affected by the initial process
- Storage parameters may be assayed after the date of implementation of routine production, provided data 'keep ahead' of the age of any clinical product which might be issued.

In vitro evaluation of novel cryoprecipitate

It is assumed that this will be produced from a 'novel' start plasma so that investigators will be aware of any specific losses of clotting factors which should be particularly considered.

Assays to be performed before and after production, and during storage: fibrinogen, FVIII:C.

Cryosupernatant

This component is increasingly used for plasma exchange procedures for patients with thrombotic thrombocytopenic purpura. Analysis of von Willebrand factor multimers and cleaving protease is therefore appropriate. vWf multimeric and cleaving protease analysis should be performed in a laboratory recognised to be proficient in this technique and which is performing the assay regularly.

In vivo studies

Whether or not *in vivo* studies are needed depends on the degree of novelty of the component, e.g. this may not be necessary for plasma which has been leucocyte depleted in the course of producing leucocyte depleted red cells, but would certainly apply in the case of a novel pathogen reduced plasma which had been exposed to chemicals. Unlike red cells and platelets, administration to normal volunteers has not been traditional. Suitable patient groups to consider would be:

For FFP:

- correction of prolonged INR prior to liver biopsy
- liver transplant recipients

- plasma exchange for TTP
- DIC.

It is difficult to get permission to study neonates and usually considerable experience has to have been gained with the product in adults.

A randomised design is preferred, with standard FFP as control.

For cryoprecipitate:

- DIC
- liver disease/transplant
- congenital hypofibrinogenaemia, if maintained on cryoprecipitate.

Table 9.5 Evaluation of novel plasma components

	Fresh Frozen Plasma						**Cryo-precipitate**	**Cryo-supernatant**
	Novel filter	**New centrifuge/ component extractor**	**Novel anticoagulant**	**Novel apheresis system**	**Novel apheresis + anticoagulant**	**Pathogen reduction**		
Volume	✔	✔	✔	✔	✔	✔	✔	✔
Leucocyte content	✔	✔	✔	✔	✔			
FVIII:C	✔	✔	✔	✔	✔	✔	✔	
Platelets	✔	✔	✔	✔	✔	✔	–	–
PT ratio	✔	–	✔	–	✔	✔	–	–
APTT ratio	✔	–	✔	–	✔	✔	–	–
Fibrinogen	✔	–	✔	–	✔	✔	✔	
II, V, VII, IX, X, XI, XIII	✔	–	✔	–	✔	✔	–	–
vWf: Ag	✔	–	✔	–	✔	✔		
vWf: RiCof	✔	–	✔	–	✔	✔		
AT III, Prot C, Prot S	✔	–	✔	–	✔	✔	–	–
TAT/Frag1.2/FPA + FXIIa	✔	–	✔	✔	✔	✔	Omit if not elevated in source plasma	
C3a + C5a	✔	–	✔	✔	✔	✔		
C1 inhibitor	✔	–	✔	–	✔	✔		
vWf Multimers	✔	–	✔	–	✔	✔		✔
vWF cleaving protease activity	✔	–	✔	–	✔	✔		✔
alpha-2 anti-plasmin	✔	–	✔	–	✔	✔		
Pathogen reduction*						✔		
PrP^c/microvesicles	?	–						
Clinical trial	–	–	#	#	#	✔	#	#

Key: ✔ recommended. – = not needed. # = consider individually. * = normally undertaken by the manufacturer.

9.5 Generic protocol for the evaluation of apheresis equipment

This protocol sets out the minimum requirements for new apheresis equipment and, in a generic form, the mechanism for assessing acceptability of the equipment hardware, the software and the associated apheresis sets. The specific validation or trial of apheresis collections from new equipment is covered in Section 9.6. Novel components, as defined in Section 9.1, produced as a result of new equipment will be assessed as detailed in other sections of this chapter.

Minimum requirements

General

Equipment should be CE-marked or the Blood Service should participate to facilitate CE Marking.

Manufacturers must comply with Good Automated Manufacturing Practice (GAMP).

Equipment hardware

Equipment should contain the following:

- manual override system
- blood flow monitor
- in-line air detector
- integral blood filter
- anticoagulant flow indicator
- collection volume preset device
- visual audible alarm for procedure completion
- automatic standby mode for power failure
- power up self-check to include all critical safety and operational procedures.

Equipment software

Software should provide parameters for

- accepted total blood volume calculation algorithm
- accepted citrate reinfusion rate calculation algorithm
- fixed upper limit citrate reinfusion (see Chapter 6)
- programmable upper limit total collection volume
- must not exceed predetermined fluid reinfusion limits (e.g. citrate, saline)
- alarm and prevent use of incorrect set (incongruent) for programmed procedure
- prevent procedure where predicted post-collection parameters fall outside programmable safety limits as defined in Chapter 6
- for other measures see Chapter 6.

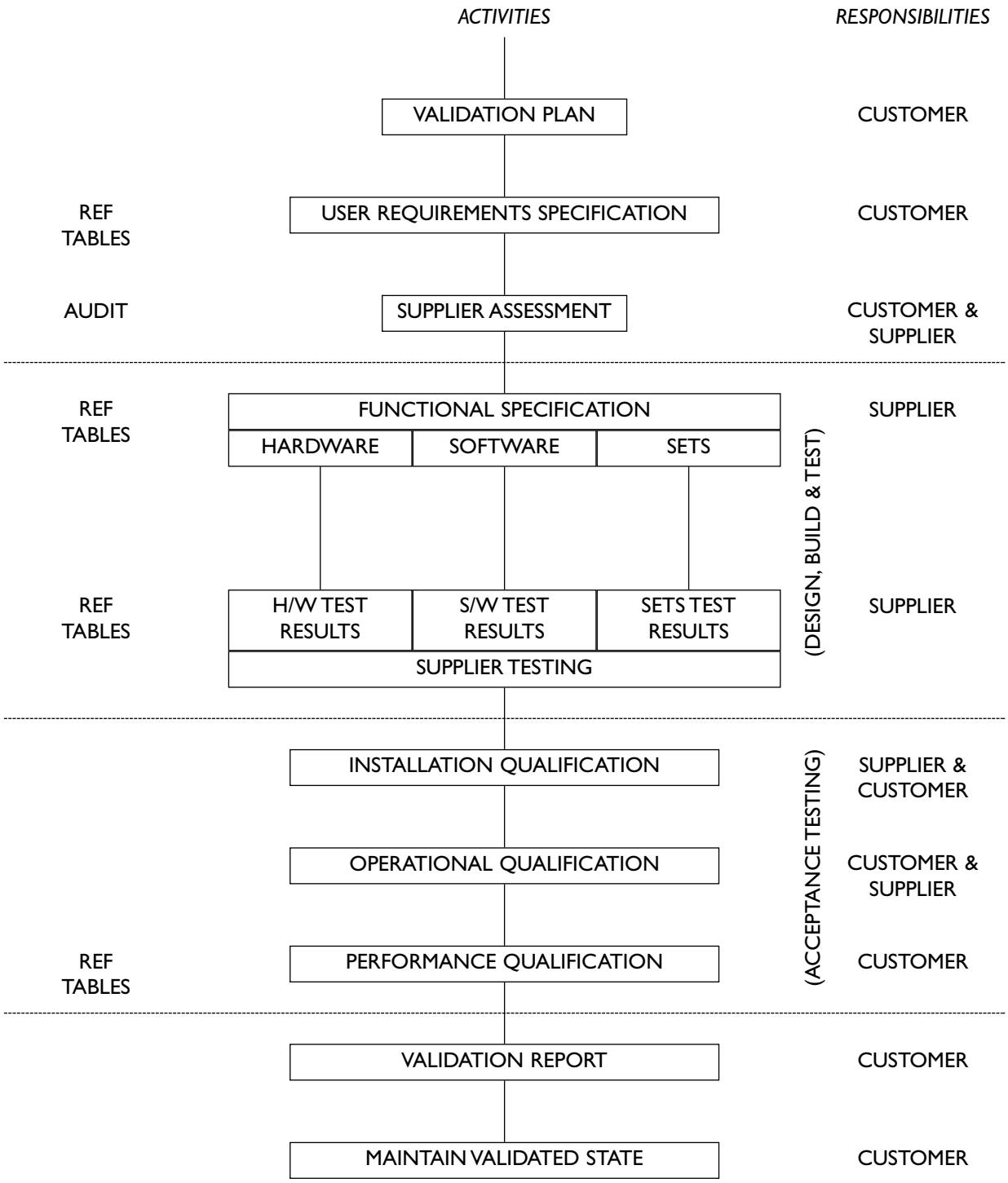
Apheresis sets

Apheresis sets should have

- closed system
- visual system to minimize risk of transposition of fluid lines
- microbial filter on 'spiked' lines
- diversion line and pouch for sampling
- consideration should be given to the incorporation of a pouch on the final pack to facilitate bacterial contamination testing
- for other measures see Chapter 6.

The overall mechanism for equipment acceptance is given in Table 9.6 for reference. Validation, installation qualification, operational qualification and performance qualification would be defined by the Blood Service, taking account of the advice within these Guidelines.

Table 9.6 Generic flowchart of apheresis equipment acceptance



9.6 Generic protocol for the evaluation of blood packs for whole blood donations and apheresis collections

Introduction

This protocol sets out in generic form the essential features of blood pack evaluations as required by the UK Blood Transfusion Services. National Services should exercise discretion in the extent to which the protocol should be applied. It may be appropriate to consider an abbreviated format, e.g. when the change to be evaluated represents the attachment of a filter to a pack assembly that is already in routine use, or where the change consists of a modified port access design.

The protocol is not intended for use with packs for stem cell collection and storage, although the principles outlined may be helpful. The principles of this section apply to components produced from whole blood donations as well as whole blood itself.

General principles

Each trial will be fully documented and will have a unique trial reference number. The key requirements are as follows:

- an evaluation outline – what type of pack is being investigated, where, when and the standards against which the assessment will be based
- the evaluation objective – to demonstrate the packs are and remain free from defects and are suitable for the production and storage of components that meet current guidelines
- identification of any restrictions, e.g.
  - situations where an evaluation would be required
  - agreement on ownership and release of the evaluation report with the supplier/ manufacturer
  - limitations of the report and its distribution
- how the trial will be controlled, e.g.
  - the identity of the person/persons responsible for the trial and their reporting lines
  - sign-off procedures and authorities including concessionary changes
  - the trial protocol will be agreed with the supplier and any concessionary changes will require agreement in accordance with local procedures.
  - trials will be conducted in three phases. Satisfactory performance and sign off in Phase 0 is a prerequisite to progression to Phase 1 and satisfactory performance in Phase 1 is a prerequisite to progression to Phase 2
  - blood collected in Phase 0 will not be used for transfusion
  - all components prepared in Phase 1 will be subject to routine quality monitoring tests
  - a minimum of 1% of components prepared in Phase 2 will be subjected to routine quality monitoring tests
  - any testing that exceeds the minimum set out herein must be fully incorporated within the report
- confidentiality: any data collected will normally be the property of the organization performing the trial; blood pack suppliers/manufacturers who wish to release information arising from the trial will require confirmation in writing from the organization performing the trial that they may do so

- quality monitoring: it is expected that packs evaluated under the trial protocol will be subject to routine quality monitoring and reporting procedures, e.g. pack faults, compliance with component specifications, etc. It follows that any adverse findings during the trial would generate a corrective action.

A summary of the numbers to be tested for each evaluation or validation phase is given in Table 9.7. The numbers given are the minimum required. More detail is given in the relevant sections below.

Table 9.7 Summary of testing numbers required for evaluations and validations

Process	Testing	Phase 0	Phase 1	Phase 2	Local process qualification
Whole blood	Component evaluation	10 See Tables 9.2 to 9.5	None	None	None
	Quality monitoring	10 100% tested	125 100% tested	2000 from each of 2 batches Minimum 1% tested	125 100% tested
Apheresis collection	Component evaluation	10 See Tables 9.2 to 9.5	None	None	None
	Quality monitoring	10 100% tested	125 100% tested	300 100% tested	10 (each machine) 100% tested

Phase 0: evaluation

After an initial familiarization with novel bag/filters (pre-Phase 0) the purpose of Phase 0 studies is to

- assess suitability to progress to Phase 1
- determine suitable quality monitoring parameters
- disclose any quality problems that might prevent components collected or prepared in these packs from being used for transfusion.

Phase 0 evaluation comprises the preliminary evaluation process and normally will require no fewer than 10 packs at the Centre undertaking the trial. Deviations from this number must be agreed in accordance with local procedures.

Processing conditions used in the Phase 0 evaluation should be the same as those applied to Phase 1 and 2 evaluations.

Component quality monitoring

Starting donations and all final components will be tested for compliance with relevant parameters listed in the component specifications in this book. Where relevant, additional assays should be performed as specified in the 'Red Book' generic evaluation protocols for new or novel blood components.

Goods inward inspection

- Check that appropriate storage information is shown on the packaging
- Check the condition of packaging on receipt. Document damaged cartons and examine contents to assess the extent of any damage.

QA pack conformance inspection

Unless otherwise indicated, the following inspection will be performed and documented for all packs to be used in Phase 0 of the trial:

- pack batch number (eye-readable and machine-readable)
- pack type number (eye-readable and machine-readable)
- base label integrity and compliance with the current UKBTS/NIBSC specifications for the uniform labelling of blood and blood components
- base label adherence (a sample of 20 at each temperature)
  - +22°C for 1 week
  - +4°C for 1 week
  - +4°C for 1 day, followed by –35°C for 1 week
- donation number, component type and blood group label adherence (a sample of 20 at each temperature)
  - +22°C for 1 week
  - +4°C for 1 week
  - +4°C for 1 day, followed by –35°C for 1 week
- seals, seams and welds satisfactory
  - absence of leaks
  - anticoagulant/additive free from turbidity, particulate matter and inclusions
- if the inspection requires removal of packs from their overwraps, either repackage and use according to the manufacturer's instructions or perform the examination immediately prior to donation
- check for acceptable handling and storage characteristics of unopened cartons of packs from receipt, through storage to use at sessions.

Checks to be performed by collection teams

Collection teams will follow routine procedures for recording pack faults, but additionally should comment on

- ease of overwrap opening
- integrity of overwrap
- accuracy of instructions for use at time of collection
- acceptability of needle characteristics
- suitability of tubing (length and flexibility)
- general suitability.

Checks to be performed by processing team

The processing team will follow routine procedures for recording pack faults, but may additionally wish to comment on

- breakage rates following freezing
- heat seal failures (in house seals)
- suitability of tubing (length and flexibility)
- ease of cannula breakage

- ability to sterile dock (during secondary processing)
- integrity of join, following local, current, procedure
- compatibility with instructions for device for sterile connection
- assess packaging of frozen packs
- inspection of packs after overnight storage at 4°C.

When the minimum number of packs has been evaluated, the individual or group responsible for the trial will prepare and submit a Phase 0 report.

Phase 1: validation

The purpose of this phase is to allow:

- staff to familiarize themselves with the packs and any associated equipment
- the generation of quality monitoring data
- the development of an appreciation of the suitability of the packs for routine use, i.e. progression to Phase 2 trial.

Phase 1 of the validation process normally will require not fewer than 125 packs to be tested at the Centre undertaking the trial. Deviations from this number must be agreed in accordance with local procedures.

It is expected that a smaller number of packs will be used for familiarization in other Centres.

This phase will include the finalization of SOPs for use in Phase 2.

Blood components produced during Phase 1 may be used therapeutically where they comply with appropriate release criteria.

Component quality monitoring

Starting donations and all final components will be tested for compliance with relevant parameters listed in the component specifications in this book.

Goods inward inspection

- Check that appropriate storage information is shown on the packaging
- Check the condition of packaging on receipt. Document damaged cartons and examine contents to assess the extent of any damage.

QA pack conformance inspection

Unless otherwise indicated, the following inspection will be performed and documented for all packs to be used in Phase 1 of the trial:

- pack batch number (eye-readable and machine-readable)
- pack type number (eye-readable and machine-readable)
- base label integrity
- seals, seams and welds satisfactory
    - absence of leaks
    - anticoagulant/additive free from turbidity, particulate matter and inclusions
- if the inspection requires removal of packs from their overwraps, either repackage and use according to the manufacturer's instructions or discard

- check for acceptable handling and storage characteristics of unopened cartons of packs from receipt, through storage to use at sessions.

Checks to be performed by collection teams

Collection teams will follow routine procedures for recording pack faults, but additionally should comment on

- ease of overwrap opening
- integrity of overwrap
- accuracy of instructions for use at time of collection
- acceptability of needle characteristics
- suitability of tubing (length and flexibility)
- general suitability.

Checks to be performed by processing team

The processing team will follow routine procedures for recording pack faults, but may additionally wish to comment on

- breakage rates following freezing
- heat seal failures (in house seals)
- suitability of tubing (length and flexibility)
- ease of cannula breakage
- ability to sterile dock (during secondary processing)
- integrity of join, following local, current, procedure
- compatibility with SCD instructions
- assess packaging of frozen packs
- inspection of packs after overnight storage at 4°C.

End users

Set up a process by which users will feedback information on acceptability of the packs for use. This would involve blood bank and ward/theatre staff. Obtain details on:

Blood bank issues:

- acceptability to end users
- acceptability of number and condition of bleed line samples
- crossmatch/other label adherence
- leak and breakage rates.

End user issues:

- general acceptability
- accessibility of ports for giving sets
- leak and breakage rates.

When the minimum number of packs has been evaluated, the individual or group responsible for the trial will prepare and submit a Phase 1 report.

Phase 2: evaluation

A minimum of 2000 packs from each of two batches for whole blood collection processes or 300 sets for apheresis collection will be used in this phase to allow data on consistency of manufacture to be collected.

Relevant SOPs will be available before commencing Phase 2. Customer communication and any associated training will also have been done by this date.

Blood components produced during Phase 2 may be used therapeutically where they comply with the normal release criteria.

Goods inward inspection

- Check that appropriate storage information is shown on the packaging
- Check the condition of packaging on receipt. Document damaged cartons and examine contents to assess the extent of any damage.

QA pack conformance inspection

Unless otherwise indicated, the following inspection will be performed and documented for packs to be used in Phase 2 of the trial:

- pack batch number (eye-readable and machine-readable)
- pack type number (eye-readable and machine-readable)
- base label integrity
- seals, seams and welds satisfactory
  - absence of leaks
  - anticoagulant/additive free from turbidity, particulate matter and inclusions.

Checks to be performed by collection teams

Collection teams will follow routine procedures for recording pack faults, but additionally should comment on:

- ease of overwrap opening
- integrity of overwrap
- accuracy of instructions for use at time of collection
- acceptability of needle characteristics
- suitability of tubing (length and flexibility)
- general suitability.

Checks to be performed by processing team

The processing teams will follow routine procedures for recording pack faults, but may additionally wish to comment on:

- breakage rates following freezing
- heat seal failures (in house seals)
- suitability of tubing (length and flexibility)
- ease of cannula breakage
- ability to sterile dock (during secondary processing)
- integrity of join, following local, current, procedure

- with SCD instructions
- assess packaging of frozen packs
- inspection of packs after overnight storage at 4°C.

Component quality monitoring

A minimum of 1% of components produced for whole blood collection processes or 300 of each component (one of each relevant component per procedure) for apheresis collection will be subjected to routine quality monitoring for parameters specified in this book.

End users

Set up a process by which users will feedback information on acceptability of the packs for use. This would involve blood bank and ward/theatre staff. Obtain details on:

Blood bank issues:

- acceptability to end users
- acceptability of number and condition of bleed line samples
- crossmatch/other label adherence
- leak and breakage rates.

End user issues:

- general acceptability
- accessibility of ports for giving sets
- leak and breakage rates.

On completion, the individual or group responsible for the trial will prepare and submit a Phase 2 report on the suitability for use of the blood pack system within the Service undertaking the trial. Deviations from this number must be agreed in accordance with local procedures.

References

1. Murphy, S, Rebulla, P, Bertolini, F, Holme, S, Moroff, G, Snyder, E, Stromberg, R (1994). In vitro assessment of the quality of stored platelet concentrates. The Biomedical Excellence for Safer Transfusion (BEST) Task Force of the International Society of Blood Transfusion. *Transfusion Medicine Review*, **8**(1), pp29–36.
2. Recommended methods for radioisotope platelet survival studies: by the panel on Diagnostic Application of Radioisotopes in Hematology, International Committee for Standardization in Hematology (1977). *Blood*, **50**(6), pp1137–1144.

Chapter 10

Microbiology tests for donors and donations: general specifications for laboratory test procedures

These guidelines specify the tests required for blood donations in the UK. Individual testing centres must have standard operating procedures for the testing process.

10.1 General requirements

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

- blood donations, components and their laboratory samples are correctly identified by barcoded and eye-readable numbers
- donations can be linked to their donor
- a donor's record is reviewed every time he/she donates
- donor samples are suitably stored under appropriate conditions of time and temperature to preserve the properties for which they will be tested
- tests are appropriately performed and controlled using validated procedures and the results recorded
- test results and other relevant test information are archived.

Test reagents, kits and equipment

Unless validated for alternative techniques, test kits and reagents should be stored and used according to the manufacturer's instructions.

All test procedures should be documented and an inventory maintained of kits and reagents in stock.

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

Test equipment should be validated, calibrated and maintained. Appropriate records for these activities should be made and retained.

Appropriate reactivity with control samples must be demonstrated with every series of tests.

A series of tests is defined as the number of tests set up at the same time, under the same conditions and processed in a similar manner. Where a microplate format is used for microbiological testing, each plate constitutes a series even if only a few wells are used.

Recording and reporting of results

The laboratory report should indicate the result of each and every test, preferably by a system that provides positive sample identification. Each test results should be recorded by a computerized system that does not require transcription. If a manual system is used it must be thoroughly documented and controlled. Reporting a series of tests, particularly those of a microbiological nature, by an 'assumed negative' procedure is potentially dangerous and not acceptable.

Release of tested components

Standard procedures must ensure that blood and blood components cannot be released for issue until all the required laboratory tests (mandatory and additional) have been completed, documented and approved within a validated system of work. Compliance with this requirement should normally be achieved by the use of a validated computerized system that requires the input of valid and acceptable test results for all the mandatory and required laboratory tests to permit the release of each individual unit.

Where a computer-based system is not used, either routinely or due to the temporary equipment or system failure, there must be an alternative system that guarantees documented approval for the release of each individual unit by a designated person.

Laboratory test categories

Laboratory tests are:

Mandatory

Required by the Departments of Health for release of all blood donations and components for clinical use:

- HBsAg, anti-HIV 1 and 2, anti-HCV, HCV NAT, anti-HTLV I/II and syphilis antibodies.

Additional

Undertaken under special circumstances:

- to increase the safety of transfusion for particular individuals or patient groups e.g. anti-CMV negative components.

Although not required for all blood donations or components, when such tests are performed to meet a specific need the results are an essential part of the criteria for release of that component.

10.2 Mandatory testing of blood donations

Blood and blood components must not be released to stock unless they have been tested (in addition to blood group serology requirements) and found negative for mandatory microbiological tests (HBsAg, anti-HIV, anti-HCV, HCV NAT, anti-HTLV and syphilis antibodies).

CE-marked assays must be used. These assays must have been assessed (in respect of sensitivity and specificity) and deemed suitable by the UK Blood Transfusion Services for the detection of markers identified above in each blood donation. Additionally, testing sites must ensure that the expected standard of performance of assays is being achieved, by using appropriate assay batch pre-acceptance testing and statistical monitoring of test results on defined quality control samples.

Normally the presence or absence of the microbiology markers described above should be determined by testing the serum of the donor. However, plasma is usually the preferred analyte for HCV NAT, anti-HTLV and syphilis antibody testing. Whenever plasma is used for testing, it must be handled according to the instructions accompanying the test kit. If there is a deviation from the kit manufacturers' instructions, the variation must be validated to ensure it meets the required specificity and sensitivity criteria and formally approved by the manufacturer.

Initial screen reactive samples

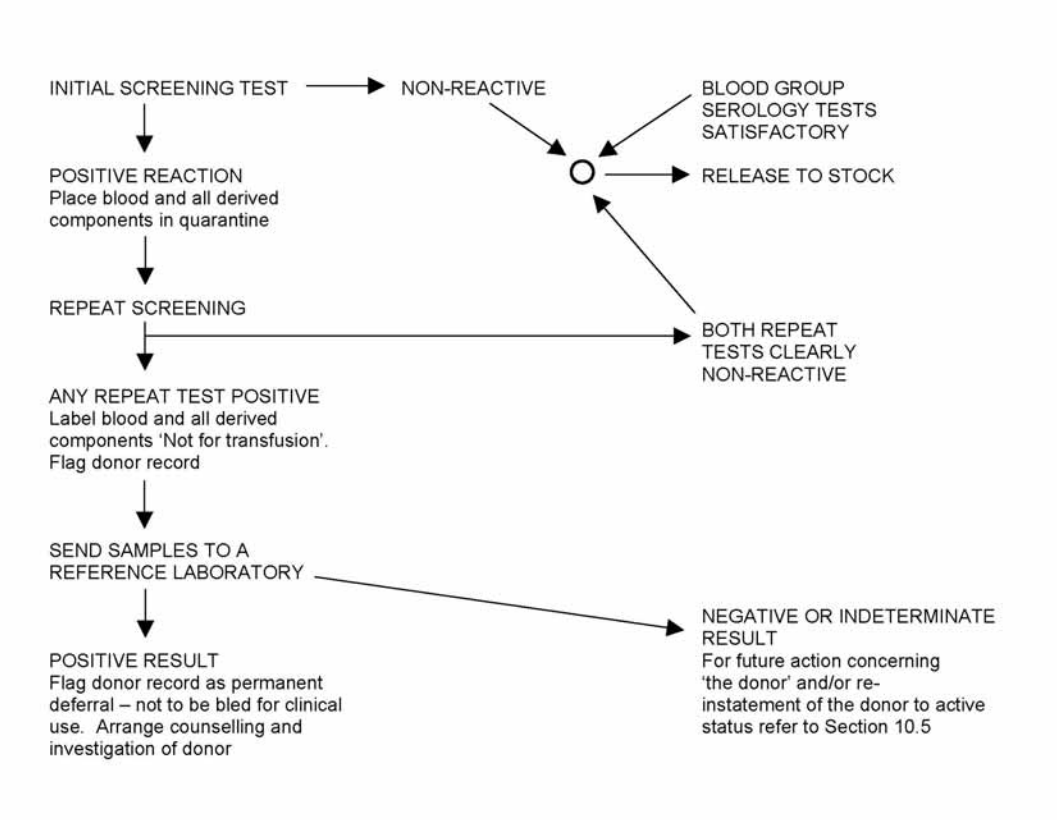


Figure 10.1 Algorithm for mandatory microbiological testing

All initially reactive samples (see Figure 10.1) must be retested in duplicate, using the same assay as that used in the original test. This is an extremely important area of work and requires particular attention to ensure that:

- the correct sample is retrieved for repeat testing
- the actual sampling procedure for repeat testing is undertaken with due care, e.g. some samplers do not accept the same barcode number twice
- the results are carefully verified
- the overall integrity of the information transfer system is maintained.

If both the repeat screening tests are clearly non-reactive, the blood and any derived components can be released to stock.

Repeat reactive samples

If one or both of the repeat screening tests are reactive, the blood and any derived components must be labelled 'Not for transfusion'. The guidance listed above applies.

If the donor is reactive for any of the mandatory microbiology tests described above, samples from the donor/donation must undergo confirmatory testing at a designated reference laboratory.

- If a positive result is confirmed, the donor record must be flagged as 'permanent exclusion risk – not to be bled for clinical use' or equivalent. Arrangements should be made to counsel and take repeat samples from the donor to confirm infection in the donor.

- If a negative or indeterminate result is reported following confirmatory testing, the procedure for reinstatement of such donors to active status is covered below.

10.3 Specific assays

HBsAg

- Specification: the UK specification for the minimum level of sensitivity for the performance of HBsAg screening is 0.2 IU/mL. A UK HBsAg working standard containing 0.2 IU/mL is available (from NIBSC) and must give a positive reaction in each series of HBsAg screening tests for the results of those tests to be valid.

- Quality control of HBsAg screening: each batch of HBsAg test kits should be shown to conform with nationally established minimum criteria for specificity and sensitivity.

- In addition to the test kit manufacturer's controls, the UK working standard must be included in each series of tests to demonstrate acceptable sensitivity of the test method.

    - No series of tests should be considered acceptable unless the result of the test kit manufacturer's and the additional quality control samples have satisfied the criteria laid down.

The designated reference laboratory must, as a minimum, perform specific neutralization tests for HBsAg and determine the anti-HBc status on repeat reactive samples. Those samples containing neutralizable HBsAg, with or without anti-HBc, indicate infection with HBV (except in cases where this may be due to recent immunization with HBsAg). Anti-HBc IgM may be useful in identifying a recent infection.

Anti-HIV 1 and 2

- Specification: the UK specification for the minimum level of sensitivity for the performance of anti-HIV 1 and 2 screening has not yet been defined beyond the requirement that in each series of tests a positive result should be obtained with the UK anti-HIV-1 working standard (available from NIBSC) or a dilution thereof, specifically prepared for a particular test system.

- Quality control of anti-HIV 1 and 2 screening:

    - each batch of anti-HIV 1 and 2 test kits should be shown to conform with locally established minimum criteria for specificity and sensitivity

    - in addition to the test kit manufacturer's controls, quality control measures should be taken to demonstrate acceptable sensitivity of the test method

    - no series of tests should be considered acceptable unless the result of the test kit manufacturer's and the additional quality control samples have satisfied the criteria laid down.

- Where fourth generation HIV (Ag/Ab) combination screening tests are used, UK Anti-HIV-2 and HIV p24 antigen working standards should be tested within batch pre-acceptance testing and show positive results.

Anti-HCV

- Specification: the UK specification for the minimum level of sensitivity for the performance of anti-HCV screening has been defined to require that in each series of tests a positive result must be obtained with the UK anti-HCV working standard

(available from NIBSC) or dilution thereof, specifically prepared for a particular test system, for the results of those tests to be valid

- Quality control of anti-HCV screening:
  - each batch of anti-HCV test kits should be shown to conform with locally established minimum criteria for specificity and sensitivity
  - in addition to the test kit manufacturer's controls, quality control measures should be taken to demonstrate acceptable sensitivity of the test method
  - no series of tests should be considered acceptable unless the result of the test kit manufacturer's and the additional quality control samples have satisfied the criteria laid down.

Hepatitis C virus nucleic acid testing (HCV – NAT)

- According to the requirements of the European Pharmacopoeia all manufacturing pools for the production of medicinal products derived from human plasma should be tested for HCV – RNA using a validated NAT assay which includes a suitable run control.
- Quality control of HCV NAT testing: for release of blood components, the NAT assay should detect a run control, defined for a single donation of 5000 IU/mL (as defined by WHO standards). For example, if donations are tested in mini-pools of 100, a run control of 50 IU/mL should be detected in each assay run.

Anti-HTLV I/II

- Specification: the specification for the minimum level of sensitivity for the performance of HTLV antibody screening has been defined to require that in each series of tests a positive result must be obtained with the UK anti-HTLV working standard (available from NIBSC) for the results to be valid. Although donor screening can be performed in mini-pools of up to 48 donations, it is expected that all individual donations in reactive pools would be individually tested according to manufacturer's instructions.
- Quality control of anti-HTLV screening:
  - each batch of anti-HTLV tests should be shown to conform with locally established minimum criteria for specificity and sensitivity
  - in addition to the test kit manufacturer's controls, quality control measures should be taken to demonstrate acceptable sensitivity of the test method
  - no series of tests should be considered acceptable unless the result of the test kit manufacturer's and the additional quality control samples have satisfied the criteria laid down.

Syphilis antibody

- Specification: the specification for the minimum level of sensitivity for the performance of syphilis antibody screening has not yet been defined beyond the requirement in each series of tests that a positive result must be obtained with the national syphilis antibody working standard when it becomes available.
- Quality control of syphilis antibody screening:
  - each batch of syphilis antibody test kits should be shown to conform with locally established minimum criteria for specificity and sensitivity

- in addition to the test kit manufacturer's controls, quality control measures should be taken to demonstrate acceptable sensitivity of the test method
- no series of tests should be considered acceptable unless the result of the test kit manufacturer's and the additional quality control samples have satisfied the criteria laid down.

10.4 Additional microbiological testing of selected donations

There is an increased risk of system error affecting tests that are not performed routinely on donors/donations. Reliable systems must be in place that ensure that errors do not occur where such tests are to be used as a basis for product release.

Antibody to cytomegalovirus (anti-CMV)

The presence or absence of anti-CMV should be determined by examination of the serum or plasma of the donor. The UK specification for the minimum level of sensitivity for the performance of anti-CMV screening has not yet been defined beyond the requirement that in each series of tests a positive result be obtained with the national anti-CMV working standard when it becomes available.

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

- Quality control of anti-CMV tests:
  - each batch of anti-CMV test kits should be shown to conform with locally established minimum criteria for specificity and sensitivity
  - in addition to the test kit manufacturer's controls, quality control measures should be taken to demonstrate acceptable sensitivity of the test method
  - no series of tests should be considered acceptable unless the result of the test manufacturer's and the additional quality control samples have satisfied the criteria laid down.

Tests for malarial antibodies

The exclusion period for donors from malarial areas is given in the JPAC *Donor Selection Guidelines*[1]. The JPAC *Donor Selection Guidelines* specify some situations where donations may only be released if a test for malaria antibody is negative. Such testing must only be undertaken using a test that has been validated for use in this setting. The presence or absence of malarial antibodies should be determined by examination of the serum or plasma of the donor. No series of tests should be considered acceptable unless the manufacturer's control tests have satisfied the criteria laid down.

Tests for antibodies to *Trypanosoma cruzi*

The deferral criteria for donors from *T. cruzi* areas are given in the JPAC *Donor Selection Guidelines*[1]. Donors at risk of *T. cruzi* must be tested for *T. cruzi* antibodies and negative results obtained prior to the release of any blood component for clinical use. Such testing must only be undertaken using a test which has been validated for use in this setting.

The presence or absence of *T. cruzi* antibodies should be determined by examination of the serum or plasma of the donor.

No series of tests should be considered acceptable unless the manufacturer's control tests have satisfied the criteria laid down.

Tests for West Nile Virus (WNV)

The exclusion criteria for donors from a WNV risk area is given in the JPAC *Donor Selection Guidelines*[1]. The JPAC *Donor Selection Guidelines*[1] specify some situations where donations may only be released if a test for antibody to WNV is negative. WNV NAT tests can be performed on donations provided by donors within the exclusion period and negative results obtained prior to the release of any blood component for clinical use. Such testing must only be undertaken using a test that has been validated for use in this setting. Normally plasma from the donor would be examined for the presence of WNV RNA. Any reactive donor would be permanently deferred. No series of tests should be considered acceptable unless the manufacturer's control tests have satisfied the criteria laid down.

Tests for antibodies to hepatitis B core (anti-HBc)

The exclusion period for donors who have had body piercing, acupuncture, etc. are given in the JPAC *Donor Selection Guidelines*.[1] Certain of these categories may require donations to be tested for anti-HBc and negative results obtained prior to release of any blood component for clinical use. Such testing must only be undertaken using a test that has been validated for use in this setting. The presence or absence of anti-HBc should be determined by examination of the serum or plasma of the donor. No series of tests should be considered acceptable unless the manufacturer's control tests have satisfied the criteria laid down.

Antibody to hepatitis B surface antigen (anti-HBs)

Donations found to be reactive for anti-HBc should be anti-HBs tested and those with levels <100 IU/L are deemed unsuitable for use; whereas those with levels >100 IU/L can be considered safe. Such testing must only be undertaken using a test that has been validated for use in this setting. The level of anti-HBs should be determined by examination of the serum or plasma of the donor. No series of tests should be considered acceptable unless the manufacturer's control tests have satisfied the criteria laid down.

10.5 Reinstatement of donors

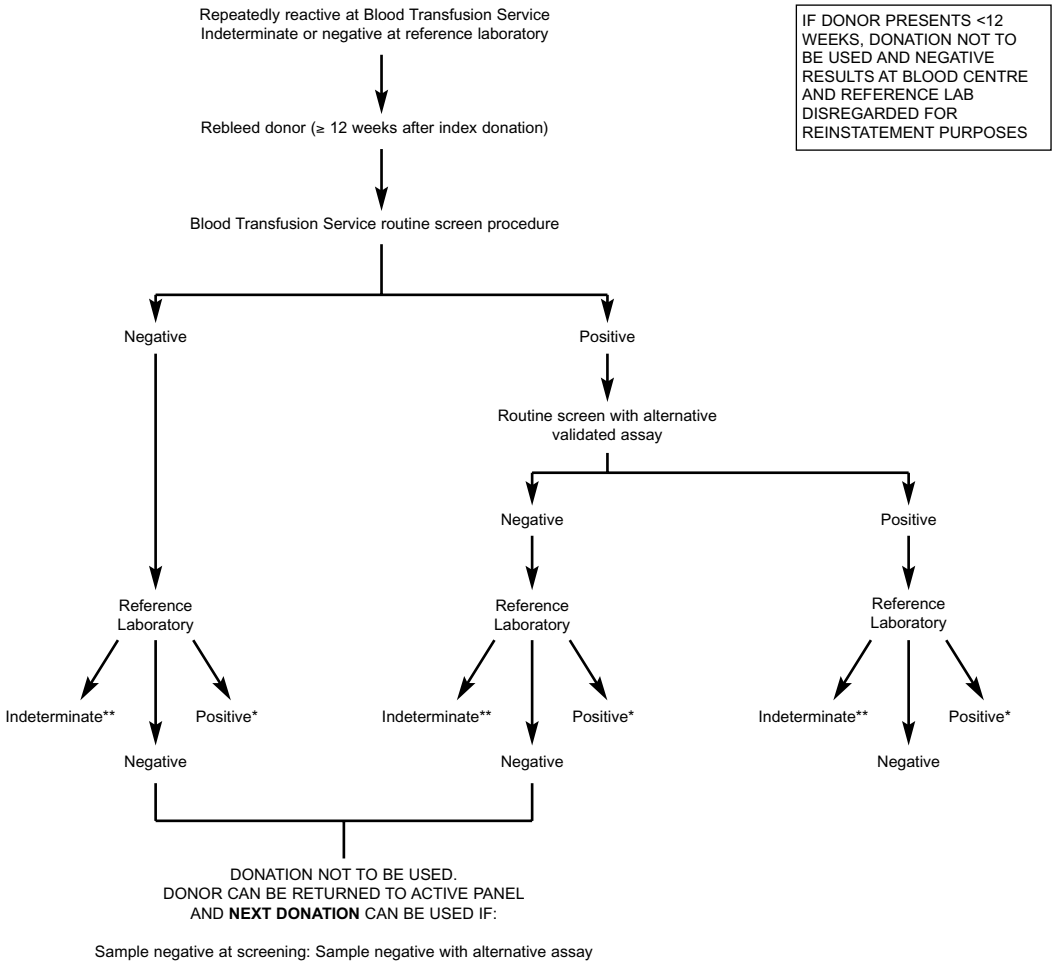
Reinstatement of donors (see Figure 10.2) whose serum has been confirmed to be falsely reactive in a microbiology assay.

- Where an initial sample taken at donation, and tested at a blood centre, is found repeatedly reactive (RR), materials from that donation must not be used for transfusion, the donor's record must be flagged in accordance with standard operating procedures and the donor removed from the active panel. No further material from the donor must be used for clinical purposes until the donor has been returned to the active panel.

- A specimen of the RR sample must be sent for confirmatory testing at a designated reference laboratory. If the specimen is considered to have been falsely reactive, reinstatement may be considered after a period of follow-up. At least 12 weeks must elapse from the date of the first sample before the donor can be retested for consideration for reinstatement.

- The specimen, taken at least 12 weeks after the initial bleed, must be sent to a designated reference laboratory. A number of options exist for reinstatement to the active panel depending upon the results of testing the 12-week follow-up sample at the blood centre and the designated reference laboratory. These are:

    - sample now non-reactive in the current screening assay at the blood centre, and confirmed negative at the designated reference laboratory. *Action – return to active panel as eligible for future donations. The next donation may be used if a negative result is obtained in the current screening test*



* Flag donor record (recorded as permanent deferral risk — not to be bled for clinical use. Arrange counselling and investigation of donor).
** Index donation for possible future reinstatement.

Figure 10.2 Action chart – donor reinstatement

- sample now non-reactive in the current screening assay at the blood centre, discordantly reactive but considered falsely so at the designated reference laboratory. *Action – return to active panel as eligible for future donations. The next donation may be used if a negative result is obtained in the current screening test*

- sample still reactive in the current screening assay used at the blood centre, but non-reactive in an alternative assay at blood centre and either non-reactive or discordantly reactive but considered falsely so at the designated reference laboratory. *Action – return to active panel as eligible for future donations if negative on an alternative screening test screening test (see next paragraph and Figure 10.2)*.

In order to reinstate a donor whose serum/plasma remains falsely reactive in the original screening test, the blood centre must run a different assay either as routine practice or as a specific screen for flagged donors whose sera previously have been shown to give a false reaction in one particular assay. The following conditions must be met for this to be acceptable:

- at least 12 weeks must elapse between the date of the first RR sample and the follow up sample tested in the alternative assay

- the alternative assay must be of equivalent sensitivity to the first assay in which the original serum/plasma gave a repeatable reaction and conform to requirements of microbiology tests, see bullet point two above

- the designated reference laboratory must have confirmed the false nature of the serum/plasma reactivity on a sample taken at least 12 weeks after the index donation

- where archival samples are held on a donor, the blood centre may adopt the strategy of testing samples taken at least 12 weeks, but not exceeding 12 months, apart in the alternative assay in order to fulfil the criteria retrospectively

- donations taken subsequent to the return of the donor to the active panel may be used provided that the donation is non-reactive by the alternative assay. The donor's record must remain flagged with the information identifying previous false reactivity for the marker.

Specific requirements for HBsAg

The designated reference laboratory must, as a minimum, perform specific neutralization tests for HBsAg and determine the anti-HBc status. Samples containing neutralizable HBsAg, with or without anti-HBc, indicate infection with HBV (except in cases where this may be due to recent immunization with HBsAg). Anti-HBc IgM may be useful in identifying a recent infection. Donors whose samples are falsely reactive for HBsAg but which contain anti-HBc cannot be considered for reinstatement unless anti-HBs is also present, or has previously been documented, at more than 100 IU/L.

10.6 Recommended standards for the reduction of bacterial contamination of platelets: donor arm cleansing, diversion of donation and testing of donation

Arm cleansing

There should be an effective, specified and validated method of arm cleansing, using an approved skin cleaning system, which shall be used according to the manufacturer's instructions. Evidence suggests that a suitable preparation is an alcoholic chlorhexidine gluconate solution.

- Adherence to the principles, protocols, and practices relating to the correct use of the specified skin cleaning system shall be regularly audited, and corrected if found to be lacking.

- A minimum of 20 mL of the first part of every blood donation should be diverted into a side-arm pouch, in order to minimize the level of bacterial skin contaminants in the collection bag. This diverted volume can be used as a source of blood samples for mandatory and other testing of the donation.

There should be a means of detecting bacterial contamination of platelet concentrates, using validated methods. The detection method should be sufficiently sensitive and used at a time appropriate to prevent clinically significant infection. Protocols must be in place for the management of reactive results.

References

1. Joint UKBTS/NIBSC Professional Advisory Committee's (JPAC) *Donor Selection Guidelines* available at www.transfusionguidelines.org.uk.

Chapter 11

Investigation of suspected transfusion-transmitted infection

11.1 General considerations

The guidelines in this section apply to reports of possible transfusion-transmitted infection (TTI) arising from blood or blood components supplied by the UK Blood Transfusion Services. Any suspected cases of TTI should be documented and fully assessed to determine whether further investigation of donors and/or donation samples is required or warranted. The guidance contained within this section covers the action to be taken at the Blood Centre in such cases.

Because TTI may be asymptomatic, cases may not be recognized or detected until months or years after the transfusion. Many cases come to light through incidental screening or specific testing on development of late clinical features of the infection in question. Cases may therefore be notified by sources other than the hospital blood bank, but close liaison will be required with the hospital blood bank that supplied the blood for transfusion and the reporting clinician.

Documentation

Reports of possible TTI must be recorded and retained. Wherever possible, details of the notification should be confirmed in writing.

For each report, confirmation of clinical and laboratory details will be required. Ideally, these should take the form of copies of the relevant recipient blood tests and computer printouts of transfusion records. Other forms of reporting of transfusion history (by letter, typed lists, etc) should be avoided in view of the risk of transcription errors.

11.2 Assessment of validity of the possible diagnosis of TTI

Clinical and laboratory details of the case should be reviewed to assess the validity of a diagnosis of possible TTI. Further information or test results may be required and requested at this stage.

Every effort should be made to collect all necessary information before taking any action with respect to contact with or additional investigation of donors. However, once a full assessment has taken place action may be considered to prevent issue of any further donations from involved blood donors. Similarly, a search for in date components from recent donations should be carried out.

When a decision has been made to commence an investigation into the case, consideration should be given as to which donors require further investigation, and whether this can be

satisfactorily carried out with samples already available at the blood centre from the index or any subsequent donation. This decision is dependent on the premise that subsequent samples may conclusively demonstrate the development of infectious markers (e.g. antibodies) in one of the implicated donors. It is expected that blood establishments will retain samples from each donation for a minimum period of three years in a suitable frozen archive. The retrieval of samples from this archive must be fully documented and be restricted mainly to such investigations.

If further investigation is required, and suitable blood samples are not available from the donor, then the decision may be made to contact the donor and request further samples.

Decisions for each case and each donor will be on an individual basis depending upon the circumstances, timing, assessed likelihood of TTI, and resources required. In cases of doubt, there should be a mechanism to ensure that there is a system for review and agreement on the way forward.

It will not always be necessary to carry out further investigations on the donor and/or donations, as testing of subsequent donations may provide sufficient information.

11.3 Identification of possible infectious donations

When investigations result in the identification of a possible or likely TTI, the donor should be removed from the donor panel and informed accordingly.

When a possible/likely TTI is identified, follow-up of components from the same donation and from any subsequent donation which could have been capable of transmitting infection must be carried out and the results recorded in the investigation file. If such components have been transfused, then a lookback investigation will be necessary. This should be carried out with the involvement of the relevant hospital haematologist and/or clinician.

Similarly, if there is a risk of transmission of infection from previous donations, tracing of these donations through to recipients (lookback) should be performed. This will generally be restricted to untested donations and to the last seronegative donation before seroconversion. Lookback for other potentially infectious donations or in any other situation should be undertaken only after discussion with a senior consultant.

11.4 Closing TTI enquiries

Each TTI investigation should be formally closed, with a conclusion and written notification to the reporter and any other interested party. It must be remembered that anonymity of donors is absolute.

Each case of possible TTI must be reported to the appropriate surveillance system (NBS/HPA in England and Wales; SNBTS National Microbiology Reference Unit in Scotland; and Northern Ireland Blood Transfusion Centre for Northern Ireland). These reports are collated and published in the annual Serious Hazards of Transfusion (SHOT) report.

11.5 Lookback investigations

Lookback investigations are initiated on recognition of a potential infectious donor. Such a situation may arise in the following circumstances:

- donors identified as infected through the introduction of a new screening test applied to all donations
- donors identified to be infected through seroconversion during their blood donation career
- donors identified to be responsible for transmission of infection to a recipient.

General principles for lookback investigations

National lookback investigations, precipitated through introduction of a new screening test, should be managed through a generic system which incorporates the following steps:

- identification of potentially infectious donations
- identification of blood components prepared from those donations
- documentation of the fate of the blood components
- notification of hospital transfusion laboratories of receipt of involved blood components
- identification of the fate of the component at the hospital, including details of any identified recipient
- for recipients not known to be dead, a procedure for notification, generally following notification of the GP/hospital clinician
- a protocol for management of the recipient notification and testing (if required)
- notification of recipient test results to recipient and other interested parties.

Lookback investigations precipitated by identification of a donor who has seroconverted and/or been responsible for transmission of infection and/or is identified through post-donation information should be carried out using the same principles.

Wherever possible, retrospective testing of stored samples should be carried out in order to identify those donations which must be included in the lookback. If samples can be tested, lookback should be performed including the last seronegative donation.

If retained samples are not available for testing, then case by case decisions on the number of donations to be included in the lookback will be influenced by the dates of donations and the availability of the particular hospital transfusion records.

Documentation and reporting

All cases of lookback should be documented in the same fashion as investigation of TTI. There should be a full audit trail of decisions made and actions taken.

Where lookback results in the identification of infected recipients, a report should be made to the surveillance system as appropriate, and cases included in the annual SHOT report.

In instances where there is doubt over potential TTI, specialized molecular genotyping of both implicated donor and infected recipient may be necessary to prove conclusively whether TTI did indeed occur.

Chapter 12

Reagent manufacture

12.1 Guidelines for reagent manufacture

12.1.1 Introduction

All reagents used to determine the group of human red cells and to detect red cell antibodies must comply with Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on *in vitro* Diagnostic Medical Devices.

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

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

12.1.2 Reference preparations

The following reference preparations will be available for use with these Guidelines:

- Anti-A (Minimum Potency Preparation) 88/722
- Anti-B (Minimum Potency Preparation) 88/724
- Anti-D (IgM) (International Standard for Minimum Potency Preparation 99/836)
- ICSH/ISBT Anti-Human Globulin Standard 96/666
- ICSH/ISBT Papain Reference Preparation 92/658
- ICSH/ISBT anti-D 91/572 (for use with Papain Reference Preparation 92/658).

See Section 12.3 for further information.

12.1.3 Definitions

Antibody identification is a test or combination of tests designed to determine the specificity of irregular antibodies.

Antibody screening is a test or combination of tests designed to detect irregular antibodies.

A **batch** of reagent is a defined quantity of material or of bulk, intermediate or finished product that is intended or purported to be uniform in character and quality, and which

has been produced during a defined cycle of manufacture. A batch may be divided into sub-batches. A batch is sometimes described as a 'lot'.

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

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

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

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

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

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

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

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

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

Irregular blood group antibodies are those of specificity other than anti-A or anti-B.

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

The **manufacturer** is the natural or legal person with the responsibility for placing the device on the market under his or her own name, regardless of whether he or she has designed, manufactured, packaged, or labelled the device.

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

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

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

Polyspecific anti-human globulin reagent should be the name for a reagent which contains anti-human IgG and anti-human complement (C3d) activity, and is recommended by the manufacturer for use in both the direct and indirect anti-human globulin techniques, i.e. for the detection of red cell bound human IgG, and C3 complement in the form EiC3b and EC3d irrespective of the presence of other human immunoglobulin or human complement specificities.

Potency titre is a term used to describe the highest dilution of a reagent that effects a **grade 2** end-point reaction.

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

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

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

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

Shelf life is the period until expiry date.

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

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

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

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

An **unequivocal** reaction in a test system is a reaction that is unambiguous. In the manual tube test, this is defined as a reaction of **grade 3** or greater.

12.1.4 General manufacturing considerations

a) Good manufacturing practice

Reagents for blood group serology must be manufactured in accordance with Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on *in vitro* Diagnostic Medical Devices.

Guidance on the principles of Good Manufacturing Practice can be obtained from *Rules and Guidance for Pharmaceutical Manufacturers and Distributors* (TSO, London).

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

b) Risk management

Risk management should be performed in accordance with:

- BS EN ISO 14971:2001 Medical Devices – Application of Risk Management to Medical Devices.
- BS EN 13641:2002 Elimination or Reduction of Risk of Infection Related to *in vitro* Diagnostic Reagents.

c) Performance evaluation

Performance evaluation should be performed in accordance with:

- BS EN 13612:2002 Performance Evaluation of *in vitro* Diagnostic Medical Devices.
- Reagents listed in Annex II, List A, of the EU *in vitro* Diagnostic Medical Device Directive must also comply with the Common Technical Specifications for *in vitro* Diagnostic Medical Devices (2002/364/EC).

d) Stability data

Stability testing should be performed in accordance with:

- BS EN 13640:2002 Stability Testing of *in vitro* Diagnostic Reagents.

e) Date of manufacture

- For blood grouping reagents the date of manufacture is the date of commencement of the last potency test on the batch or sub-batch that indicates attainment of the required specification.
- For reagent red cells the date of manufacture is the date of collection from the donor. Where reagent red cells are prepared from more than one donor, the date of collection of the first donation should be recorded as the date of manufacture.
- Where a freezing process is used to preserve red cells before their preparation for issue as reagent red cells, the date of manufacture is the date of recovery from the frozen state.

f) Colour coding of reagents

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

- Polyspecific anti-human globulin reagents may be coloured green, anti-A may be coloured blue, anti-B may be coloured yellow.
- The colourant should not interfere with the observation of the test result.

g) Freedom from microbial contaminants

- Reagents should be prepared using validated processes to produce a final product free from microbial contaminants that adversely affect the unopened product during storage at the recommended temperature. The manufacturer should routinely monitor the efficacy of the process used in the manufacture of the reagent.

- A preservative may be included in the reagent to minimize the effects of contamination during use if the preservative has been shown not to adversely affect the product during storage or use.

- Other than reagent red cells, all reagents for blood group serology recommended by the manufacturer for storage in the liquid state, should be filtered through a sterile filter of pore size not exceeding 0.22 μm. All reagents should be dispensed into the immediate container under aseptic conditions.

- Tests for contamination do not give absolute assurance of freedom from microbial contaminants. Bactericidal agents in common use for blood grouping reagents do not guarantee the absence of microbial agents after opening of the container.

h) Retained samples

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

- A minimum of two final containers of each batch of reagent red cells should be retained and stored as recommended by the manufacturer, to enable analysis of reported defects. Such samples should be retained for at least ten days beyond the expiry date.

i) Tests required

The manufacturer should test, as described in these guidelines, each lot of a reagent obtained from the immediate container to be supplied for use (see Section 12.2.1).

j) Human source material

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

Samples/donations that are obtained specifically for reagent purposes, will require additional consenting of the donor, and must have appropriate ethical approval. Donor materials that are obtained and retained for genomic or Nucleic Acid Testing must comply with the regulations laid down by The Human Tissue Act 2004 (except Scotland).[1]

Residual samples retained from patient testing laboratories may be used without further explicit consent, if anonymized.[2] Additional samples taken from patients specifically for reagent use will require ethical approval and explicit consent. All patient samples acquired and retained must comply with the regulations laid down by the Human Tissue Act (2004).

Each individual donation or sample of human material in a reagent for blood group serology shall be tested and found negative for mandatory microbiological tests required by the UK Blood Transfusion Services for blood donations (see Chapter 10). A statement is required in the 'instructions for use' to this effect.

To ensure retrospective microbiological testing, an appropriate sample, collected at the same time as the donation used in the formulation of a particular reagent, should be archived until at least six months after the expiry date of the last batch of the reagent made from that material.

k) Label requirements

The label must conform to the requirements of:

- BS EN 375:2001 Information Supplied by the Manufacturer with in vitro Diagnostic Reagents for Professional Use.

In addition the instructions for use should contain the following:

- The label fixed to the immediate container of a reagent should leave uncovered sufficient area of the full length or circumference of the container to allow ready visual inspection of the contents.
- The specificity of the reagent for blood group serology should be of a print size which is clearly legible. The print size of other information on the label should not exceed that used for the specificity of the reagent.
- The typeface used should clearly differentiate between antigens and related antibody specificities represented by upper and lower case characters, e.g. C/c, S/s and K/k.
- For products needing to be prepared in the final form by the user following the instructions of the manufacturer and to be retained in the manufacturer's immediate container, a space should be available on the container label for the user to write the expiry date of the prepared product when stored as recommended by the manufacturer.
- The main panel of labels of enzyme-treated reagent red cells may be coloured pink in order to be distinguishable from non-enzyme-treated reagent red cells. Pantone colour reference 223 is recommended.
- For other reagents, any colour appearing on the main panel of the label should comply with FDA regulations (21 CRF 660.28) as shown in Table 12.1.

Table 12.1 Label colour coding

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

l) Instructions for use (package insert)

The instructions for use must conform to the requirements of:

- BS EN 375:2001 Information Supplied by the Manufacturer with *in vitro* Diagnostic Reagents for Professional Use.

In addition:

- For blood grouping reagents containing monoclonal antibodies, the identity of the cell line(s) from which the monoclonal antibodies have been derived.
- For reagent red cells for antibody screening and for identification, the 'antigen profile' of the component cell samples is part of the instructions for use and should have the lot number and expiry date of the reagent to which it refers.

- A statement that loss of reactivity may occur during the stated shelf life of the red cells and that since this loss is partly determined by characteristics of individual blood donations or donors, which cannot be predicted or controlled, the conditions of storage and use recommended by the manufacturer should be rigidly applied.
- For enzyme-treated reagent red cells, information should be given concerning those antigens which are rendered inactive or less active by the enzyme treatment used.

12.2 Specifications, performance evaluation and quality control of blood grouping reagents

12.2.1 Blood typing antisera

a) General requirements

- It is essential that blood grouping reagents are prepared using reliable manufacturing procedures that are consistently capable of producing safe and efficacious products. The products must comply with requirements of the EU Directive (98/79/EC) on *in vitro* diagnostic medical devices and other relevant international standards detailed in Section 12.3.
- The term weak D (D^u) is used in these Guidelines to indicate a weakened expression of a normal D antigen. The term partial D is used in these recommendations to indicate the expression of only a part of the normal D antigen. The reactivity of RhD blood grouping reagents against partial D red cells is determined by the nature of the D variant, the anti-D reagent and the technique used.
- The blood grouping reagent is satisfactory if an unequivocal positive result is obtained with all the red cell samples having the antigen corresponding to the blood grouping reagent being assessed, by all the methods recommended for use by the manufacturer.
- If reactivity is claimed by the manufacturer against weak variants or sub-groups of a particular antigen, red cells from at least two confirmed/reference samples should be tested (see Table 12.3).
- The following grading system (see Table 12.2) is used throughout these Guidelines for manual tube/microplate 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.

Table 12.2 Grading system for serological tests

Reaction Grade	Description	Titration Score
Grade 5	Cell button remains in one clump or dislodges into a few large clumps, macroscopically visible	12
Grade 4	Cell button dislodges into numerous large clumps, macroscopically visible	10
Grade 3	Cell button dislodges into many small clumps, macroscopically visible	8
Grade 2	Cell button dislodges into finely granular but definite, small clumps, macroscopically visible	5
Grade 1	Cell button dislodges into fine granules, microscopically visible	3
Grade 0	Negative result	0

Unless otherwise stated, an unequivocal manual tube reaction is defined as a grade 3 or greater.

b) Performance evaluation

Performance evaluation should be performed in accordance with:

- BS EN 13612:2002 Performance Evaluation of *in vitro* Diagnostic Medical Devices.
- Reagents listed in Annex II, List A, of the EU *in vitro* Diagnostic Medical Device Directive must also comply with the Common Technical Specifications for *in vitro* Diagnostic Medical Devices (2002/364/EC).

Stability testing should be performed in accordance with:

- BS EN 13640:2002 Stability Testing of *in vitro* Diagnostic Reagents.

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

- In the case of polyclonal antibodies, contaminating antibodies to antigens having a prevalence of greater than 99% in the general population of the UK should be excluded by negative results in tests using samples of red cells from four different individuals who lack the antigen corresponding to the antibody specificity under test. Tests for the presence of contaminating ABO antibodies should be performed with red cells from a minimum of two individuals of group A_1 and two of group B who lack the antigen corresponding to the antibody specificity under test.

- If tests using all methods recommended for use by the manufacturer do not exclude the presence of antibodies to the following antigens, these antibody specificities should be stated in the package insert as not having been excluded in specificity testing:

  Xg^a; Do^a; Yt^a; Co^b; Wr^a; and V^w.

- A blood grouping reagent recommended for use by a direct agglutination method should be tested against red cells lacking the antigen corresponding to the antibody specificity but coated with IgG blood group antibody to effect a grade 5 reaction in the anti-human globulin technique. Polyclonal IgG blood group antibodies from at least four individuals should be tested separately.

- Blood grouping reagents which are chemically modified, and/or contain in their formulation a potentiator of agglutination, or require the user to add a potentiator, shall be tested, by all methods recommended by the manufacturer with red cells lacking the antigen corresponding to the antibody specificity under test but sensitized with an IgG antibody to effect a grade 5 reaction in the anti-human globulin technique.

- Potentiated blood grouping reagents producing agglutination by those methods recommended by the manufacturer, should be supplied with a reagent control that has been shown to effect a degree of non-specific reaction with IgG coated red cells similar to the corresponding blood grouping reagent.

- Blood grouping reagents recommended for use by a direct agglutination method should not contain antibodies reactive against red cells coated with IgG when used by direct agglutination methods recommended by the manufacturer.

c) Batch release testing requirements

Specificity tests

- The manufacturer should test the blood grouping reagent as a final product, by all methods recommended by the manufacturer for the specificity and reactivity claimed. Specificity should be determined by testing the reagent in accordance with the requirements outlined in Table 12.3.

- If a range of incubation times or incubation temperatures is recommended by the manufacturer, the range(s) should be used in these test procedures.

Requirements

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

Table 12.3 Requirements for conventional blood typing reagents

Antibody specificity	Specification	Performance evaluation As a minimum, two examples of the following reference cells should be included*	Batch release testing					
			Specificity				Potency	
			Positive reactors		Negative reactors			
			Cell type	No.	Cell type	No.	Cell type	No.
Anti-A	Normally blue coloured	Ax, A_3	A_1	2	B	2	A_1	1
	Should equal or exceed potency of reference preparation(s)	A cord cells	A_2B	2	O	2	A_2B	2
	Should detect variants and subgroups as detailed in the manufacturer's instructions for use		Ax*					
Anti-B	Normally yellow coloured	Bx, B_3, Bv	B	2	A_1	2	B	1
	Should equal or exceed potency of reference preparation(s)	B cord cells	A_1B	2	O	2	A_1B	2
	Should detect variants and subgroups as detailed in the manufacturer's instructions for use							
Anti-A,B	Normally clear coloured	A_1, A_2, B, A_1B, A_2B	A_1	1	O	4	A_1	1
	Should equal or exceed potency of reference preparation(s)	Ax, A_3	A_2	2			A_2	2
	Should detect variants and subgroups as detailed in the manufacturer's instructions for use	Bx, B_3	B	2			B	2
		A and B cord cells	Ax	2				
Anti-A_1	Normally clear coloured		A_1	2	A_2	2	A_1	2
	Should equal or exceed potency of reference preparation(s)		A_1B	2	A_2B	2		
	Should detect variants and subgroups as detailed in the manufacturer's instructions for use				B	2		
					O	2		
Anti-D	Normally clear coloured	Weak D (500 sites/cell)	R_1r	2	r′r	1	R_1r	2
	Should equal or exceed potency of reference preparation(s)	C^w, C^x	R_2r	2	r″r	1		
	Should detect variants and subgroups as detailed in the manufacturer's instructions for use	D^{VI} type 1, D^{VI} type 3, D^{IV}, D^V, D^{VII}, DFR, DBT, $R_O{}^{HAR}$	Weak D*	2	rr	1		

Table 12.3 Continued

Antibody specificity	Specification	Performance evaluation As a minimum, two examples of the following reference cells should be included*	Batch release testing					
			Specificity				Potency	
			Positive reactors		Negative reactors			
			Cell type	No.	Cell type	No.	Cell type	No.
Anti-C	Normally clear coloured Potency titre greater than 4 vs by techniques detailed in manufacturer's instructions for use Should detect variants and subgroups as detailed in the manufacturer's instructions for use	C^w, Cx, r'^S R_2R_Z	R_1r R_1R_2 $r'r$	1 2 1	R_2R_2 $r''r$ rr	1 1 1	R_1r	2
Anti-E	Normally clear coloured Potency titre greater than 4 vs by techniques detailed in manufacturer's instructions for use Should detect variants and subgroups as detailed in the manufacturer's instructions for use	R_1R_Z E^w	R_2r R_1R_2 $r''r$	1 2 1	R_1R_1 $r'r$ rr	1 1 1	R_2r	2
Anti-c	Normally clear coloured Potency titre greater than 4 vs by techniques detailed in the manufacturer's instructions for use Should detect variants and subgroups as detailed in the manufacturer's instructions for use	R_1R_Z, $R_1{}^wR_1$	R_1r R_1R_2 $r'r$	2 1 1	R_1R_1	3	R_1r	2
Anti-e	Normally clear coloured Potency titre greater than 4 vs by techniques detailed in the manufacturer's instructions for use Should detect variants and subgroups as detailed in the manufacturer's instructions for use	R_2R_Z	R_2r R_1R_2 $r''r$	2 1 1	R_2R_2	3	R_2r	2
Anti-C^w	Normally clear coloured Potency titre greater than 4 vs by techniques detailed in the manufacturer's instructions for use	$R_1{}^wR_1$, r'^wr, $R_1{}^wr$	$R_1{}^wr$ or $R_1{}^wR_2$ r'^wr	2 2 1	R_1r R_1R_1 $r'r$	1 1 1	$R_1{}^wr$	2

Table 12.3 Continued

Antibody specificity	Specification	Performance evaluation As a minimum, two examples of the following reference cells should be included*	Batch release testing					
			Specificity				Potency	
			Positive reactors		Negative reactors			
			Cell type	No.	Cell type	No.	Cell type	No.
Anti-K	Normally clear coloured Potency titre greater than 4 vs by techniques detailed in the manufacturer's instructions for use Should detect variants and subgroups as detailed in the manufacturer's instructions for use	Kk Kp (a+b–) Kk Kp (a–b+)	Kk	4	kk	4	Kk	2
Anti-k	Normally clear coloured Potency titre greater than 4 vs by techniques detailed in the manufacturer's instructions for use Should detect variants and subgroups as detailed in the manufacturer's instructions for use	K+k+Kp(a+)	Kk Kk Kp(a–) Kk Kk Kp(a+b+)	4 2 2	KK	4	Kk	2
Anti-Fy^{a}	Normally clear coloured Potency titre greater than 4 vs by techniques detailed in the manufacturer's instructions for use Should detect variants and subgroups as detailed in the manufacturer's instructions for use	Fy^{x}	Fy(a+b+)	4	Fy(a–)	4	Fy(a+b+)	2
Anti-Fy^{b}	Normally clear coloured Potency titre greater than 4 vs by techniques detailed in the manufacturer's instructions for use Should detect variants and subgroups as detailed in the manufacturer's instructions for use		Fy(a+b+)	4	Fy(b–)	4	Fy(a+b+)	2
Anti-Jk^{a}	Normally clear coloured Potency titre greater than 4 vs by techniques detailed in the manufacturer's instructions for use Should detect variants and subgroups as detailed in the manufacturer's instructions for use		Jk(a+b+)	4	Jk(a–)	4	Jk(a+b+)	2

Table 12.3 Continued

Antibody specificity	Specification	Performance evaluation As a minimum, two examples of the following reference cells should be included*	Batch release testing					
			Specificity				Potency	
			Positive reactors		Negative reactors			
			Cell type	No.	Cell type	No.	Cell type	No.
Anti-Jk^{b}	Normally clear coloured Potency titre greater than 4 vs by techniques detailed in the manufacturer's instructions for use Should detect variants and subgroups as detailed in the manufacturer's instructions for use		Jk(a+b+)	4	Jk(b–)	4	Jk(a+b+)	2
Anti-S	Normally clear coloured Potency titre greater than 4 vs by techniques detailed in the manufacturer's instructions for use Should detect variants and subgroups as detailed in the manufacturer's instructions for use	SS, Ss, ss	Ss	4	ss	4	Ss	2
Anti-s	Normally clear coloured Potency titre greater than 4 vs by techniques detailed in the manufacturer's instructions for use Should detect variants and subgroups as detailed in the manufacturer's instructions for use		Ss	4	SS	4	Ss	2
Anti-M	Normally clear coloured Potency titre greater than 4 vs by techniques detailed in the manufacturer's instructions for use Should detect variants and subgroups as detailed in the manufacturer's instructions for use	NN He+	MN	4	NN	4	MN	2
Anti-N	Normally clear coloured Potency titre greater than 4 vs by techniques detailed in the manufacturer's instructions for use Should detect variants and subgroups as detailed in the manufacturer's instructions for use		MN	4	MMS	4	MN	2

Table 12.3 Continued

Antibody specificity	Specification	Performance evaluation As a minimum, two examples of the following reference cells should be included*	Batch release testing					
			Specificity				Potency	
			Positive reactors		Negative reactors			
			Cell type	No.	Cell type	No.	Cell type	No.
Anti-P_1	Normally clear coloured Potency titre greater than 4 vs by techniques detailed in the manufacturer's instructions for use Should detect variants and subgroups as detailed in the manufacturer's instructions for use		P_1 strong P_1 weak	4 4	P_1 (–)	4	P_1 (+)	2
Anti-Le^a	Normally clear coloured Potency titre greater than 4 vs by techniques detailed in the manufacturer's instructions for use Should detect variants and subgroups as detailed in the manufacturer's instructions for use		Le(a+b–)	4	Le(a–)	4	Le(a+)	2
Anti-Le^b	Normally clear coloured Potency titre greater than 4 vs by techniques detailed in the manufacturer's instructions for use Should detect variants and subgroups as detailed in the manufacturer's instructions for use	A_1B Le (a–b+)	A_1B Le(a–b+)	4	Le(b–)	4	A_1B Le(b+)	2

* For reagents where reactivity against the antigen is claimed.

- Rouleaux formation, prozone or haemolysis should not occur in tests using any of the methods recommended by the manufacturer.

Potency tests – tube or microplate methods

- Potency titrations should be performed in accordance with the manufacturer's recommended method of use using an appropriate diluent.
- Manufacturers should compare the potency titre of each batch of reagent with an appropriate reference preparation (see Section 12.3).

Requirements

- Potency titrations for each batch tested should equal or exceed any existing British or International reference preparations.

12.2.2 Anti-human globulin reagents

a) Introduction

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

b) General requirements

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

c) Performance evaluation

Performance evaluation should be performed in accordance with:

- BS EN 13612:2002 Performance Evaluation of *in vitro* Diagnostic Medical Devices.
- Reagents listed in Annex II, List A, of the EU *in vitro* Diagnostic Medical Device Directive must also comply with the Common Technical Specifications for *in vitro* Diagnostic Medical Devices (2002/364/EC).

Stability testing should be performed in accordance with:

- BS EN 13640:2002 Stability Testing of *in vitro* Diagnostic Reagents.

d) Batch release testing requirements

Specificity testing

Tests for IgM or IgG red cell heterospecific antibodies

- Heterospecific antibodies can cause haemolysis or agglutination of unsensitized red cells in the indirect anti-globulin test. Full details of tests for heterospecific antibodies are outlined in Section 12.4.

Requirements

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

Tests for unwanted positive reactions

- These test for excess anti-C3d and anti-C3c, which can cause unwanted positive reactions in the indirect anti-globulin test, and for the presence of any undesirable antibodies in the reagent. Full details of tests are outlined in Section 12.4.

Requirements

- All reactions should be negative on macroscopic examination.

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

- The reference reagent (ICSH/ISBT 96/666) should be tested in parallel with the test reagent, each being titrated against red cells sensitized with potent IgG anti-D antibody.

Requirements

- The potency titre of the test anti-human globulin or anti-IgG reagent should be at least equal to that of the reference reagent (ICSH/ISBT 96/666).

Potency tests

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

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

Full details of tests are outlined in Section 12.4.

Requirements

- The anti-human globulin reagent or anti-IgG reagent is satisfactory if the reaction grade at all dilutions attains or exceeds that of the reference reagent (ICSH/ISBT 96/666) without significant prozone, against red cells sensitized 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 antihuman globulin reagent undiluted and 1 in 2.

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

- Test AHG or anti-complement reagents against a selection of complement coated red cells to determine the optimum potency. C3 and C4 complement-coated red cells should be prepared as described in Section 12.4. In addition, anti-complement activity may be evaluated by tests with complement-fixing antibodies, such as anti-Jk^a.

Requirements

- The anti-human globulin reagent should have an anti-C4c titre of 1 in 2 or less.
- The anti-human globulin reagent should not affect a macroscopic reaction with EC4d red cells.
- The reagent should attain the potency titre of the reference reagent (ICSH/ISBT 96/666).
- Conventional (polyclonal) anti-human globulin or anti-human globulin containing monoclonal IgG anti-C3d that attain adequate reactivity with an optimal incubation period different from that recommended for the detection of IgG antibody, should state in the instructions for use the appropriate incubation period required for the optimum detection of red cell bound C3c/d complement components.

Tests for unwanted positive reactions

- These test for excess anti-C3d and anti-C3c, which can cause unwanted positive reactions in the indirect anti-globulin test, and for the presence of any undesirable antibodies in the reagent. Full details of tests are outlined in Section 12.4
- All test results should be negative as defined by the manufacturer in the 'instructions for use'.

Instructions for use

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

- Inadequate washing of red cells in the anti-human globulin test may result in neutralization of the anti-human globulin reagent.
- Following completion of the wash phase in the anti-human globulin test, excess residual saline may dilute the anti-human globulin reagent, when added, beyond that in the manufacturer's assessment.
- No single test is capable of detecting all clinically significant antibodies.
- For each batch of antibody screening being undertaken by an anti-human globulin test, a positive and negative control should be included. The positive control should be a weak anti-D (0.1 IU/mL); the negative control an inert serum, tested against the antibody screening cells being used.

12.2.3 Reagent red cells

a) 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 red cell allo-antibodies.

b) General guidelines for reagent red cell manufacture

- When testing reagent red cells, in order to confirm the presence or absence of antigens listed in the antigen profile, a sample from each individual should be tested whenever possible, with a minimum of two antisera for each specificity prepared from different donors/cell lines.

- Where such testing produces conflicting results, repeat and further testing with at least one additional example of the relevant antibody(ies) should be undertaken to confirm the antigenic status of that cell.
- Where such testing has been performed with only one example of any blood grouping reagent, this information should be stated in the antigen profile included within the package insert.
- Reagent red cells should be shown not to produce unwanted positive reactions by the methods recommended for use by the manufacturer.
- Except for IgG-sensitized and C3-sensitized red cells, reagent red cells should be negative in the direct anti-human globulin technique with anti-IgG, anti-complement and polyspecific anti-human globulin reagents.
- With the exception of umbilical cord blood, red cells used to test patient's samples for irregular antibodies should not be pooled.
- Reagent red cells should be processed by a method and suspended in a medium that consistently ensures stability of the antigens specified in the antigen profile included within the package insert.
- All red cells reagents should be free of ABH specific blood group substances and blood group antibodies, including anti-A and anti-B, demonstrable by the manufacturer's recommended methods of use.
- The method of manufacture should ensure that white cells are removed from donations of red cells before the white cells lyse and release enzymes, which may adversely affect the properties of the red cells.

c) Immediate container label and instructions for use sheet

The immediate container and instructions for use sheet for reagent red cells should also include:

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

d) Reagent red cells for use in ABO and RhD grouping

- Reagent red cells should be groups A_1 and B. In addition, A_2 B or O red cells may be included.
- At least one of the set should be RhD positive and one RhD negative.

e) Reagent red cells for use in antibody screening

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. Reagent red cells of a lesser specification may be used when performing antibody screening tests on blood donor samples.

In general the following should apply:

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

f) Reagent red cells for use in antibody screening of patient samples

- As a minimum the following antigens should be expressed on the reagent red cells for antibody screening:

  C; c; D; E; e; K; k; Fy^a; Fy^b; Jk^a; Jk^b; S; s; M; N; P_1; Le^a and Le^b.

- As a minimum, reagent red cells from two individuals should be provided. These red cells should not be pooled. One reagent red cell should be R_2R_2; the other R_1R_1 (or $R_1{}^wR_1$).
- Apparent homozygous expression of the following antigens is desirable:

  Fy^a; Fy^b; Jk^a; Jk^b; S and s.

g) Reagent red cells for use in antibody screening of donor samples

- Reagent red cells may be:
  - provided unpooled from a minimum of two individuals; or
  - as a pool of red cells in equal proportions from no more than two donors; or
  - red cells from a single donor.
- Pooled reagent red cells for antibody screening should be used only for testing samples from blood donors; not samples from patients.
- As a minimum the following antigens should be expressed:

  D; C; c; E; e; K.

h) Reagent red cells for use in antibody identification

- Reagent red cells for use in the identification of irregular antibodies should be confirmed as group O by an ABO blood grouping procedure which is capable of demonstrating the A_x phenotype.
- Where practicable, red cells from individuals known consistently to effect troublesome reactions with HLA antibodies should not be used in reagent red cells for antibody identification.
- The antigen profile of reagent red cells for antibody identification should permit the identification of frequently encountered antibodies, for example anti-D, anti-E, anti-K, anti-Fy^a, and of commonly encountered alloantibody mixtures, for example, anti-D+K.
- A red cell antibody identification panel comprises cells from the eight or more individuals which should between them express the following antigens:

C; C^w; c; D; E; e; K; k; Kp^a; Fy^a; Fy^b; Jk^a; Jk^b; S; s; Le^a; Le^b; M; N; P_1 and Lu^a.

- Red cells from one individual should be R_1R_1 and from another $R_1{}^WR_1$ and between them should express the antigens:

  K; k; Fy^a; Fy^b; Jk^a; Jk^b; S and s.

- Red cells from one individual should be R_2R_2, another r″r and those from another r′r.

- Red cells from a minimum of three individuals should lack the Rh antigens C, E and D. One of these three individuals should be K positive. Between them, red cells from these individuals should exhibit apparent homozygous expression of the antigens:

  c; k; Fy^a; Fy^b; Jk^a; Jk^b; S and s.

i) Reagent red cells (IgG coated) for use in the control of the anti-human globulin technique

- To ensure that the anti-IgG activity in negative antiglobulin tests has not been fully or partially neutralized, control red cells 'sensitized' with IgG antibody are added to negative tests.

- Group O RhD positive red cells are sensitized with sufficient anti-D to render an indirect antiglobulin test negative when a volume of these sensitized red cells and a volume of serum diluted 1 in 1000 are added, but remains positive if a volume of saline instead of diluted serum is added.

j) Other reagent red cells

These reagent red cells should be manufactured in accordance with the relevant guidelines above.

12.2.4 Miscellaneous reagents

a) Fetal calf serum and bovine serum albumin

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

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

- red cells to become T/Tk etc. transformed

- inhibition of antigen:antibody reactions

- false positive reactions or rouleaux.

b) Proteolytic enzyme preparations

The activity of each batch of proteolytic enzyme should be assessed to ensure batch-to-batch consistency using a biochemical assay, e.g. azo-albumin technique.[3]

For manual antibody detection techniques, red cells treated with the enzyme should achieve activity comparable to that of the reference enzyme preparation 92/658 and associated reference anti-D 91/562.

For automated antibody detection techniques for patient pre-transfusion samples rbcs treated with the enzyme should readily detect a weak anti-D of 0.1 IU/mL, e.g. anti-D 95/784.

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

c) Water

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

d) Saline

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

e) Low ionic strength solution

The term low ionic strength solution (LISS) should not be used to denote a low ionic strength formulation other than that described by Moore and Mollison.[4] LISS should not be used in place of preparations designed for a particular technology.

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

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

f) Weak antibodies for use as controls in antibody detection techniques

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

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

These weak antibodies should:

- when used undiluted give a grade 2–4 reaction with red cells with homozygous antigen expression and have a mean IAT titre of 4 with the same cells
- for weak anti-D the antibody activity should be expressed in IU/mL.

12.3 Reference preparations

12.3.1 Introduction

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

12.3.2 British Minimum Potency Reference Preparations for Anti-A (88/722) and Anti-B (88/724)

These anti-A and anti-B preparations are the lyophilized residues of culture supernatants from murine monoclonal hybridomas BRIC 131 and ES4 respectively. The preparations, when reconstituted and diluted according to the supplied instructions, define the minimum acceptable potency of manufactured anti-A, anti-B, anti-A,B and anti-A+B blood grouping reagents, i.e. the titre of the grouping reagent should be at least equal to that of the appropriate minimum potency reference preparation. These preparations will be replaced by International Standards in 2006.

12.3.3 International Standard for Minimum Potency of Anti-D blood grouping reagents (99/836) for use in direct tests

This preparation is the lyophilized residue of culture supernatant from a human-murine monoclonal heterohybridoma secreting an IgM anti-D (RUM-1). When reconstituted and diluted according to the supplied instructions, 99/836 defines the minimum acceptable potency of anti-D grouping reagents in direct tube tests ie the titre of the grouping reagent should be at least equal to that of preparation 91/592 in tube tests using unmodified red cells and without additional agents.

12.3.4 International Council for Standardization in Haematology/International Society of Blood Transfusion (ICSH/ISBT) Reference Preparations for Papain (92/658) and Anti-D (91/562)

The intended use of 92/658 and 91/562 is to ensure adequate sensitivity combined with freedom from false-positive reactions of manufacturers' enzyme preparations and techniques. The recommended procedure is to test 92/658 in conjunction with a titration series of 91/562 for sensitivity, and a series of inert sera for false-positive reactions, according to a specified two-stage reference method and compare the titration scores with those obtained from testing the manufacturer's enzyme preparation in its recommended technique with 91/562 and the inert sera.

12.3.5 ICSH/ISBT Standard for Anti-Human Globulin (96/666)

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

12.3.6 UK BTS/NIBSC Anti-D Reference Preparation (95/784)

This preparation consists of lyophilized human plasma with a reconstituted anti-D potency of 1.0 International Units (IU)/mL. At 1 in 8 dilution, it is intended to be used to assure the efficacy of red cell washing prior to the addition of an anti-globulin reagent. At 1 in 16 dilution, it is intended to be used in intra-laboratory monitoring to assess test operator variability in the detection of weak, macroscopic agglutination in the spin-tube anti-globulin test.

12.4 Recommended serological techniques for reagent testing

12.4.1 Potency titrations

a) Introduction

The use of a semi-automatic pipette is recommended; one volume being in the order of 40μl.

A separate pipette tip should be used for each reagent.

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

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

The potency titre is the reciprocal of the highest dilution of the reagent that effects a grade 2 reaction using the required technique.

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

b) Potency test methods for manual and microplate blood grouping reagents

I Manual method. Direct test

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

II Manual method. Indirect anti-human globulin test

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

III Microplate method

Equipment

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

Method

- Using a microplate, add one volume (25–50 μl) of each dilution of the reagent to one volume of 2–3% test red cells.
- Mix the contents of the wells using a microplate shaker. Incubate at 19–25°C for 15 minutes.
- Centrifuge the microplate at l00g for 40 seconds. Gently dislodge the red cells from the bottom of the wells using a microplate shaker.
- Determine the reaction grade using a concave mirror or automatic plate reader.

c) Avidity determination

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

- Determine the time from mixing at which macroscopic agglutination first appears and record the reaction grade at one minute.

d) Test used in performance evaluation and batch release testing of AHG

i) Tests for IgM and IgG red cell heterospecific antibodies

- These test for heterospecific antibodies which can cause haemolysis or agglutination of unsensitized red cells in the indirect anti-globulin test.

Method

- Divide 12 test tubes into 2 sets of 6.
- Into each of the first set of tubes, add one volume of washed 2–3% untreated red cells in saline from two group A_1 RhD positive, two group B RhD positive and two group O RhD positive individuals.
- Into each of the second set of tubes add one volume of washed 2–3% enzyme-treated red cells (papain, bromelin or ficin) in saline from the same group A_1 RhD positive, group B RhD positive and group O RhD positive individuals.
- Add two volumes of the anti-human globulin reagent as intended to be supplied for use, to each test tube. Mix thoroughly. Incubate the reactants for five minutes at 19–25°C.
- Centrifuge the tubes.
- Determine the reaction grade.

ii) Control of enzyme treatment

Weak IgG anti-D, known to be reactive with enzyme-treated red cells should effect a positive reaction with each washed, enzyme-treated, red cell sample by the following method:

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

iii) Tests for unwanted positive reactions

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

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

- Select integral segment lines from two packs of group A_1, two packs of group B and two packs of group O blood stored at 2–6°C for at least ten days.
- Wash each of the red cell samples with saline sufficient to remove serologically reactive traces of plasma.
- Prepare suspensions of each red cell sample as 2–3% in saline and as 1.5–2% in LISS.

Incubation of red cells and fresh group-compatible serum.

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

Tests with anti-human globulin reagents

- For each anti-human globulin reagent, prepare two sets of six tubes. To each of the first set of six tubes, add in sequence one volume of the 2–3% suspension of washed red cells from the saline test above.
- To each of the second set of six tubes, add in sequence one volume of the washed 2–3% suspension of washed red cells from the LISS tests above.
- Add two volumes of undiluted anti-human globulin, as supplied for use, to each of the 12 tubes. Mix thoroughly.
- Centrifuge the tubes.
- Determine the reaction grade.

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

The anti-IgG reference reagent (see Section 12.3.5) should be tested in parallel with the test reagent, each being titrated against red cells sensitized with potent IgG anti-D antibody.

Method

Test cells

- A 2–3% suspension in saline of washed pooled group O R_1r red cells is prepared from four individuals.

Anti-D

- Anti-D suitable for use in this application should have a potency titre of greater than 512.
- To 4 mL of the potent IgG anti-D add 2 mL of the 2–3% suspension of pooled group O R_1r red cells.
- Mix and incubate at 37°C for 45 minutes.
- Wash the red cell sample with saline sufficient to remove serologically reactive traces of serum. Prepare suspensions of each red cell sample as 2–3% in saline.

Technique

- Prepare 1 mL volumes of twofold serial dilutions of the test anti-human globulin reagent and anti-IgG reference preparation from 1 in 8 to 1 in 4096 (10 tubes).
- Prepare a set of ten tubes for each anti-human globulin reagent to be assessed.
- Place two volumes of each dilution into each of the series of ten tubes.
- Add one volume of the 2–3% suspension of pooled sensitized R_1r red cells to each tube, mix and centrifuge.
- Determine the potency titre.

Controls

The washed, strongly sensitized 2–3% 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.)

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

Selection of weak IgG antibody preparations

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

The following are selected:

- an IgG anti-D to give an anti-human globulin potency titre of 8–32 using a pool of group O R_1r red cells from four individuals
- an IgG anti-K containing a final concentration of 0.014M EDTA neutralized to pH 7, to give an anti-human globulin potency titre of 8–32 using Kk red cells
- an IgG anti-Fy^a containing a final concentration of 0.014M EDTA neutralized to pH 7, to give an anti-human globulin potency titre of 8–32 using Fy(a+b+) red cells.

Test cells

Prepare 10 mL of a 2–3% suspension of washed R_1r red cells pooled in equal proportions from four individuals. Similarly, prepare 10 mL of a 2–3% suspension of washed Kk red cells and 10 ml of a 2–3% suspension of washed Fy(a+b+) red cells.

Sensitization of test cells

Anti-D

Using a set of five containers each of 20 to 25 mL volume, prepare 4 mL volumes of serial twofold dilutions of the anti-D from undiluted to 1 in 16.

- Add 2 mL of the 2–3% suspension of pooled R_1r red cells in saline to each container. Mix and incubate at 37°C for 45 minutes.
- Wash the red cells four times with 20 mL volumes of saline at each wash and remove the last supernatant.
- Add 2 mL of saline to the packed washed red cells to prepare the 2–3% suspensions of sensitized red cells.

Anti-K

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

Anti-Fy^a

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

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

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

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

Anti-D sensitized red cells

- Prepare five sets of five tubes for each anti-human globulin reagent under test and the anti-IgG reference reagent.
- Place two volumes of the anti-human globulin reagent, undiluted to 1 in 16 in the appropriate tubes for each of the five sets of five tubes.
- Using the 2–3% suspension of red cells sensitized with the undiluted anti-D for the first set of five tubes, the 2–3% suspension of red cells sensitized with the anti-D diluted 1 in 2 for the second set of five tubes, and so on, finishing with the 2–3% suspension of red cells sensitized using the anti-D diluted 1 in 16 for the fifth set of five tubes, add one volume of the washed red cells to each of the sets of anti-human globulin dilutions (see Table 12.4).

Table 12.4 Chequerboard test format

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

- Mix thoroughly. Centrifuge the tubes, appropriately.
- Determine the reaction grade.

Anti-K sensitized red cells

As above, but using the anti-K sensitized Kk cells.

Anti-Fy^a sensitized red cells

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

Controls

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

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

Preparation of the complement sensitized red cells

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

The C3 and C4 activation states produced on red cells by the various methods are shown in Table 12.5.

Table 12.5 Complement C3 and C4 activation

Method of preparation	Initial state	State after trypsin treatment
Very low ionic strength medium* 37°C	iC3b/C4b	iC3d/C4d
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 strength solution (LISS).

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

Anti-C4b potency

Method

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

Controls

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

Anti-C4d potency

Method

- Place two volumes of undiluted anti-human globulin in a tube.
- Add one volume of 2–3% EC4d red cells. Mix thoroughly. Incubate for five minutes at 19–25°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-human globulin does not agglutinate unsensitized red cells that have been trypsin-treated, using the direct anti-human globulin technique.

Anti-C3d potency

Method

- Prepare a set of seven tubes for each anti-human globulin under test and the anti-C3d reference reagent (see Section 12.3.5) which is tested in parallel, at the dilution for the 'immediate test' stated in its accompanying instructions for use.
- Place two volumes of each anti-human globulin dilution in each of the tubes (undiluted, that is as intended to be supplied for use, to 1 in 64).

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

Controls

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

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Chapter 13

Donation testing (red cell immunohaematology)

13.1 Scope

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

13.2 General requirements

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

13.2.1 Specific procedures are written in the form of standard operating procedures.

13.2.2 Blood donations, components and their laboratory samples are correctly identified by barcoded and eye-readable numbers.

13.2.3 Donations can be linked to their donor.

13.2.4 A donor's record is reviewed every time they donate.

13.3 Samples

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

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

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

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

- haemolysis
- lipaemia
- clots
- volume
- cell:plasma(serum) ratio

- consider the buffy coat layer (note: a large buffy coat layer in the sample may give rise to erroneous results).

13.3.5 Labels should be examined for defective labelling.

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

13.4 Reagents and test kits

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

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

13.4.3 Reagents and test kits out with these instructions must be validated.

13.4.4 Reagent antisera must be validated and assured for specificity and potency as per Table 12.3.

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

- lot number
- expiry date
- supplier
- stock levels.

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

13.5 Equipment

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

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

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

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

- service contract details
- downtime
- faults
- maintenance
- calibration.

These logs must be retained.

13.6 Test procedure

Test procedures must:

13.6.1 Be validated before being introduced into routine use.

13.6.2 Be written in the form of standard operating procedures.

13.6.3 Be performed in compliance with the standard operating procedures.

13.6.4 Be monitored and reviewed.

13.6.5 Be performed by trained staff and the training records must be maintained.

13.6.6 Include the recording of test results.

13.7 Reporting of results

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

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

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

13.7.4 Information must be archived.

13.8 Release of tested components

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

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

13.9 Laboratory test categories

Laboratory tests include the following categories:

13.9.1 Mandatory tests – required as part of the criteria for release of all blood donations and components for clinical use. Currently these are ABO and D blood grouping and irregular red cell antibody screening.

13.9.2 Additional tests – undertaken in special circumstances:

- increase the safety of transfusion for susceptible patients or clinical effectiveness of specific transfusions, e.g. by providing HbS screened red cells
- whilst not required for all blood donations or components, when such tests are performed to meet a specific need the results are an essential part of the criteria for release of that component.

13.10 Mandatory testing of blood donations

13.10.1 Blood groups shall be determined using reagents that comply with Chapter 12 of these guidelines.

13.10.2 All mandatory tests must be performed using an automated test system in the first instance (see Section 13.13). Any persistent failures may be resolved using manual methods (see Section 13.14).

13.10.3 ABO blood grouping

- The ABO blood group must be determined on each blood donation
- A donor whose ABO blood group is unknown to the test centre, e.g. a first-time donor, the ABO blood group must be determined by testing the plasma/serum with group A1, and B red cells. The red cells of the donation must be tested twice with anti-A and anti-B as a minimum. The ABO group can only be accepted if the results are in agreement
- If the security of sampling analysis and data transfer is assured, it is sufficient to test the red cells from previously tested donors with anti-A and anti-B once. There is no requirement to test the plasma. The ABO blood group shall be accepted only if the results are in agreement with those of previous tests.

13.10.4 Quality control of ABO blood grouping

- Quality control procedures recommended by reagent and equipment manufacturers should be followed
- The following minimum test monitors are required for each batch of ABO blood grouping tests:
  - anti-A, anti-B, anti-A,B and anti-A + B, must give appropriate reactions with A_1, A_2, A_2B, B and O cells
  - reagent red cell samples must give appropriate reactions with anti-A, anti-B, anti-A,B and/or anti-A+B.

13.10.5 D grouping

- The D blood group must be determined on each donation of blood.
- In the testing of donors being grouped for the first time, two anti-D blood grouping reagents should be used capable of detecting between them D^{IV}, D^{V} and D^{VI} antigens. If two monoclonal anti-Ds are used, they should be from different clones.
- Donors whose blood gives an unequivocal positive reaction with both anti-D reagents should be regarded as D positive.
- Donors whose blood is unequivocally negative with both anti-D reagents should be regarded as D negative.
- If the results with the anti-D reagents are discordant or equivocal, the tests should be repeated. Where the D group is in doubt it is safer to classify such donors as D positive.
- For known (repeat) donors one anti-D reagent, or blended reagent, that detects weak D, D^{IV}, D^{V} and D^{VI} can be used.

13.10.6 Quality control of D grouping

- Quality control procedures recommended by reagent and equipment manufacturers should be followed.
- The following minimum test monitors are required for each batch of D grouping tests:
  - Each series of D blood grouping tests must obtain appropriate reactions with R_1r red cells as a positive and with r′r or rr red cells as a negative
  - Appropriate reactivity with test monitor red cell samples expressing weak D should also be assured during use, although not necessarily with each batch of tests.

13.10.7 Antibody screening

Blood and blood components with antibodies of probable clinical significance may be released, as shown in Table 13.1.

- Routine antibody screen
  - All donations must be tested for the presence of red cell antibodies. This is achieved by testing the donor's serum or plasma using a validated technique capable of detecting, anti-D at 0.5 IU/mL or lower
  - Reagent red cells for routine antibody screening may be:
    - provided from a minimum of two individual donations (not pooled); or
    - as a pool of red cells in equal proportions from no more than two donations; or
    - red cells from a single donation
    - as a minimum the following antigens should be expressed: D; C; c; E; e; K.
  - each batch of tests must include a test monitor of ≤0.5 IU/mL anti-D
  - Donations found to be reactive in the routine antibody screen should be further tested by an indirect antiglobulin test to determine the fate of the products as specified in Table 13.1.
- Antibody screen for blood for neonates
  - Blood for neonatal use must be screened and found negative for antibodies by an indirect antiglobulin test, performed using a two cell panel expressing the following antigens as a minimum: C; C^w; c; D; E; e; K; k; Kp^a; Fy^a; Fy^b; Jk^a; Jk^b; S; s; M; and Lu^a.

Table 13.1 Minimum release criteria for blood products with antibodies of probable clinical significance

Product	Antibody screen for blood for neonates	Donation plasma sample diluted 1 in 10	Donation plasma sample diluted 1 in 50
For neonatal use	Negative	Not applicable	Not applicable
Red cells in SAG-M	Not applicable	Not applicable	Negative
All other products	Not applicable	Negative	Not applicable

13.11 Additional testing

13.11.1 Antibody identification: donations found to be reactive in the routine antibody screen may be further investigated for specificity.

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

- Red cells, platelets and Fresh Frozen Plasma from group O donors with high titres of anti-A, anti-B and/or anti-A,B can result in haemolytic transfusion reactions when given to non-group O patients. Such group O donors are generally termed 'high-titre group O donors'
- Reactions are more likely to occur when
  - the serological titre of the anti-A, anti-B and/or anti-A,B in the component is high
  - the plasma volume of the transfused product is high
  - the blood volume of the recipient is small
- Each blood establishment should have a testing and issuing policy to avoid the use of high-titre anti-A and/or anti-B in instances where a significant adverse clinical reaction is likely. The policy should cover the following components:

- whole blood and red cells
- fresh frozen plasma
- apheresis platelet donations
- pooled platelets containing plasma from a single 'high-titre' group O donor.
- blood/components for neonatal use

- Where high-titre anti-A/B testing is deemed necessary, a saline agglutination test, performed as detailed in Chapter 12) should give a negative result, at a dilution of 1/128; or an equivalent dilution by other techniques
- There should be a procedure in place to collect and review testing and patient outcome data and to implement changes in policy in the light of continuing clinical experience with the plasma containing blood products issued
- Components from group O donors with 'low titres' of anti-A, anti-B and/or anti-A,B can cause intravascular haemolysis in non-group O recipients if given in sufficiently large volumes.

13.11.3 Additional phenotyping

- Red cell components should only be labelled with confirmed extended phenotypes
- A confirmed phenotype is one where the typing has been carried out in duplicate on the current donation or once on the current donation and is in agreement with historic data from previous donations
- Red cell phenotype information should not be based on historic data alone.

13.11.4 Quality control of additional phenotyping

- Quality control of procedures recommended by reagent and equipment manufacturers should be followed
- The test monitors shown in Table 13.2 are required for each batch of tests
- Within some test procedures reagent cross contamination may occur. Test monitors should be selected in order to maximize, the detection of such contamination.

Table 13.2 Test monitor red cell samples

Blood grouping reagent	Test monitor red cell samples	
	Positive	**Negative**
Anti-C	R_1r	rr or R_2r
Anti-E	R_2r or r″r	rr or R_1r
Anti-c	R_1r or r′r	R_1R_1
Anti-e	R_2r or r″r	R_2R_2
Anti-K	K+k+	K–k+
Other specificities	Heterozygous positive	Antigen negative

13.12 Donations found to have a positive direct antiglobulin test

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

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

13.12.2 Non-red cell components may be prepared and issued from DAT positive red cell donations

13.12.3 Red cell units may be prepared and issued from DAT positive red cell donations provided that

- the ABO and RhD groups are confirmed
- red cell antibodies have been excluded as per the mandatory antibody screening (see Table 13.1).

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

13.13 Automated testing

An automated system as a minimum must accomplish the following:

13.13.1 Positive sample identification, reading and interpretation of results.

13.13.2 Matching of results to sample identification.

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

13.14 Manual testing

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

13.14.2 Manual testing can be used to resolve anomalous results.

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

13.14.4 Manual tests must be performed and controlled according to manufacturers' instructions.

13.14.5 Test results and controls must be recorded.

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

Chapter 14

Patient testing (red cell immunohaematology)

14.1 Scope

These specifications provide guidance on the tests required for patient testing, including antenatal patients, and extended testing of donor samples within Red Cell Immunohaematology (RCI) in the UK blood services. For brevity the word 'patients' has been used throughout except where differences in the testing of donors and patients exist. These guidelines should be read in conjunction with those of the British Committee for Standardization in Haematology (BCSH), where they pertain to patient testing.

14.2 General requirements

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

14.2.1 Specific procedures are written in the form of standard operating procedures (SOPs).

14.2.2 A document control process should ensure that only current versions of SOPs are in use.

14.2.3 Secure and effective procedures should be in place to ensure that:

- samples for pre-transfusion testing are labelled as described in the BCSH 'Guidelines for the administration of blood and blood components and management of the transfused patient'
- any variation from the above guideline is covered by a risk assessment
- both barcoded and eye-readable numbers identify all samples.

14.2.4 Reagents, test kits and equipment should be used according to manufacturers' instructions unless otherwise validated. Refer to Chapters 12 and 13 for details.

14.2.5 Test procedures and equipment should be validated prior to introduction into routine use and should be subject to periodic monitoring and review.

14.2.6 Test procedures and equipment operating instructions should be written in the form of and performed in compliance with SOPs.

14.2.7 Only staff who are appropriately qualified may perform laboratory tasks, and records of training to the required standard should be maintained.

14.2.8 Laboratories where patient referrals are investigated should be subject to external accreditation.

14.2.9 All patient records and test results should be maintained according to the requirements of the Caldicott Report (1997)[1] and Data Protection Act (1998).

14.3 Requests

14.3.1 All samples should be accompanied by a request form specifying the tests or investigations required (where appropriate) and the signature and name of the person making the request.

14.3.2 There should be a written policy for accepting blood transfusion requests. Requests for compatibility testing should include the date and time that the blood is required, the number or volume and type of component required and any other specific requirements, e.g. CMV negative or irradiated products.

14.3.3 There should be a policy for documenting telephone or fax requests which should include amendments to a written request.

14.3.4 Telephone or fax requests should be made by a designated individual and their identity documented. The date and time of request should also be recorded. The information given by the requester should be confirmed by the receiving laboratory.

14.3.5 All requests should contain the following information as a minimum:

- surname/family name and first name(s) in full
- date of birth (not age or year of birth)
- NHS number or address or other unique patient identifier
- reason for the request.

14.4 Samples

14.4.1 Patient samples should be clearly identified and given a unique patient identification number by the laboratory. Duplicate records should be avoided to prevent previous transfusion or obstetric history being overlooked.

14.4.2 All samples should display the following information as a minimum:

- surname/family name and first name(s) in full
- date of birth (not age or year of birth)
- NHS number or address or other unique patient identifier.

14.4.3 Reconciliation checks of all samples and patient information should be completed prior to testing.

14.4.4 An SOP should detail the procedure to follow in the event of receiving inadequately or inappropriately labelled samples or incorrect samples for the tests requested.

14.4.5 Labels pre-printed away from the bedside (addressograph labels) should not be used on samples for pre-transfusion testing.

14.4.6 Samples for pre-transfusion testing should: have handwritten labels that should be dated and signed by the individual taking the blood; or have labels generated and attached at the bedside from scanning barcoded wristbands.

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

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

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

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

14.4.9 Validated protocols for sample storage and preparation should be available.

14.4.10 The timing of samples for pre-transfusion testing should be taken in accordance with the guidelines given in Table 14.1.

Table 14.1 Timing of pre-transfusion samples

Patient transfused within	Sample to be taken not more than
3–14 days	24 hours before transfusion
15–28 days	72 hours before transfusion
29 days–3 months	1 week before transfusion

14.4.11 In situations where patients are being repeatedly transfused, a daily sample is not a requirement. These patients should be screened for irregular red cell antibodies every 72 hours. This interval is considered both practical and safe.

14.4.12 Samples, other than those for pre-transfusion testing, should be received by the laboratory with sufficient time to allow the completion of routine testing within seven days of venepuncture.

14.4.13 Whole blood samples will deteriorate over a period of time. Problems associated with storage include red cell lysis, bacterial contamination, loss of complement in serum and decrease in the potency of antibodies, particularly IgM antibodies. The suggested working limits are shown in Table 14.2.

Table 14.2 Suggested maximum storage time of samples

	18–25°C	4°C	–30°C
EDTA whole blood	Up to 48 hours	Up to 7 days	N/A
Separated plasma/serum	N/A	Up to 7 days	Up to 6 months

14.4.14 Although antibodies are probably stable for up to six months in frozen storage, the risk of intervening transfusion or pregnancy and the risk associated with incorrect sample identification of separated plasma/serum samples should be assessed before considering the use of stored samples for pre-transfusion testing.

14.5 Test reagents and test systems

14.5.1 Where manufacturers define protocols for the preparation and storage of reagents and test kits these should be followed.

14.5.2 Verification testing should be performed on each batch of reagents and test kits.

14.5.3 There should be procedures in place to ensure the traceability of all batches of reagents and test kits used.

14.5.4 CE marked reagents should be used where appropriate.

14.5.5 Test equipment should be re-evaluated following any mechanical alterations or software upgrades.

14.5.6 Test equipment should be used, cleaned, calibrated and maintained in accordance with manufacturers' instructions and written procedures.

14.5.7 Any deviations from manufacturers' instructions should be validated and documented.

14.5.8 Service contracts for the maintenance of equipment should be in place.

14.5.9 An equipment log should be maintained detailing service contracts, any faults and all maintenance visits.

14.5.10 A standard grading system for manual tube serological testing is detailed in Table 12.2. The manufacturers' recommendations should be used for the grading of serological tests in alternative test systems.

14.5.11 The requirements for automated test systems in Section 13.13 should be met. Note also that the ability of blood grouping automation to handle different sample sizes varies, with 6.5mL EDTA samples being the most widely used.

14.5.12 Column agglutination cards/cassettes should be inspected for evidence of bubbles and drying before use: if there is evidence of this, the manufacturer's instructions should be followed.

14.5.13 Documented procedures should be in place for dealing with discrepant results.

14.5.14 Where test reagents need to be stored, or test equipment operated, within a prescribed temperature range, then ambient temperatures should be monitored and recorded, and action taken where necessary.

14.6 Authorizing and reporting results

14.6.1 Authorizing and reporting of routine test results should be the responsibility of designated laboratory personnel. Non-routine and discrepant results should be authorized by consultant grade staff, or other senior staff designated by the consultant.

14.6.2 There should be documented procedures for reporting written, telephone or faxed results.

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

14.6.4 Test results reported by telephone should be given to a designated individual. Records should be kept of results given over the telephone, which should include as a minimum:

- date and time of call
- details of requester
- patient details given by requester to confirm patient identification (see Section 14.3.4)
- results given
- signature of person giving the results.

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

14.6.6 Where electronic data interchange is in place either direct to surgeries/hospitals or onto a web browser the system should be based on the principles of the Caldicott Report (1997). The system should be validated and password controlled with clearly defined access levels. Data should be encoded with an electronic signature to ensure that the information cannot be altered and can only be viewed by designated individuals.

14.6.7 Test results and other relevant test information should be archived and maintained in accordance with legislation (currently 30 years).

14.7 ABO and D grouping

14.7.1 Introduction and general requirements

ABO grouping is the most important serological test performed pre-transfusion.

- The ABO and D group should be determined on all pre-transfusion and antenatal red cell samples as an aid to sample security.
- Errors in D grouping can lead either to unnecessary prophylactic anti-D being given to a woman, or to the sensitization of females, with potential future morbidity of a fetus or infant.
- The sensitivity, accuracy and security of the systems used should not be compromised.
- The introduction of monoclonal blood grouping reagents has greatly increased sensitivity for standard A, B and D antigens but the detection of some A, B and D variants including weak D may be compromised unless reagents are carefully selected.
- Fully automated ABO and D grouping procedures have significantly improved the accuracy and security of results, and should be used wherever practical and appropriate.
- Wherever a manual ABO and D grouping test is performed, a procedure designed to obviate individual operator error should be in place.
- The results of ABO and D grouping tests should be checked against any available historical records. A suitable historical record is an unambiguous grouping result performed as part of a validated system.
- Clotted or EDTA samples can be used for ABO and D grouping but for fully automated procedures, EDTA samples are essential.

14.7.2 Test procedures

- The patient's red cells should be tested in a cell (forward) group using monoclonal anti-A and anti-B reagents.
- The anti-B reagent should not react with red cells possessing acquired B antigens.
- Anti-A,B or anti-A+B reagents can be used in conjunction with anti-A and anti-B but are not essential.
- Reverse grouping should be performed using A_1 and B reagent red cells.
- In the grouping of samples from patients where there is no historical record of ABO and D grouping, the grouping procedure should consist, as a minimum, of two elements:

  (a) an ABO cell (forward) group and D group; AND

  (b) a second ABO group and D group.

  The second ABO group may consist of either a second cell (forward) group OR a reverse ABO group; and the second D group may use the same or a different D grouping reagent from the first D group.

In manual grouping, these two elements (a) and (b) should involve two independent, suitably qualified testers sampling from the original sample separately, except in urgent situations. The results should be read by the same two testers and then recorded in such a way that neither tester is aware of the other's result until all of the test results have been recorded.

- If the sample is from a neonate then two ABO cell groups and two D groups should be performed as above for manual or automated grouping.
- Where there is a historical record of ABO and D grouping, an ABO cell (forward) group and D group should be performed as a minimum.
- In the absence of a reverse group a diluent control reagent should always be included in the grouping procedure. If positive (even weakly) the results should be disregarded and a procedure for grouping discrepancies (see Section 14.7.6) should be followed.
- Interpreted test results should be checked, electronically wherever possible, against historical results.
- A senior member of staff should review the results of manual tests entered into the computer system by checking against the original worksheets of all testers, and against the results of all tests from other sources (i.e. referring/transferring laboratory) if available.
- It is recommended that a risk assessment is carried out before reverse grouping is omitted from the testing protocol. For example, if there is only one historical group, there is a risk that a sample, which may be from the wrong patient, could be interpreted as group O when the patient is actually group A. If the patient is then transfused group O blood prior to the next sample, the mixed field reactions obtained on the second sample may be misinterpreted as group O, thus confirming the erroneous result.
- If serum is used for the reverse group it is recommended that the A_1 and B grouping cells should be resuspended in a diluent containing EDTA to prevent misinterpretation of results due to haemolysis.

14.7.3 Controls for manual and automated ABO and D grouping

See Table 14.3.

Table 14.3 Controls for manual and automated ABO and D grouping

Reagent	Positive control	Negative control
Anti-A	A	B
Anti-B	B	A
Anti-D	D positive	D negative
A_1 grouping cells	Anti-A	Anti-B
B grouping cells	Anti-B	Anti-A

a) Manual ABO and D grouping

- If manual testing is performed using column agglutination technology on small numbers of samples only, and if the guidelines elsewhere in Chapter 14 are rigorously followed (particularly the verification of reagents received, and the monitoring of storage conditions), it is not mandatory to include controls with each batch of tests. The frequency of controls in this situation will depend on a number of factors, e.g. whether the same batch of cards is used regularly in an automated procedure (see below).
- If manual testing is performed using a tube or microplate method, controls should be used, as a minimum, with each batch of tests, reagents or pre-dispensed microplates.

b) Automated ABO and D grouping

- When using fully automated systems, whole blood control samples should be loaded onto the machine in the same way as the patients' samples. Controls should be included at least twice daily: the frequency should be adjusted to take into account the length of time the reagents have been in use on the machine.
- When using a column agglutination or microplate method that is not fully automated, controls should be used, as a minimum, with each batch of tests, reagents or pre-dispensed microplates.

14.7.4 Interpretation of results

- There is a high risk of error during the manual reading and interpretation of ABO and D groups with potentially catastrophic results. The risk of error can be minimized by separating the procedure into two distinct tasks e.g. forward and reverse group (see Section 14.7.2).
- An optical or image analysis system should be used wherever possible to interpret the results of tests performed using microplate or column agglutination methods. The system used should be validated against manual reading prior to use. If the system does not have a high degree of automation (e.g. there is no integrated barcode reader) a double-checking procedure of cards or microplates, using different staff members, should be employed.
- A visual inspection should be made if an automated reader cannot interpret a test result.
- Where a manual edit of a misinterpreted or non-interpreted result needs to be made, this should be checked by two independent members of staff. If the frequency of editing is high then system performance should be checked, and the system revalidated if equipment or procedural changes are made.
- Algorithms for editing and re-testing should be documented.

14.7.5 Authorization of results

As part of the authorization process:

- The personal identification number attached to the sample should be checked against the referral form/computer-generated worksheet to ensure that no errors have been made at the sample number labelling and sample registration stages.
- The ABO and D group should be verified, electronically wherever possible, against any available previous results. All discrepancies should be resolved prior to transfusion (see also Sections 14.7.2, 14.7.6 and 14.7.7).
- The laboratory should have a policy with respect to the seniority of staff permitted to manually edit and authorize test results.

14.7.6 Dealing with grouping discrepancies

- ABO and/or D groups should be repeated when a discrepancy is found.
- Repeat tests should be performed using washed red cells.
- It is important to prevent the perpetuation of errors, e.g. by ensuring that red cells for retesting are taken from the original sample.
- Repeatable anomalous results should be referred to a senior staff member.
- It may be necessary to request a fresh sample.
- A further sample is essential where verification checks against historical results reveal a discrepancy.

- If the cause of the discrepancy is a failure to obtain a reliable reverse group due to hypogammaglobulinaemia or insufficient sample, the cell group should be repeated.

a) Unexpected mixed-field reactions

Any groups showing unexpected mixed-field reactions should be repeated and/or investigated prior to the authorization of the group. These reactions may represent an ABO/D incompatible transfusion (planned or unplanned), bone marrow/stem cell transplant, an A_3 or B_3 or a twin chimera (extremely rare).

b) A/B variants

Variants of groups A and B may give much weaker than normal reactions with monoclonal anti-A and anti-B. For example A_x or B_x give very variable reaction strengths with different reagents, with some anti-A and anti-B reagents failing to react with A_x and B_x cells. The most likely finding is a negative reaction with anti-A or anti-B and a missing agglutinin on the reverse group (though anti-A_1 is commonly detected in A variants). Absorption/elution studies with anti-A or anti-B may be beneficial in identifying the variant. Due to the difficulties in identifying weak A/B antigens it is prudent to transfuse these patients with group O red cells.

c) Acquired B

Some anti-B reagents may react strongly with the acquired B antigen. This usually leads to a discrepancy between cell and reverse groups. Anti-B reagents found to react with acquired B cells should not be used in routine ABO grouping.

d) Intrauterine transfusions

For several months post-delivery, neonates who have received intrauterine transfusions may appear to be the same ABO and D group as the transfused cells due to bone marrow suppression.

e) Presence of cold reacting alloantibodies

An unexpected reaction with the reverse grouping cells may be observed if these cells carry an antigen to a cold reactive alloantibody (other than anti-A and anti-B) that is present in the patient's plasma. In these cases, the reverse group should either be repeated at 37°C with the same cells or tested with A_1 and B cells that lack the implicated antigen.

f) Presence of cold autoantibody

If a sample shows evidence of strong autoagglutination, washing the cells in pre-warmed saline may be of benefit. In severe cases pre-warming the patient cells, serum/plasma and grouping reagents prior to mixing and incubation of the tests at 37°C may be the only option.

14.7.7 Weak D and Partial D

- It is important to note that monoclonal anti-D reagents vary widely in their ability to detect both partial D and weak D. When two different reagents are used it is helpful to use those of a similar reactivity with partial D and weak D red cells, in order to reduce the number of discrepancies. If a discrepancy occurs the patient should be treated as D negative until the D status is resolved.
- Patients should not be classified as D positive on the basis of a weak reaction with a single anti-D reagent. If clear positive results are not obtained with two monoclonal anti-D reagents it is safer to classify the patient as D negative.
- Patients of category DVI are the most likely to produce anti-D.
- Reagents used for D grouping patients should not detect category DVI.
- Patients with known partial D status should be regarded as D negative.
- Reagents used for D grouping donors should detect category DVI.

- Donors with known partial D status should be regarded as D positive.
- It is useful when investigating patients with suspected weak D or partial D to test the patients' cells against an identification kit containing monoclonal antibodies directed against the different epitopes of the D antigen. Kits usually contain a mixture of IgG and IgM antibodies and can be used to identify the majority of the known partial D antigens. The kit may also be useful for the confirmation of weak D when used as described by the manufacturer.

14.8 Antibody screening

14.8.1 Antibody screening (and/or antibody identification, where appropriate – see Section 14.9) should be performed on all samples used for pre-transfusion testing and antenatal testing. For pre-transfusion testing, note that:

- Where the volume of blood transfused in any 24-hour period is equivalent to the patient's own blood volume, blood that is ABO group compatible can be issued without the need for a serological crossmatch.[2, 3] ABO incompatibility should be excluded using serological testing or electronic issue. If ABO non-identical blood has to be transfused, blood of the same group as the patient should be used as soon as possible after the first transfusion.
- Where fetal transfusion is to be performed, group O blood (or ABO identical with the fetus if known) should be crossmatched against the maternal plasma, which should be screened for the presence of clinically significant red cell antibodies.
- Where neonatal transfusion is to be performed, blood ABO compatible with maternal plasma may be given without prior crossmatching provided that:

  (a) there are no atypical red cell alloantibodies in the plasma of the mother or neonate; and

  (b) the DAT of the neonate is negative.

  See Chapter 8 for the specification of blood components.

14.8.2 An indirect antiglobulin test (IAT) should be used as the primary method for the screening of patients' plasma for the presence of clinically significant red cell antibodies.

14.8.3 Test systems using tubes (liquid-phase), microplates (liquid- or solid-phase), or cards/cassettes (column agglutination) are suitable. If a tube method is used for antibody screening then this should involve the suspension of red cells in a low ionic strength solution (LISS).

14.8.4 The requirements in Section 4.1 for reagent red cells, whether they are used as part of an in-house test system or as part of a proprietary test system, should be followed.

14.8.5 An autologous control or direct antiglobulin test (DAT) need not form a part of antibody screening.

14.8.6 A weak anti-D control serum or reagent (containing anti-D at a level of approximately equal to or less than 0.1 IU/mL) should be used on a regular basis to assure the efficacy of the whole test procedure, including antigen expression on screening cells.

14.8.7 The use of further control sera or reagents, containing weak examples of specificities known to be clinically significant when present in patients' plasma (e.g. anti-Fy^a) is also recommended to assure the sensitivity of the test procedure and the integrity of antigen expression of reagent red cells during storage.

14.8.8 IgG weakly sensitized red cells should be used to control the washing phase of the liquid-phase IAT. Haemagglutination of these sensitized red cells when added to tubes in which a negative IAT result has been recorded indicates the presence of free anti-IgG, thus validating the test result.

14.9 Antibody identification

When an alloantibody is detected in the screening procedure, the specificity should be determined and its likely clinical significance assessed. It is essential to determine the specificity of all clinically significant antibodies present. It is important to employ a systematic approach to antibody exclusion in the process of antibody identification. Several reagent red cell panels may be needed to identify some combinations of antibodies.

14.9.1 Principles of antibody identification

- The patient's serum or plasma should be tested by an appropriate technique against an identification panel of reagent red cells. As a starting point, the technique by which the antibody was detected during screening should be used. Inclusion of the patient's own red cells may be helpful, for example, in the recognition of an antibody directed against a high frequency antigen or the presence of an autoantibody.

- The specificity of the antibody should only be assigned when it is reactive with at least two examples of reagent red cells carrying the antigen and non-reactive with at least two examples of reagent red cells lacking the antigen. Note that, wherever possible, the presence of anti-Jk^a, -Jk^b, -S, -s, -Fy^a, and -Fy^b should be excluded using red cells having homozygous expressions of the relevant antigen.

- A weak anti-D control serum or reagent (containing anti-D at a level of approximately or less than 0.1 IU/mL) should be used on a regular basis to assure the efficacy of the whole test procedure, including antigen expression on screening or panel cells.

- When one antibody specificity has been identified, it is essential that the presence of additional clinically significant antibodies has not been missed. Multiple antibodies can only be confirmed by choosing cells antigen negative for the recognized specificity, but positive for other antigens to which clinically significant antibodies may arise. Knowing the phenotypes of the patient can aid in cell selection for the identification and exclusion process. The requirements detailed above should be met for each antibody detected but in some circumstances this may not be possible.

- The use of a panel of enzyme (e.g. papain) treated cells is strongly recommended for antibody identification, particularly when an antibody weakly reactive by the antiglobulin technique, or a mixture of antibodies, is present. The use of monospecific antiglobulin reagents in place of polyspecific reagent is beneficial when determining the presence of IgG antibodies in serum samples containing complement-binding antibodies. The use of room temperature saline techniques may also be helpful.

14.9.2 Reagent red cells for use in antibody identification

See Section 12.2.3 for details.

- If the patient is group A, B or AB and all reagent cells react with the test plasma, it may be useful to include cells of the same ABO group as the patient to exclude the possible presence of anti-HI.

- When the phenotypes of the patient are known, it is not necessary to look for any specificity where the patient is positive for the antigen. Where the patient is negative there should be at least two examples of cells with phenotypes lacking, and at least two examples of phenotypes carrying, expression of the corresponding antigen. The panel should be able to resolve as many likely antibody mixtures as possible.

14.9.3 Red cell phenotyping in antibody identification

- When an antibody has been identified, the patient's red cells should be phenotyped using a reagent of the same specificity as the assigned specificity. Suitable controls should be used, i.e. antigen positive (from a heterozygote) and antigen negative.

- The patient's red cells, as determined during phenotyping, will normally be expected to lack the antigen(s) corresponding to the antibody specificity (specificities) assigned. If this is not the case:

  (a) the antibody may be an autoantibody (in which case the patient's cells will normally be DAT positive), and/or

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

  (c) the assignation of antibody specificity may be incorrect

  (d) the patient may have been recently transfused.

- The incorporation of a reagent control or AB serum control used by the same technique as the phenotyping reagent is recommended, unless the reagent is known to contain unpotentiated IgM antibody. A positive result in this control test will invalidate the phenotyping test results.

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

  (a) the plasma contains an antibody to a low frequency antigen

  (b) the red cells in the donation are DAT positive

  (c) blood of the wrong ABO group has been selected for crossmatching.

14.9.4 Extended red cell phenotyping

- It may be useful to perform extended red cell phenotyping to identify the potential antibodies that could be produced. This may not be possible to determine if the patient has been recently transfused. The following antigens should be tested for:

  C, c, D, E, e, M, N, S, s, K, k, Fy^a, Fy^b, Jk^a, Jk^b.

14.9.5 Investigation of a broadly reacting plasma sample

- If all cells are positive, with reactions of equal strength:

  (a) An antibody to a high incidence antigen should be considered. The use of an enzyme (e.g. papain) treated panel is particularly useful as reactivity or non-reactivity of the test plasma may exclude certain specificities immediately, and save time and the unnecessary use of rare typing reagents.

  (b) The cells of the patient should be typed for as many high incidence antigens as possible and, if a negative is found, cells lacking that antigen should be matched against the patient's plasma. Where possible the antigen negative cells should include antigens that the patient lacks in order to exclude the presence of further, underlying, alloantibodies.

- If all cells are positive with varying degrees of strength:

  (a) When cells give different strength reactions and a mixture of antibodies cannot be confirmed antibodies to Ch, Rg, Cs^a, Kn^a / McC^a (CR1-related) should be considered. Ch and Rg are antigens which reside on the C4d component of the complement protein C4 and the serological activity in IAT tests is due to the small amount of C4 adsorbed on to the surface of the cells *in vivo*. Coating reagent red cells with C4 *in vitro* in a low ionic strength medium enhances the serological reactivity of anti-Ch and anti-Rg. Plasma inhibition may also be used to confirm Ch and Rg specificities but dilution of the test plasma may be needed for complete inhibition to be observed.

(b) Because of the heterogeneity of the CR1 protein not all examples of Kn^a-related antibodies have the same specificity. Therefore, the cells of different antibody makers may not be mutually compatible.

- Use of an extended range of enzyme (trypsin or chymotrypsin) treated and chemically (AET or DTT) modified cells can aid in the identification process if the specificity cannot be determined or is in doubt. Reactivity or non-reactivity of the test plasma may indicate a specificity within a certain blood group system.
- An eluate prepared from group O, antigen matched, cells and sensitized with antibody from the test plasma, may be helpful in antibody identification. The eluate will isolate any antibody (present in the test plasma) to a high incidence antigen and will enable the matching of rare phenotype cells, including null phenotype cells, of any ABO group.
- In a complex mixture of antibodies individual antibodies can be isolated by adsorption of one antibody on to red cells positive for the corresponding antigen and lacking the antigens for the other antibodies. An eluate may be prepared from the first aliquot of cells to confirm the specificity of the adsorbed antibody. Both the aliquots of absorbed sera and eluates can be tested for individual specificities. This procedure is useful when there are insufficient fully typed reagent panel cells available to detect each specificity individually in the raw serum.

References

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Chapter 15

HLA typing and HLA serology

Preface

For the seventh edition we have made significant changes in both the structure and content to the previous version of the chapter concerning HLA typing and antibody testing. This obviously reflects the continuing scientific and technical development in the field. DNA-based testing for HLA alleles now predominates over serological phenotyping and antibody detection and characterization increasingly involve non-cell based methods. To reflect this, much of the guidance relating to HLA serology in the sixth edition has been removed. This HLA section is now constructed from three main parts, concerning reagents (Section 15.1), testing (Sections 15.2, 15.3 and 15.4), and application to donor and patient investigations (Section 15.5), respectively. For certain patient or donor investigations there is, of course, an overlap with the guidance given in this and the Granulocyte or Platelet immunology chapters (Chapters 16 and 17 respectively). This is particularly relevant to the laboratory investigations of platelet refractoriness and TRALI, so diagrams are included (Figures 15.1 and 15.2) to indicate how the different guidelines relate to each other.

Introduction

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

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

HLA typing or antibody investigations may be undertaken for diagnostic purposes or to investigate harmful consequences of transfusion. Thus the diagnosis of immune refractoriness requires the demonstration of HLA-specific antibodies (or other, platelet-specific antibodies) in the donor. As part of the investigation of transfusion-related acute lung injury (TRALI), implicated donors are screened for HLA (and HNA) -specific antibodies and the patient HLA typed if possible. The investigation of transfusion associated graft versus host disease (TAGvHD) requires HLA typing of patient and donors.

The European Federation for Immunogenetics (EFI) has established standards (currently version 5.3, www.efiweb.org)[1] for histocompatibility testing and where appropriate these

guidelines will defer to the relevant EFI standard and these will be stated in the text. In general guidance for practice is indicated by the term 'should'. The use of the term 'must' is mostly limited to circumstances where an EFI standard applies.

Terminology and nomenclature

All assignments, irrespective of the method, must comply with the current WHO Nomenclature Committee for Factors of the HLA System report,[2] *Nomenclature for Factors of the HLA System*, 2004. Marsh SGE *et al*[3] (and see EFI standards D1.000-D1.300, inclusive). Examples of acceptable HLA assignments are as follows:

- HLA-B12, HLA-B44, HLA-B-44(12), HLA-B*44, HLA-B*4409
- HLA may be serologically typed (to determine the phenotype) or by DNA analysis. The term genotype is properly used to describe the genetic (DNA) constitution determined by the pattern of inheritance (EFI standard D1.230).

Traditionally HLA typing has been performed using serological typing techniques using panels of alloantibodies derived primarily from multiparous women. This has allowed for the typing of HLA serological antigen specificities. Some HLA specificities, termed 'broad', have, since their first discovery, been further 'split' into two or more subtypes. For example, the 'broad' HLA-A9 specificity can be split into the A23 and A24 types, and similarly HLA-B15 can be split into B62, B63, B75, B76 and B77. In addition two HLA specificities, Bw4 and Bw6, have been defined which refer to public epitopes present as alternatives on all HLA-B antigens. Bw4 is also found on certain HLA-A antigens.

More recently HLA typing has been performed by DNA based molecular techniques, which employ either DNA based probes or primers, to type for the presence or absence of sequence motifs. Kits using this technology are able to define the HLA alleles present in an individual to a variable level of resolution dependent on a number of factors which may include; the number of probes or primers employed; the number of alleles defined for a given specificity; the HLA alleles present in the individual. Although it is possible to achieve a high resolution or allele level typing using these methods, it is common practice to type only to a low or medium level of resolution, and in so doing to present HLA typing results that include some ambiguity in the interpretation.

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

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

15.1 Reagents

15.1.1 General guidelines

HLA reagents prepared from human source material should comply with the guidelines in Section 12.1.4.j.

Exceptionally, reagents not tested at source as required in Section 12.1.4.j, and for which no alternative exists, may be supplied for use with the expressed approval of the user and with the understanding that the reagent must be regarded as potentially infectious.

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

The instructions for use of these reagents should indicate that the reagent(s) has not been tested at source as required in Section 12.1.4.j, and that the reagents are to be considered as potentially infectious. In addition the package insert should give information on the safe disposal of the material and the container, multi-well tray or reservoir.

Immediate container label

HLA reagents issued separately: the label should conform to the requirements of BS EN 375:1992. In addition, the body of a container presented in sealed bags or foiled pouches, should be marked with a unique identifier to enable identification and traceability.

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

The instructions for use should contain the information required by BS EN 375: 1992, should comply, where applicable, with the requirement of Section 12.1.4.l.

15.1.2 Serological typing reagents

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

- the claimed HLA specificity(ies) of the reagent, the percentage of specific reactions giving a cytotoxicity score of 80% to 100% cell death, the values of the correlation coefficient r obtained by the pretesting of the reagent against a well characterised cell panel, and the reaction score
- the manufacturer should provide information of the incidence of equivocal cytotoxicity scores within the package insert
- HLA typing sets should include a representation of the multi-well tray or reservoir layout indicating the position, HLA specificity(ies) and batch (or sub-batch) reference of the HLA typing reagent contained in each well
- monoclonal antibodies should be identified as such
- an instruction that thawing and refreezing of the HLA typing reagents should be kept to the absolute minimum from the date of manufacture to the date of use. HLA typing sera frozen in micro-well trays should be used within one hour of thawing. Sera supplied freeze-dried in micro-well trays should be used within one hour of their reconstitution; unused trays should not be refrozen for later use
- when reagents are supplied as a HLA typing set for the detection of a single antigen, the instructions for use should indicate which controls are appropriate to demonstrate specificity and cross-reactivity
- for HLA typing sets, a list should be provided of those specificities that cannot be adequately defined in the presence of other specified specificities
- each HLA typing set for Class I or Class II phenotyping should contain at least one positive control antibody preparation, previously shown to react with all target cells, and should include at least one negative control preparation, previously shown to lack antibody activity or be from a male with no history of blood transfusion.

Preservation

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

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

Stability and expiry date

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

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

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

15.1.3 Requirements for phenotype assignment

HLA Class I and Class II serological typing must comply with EFI standards E1.000 to E3.640 inclusive. HLA reagents and kits to be used for phenotyping lymphocytes by cytotoxicity should comply with the following:

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

15.1.4 Rabbit complement for use in HLA serology

General guidelines

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

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

Immediate container label

The label of the immediate container of rabbit complement for use in HLA serology should conform to the specifications in Section 12.1.4.k.

Instructions for use

The instructions for use supplied with rabbit complement for use in HLA serology should conform to the specifications in Section 12.1.4.l.

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

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

Potency tests on rabbit complement for use in HLA serology

The following UK reference reagents are available for use in assessing rabbit complement:

- for HLA Class I serology:
  - rabbit complement (HLA Class I serology, 02/314)
  - anti-HLA-A2 serum (90/692)
- for HLA Class II serology:
  - rabbit complement (HLA Class II serology, 02/314)
  - anti-B lymphocyte (monoclonal) preparation (92/556).

Method

Manufacturers should determine the potency titre of the appropriate UK reference antibody reagents (anti-A2 serum for HLA Class I Serology; anti-B lymphocyte (monoclonal) for HLA Class II Serology) using the respective UK reference rabbit complement and their own 'test' complement as detailed in the following guidelines:

- Each batch of rabbit complement should be tested by the following procedure, which is the description for the current reference preparations (for complement used in HLA Class I: 02/314 and anti-HLA-A2: 90/692; complement used in HLA Class II: 02/314 and anti-B lymphocyte: 92/556). The instructions for use with these reference preparations, or their successors, should be followed.

Reconstitute the appropriate UK reference antibody reagent in 0.5ml sterile deionised water (see Section 12.2.4c).

- For HLA Class I, prepare successive dilutions of the UK reference anti-A2 serum of: undiluted (N), 1 in 2, 1 in 3, 1 in 4, 1 in 8, 1 in 16, 1 in 32 and 1 in 64 in RPMI 1640 or similar tissue culture medium, supplemented with 100 ml/L of inactivated fetal bovine serum
- For HLA Class II, add 100 µl of reconstituted anti-B lymphocyte (monoclonal) preparation to 1900μl of RPMI 1640 or similar medium, supplemented with 100 ml/L fetal bovine serum, to give 2 ml of a 1 in 20 dilution. Double dilute the 1 in 20 prepared dilution to 1/128 (of the initial 1 in 20 dilution) with RPMI 1640 or similar medium, containing 100 ml/L fetal bovine serum
- Add 1μl of each dilution of antibody to wells in rows A to F, columns 1 to 8 (see Table 15.1) of an oiled microwell tray as shown above. Add 1 μl of diluent material to the remaining wells in rows A to F, columns 9 to 10, as medium control
- For HLA Class I, add l μl of a suspension of 1–2 × 10^6 ml^{-1} of normal peripheral lymphocytes, separated from an individual known to possess the HLA-A2 antigen, to all the wells, taking care to avoid carry-over
- For HLA Class II, prepare B lymphocytes from a fresh blood sample by a Class II immunomagnetic bead positive selection method. 100 μl at 1–2 × 10^6 ml^{-1} will be sufficient for each complement under test. Add 1 μl of isolated B cells to all wells of the tray (do not separate the beads from the B cells). Incubate at 20°C–22°C for 30 minutes
- Five minutes before the complement is required to be added, reconstitute the appropriate UK reference reagent for rabbit complement as described in the corresponding instructions for use. Thaw or reconstitute the complement under test. Keep on melting ice until required

- Add 5 μl of UK reference reagent for rabbit complement to each well in rows D to F and 5 μl of test complement to each well in rows A to C, avoiding carry-over at all times
- Reincubate at 20°C–22°C for 60 minutes
- Assess cell death by staining and microscopy. Note, for HLA Class II serology use a fluorescent microscopy method
- Record percentage cell death for each well. Compare the degree of cell death observed in the three replicate tests of each dilution of the UK reference antibody reagent obtained with the UK reference complement and the complement under test.

Table 15.1 Dilution of UK reference reagent

Complement	N[a,b]	1 in 2[a,b]	1 in 3[a] 1 in 4[b]	1 in 4[a] 1 in 8[b]	1 in 8[a] 1 in 16[b]	1 in 16[a] 1 in 32[b]	1 in 32[a] 1 in 64[b]	1 in 64[a] 1 in 128[b]	Medium controls	Column
Reference										F
Reference										E
Reference										D
Test										C
Test										B
Test										A
Row	1	2	3	4	5	6	7	8	9	10

[a]Dilutions of UK reference anti-HLA-A2 serum for HLA Class I serology
[b]Dilutions of UK reference anti-B lymphocyte (monoclonal) preparation for HLA Class II serology

Requirements

Rabbit complement for use in HLA Class I and II serology should enable all dilutions of the UK reference antibody reagent to attain or exceed the percentage cell death obtained with the UK reference complement.

Toxicity tests on rabbit complement for use in HLA serology

Rabbit complement recommended for use should not cause cell death in the absence of HLA antibody when tested against serum collected from five non-transfused male volunteers using samples of appropriate lymphocytes preparations from 10 random donors in the one and one half-hour incubation NIH cytoxicity test.

Rabbit complement recommended for use with monoclonal HLA typing reagents should not cause cell death in the absence of human serum when tested against RPMI 1640 or similar medium, supplemented with 100 ml/L fetal bovine serum, using samples of appropriate lymphocyte populations from 10 random donors in the one and one half-hour incubation NIH cytotoxicity test.

15.1.5 DNA typing reagents

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

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

Instructions for use

In addition to Section 12.1.4.l of these Guidelines, the instructions for use must adhere to the EFI standards for Nucleic Acid Analysis (Section L) and should include the following:

- an explanation of the test and intended application of the kit should be stated
- the principle of the procedure
- reagents and equipment required to perform the test
- detailed instructions for all components of the test
- the gene targeted as a PCR amplifcation control (PCR-SSP)
- the specificity and nucleotide sequence of all primers and probes used in the HLA typing kit
- a table or diagram indicating the location of the probes and or primers utilised in the test
- a list of ambiguous combinations of alleles defined for each test kit – this may also be given as part of interpretative software
- the HLA alleles which are claimed to be detected by the HLA typing kit. These alleles should be further divided into the following groups:
  - those HLA alleles which have been detected in appropriately controlled validation tests
  - those HLA alleles which have not been directly detected in validation tests but where the reactivity of the allele is expected to be detected
  - those HLA alleles which have not been directly detected in validation tests and whose reactivity cannot be assumed to be detected by the kit
  - those HLA alleles that are known to produce known weak or unreliable signals in the output systems
- the date and the source of the sequence information used in the kit design and a statement that new alleles described following the date of design may not be detected by the kit
- the control tests to be performed to check for contamination (negative control) of the test system
- the control DNA to be included to check for quality of sample DNA used
- the control test to be performed to generate a true positive signal
- acceptable limits of signal intensity should be specified for positive and negative results. All computer software assisted interpretation of results should be validated on control DNA
- the chemical components of the kits should be listed and reference made to any toxic substances included in the kit with recommendations for their safe disposal. Reference to material safety data sheets should be given.

Requirements

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

15.2 Testing of HLA genes and gene products

DNA-based methods must identify all HLA alleles included in the most recent WHO Nomenclature Committee for Factors of the HLA System report,[2] *Nomenclature for Factors of the HLA System, 2004.*[3] Alleles should be reported either as individual alleles with four digits resolution or as allele groups with two digits. HLA typing at high resolution requires DNA-based analysis and must give unambiguous allele assign to a minimum of four digits, for example A*0201, A*0301. A low resolution HLA typing may be given as A*02, A*03.

The minimum level of resolution by serological typing is given in Table 15.2. Typing to the level of broad specificities is acceptable antigens but the higher level to include the split specificities, as indicated, is recommended. HLA-C types Cw12 and Cw14 to Cw18 have not been formally designated as recognised antigens and may not be identified serologically.

15.3 Testing for HLA-specific antibodies

15.3.1 General guidance

HLA-specific antibody screening and characterization must comply with EFI standards section F (Antibody Screening), Section M (Flow Cytometry), M1.0000 to M2.4200 if performing antibody screening by flow cytometry and section N (Enzyme Linked Immunosorbent Assay) if performing antibody screening by ELISA.

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

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

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

15.3.2 Characterization of antibody specificity

It has become obvious that the characterization of sera containing of HLA-specific antibodies is method dependent. This is most apparent in the results of recent UK NEQAS schemes, but while certain differences may be attributable to disparity in sensitivity for different immunoglobulin isotypes, the inconsistencies between methods are yet to be fully explained. It is therefore advisable, at the moment, to use more than one method to achieve a complete identification.

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

Panels of HLA typed cells or purified HLA molecules are used for identification. The composition of the panel should be sufficient to discriminate the specificities (Class I, Class II, or both as appropriate) given in Table 15.3. The full list of antigens comprising a panel should be supplied and typed to the higher level of resolution shown in Table 15.2.

Table 15.2 HLA antigens that are defined by serological typing

HLA-A	HLA-B	HLA-C	HLA-DR	HLA-DQ
A1	**B7**	**Cw1**	**DR1**	**DQ5 (1)**
A2	**B8**	**Cw2**	**DR15**	**DQ6 (1)**
A3	**B13**	**Cw9 (Cw3)**	**DR16**	**DQ2**
A11	**B18**	**Cw10 (Cw3)**	**DR17 (3)**	**DQ7 (3)**
A23 (A9)	**B27**	**Cw4**	**DR18 (3)**	**DQ8 (3)**
A24 (A9)	**B35**	**Cw5**	**DR4**	**DQ9 (3)**
A25 (A10)	**B37**	**Cw6**	**DR11**	**DQ4**
A26 (A10)	**B38 (B16)**	**Cw7**	**DR12**	
A29	**B39 (B16)**	**Cw8**	**DR13**	
A30 (A19)	**B41**	Cw12	**DR14**	
A31 (A19)	**B42**	Cw14	**DR7**	
A32	**B45**	Cw15	**DR8**	
A33	**B44**	Cw16	**DR9**	
A34 (A10)	**B46**	Cw17	**DR10**	
A36	**B47**	Cw18	**DR103**	
A43	**B48**			
A66 (A10)	**B49 (B21)**		**DR51**	
A68 (A28)	**B50 (B21)**		**DR52**	
A69 (A28)	**B51**		**DR53**	
A74 (A19)	**B52**			
A80	**B53**			
	B54 (B22)			
	B55 (B22)			
	B56 (B22)			
	B57 (B17)			
	B58 (B17)			
	B59			
	B60 (B40)			
	B61 (B40)			
	B62 (B15)			
	B63 (B15)			
	B64 (B14)			
	B65 (B14)			
	B67			
	B71 (B70)			
	B72 (B70)			
	B73			
	B75 (B15)			
	B76 (B15)			
	B77 (B15)			
	B78			
	B81			
	Bw4			
	Bw6			

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

For DNA typed donors the types should be supplied at the four-digit level (eg HLA-A*0201) and null alleles identified.

a) HLA antibody characterization by complement dependent cytotoxicity

Rabbit complement

Rabbit complement used for detection of HLA antibodies by complement dependent cytotoxicity should comply with guidelines in Section 15.1.4.

Instructions for use

In addition to the information required in Section 15.1.2, the instructions for use should include the following information on each individual preparation of HLA reagent lymphocytes or set of HLA reagent lymphocytes:

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

Reagent lymphocytes

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

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

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

Reagent lymphocytes supplied as previously frozen in test trays should contain 1,000 to 2,000 lymphocytes per well, after recovery following manufacturers instructions.

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

b) HLA antibody characterization by solid-phase and particle-bound methods

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

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

Human material

If a product is prepared from human source material then the guidance in Section 12.1.4.j must be followed.

Instructions for use

The instructions for use must comply with the requirements of BS EN 375:1992 and the information required in Section 15.1.2. In addition the instructions for use should include the following information on each individual preparation or component of a set of HLA screening product:

- the HLA antigens represented in each container
- the nature of the HLA screening product (see Section 15.3.2 a, 'Instructions for use')
- the concentration of any cells or particles in suspension should be stated in the instructions for use of HLA screening product issued in individual immediate containers or on the antigen information table of batches issued as multi-immediate container products or multi-well trays or reservoirs
- HLA screening products issued in multi-well trays should include a representation of the tray or reservoir layout indicating the location of the HLA antigens in the wells of the tray
- the expiry life of the HLA screening product following reconstitution or preparation and subsequent storage in conditions recommended by the manufacturer should be stated
- when components of an HLA screening product contains preservatives the name of the chemical preservatives and the components which contain them should be stated.

15.4 Leucocyte crossmatching in blood transfusion

Crossmatching may be used in the management of patients refractory to random donor platelet transfusion and in the diagnosis of transfusion-related acute lung injury (TRALI) and the treatment of HLA or HNA sensitised patients with granulocyte transfusions.

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

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

15.4.1 Lymphocytotoxic crossmatch

Assessment of leukocyte crossmatches must comply with EFI standard C5.130 and the standards for serological investigation given above.

Cytotoxic crossmatch requirements

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

A negative control serum derived from a male untransfused human donor or a pool of sera that has been previously shown not to react with lymphocytes by CDC should be used.

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

Table 15.3 Characterization of HLA-specific antibodies

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

For the investigation of antibody specificity the minimum standard should be the ability to resolve those specificities shown in bold type.

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

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

15.4.2 Flow cytometric crossmatch

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

A two-colour FCXM should be used with one antibody directed against human IgG heavy chain conjugated to a fluorochrome, e.g. fluorescein isothiocyanate (FITC). A second antibody conjugated to a different fluorochome e.g. Phycoerythrin (PE), should be used to identify the cell lineage under investigation unless a purified cell population is used or the target population can be resolved by physical parameters (ie forward scatter, FSC and/or side scatter, SSC). Otherwise there must be compliance with EFI standards M2.0000-M2.1300 and M2.000-M2.2500.

FCXM requirements

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

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

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

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

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

15.5 Donor and patient testing

HLA Class I typed platelets should normally be provided for refractory patients on the basis a lack of mismatched Class I antigens. In the absence of a zero mismatched donor, a compatible donor can be selected on the basis of a lack of antigens or alleles corresponding to the antibody specifities identified in the patient. Where a patient's antibodies have not been characterised a crossmatch can be performed.

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

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

Potential HSC donors are typed for both HLA Class I and Class II for registration on one of the national donor panels.

Transfusion Associate Graft versus Host Disease (TAGvHD) can be a consequence of using immunocompetent blood products from donors that share HLA alleles with the patient, particularly where the donor carries no mismatched alleles.

HLA and/or granulocyte specific antibodies present in donor plasma have been implicated in nearly 90% of TRALI cases (patient leucocyte antibody or inter-donor reactions in pooled products have also been reported as causes of TRALI). The identification of leucocyte-specific antibodies in implicated donors provides support for the diagnosis of TRALI, which has similar features to acute respiratory distress syndrome (ARDS).

15.5.1 Apheresis platelet donors

All potential plateletpheresis donors used for the provision of HLA selected platelets should be typed for the HLA-A, -B and Cw. If serological typing is used the minimum level of typing should be for the HLA Class I specificities listed in Table 15.2. Resolution to the level of broad antigen group, shown in brackets, is acceptable. For all donors HLA-Bw4 or -Bw6 should be assigned.

If DNA-based typing is performed on donors a typing strategy should be employed that allows for HLA alleles to be defined to at least the two-digit level of resolution. Typing should also be capable of determining the presence of the Bw4 and Bw6 epitopes encoded by the HLA-B alleles and Bw4 for HLA-A.

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

15.5.2 Haemopoietic stem cell donors

All potential haemopoietic stem cell donors must be typed for the HLA-A, HLA-B and HLA-DR.

If serological typing is used the minimum level of typing should be for the HLA Class I specificities listed in Table 15.2. Resolution to the level of broad antigen group, shown in brackets, is acceptable. If DNA-based typing is performed on donors a typing strategy should be employed that allows for HLA alleles to be defined to at least the two-digit level of resolution.

If HLA-DQ or HLA-Cw are tested they should be typed to the resolution shown in Table 15.2 if a serological method is used. For DNA typing resolution should be to the two-digit level. DNA typing for HLA-DQ can be limited to analysis of DQB1.

15.5.3 Investigation TAGvHD

Full HLA typing should be completed for the patient and all implicated donors. If serological typing is used the minimum level of typing should be for the HLA Class I and II specificities listed in Table 15.2. If DNA-based typing is performed on donors a typing strategy should be employed that allows for HLA alleles to be defined to at least the two-digit level of resolution.

15.5.4 Investigation of refractoriness

The investigation of refractoriness (see Figure 15.1) and the provision of selected platelets in such cases should comply with the *BCSH Guidelines for the Use of Platelet Transfusions*.[5] Serological investigation of suspected immune refractoriness requires screening for HLA Class I-specific antibodies only, but the screening technique must detect HLA-A, B, and C-specific antibodies. Any screen positive patient should be tested further for specificity to include all the Class I antigens listed in Table 15.3.

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

15.5.5 Investigation of TRALI

Sera from all implicated donors must be screened for both HLA Class I and Class II-specific antibodies. Any screen positive serum should be further characterised for HLA class I and class II specificities to include all those listed in Table 15.3. See also Figure 15.2.

If any of the implicated donors are shown to have HLA-specific donors the patient should be typed for HLA Class I and Class II (Table 15.2) to determine the presence of alleles corresponding to the antibody specificities found in the donor(s).

If a donor serum has HLA-specific antibodies that cannot be completely characterised or a specificity corresponding to any of the patient's HLA antigens cannot be excluded then a crossmatch between donor and patient should be performed as described in Section 15.4.

15.5.6 Investigation of febrile transfusion reactions

If an investigation is requested, sera from patients should be screened for both HLA Class I- and Class II-specific antibodies. Any screen-positive serum should be further characterised for HLA Class I and Class II specificities to include all those listed in Table 15.3.

References

1. The European Federation for Immunogenetics (EFI) standards available at www.efiweb.org.
2. *WHO Nomenclature Committee for Factors of the HLA System Report* (see www.anthonynolan.org.uk).
3. Marsh, SGE et al (2004) *Nomenclature for Factors of the HLA System, 2004. Tissue Antigens*, **65**, pp301–68.
4. Schreuder, G, M, Th et al (2004) The HLA dictionary 2004: a summary of HLA-A, -B, -C, -DRB1/3/4/5 and -DQB1 alleles and their association with serologically defined HLA-A, -B, -C, -DR and -DQ antigens. *Tissue Antigens*, **65**, pp1–55.
5. British Committee for Standards in Haematology (2003) Guidelines for the Use of Platelet Transfusions. *British Journal of Haematology*, **122**, pp10–23.

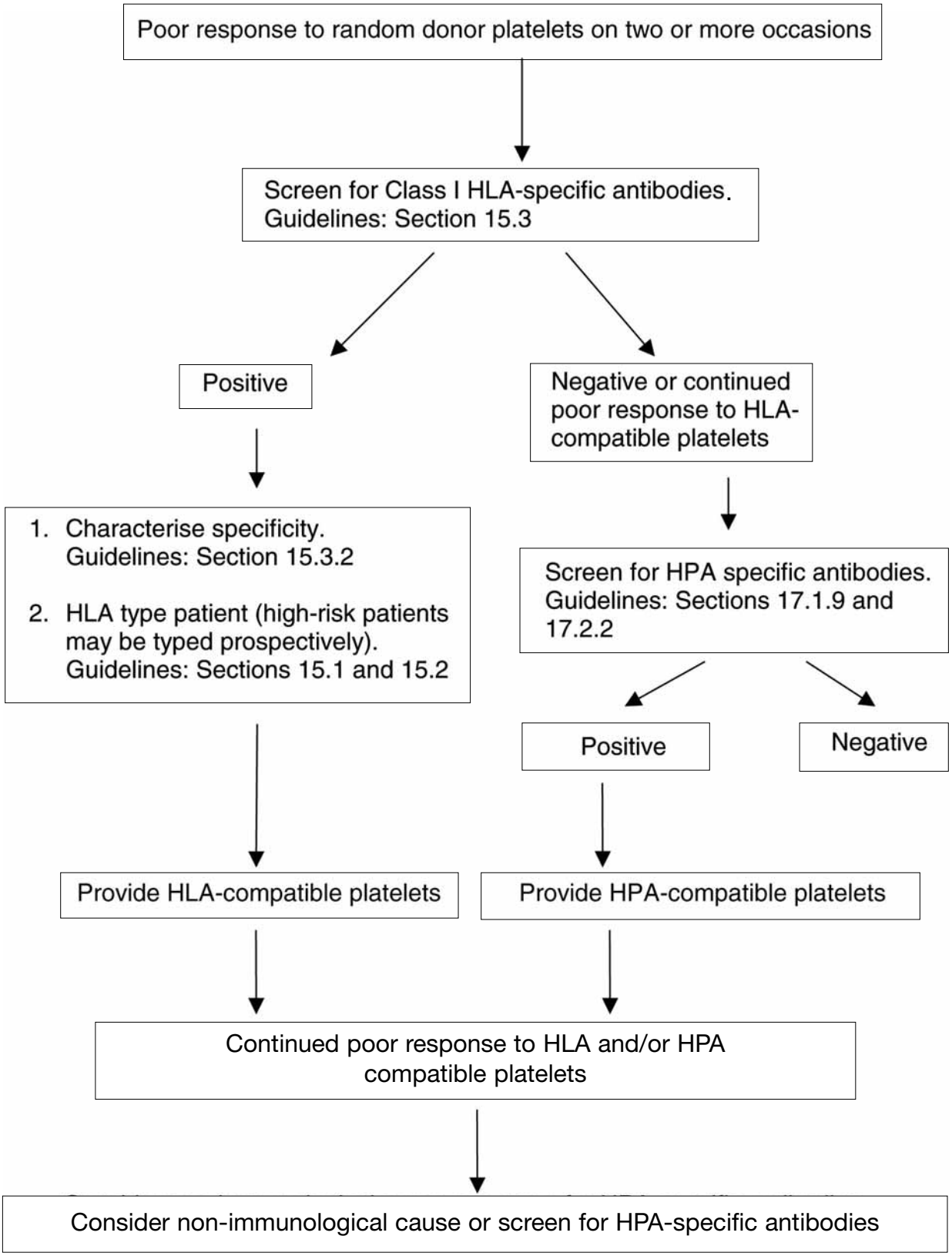

Figure 15.1 Algorithm for laboratory investigation of platelet refractoriness

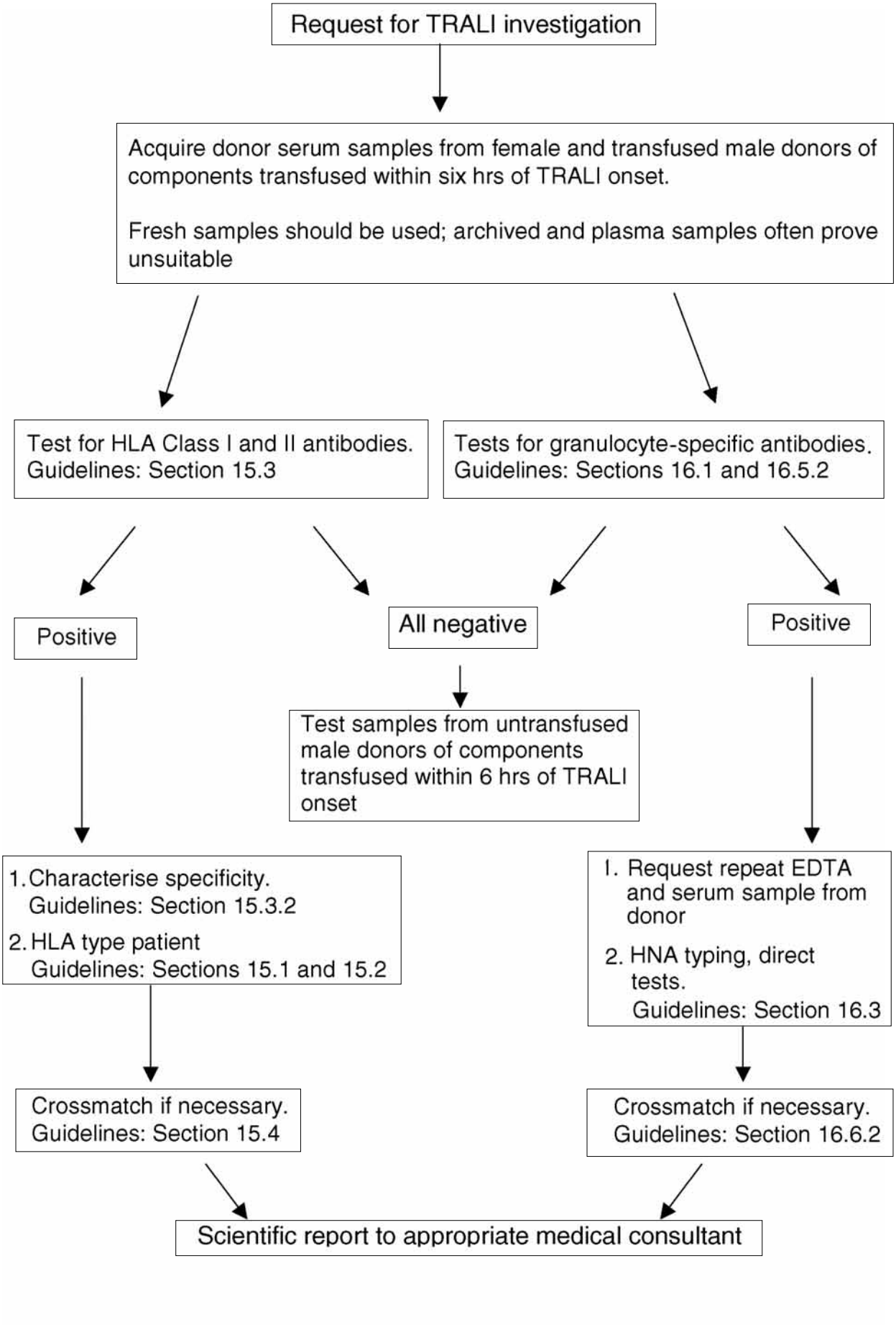

Figure 15.2 Algorithm for laboratory investigation and reporting of TRALI case

Chapter 16

Granulocyte immunology

16.1 Reagent manufacture/reference preparations

16.1.1 HNA typing reagents

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

HNA typing reagents prepared from human source material should comply with the guidelines in Section 12.1.4.

An instructions for use sheet (package insert) should be prepared and supplied with antibody typing reagents, see Section 12.1.4. Information in the instructions for use sheet should further indicate the immunoglobulin class of the antibodies and the presence of any other contaminating antibodies reactive by the recommended methods.

HNA typing reagents used in genomic DNA and PCR-based techniques should comply with the guidelines in Chapter 18.

16.1.2 Composition of granulocyte cell panel for HNA antibody detection

It is recommended that laboratories make all reasonable efforts to include cells in their panel that will aid the detection and identification of clinically significant HNA antibodies. The panel should consist of granulocytes typed at a minimum for HNA-1a, 1b, 1c, 2a(+), 2a(-), 3a(+) and 3a(-) by validated HNA typing techniques. Ideally, the panel should contain granulocytes that are homozygous for HNA-1a and -1b and be from Group O donors.

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

16.1.3 The preparation of granulocytes/lymphocytes

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

- granulocytes/lymphocytes should be prepared from donors/patients within 24 hours of venesection. Precautions must be taken to minimize activation of granulocytes during isolation

- granulocyte/lymphocyte preparations should be essentially free from red cells that would otherwise interfere with the technique or its reading
- the viability of isolated granulocytes should be sufficient as to not interfere in the technique or its reading.

16.1.4 Control sera

a) Selection of normal control sera

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

b) Selection of positive control sera

At least one positive control should be included in each assay. The selection and number of positive control sera will depend on the technique and the HNA type of the granulocytes being used. In glycoprotein specific assays, a positive control for each glycoprotein used should be included as a minimum.

c) Quality control schemes

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

16.2 Nomenclature

The current nomenclature for Human Neutrophil Antigens (HNA) and corresponding antibodies must be used for recording granulocyte specific alloantigen and alloantibody specificities;[1] see Table 16.1.

Table 16.1 Current nomenclature for human neutrophil antigens and corresponding antibodies

System	Antigen	Original name	Glycoprotein	CD
HNA-1	HNA-1a	NA1	$Fc_{\gamma}RIIIb$	CD16
	HNA-1b	NA2	$Fc_{\gamma}RIIIb$	CD16
	HNA-1c	SH	$Fc_{\gamma}RIIIb$	CD16
HNA-2	HNA-2a	NB1	GP 56-64kDa	CD177
HNA-3	HNA-3a	5b	GP 70-95kDa	
HNA-4	HNA-4a	MART[a]	CD11/18	CD11b
HNA-5	HNA-5a	OND[a]	CD11/18	CD11a

16.3 HNA typing methods

HNA types should be determined using antibody-based and DNA/PCR-based techniques that have been validated in the laboratory.

Polyclonal human anti-HNA antisera used in serological techniques should be well characterized. There is no requirement to use typing antisera that are ABO compatible with the granulocytes since the available evidence suggests that granulocytes either do not express ABH antigens or do so very weakly.

16.4 HNA antibody detection methods

There are several techniques for the detection of HNA-reactive antibodies. These techniques can be divided into non-specific (where intact granulocytes are used, e.g.

Granulocyte Immunofluorescence test, Granulocyte agglutination test) and specific assays (where glycoprotein capture, or purified glycoproteins or recombinant antigens are used, e.g. Monoclonal Antibody Immobilization of Granulocyte Antigen test). Laboratories should use tests with adequate sensitivity for the detection and identification of HNA-reactive antibodies.

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

i) The detection of clinically significant HNA-reactive alloantibodies to the antigens of the HNA-1, HNA-2 and HNA-3 systems.

ii) The detection and identification of HNA-reactive antibodies in samples containing a mixture of HNA- and HLA-reactive antibodies, including antibodies to HNA-3a, which is expressed on both granulocytes and lymphocytes.

iii) The identification of the specificities in samples containing mixtures of alloantibodies against several HNA antigens (e.g. masking of certain HNA specificities by composition of the panel).

iv) Techniques should be available to detect cytotoxic and non-cytotoxic anti-lymphocyte antibodies and thereby aid the distinction between granulocyte-specific, lymphocyte reactive and HLA class I and class II antibodies.

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

16.5 Donor testing

16.5.1 HNA typing

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

16.5.2 Investigation of HNA antibodies

HNA antibody specificities should only be assigned when the sample investigated has been tested and a minimum of three positive and three negative reactions obtained. An antibody report can be issued at this stage. A donor with a HNA alloantibody should receive a HNA antibody card and an information leaflet. However, before a HNA antibody card and information leaflet is issued, the donor should be typed (on one occasion but ideally by two methods) and found negative for that antigen.

16.6 Patient testing

16.6.1 HNA typing

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

16.6.2 Investigation of HNA antibodies

Patients should be investigated for HNA antibodies following the guidelines for donor investigation. However, laboratories providing diagnostic tests for Neonatal Alloimmune Neutropenia (NAIN) are advised to investigate cases with a clinical diagnosis of possible

NAIN and a negative HNA antibody screen for antibodies against low frequency or 'private' antigens. An effective approach is to use granulocytes from the child's father as an additional panel cell (paternal granulocytes should be HNA typed as a 'patient sample'). Alternatively, laboratories may refer such cases to a reference laboratory.

In the investigation of Transfusion Related Acute Lung Injury (TRALI) both patient samples (pre and post-transfusion) and donor samples should be investigated for the presence of both HNA and HLA class I and class II antibodies (see algorithm in Table 15.2). Where antibody specificities are identified, the donor and patient should be typed to determine the presence or absence of the cognate antigen. Wherever possible, a crossmatch should also be performed between the implicated donor serum samples and granulocytes/lymphocytes from the patient to determine the clinical relevance of any antibodies and the presence of any low frequency antibodies. When 'pooled' platelet products are implicated in a case of TRALI, consideration should also be given to the possibility of the formation of inter-donor immune complexes. In such cases, all the donors who contributed to the pool should also be HNA and HLA typed. In a small proportion of TRALI cases, patient antibodies may react with infused donor cells/antigens and it may be necessary to incubate the patient's serum with granulocytes/lymphocytes from the donor.

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

A patient or donor with HNA antibodies should receive a HNA antibody card and an information leaflet.

16.6.3 Controls for direct tests for granulocyte bound immunoglobulins

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

References

1. Bux, J. (1999) Nomenclature of granulocyte alloantigens. ISBT Working Party on Platelet and Granulocyte Serology, Granulocyte Antigen Working Party. International Society of Blood Transfusion. *Transfusion*, **39**(6), pp662–3.

Chapter 17

Platelet immunology

17.1 Reagent manufacture /reference preparations

17.1.1 HPA typing reagents

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

HPA typing reagents prepared from human source material should comply with the guidelines in Section 12.1.4.

An instructions for use sheet (package insert) should be prepared and supplied with antibody typing reagents; see Section 12.1.4.l. Information in the instructions for use should further indicate the immunoglobulin class of the antibodies and the presence of any other contaminating antibodies reactive by the recommended methods.

HPA typing reagents used in genomic DNA and PCR-based techniques should comply with the guidelines in Chapter 18.

17.1.2 Composition of platelet cell panel for HPA antibody detection

It is recommended that laboratories make all reasonable efforts to include cells in their panel that will aid the detection and identification of clinically significant HPA antibodies. The panel should consist of platelets typed at a minimum for HPA-1, -2, -3, -5 and -15 by validated HPA typing techniques. Ideally, the panel should contain platelets that are homozygous for HPA-1a, -1b, -2a, -2b, -3a, -3b, -5a, -5b, -15a and -15b and be from group O donors.

HPA typing of a platelet panel donor should be based on two concordant typings performed on samples obtained on different occasions. Wherever possible phenotyping for the above antigens should also be performed on one occasion.

17.1.3 Selection of normal control sera

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

17.1.4 Selection of positive control sera

At least one positive control should be included in each assay. The selection and number of positive control sera will depend on the technique and the HPA type of the platelets being used. In glycoprotein specific assays a positive control for each glycoprotein used should be included as a minimum.

17.1.5 Reference preparations

Sensitivity of techniques should be monitored on the basis of the inclusion of a 'weak positive' control. For anti-HPA-1a and -5b, the internal sensitivity control should be calibrated against the WHO International Reference Reagents for Anti-HPA-1a (NIBSC code 93/710) and Anti-HPA-5b (NIBSC code 99/666) when diluted as instructed by the manufacturer.

In-house sensitivity standards, with similar reaction strengths to the above reagents, should be prepared for anti-HPA-1 and HPA-5, and if possible, for anti-HPA-2, -3 and -15 antibodies.

17.1.6 Quality control schemes

Laboratories should take part in regular external quality control exercises such as the UK QAS for HPA antibody detection and for HPA genotyping. Effective mechanisms should be in place to correct poor performance in the quality scheme.

17.1.7 Nomenclature

The current HPA nomenclature must be used for recording platelet specific alloantigen and alloantibody specificities;[1] see Table 17.1. Any subsequent additions can be found in the Immuno Polymorphism Database (IPD) website http://www.ebi.ac.uk/ipd/hpa.[2]

Table 17.1 Current HPA nomenclature for platelet-specific alloantigen and alloantibody specificities

System	Antigen	Original names	Glycoprotein	CD
HPA-1	HPA-1a	Zw^a, PI^{A1}	GPIIIa	CD61
	HPA-1b	Zw^b, PI^{A2}		
HPA-2	HPA-2a	Ko^b	GPIbα	CD42a
	HPA-2b	Ko^a, Sib^a		
HPA-3	HPA-3a	Bak^a, Lek^a	GPIIb	CD41
	HPA-3b	Bak^b		
HPA-4	HPA-4a	Yuk^b, Pen^a	GPIIIa	CD61
	HPA-4b	Yuk^a, Pen^b		
HPA-5	HPA-5a	Br^b, Zav^b	GPIa	CD49b
	HPA-5b	Br^a, Zav^a, Hc^a		
	HPA-6bw	Ca^a, Tu^a	GPIIIa	CD61
	HPA-7bw	Mo^a	GPIIIa	CD61
	HPA-8bw	Sr^a	GPIIIa	CD61
	HPA-9bw	Max^a	GPIIb	CD41
	HPA10bw	La^a	GPIIIa	CD61
	HPA11bw	Gro^a	GPIIIa	CD61
	HPA12bw	Iy^a	GPIbβ	CD42c
	HPA13bw	Sit^a	GPIa	CD49b
	HPA14bw	Oe^a	GPIIIa	CD61
HPA-15	HPA-15a	Gov^b	CD109	CD109
	HPA-15b	Gov^a		
	HPA-16bw	Duv^a	GPIIIa	CD61

17.1.8 HPA typing methods

HPA types should be determined using antibody-based and DNA/PCR-based techniques that have been validated in the laboratory. These techniques should comply with the guidelines in Section 12.1.4 and Chapter 18.

Polyclonal human anti-HPA antisera used in serological techniques should be well characterized. When used in techniques with 'intact' platelets the antisera should be ABO compatible with the platelets to be typed. Alternatively, anti-A and anti-B antibodies may be removed by absorption or neutralization. This is not a requirement when using human antisera in glycoprotein capture assays, but reactivity against ABO incompatible platelets should be assessed. Sera shown to contain anti-A/B activity in these assays should be subject to the same requirements as those used in 'intact' platelet assays.

17.1.9 HPA antibody detection methods

There are several techniques for the detection of HPA-reactive antibodies. These techniques can be divided into non-specific (where intact platelets are used, e.g., platelet immunofluorescence test, solid phase adherence test) and specific assays (where glycoprotein capture, or purified glycoproteins or recombinant antigens are used, e.g. monoclonal antibody immobilization of platelet antigen test). Laboratories should use tests with adequate sensitivity for the detection and identification of HPA-reactive antibodies.

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

- the detection of clinically significant HPA-reactive alloantibodies in the HPA-1, HPA-2, HPA-3, HPA-5 and HPA-15 systems
- the identification of HPA-reactive antibodies and their specificity in samples containing a mixture of HPA- and HLA-reactive antibodies
- the identification of the specificities in samples containing mixtures of alloantibodies against several HPA antigens (e.g. masking of certain HPA specificities by composition of the panel).

Where HPA-reactive antibodies are detected, but the specificity cannot be determined, the samples should be referred to a reference laboratory for antibody specificity investigations. However, all reasonable efforts should be made to screen against the widest possible range of HPA antigens.

17.1.10 Validation of laboratory kits

Kits for the detection of HPA-reactive antibodies should be validated for sensitivity and specificity on a batch basis using a panel of clinically representative HPA antisera. It is recommended that for monitoring of the sensitivity of HPA antibody detection the panel of antisera should contain 'weak' reactive HPA antibodies (not obtained by dilution of strongly reactive HPA typing sera). A panel of sera shown to be inert for HPA and HLA antibodies should also be used.

Kits for HPA typing should be validated for specificity on a batch basis using a panel of at least nine donors (three of each, 'aa, ab, bb' if available) for each HPA mutation.

17.2 Donor testing

17.2.1 HPA typing

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

17.2.2 Investigation of HPA antibodies

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

17.3 Patient testing

17.3.1 HPA typing

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

- A provisional type can be issued on the basis of a genotype performed on one occasion. However, it is recommended that a second typing technique be used when quality exercises or routine practice have revealed technical problems when typing for particular polymorphisms. Typing of subsequent samples will allow a confirmed genotype to be reported.
- HPA typing of fetal amniocytes can be undertaken by molecular techniques using DNA isolated from non-cultured amniocytes and a provisional HPA genotype reported. The HPA genotype should be repeated on DNA extracted from cultured amniocytes and shown to be concordant with the first result.

17.3.2 Investigation of HPA antibodies

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

- Laboratories serving populations with non-Caucasoid patients are advised to include in their panels cells which will aid the detection and identification of additional clinically significant antibodies (e.g. HPA-4, Nak^a/GPIV)
- Laboratories providing diagnostic testing for neonatal alloimmune thrombocytopenia (NAITP) are advised to investigate cases with a clinical diagnosis of possible NAITP and a negative HPA antibody screen for antibodies against low frequency or 'private' antigens. An effective approach is to use platelets from the child's father as an additional panel cell (paternal platelets should be HPA typed as a 'patient sample'). Alternatively, laboratories may refer such cases to a reference laboratory.
- Laboratories providing diagnostic testing for platelet refractoriness (PR) should follow the algorithm for laboratory investigations of PR in Figure 15.1.

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

References

1. Metcalfe, P, *et al.* (2003) Nomenclature of human platelet antigens. *Vox Sanguinis*, **85**(3), pp240–5.
2. Immuno Polymorphism Database (IPD) website www.ebi.ac.uk/ipd/hpa.

Chapter 18

Guidelines for the use of DNA/PCR techniques in transfusion centres

18.1 Safety precautions

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

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

Operators should wear nitrile gloves when handling ethidium bromide. Liquid preparations of ethidium bromide are available from commercial sources and are preferable to handling the powder form.

18.2 Avoidance of contamination

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

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

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

i) one to prepare reagents. This is particularly important to avoid contamination of primers

ii) one dedicated to pre-PCR manipulation, e.g. DNA isolation. A Class II Laminar Flow cabinet should avoid contamination of the sample with DNA from the operator

iii) one dedicated to setting up PCR reactions

iv) one for manipulation of PCR amplified DNA. PCR amplified products should be kept away from areas used for pre-amplification manipulation and reagent preparation.

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

Examples of measures which will contribute to minimize contamination include:

i) the use of new sterilized, disposable plastic tubes or glassware for handling DNA

ii) the use of freshly prepared and sterilized materials and reagents when making up solutions for DNA samples, particularly dH2O and Tris buffers

iii) aliquotting reagents in small amounts to minimize the number of repeat samplings

iv) the change of gloves and coats when moving between the areas dedicated for pre- and post-PCR manipulations

v) the use of positive displacement dedicated pipettes or plugged tips to carry out PCR preparations

vi) routine wipe-tests of pre-amplification work areas should be performed. If an amplified product is detected, the area must be cleaned to eliminate the contamination, retested and measures taken to prevent future contamination

vii) reagents used for amplification must not be exposed to post-amplification work areas.

18.3 Working practices

- DNA should be as intact as possible. Degraded DNA should be avoided.
- An archival record (e.g. photograph) of each electrophoretic run should be retained.
- The performance of probes and primers should be fully validated and characterized before they are put into use. Others should be used only for research purposes.
- Reagents (e.g. chemicals, enzymes) should be stored and utilized under conditions recommended by the manufacturer, including for example storage temperature, test temperature, shelf life, diluent buffer and concentration for use.
- Each lot of reagents must be tested before use in routine typing.
- For reagents and kits, the source, lot number, expiration date, and storage conditions should be documented.
- Users should have procedures to ensure that periodic checks of probes and primers are carried out to detect their deteriorating performance or contamination.
- Thermal cyclers should be serviced at least annually according to the manufacturer's recommendations and a temperature calibration should be performed. A record of the service and calibration checks should be maintained.

Chapter 19

Fractionated plasma products/derivatives

Introduction

The term 'fractionated plasma products/plasma derivatives' is used to describe a range of therapeutic preparations manufactured from human blood plasma by selective separation and purification of target proteins, or groups of proteins.

19.1 Product range

A wide range of fractionated plasma products/plasma derivatives is available for clinical use in the UK. There are four main categories of plasma products. These are listed as follows:

- albumin for intravenous infusion (typically 4.5% and 20% w/v human albumin in aqueous solution)
- immunoglobulins for intravenous infusion (which may be freeze-dried, or may be presented in aqueous solution)
- immunoglobulins for intramuscular injection (normally presented as an aqueous solution). A broad range of hyperimmune immunoglobulins is available in the UK, including anti-D, hepatitis B, tetanus, varicella zoster, and rabies immunoglobulin
- coagulation factors (typically freeze-dried concentrates of the relevant coagulation factor(s), which are intended for intravenous injection after reconstitution). Products available in the UK include:
  - high purity factor VIII
  - Factor VIII/von Willebrand factor
  - high purity factor IX
  - factor II, IX and X concentrate
  - antithrombin
  - factor VII
  - factor XI
  - factor XIII
  - fibrin sealants (fibrinogen + thrombin)

  - activated prothrombin complex concentrate

- in addition, it should be noted that coagulation factors manufactured using recombinant DNA technology are also available, i.e. factor VIIa, factor VIII, factor IX and activated Protein C

- virus-inactivated plasma, prepared from pooled plasma donations, is available as an alternative to single donor FFP.

19.2 Manufacturing sites

Fractionated plasma products are manufactured in the UK by:

- Bio Products Laboratory (BPL), Elstree

- Protein Fractionation Centre (PFC), Edinburgh.

In addition, several products are also available from licensed non-UK manufacturers.

19.3 Summary of regulatory arrangements

Arrangements in the UK for the manufacture and supply of fractionated plasma products are covered by the provisions of the Medicines Act 1968 and its amending Statutory Instruments, which subsume European legislation (Directives) into UK law. This legislation is administered in the UK by the Medicines and Healthcare Products Regulatory Agency (MHRA). These arrangements differ from those which apply in blood establishments and are summarized briefly as follows:

- fractionated plasma products available in the UK will have been manufactured in an MHRA-approved facility. All licensed plasma products must be manufactured under the terms of an approved manufacturer's licence. Each individual product will be manufactured, tested and marketed in accordance with the requirements of a marketing authorization (product licence) issued by the MHRA

- products intended for clinical trial will be manufactured under an investigational medicinal products manufacturer's licence and supplied for evaluation under the terms of a clinical trial approval (CTA) document which will also be approved by the MHRA

- products for supply under named-patient provisions, will have been manufactured under the terms of a manufacturer's 'specials' licence.

Irrespective of intended use, the minimum requirements for manufacture are those defined in GMP Commission Directive 2003/94/EC 'laying down the principles and guidelines of good manufacturing practice in respect of medicinal products for human use and investigational medicinal products for human use' and guidelines detailed in the Eudralex Collection (*The Rules Governing Medicinal Products in the European Union*) Volume 4 at http://pharmacos.eudra.org/F2/home.html.

19.4 The sourcing of plasma for manufacture of fractionated plasma products

The plasma used in the manufacture of fractionated plasma products may be obtained by plasmapheresis or as plasma recovered from whole blood. Guidelines exist (see below) which address the suitability of plasma types for the manufacture of different fractionated plasma products. Based on advice from the Committee on Safety of Medicines (CSM), fractionation of UK plasma was discontinued in 1998/9, as one of several measures introduced to limit the potential for transmission of variant Creutzfeldt-Jakob disease (vCJD) by blood and blood products.

It is now a clear requirement that plasma for fractionation is derived from areas of the world where there are few cases of BSE and low risk of vCJD. It is also necessary that plasma is collected from areas whose donor populations have a low risk of viral disease. Currently plasma used by UK manufacturers is sourced from the USA and Germany.

Regular meetings are held by the European Agency for the Evaluation of Medicinal Products (EMEA) to review safety issues relating to vCJD. Part of this review process is to assess whether it is safe to fractionate plasma collected from donors resident in the UK. Any removal of the current moratorium on the use of UK plasma will depend on progress with the following three criteria:

- introduction of a reliable test to screen for the possibility of infection
- evidence is available to prove that vCJD cannot be transmitted through plasma products
- evidence is available to prove that the manufacturing process eliminates the infective agent.

None of the three requirements listed above have yet been achieved, although there are now good data to demonstrate that significant log reductions of prions can be achieved during the preparation of all plasma products.

All plasma is obtained against detailed purchasing specifications agreed with the suppliers. Plasma for fractionation used to manufacture products released in the EU will need to comply with the requirements of Directive 2002/98/EC.

Guidance on donor selection criteria is also given in Commission Directive 2004/33/EC. Other regulatory requirements relating to plasma collection are summarised in the following key monographs and guidelines:

- the *European Pharmacopoeia* (*Ph Eur*) monograph 'Human Plasma for Fractionation' – which defines the minimum specification for plasma to be used for the manufacture of fractionated plasma products which will be marketed in Europe
- the Council of Europe 'Recommendation R(95)15 on the preparation, use and quality assurance of blood components' – cross-referenced by the *Ph Eur* monograph as an appropriate source in respect of donor selection and testing for transmissible agents of infection
- the US Code of Federal Regulations (CFR), Title 21, Part 640 on 'Additional standards for human blood and blood products' – defining the minimum standards for donor suitability, and for collection of recovered and apheresed plasma ('Source Plasma') in the USA
- document of the European Medicines Agency (www.emea.eu.int) EMEA/CPMP/BWP/3794/03 provides advice to the fractionator on the construction of a 'Plasma Master File'. The text sets out EU control authority expectations of the data which industry must provide on the systems used to control the collection, processing and testing of plasma for fractionation. This system now requires fractionators to declare limits on the prevalence and incidence of markers for infectious disease in each donor population. The methods for calculating incidence and prevalence are contained in 'Guideline on epidemiological data on blood transmissible infections' (EMEA/CPMP/BWP/125/04).

19.5 Summary of key steps during the manufacture of plasma products

Plasma products are prepared from human plasma using a wide range of separation and purification technologies. In each case, the manufacturing method will have been fully validated for consistency and reliability of outcome. The final product and key processing intermediates will be evaluated for stability using defined guidelines. All processes will contain at least one (often more) processing steps designed and validated for the inactivation or removal of a broad range of viruses.

Each product type is manufactured using carefully specified manufacturing conditions which are declared in the form of a marketing authorization (product licence) which must be approved by an appropriate regulatory authority. Each batch of product must conform

with the requirements for control of reagents, raw materials (including plasma), processing conditions, manufacturing premises, laboratory testing, etc., as defined in the licence. All elements of the manufacturing procedure must be controlled and tested to defined standards. Key issues which need to be addressed will include the following:

- control of all raw materials, especially plasma. This will include the agreement of detailed specifications with the supplier. All lots will be subject to an approval procedure which may include batch testing
- careful control of the manufacturing environment, paying special attention to microbiological cleanliness
- in addition, all services, such as steam, water for injections and compressed air must be monitored regularly for compliance with defined specifications, according to appropriate *Ph Eur* monographs and various EU guidelines
- careful control of all equipment, in terms of both cleanliness and operational effectiveness. All equipment must be installed carefully and validated extensively prior to use. Equipment will also be subject to regular maintenance, calibration and revalidation
- tight control of each stage of the manufacturing process, with close monitoring of relevant in process parameters. Any processing step may have an impact on product quality, but special care is taken to ensure that the processes used for viral inactivation/removal and for product sterilization have been validated and are reliably performed on each occasion
- all intermediates and final products will be tested extensively to ensure that they meet all relevant specifications
- increasingly, data from process measurements and laboratory testing is analysed using techniques of statistical process control to detect trends in performance and to verify that the processes are fully under control
- each stage in the manufacturing process from product development to product issue will be covered by a detailed quality management system. This will include a detailed system for tracing product issued and for recall if necessary. Detailed pharmacovigilance systems must be in place to monitor and report adverse reactions to all plasma products. Serious adverse reactions are reported directly to the MHRA. Regular periodic safety update reports (PSURs) are also submitted to the MHRA detailing all adverse reactions reported for each product
- all batches will be subject to intensive scrutiny and independent approval by QA personnel. This will include formal review by a qualified person approved by the MHRA who will certify that each batch has been manufactured according to the product licence and in compliance with GMP. Final release of each batch will require certification by an official medicines control laboratory (OMCL); see below.

19.6 Release of plasma products by an official medicines control laboratory

In accordance with Article 114 of Directive 2001/83/EC, as amended by Directive 2004/27/EC, all fractionated plasma products supplied under marketing authorization (see above) are subject to 'control authority batch release'. This mechanism requires approval of release of every batch of product by an OMCL of the European Union. The procedures for OMCL batch release are coordinated and regulated by the European Department for Quality of Medicines (EDQM), and guidelines for the procedures can be found on the website http://www.pheur.org.

The purpose of this official release mechanism is to confirm that each batch complies completely with the specification contained in the marketing authorization (product licence). Release depends on the results of tests carried out by the OMCL on samples of the batch and of the plasma pools used to manufacture the batch, according to the

relevant EDQM guidelines. If the test results are satisfactory, then the batch can be approved following a review of protocols supplied by the manufacturer. In the UK the designated OMCL is the National Institute for Biological Standards and Control (NIBSC).

Many of the tests used by both manufacturers and control authorities require the use of standards within each assay. These 'working standards' should be calibrated in international units against the appropriate international standards, which are established by the WHO and distributed by NIBSC. Working standards can be prepared by manufacturers, but British working standards are also available from NIBSC for several of the coagulation factors (II, VII, VIII, IX, X) and for virology testing of plasma pools.

19.7 Regulatory guidelines and monographs on fractionated plasma products and plasma fractionation

The manufacture of fractionated plasma products is a well-regulated activity, subject to control agency inspection and, as discussed in Section 19.6, to batch release by an OMCL. The manufacturing processes are specific to individual manufacturers, in many cases involving proprietary information. A range of monographs and guidelines exists, providing minimum standards for the source material (plasma), the final products and aspects of the manufacturing operation (in particular virus inactivation/ elimination and the principles of GMP).

The following list provides the key regulatory guidelines which must be followed by plasma fractionators who manufacture products for release in the EU:

- general guidance on the manufacture of fractionated plasma products is provided by:
  - Committee for Proprietary Medicinal Products (CPMP) in its 'Note for guidance on plasma-derived medicinal products' (CPMP/BWP/269/95 Rev 3), effective January 2001
  - the EU Guide to GMP is contained in Eudralex, Volume 4: The Rules Governing Medicinal products in the European Union found at http://pharmacos.eudra.org/F2/home.html. This contains guidance on a number of key issues for plasma fractionators in the main body of the text. In addition, a number of annexes have direct relevance:
    - Annex 1: Manufacture of sterile medicinal products
    - Annex 8: Sampling of starting and packaging materials
    - Annex 13 Revision 1 of the EC guide to GMP: Manufacture of investigational medicinal products
    - Annex 14 : Manufacture of products derived from human blood or human plasma', revised September 2000
    - Annex 15: Qualification and validation
    - Annex 16: Certification by a qualified person and batch release
- more specific guidance on validation of virus inactivation/elimination procedures is provided by the EU Committee on Proprietary Medicinal Products (CPMP) in its 'Note for guidance on virus validation studies' (CPMP/BWP/268/95)
- CPMP guidance on classical CJD, vCJD and fractionated plasma products is provided in the position statements:
  - CPMP/938/95 (on sporadic, familial and iatrogenic CJD)
  - CPMP/BWP/2879/02 rev 1 (on vCJD)
- guidance on minimising the risk of transmissible animal spongiform encephalopathy agents in plasma products is provided in EMEA/410/01 Revision 2

- CPMP guidance on implementation of the NAT test of plasma pools for HCV-RNA is incorporated in CPMP/BWP/269/95 Revision 3. These were originally published separately with the following references:
  - CPMP/117/95 'Intramuscular Immunoglobulins: nucleic acid amplification tests for HCV-RNA detection'
  - CPMP/BWP/391/95 on 'Implementation of CPMP/117/95 recommendation'
  - CPMP/BWP/390/97 on 'The introduction of nucleic acid amplification technology (NAT) for the detection of hepatitis C virus RNA in plasma pools'
  - CPMP guidance on testing for alanine aminotransferase (ALT), as an adjunct to virus marker testing, is provided in CPMP/BWP/385/99 'Plasma-derived medicinal products: position paper on ALT testing'. This is also incorporated into CPMP/BWP/269/95 Revision 3
- published *Ph Eur* monographs on individual fractionated plasma products provide agreed minimum legal standards and specifications for each product type.

The monograph for anti-D immunoglobulin now includes the requirement that all plasma pools are tested by NAT for parvovirus B19 and each pool must contain less than 10^4 IU/mL of B19 DNA.

Note: CPMP became the Committee for Medicinal Products for Human Use (CHMP) in May 2004.

Chapter 20

Hospital blood banks: impact of EU Blood Directives and Better Blood Transfusion

Introduction

The safety of blood transfusion as a medical intervention depends not only on the safety of the product, but also on the safety of the clinical transfusion process and appropriate indications for transfusion. Hospital blood banks link the blood establishments (responsible for the product), the clinicians (responsible for the transfusion), and the patient who receives the blood.

The Better Blood Transfusion[1] initiatives emphasise the importance of hospital blood banks in promoting better blood transfusion and ensuring the safety of the clinical transfusion process. The 'Red Book' deals with the safety of the products; the *Handbook of Transfusion Medicine* deals with the indications for transfusion and the safety of the clinical transfusion process.

The European Union, whilst not having a competence in 2005 to influence clinical practice, has nevertheless included aspects of the work of hospital blood banks in the 'Blood Directives' in recognition of their key role in the safety of blood transfusion. It draws a clear distinction between blood establishments (the four national blood/transfusion services and the centres from which they operate) and hospital blood banks whose role it is to provide compatible blood components to clinicians for individual patients.

This chapter summarizes the regulatory impact of these Directives, transposed into UK law as the Blood Safety and Quality Regulations 2005,[2] on hospital blood banks.

The printed Seventh Edition of the 'Red Book' reflects the situation in April 2005. Future changes will be described on the website www.transfusionguidelines.org.uk and, where appropriate, in Change Notifications issued by JPAC.

20.1 EU Blood Directives

Directives 2002/98/EC[3] and 2004/33/EC[4] constitute the 'Blood Directives' in 2005. Two further Directives, one on haemovigilance/traceability and one on quality systems, are expected by 2006. Directives on tissues (see Chapter 1) are also relevant to some hospital blood banks.

The Blood Safety and Quality Regulations 2005 transpose these Blood Directives into UK law. The Regulations apply throughout the UK, including the private sector, and come into force on 8 November 2005.

An NHS Operational Impact Group has been established to provide guidance to the NHS on the implementation of the Regulations.Updates are found on www.transfusionguidelines.org.uk.

Competent authority

The Secretary of State is designated the competent authority for the purpose of the Directive. The enforcement obligations under the regulations will be discharged by the Medicines and Healthcare Products Agency (MHRA) to monitor compliance with the regulations and carry out inspections of blood establishments.

20.2 Blood Safety and Quality Regulations 2005: hospital blood bank requirements

The following are extracts of the principal requirements of the Regulations for hospital blood banks. As the regulations are complex they should be consulted in full together with advice emanating from the NHS Operational Impact group.

Hospital blood bank requirements (Regulation 9)

1) The person responsible for the management of a hospital blood bank shall -

   (a) ensure that personnel directly involved in the testing, storage and distribution of human blood and blood components for the hospital blood bank are qualified to perform those tasks and are provided with timely, relevant and regularly updated training;

   (b) establish and maintain a quality system for the hospital blood bank which is based on the principles of good practice;

   (c) ensure that all testing and procedures referred to in Parts 4 and 5 (Authorization) of the Schedule which are applicable to activities carried out by the hospital blood bank are validated;

   (d) maintain documentation on operational procedures, guidelines, training and reference manuals and reporting forms so that they are readily available for inspection under regulation 15 (Inspections);

   (e) maintain, for not less than 30 years, the data needed to ensure full traceability of blood and blood components, from the point of receipt of the blood or blood component by the hospital blood bank;

   (f) notify the Secretary of State of -

      (i) any serious adverse events related to the testing, storage and distribution of blood and blood components by the hospital blood bank which may have an influence on their quality and safety, and

      (ii) any serious adverse reactions observed during or after transfusion which may be attributable to the quality or safety of blood or blood components issued for transfusion by the hospital blood bank;

   (g) establish and maintain a procedure, which is accurate, efficient and verifiable, for the withdrawal from distribution of blood or blood components associated with any notification referred to in paragraph (f); and

   (h) ensure that the storage, transport and distribution conditions of blood and blood components comply with the requirements of Part 4 (Authorization) of the Schedule.

Requirement for hospital blood banks to provide information to the Secretary of State (Regulation 10)

(1) As soon as practicable after the end of the reporting year, the person responsible for management of a hospital blood bank shall submit an annual report to the Secretary of State, which shall -

(a) include a declaration that the hospital blood bank has in place appropriate systems to ensure compliance with the requirements of these Regulations; and

(b) provide details of the systems which it has in place to ensure such compliance.

(2) The person responsible for management of a hospital blood bank shall without delay notify the Secretary of State of any changes to the matters in respect of which evidence has been supplied pursuant to paragraph (1) which might affect compliance with the requirements of these Regulations.

Disclosure of information by blood establishments and hospital blood banks (Regulation 14)

(1) A blood establishment and the person responsible for management of a hospital blood bank shall ensure that all information which is collected for the purposes of these Regulations is held securely so that it is -

(a) available for, and may be disclosed for the purpose of, tracing donations;

(b) not otherwise disclosed except -

(i) in accordance with one or more of the requirements of paragraph (2), or

(ii) where they have been rendered anonymous so that donors are no longer identifiable;

(c) subject to safeguards against unauthorized additions, deletions or modifications.

Paragraph (2) details the exceptions and paragraphs (3) and (4) are relevant to the inspectors.

(5) The responsible person of the blood establishment and the person responsible for management of the hospital blood bank shall ensure that they put in place a procedure to ensure that any discrepancies relating to data which are brought to their attention are resolved without delay.

Inspections (Regulation 15)

(5) The Secretary of State may inspect hospital blood banks with a view to ensuring that -

(a) hospital blood banks and persons responsible for the management of such blood banks comply with the requirements of these Regulations; and

(b) problems relating to compliance with those requirements are identified.

At the time of writing the details of any inspections of hospital blood banks remain to be clarified. Hospital blood banks undertaking secondary processing of blood and blood components such as irradiation may need to seek blood establishment licences.

Pre-deposit autologous donations

Directive 2002/98/EC applies to pre-deposit autologous donations. It does not apply to all other forms of autologous transfusion practice.

Collection, testing and processing of pre-deposit autologous donations is an activity that can only be undertaken by a blood establishment. Hospitals therefore that wish to set up or continue running pre-deposit autologous donation clinics must seek blood establishment status or ask their blood establishments to undertake this activity.

Traceability/haemovigilance

Directive 2002/98/EC calls for specific technical requirements dealing with traceability and procedures for the notification of serious adverse events and reactions. Common definitions have been agreed (see Definitions). Procedures will be required to verify that each unit issued has been transfused to the intended recipient or, if not transfused, verify its subsequent disposal. Specific data on traceability as detailed in the awaited Directive will have to be retained in an appropriate and readable storage medium for 30 years.

Quality systems

Directive 2002/98/EC calls for specific technical requirements including Community standards and specifications with regard to quality systems for blood establishments. Some of these will impact on hospital blood banks.

It is expected that the definitions in the awaited Directive will be used throughout blood establishments and hospital blood banks to ensure uniformity of standards.

Blood establishments and hospital blood banks will be supported by a quality assurance function (see Definitions). That function shall be involved in all quality-related matters.

20.3 Better Blood Transfusion

The various Better Blood Transfusion initiatives (see Chapter 2) are not legally binding as are the Regulations, but they are guidelines for best practice and compliance is expected by the Department of Health.

A tool kit for improving transfusion practice in hospitals in partnership with the blood establishments can be found on www.transfusionguidelines.org.uk.

References

1. Department of Health (2002) *Better Blood Transfusion: Appropriate Use of Blood*, HSC 2002/009 available at www.transfusionguidelines.org.uk.

2. *Statutory Instrument 2005 No. 50. The Blood Safety and Quality Regulations 2005* ISBN 0110516222 available at www.opsi.gov.uk.

3. EU Directive 2002/98/EC 'setting standards of quality and safety for the collection, testing, processing, storage and distribution of human blood and blood components and amending Directive 2001/83/EC'. *OJ*, L 33, 08.02.2003, p30.

4. Commission Directive 2004/33/EC 'implementing Directive 2002/98/EC of the European Parliament and of the Council as regards certain technical requirements for blood and blood components'. *OJ*, L 91, 30.03.2004, p25.

Chapter 21

Tissue banking: general principles

Introduction: reference documents for tissue banking and haemopoietic progenitor cells

The advice contained in these Guidelines is believed to represent acceptable practice at the time of printing. It is policy to revise these Guidelines as new developments occur. However, it may not be possible to do so at the time of such change and the Guidelines should therefore be used with due regard to current acceptable practice.

The guidelines in this section apply to tissue banking activities within the Blood Transfusion Services of the UK. They must be read in conjunction with the other sections of the guidelines including those that apply to care and selection of blood donors, quality systems, quality assurance and to testing of donors.

Reference should be made to the current version Joint UKBTS/NIBSC Professional Advisory Committee's *Donor Selection Guidelines* available at www.transfusionguidelines.org.uk.

Note should also be made of various UK legal statutes and relevant documents which apply to tissue banking, and documents from the Council of Europe and the European Directives. These include the following:

UK legal statutes and documents relevant to tissue banking

Note: this list is current for 2005.

1. Department of Health. *A Code of Practice for Tissue Banks: Providing Tissues of Human Origin for Therapeutic Purposes*. DoH, February 2001. ISBN 1-84182-329-5.
2. Department of Health. *Decontamination of Medical Devices*. HSC 2000/032, 18 October 2000.
3. Department of Health. *Guidance on the Microbiological Safety of Human Tissues and Organs used in Transplantation*. Advisory Committee on Microbiological Safety of Blood and Tissues for Transplantation, DoH, August 2000. www.advisorybodies.doh.gov.uk/acmbtt.
4. Department of Health. *Variant Creutzfeldt-Jakob Disease (vCJD): Minimising the Risk of Transmission*. HSC 1999/178, 13 August 1999.
5. Department of Health. *Controls Assurance in Infection Control: Decontamination of Medical Devices*. HSC 1999/179, 13 August 1999.

6. Medical Research Council. *Operational and Ethical Guidelines for Collections of Human Tissue and Biological Samples for Use in Research*. Report of the Medical Research Council Working Group, November 1999.

7. Report of the Working Party of the Royal College of Pathologists and the Institute of Biomedical Science. Third Edition (2005) *The Retention and Storage of Pathological Records and Archives*.

8. *British Association for Tissue Banking: Association Standards* (current version). www.batb.org.uk.

9. General Medical Council booklets (available at www.gmc-uk.org):
Confidentiality: Protecting and Providing Information: April 2004
Serious Communicable Diseases: October 1997
Good Medical Practice (Third Edition): May 2001
Seeking Patients' Consent: the Ethical Considerations: November 1998
Research: the Role and Responsibility of Doctors: February 2002

10. Department of Health, *A Code of Practice for the Diagnosis of Brain Stem Death Including Guidelines for the Identification and Management of Potential Organ and Tissue Donors*. Department of Health, March 1998 available at www.dh.gov.uk.

11. *Transmissible Spongiform Encephalopathy Agents: Safe Working and the Prevention of Infection*. Advisory Committee on Dangerous Pathogens Spongiform Encephalopathy Advisory Committee. June 2003 available at www.dh.gov.uk.

12. *NHS Estates Health Technical Memoranda (HTM) 2010 Sterilization* www.nhsestates.gov.uk.

13. *NHS Estates Health Technical Memoranda (HTM) 2030 Washer-Disinfectors* www.nhsestates.gov.uk.

14. *NHS Estates Health Technical Memoranda (HTM) 2031 Clean Steam for Sterilization* www.nhsestates.gov.uk.

15. *Data Protection Act 1998*, ISBN 0-10-542998-8 available at www.opsi.gov.uk.

16. *The Anatomy Act 1984 (Commencement) Order 1988*, ISBN 0-11-086081-0 available at www.opsi.gov.uk.

17. Department of Health, The Caldicott Committee, *Report on the Review of Patient-Identifiable Information*. December 1997 available at www.dh.gov.uk.

18. Medicines Control Agency (2002) *Rules and Guidance for Pharmaceutical Manufacturers and Distributors 2002, Sixth Edition*. Norwich: The Stationery Office, ISBN 0-11-322559-8.

19. Nuffield Council of Bioethics (1995) *Human Tissues: Ethical and Legal Issues* available at www.nuffieldbioethics.org.

20. *Human Organ Transplants Act 1989 (Scotland)*, ISBN 0-10-543189-3 available at www.opsi.gov.uk.

21. *Human Tissue Act 2004 (except Scotland)*, ISBN 0-10-543004-8 available at www.legislation.opsi.uk.

22. *Coroners Act 1988*, ISBN 0-10-541388-7 available at www.opsi.gov.uk.

23. *The Human Tissue Act 1961 (Scotland)*.

24. Department of Health, HSG (93)40: *Protecting Health Care Workers and Patients from Hepatitis B and Addendum to HSG (93)40* available at www.dh.gov.uk.

25. Department of Health, *Human Bodies, Human Choices the Law on Human Organs and Tissue in England and Wales a Consultation Report*. July 2002 available at www.dh.gov.uk.

26. Department of Health, *Human Bodies, Human Choices: Summary of the Responses to the Consultation Report*. April 2003 available at www.dh.gov.uk.

27. Department of Health, *Saving Lives, Valuing Donors: A Transplant Framework for England*. July 2003 available at www.dh.gov.uk.

28. *Joint UKBTS/NIBSC Professional Advisory Committee's Position Statements* available at www.transfusionguidelines.org.uk.

Documents from the Council of Europe

29. Council of Europe, *Convention for the Protection of Human Rights and Dignity of the Human Being with Regard to the Application of Biology and Medicine: Convention on Human Rights and Biomedicine*. Oviedo, 4IV. 1997, European Treaty Services/164 www.conventions.coe.int.

30. *Solutions for Organ Preservation*. European Pharmacopoeia, monograph No. 1264 (supplement 5).

31. Council of Europe (2004) '*Guide to the safety and quality assurance for organs, tissues and cells, Second Edition*'. ISBN 92-871-4891-0 available from Council of Europe publishing at www.coe.int.

European Union Directives

These are listed in Chapter 1.

32. Directive 2004/23/EC *'on setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells'. OJ*, L 102, 07.04.2004, p48.

33. Commission Directives on Technical Aspects expected in 2005/2006.

These Directives will be legally binding throughout the UK from April 2006.

Chapter 22

Tissue banking: selection of donors

22.1 General considerations

The overall responsibility for applying the policies for the selection and care of tissue donors lies with the tissue bank designated medical officer, who must have relevant clinical experience and will be familiar with the various legal statutes and relevant documents which apply to tissue banking. The tissue bank designated medical officer must consult with relevant specialist advisors as appropriate.

The designated medical officer will rely on procedures and documentation that enable the appropriate medical and behavioural history to be acquired, to prevent microbial infection and transmission of disease (including malignant or neurodegenerative disease) to the recipient. Decisions on donor assessment should be consistent with JPAC *Donor Selection Guidelines*.[1]

The designated medical officer will be responsible for policies regarding consent and counselling.

Tissues must be procured, transported, processed, stored and distributed according to the requirements stated in these guidelines.

Procedures must be in place to document a complete audit trail from donor to recipient. Tissue banks must ensure that tissues can be traced from the donor to the point of issue. It is the responsibility of the hospital to document the fate of the tissue from its receipt to its use or discard. This will ensure that the audit trail can be followed in both directions. Clinicians caring for the recipients of tissues associated with risks identified following the issue of tissue must be informed where pertinent. Mechanisms should be in place to ensure that confidentiality is maximized.

United Kingdom Blood Transfusion Services Tissue Banks may collect tissues from donors referred to them by a third party such as a Donor Transplant Co-ordinator or another tissue bank and may also refer donors to other tissue banking agencies such as a cornea or research bank. Whenever the information regarding donor medical and behavioural history and/or consent for donation is obtained by, or on behalf of a third party this must be subject to a written agreement between the parties involved. The agreement must specify what information is required regarding the medical and behavioural history of the donor and consent for donation, the standards for obtaining this information and the responsibilities of both parties in ensuring that the information is accurate and properly documented. The information should, as a minimum, be provided in accordance with the guidance in this document and the current JPAC *Donor Selection Guidelines*.[1] It is the responsibility of the designated medical officer to determine the bank's policy for the referral of donors.

22.2 Consent

Consent must be obtained by appropriately trained professionals competent in the issues and processes of tissue donation. No coercion or inducement to donate must be applied during the consent procedure.

For living donors written consent must be obtained. These donors must be competent to give consent before donations can be accepted. Consent must cover retrieval, testing (including for HIV), discard and access to medical records. If there are circumstances where the tissue may be used for research and development, or teaching, specific consent must be obtained for this as well. Explicit information must be given if tissues are to be retrieved for specific commercial use. Where donors are not competent, the guidance of the Nuffield Council of Bioethics: *Human Tissue: ethical and legal issues*[2] must be followed.

There is no legal requirement for written consent for cadaver tissue retrieval. It is current practice to confirm and document lack of objection from the next of kin. It should be established that a surviving spouse or partner or living first-degree relative does not object. It is adequate to speak to one member of the family in order to establish the wishes of the other family members. Consent must cover retrieval, testing, discard and access to medical records. If there are circumstances where the tissue may be used for research and development, or teaching, specific consent must be obtained for this as well. Explicit information must be given if tissues are to be retrieved for specific commercial use.

Living donors and families of cadaveric donors must be informed that information relating to the donation will be stored in accordance with the Data Protection Act and may be shared with relevant healthcare professionals.

For cadaveric donors, information to be supplied to the next of kin regarding various aspects of tissue donation which forms the basis of consent should include the following:

- that reconstruction will be performed following retrieval
- explicit information on which tissue is to be retrieved and the clinical purpose to which it is to be put
- if tissue is found to be unsuitable for clinical transplantation it will be discarded via local discard policies or, if permission is granted, it may be used for research or educational purposes
- that the donor will be tested for markers of microbial infection including HIV and after individual case assessment, those relevant contacts will be informed in the event of a relevant confirmed positive result
- that details of medical and behavioural history will be sought from additional professional sources and recorded.

Where the Coroner (the Procurator Fiscal in Scotland) is in legal possession of the body, permission must be requested to undertake the retrieval.

22.3 Medical and behavioural history

For living donors

Medical and behavioural history must be sought by appropriately trained professionals and in compliance with the following guidance.

- Information must be obtained by face-to-face interview with the donor. This must allow for the exclusion of lifestyle infectious risks. During interviews, a mechanism should be in place to ensure that confidentiality is maximized.
- The interview must be conducted while the donor is free from the effect of anaesthetic, hypnotic, or narcotic medication. The donor must be mentally competent to give an accurate history. If the donor is a minor, the interview must be conducted with the parents or guardian.

- If the medical interview is not done at the time of admission for surgery, a system must be in place to capture any relevant medical and behavioural history changes that may occur in the interval between interview and donation.
- A standard questionnaire to elicit the medical and behavioural history must be used.
- Donors should be selected according to the JPAC *Donor Selection Guidelines*[1]
- The completed questionnaire must be retained as part of the tissue bank donor record.
- The medical records, if available, must be consulted to review the medical and behavioural history and the medical examination.

Further medical history may be sought, where appropriate, from:

- the general practitioner
- any other relevant medical personnel.

For cadaver donors

The cause of death and the medical and behavioural history should elicit whether the donor meets the selection criteria outlined in the JPAC *Donor Selection Guidelines*.[1] Modifications for the behavioural and medical history questions may be needed when accepting paediatric donors. Where the cadaver donor is less than 18 months of age, or breast fed within the 12-month period prior to donation, the mother's risk for transmissible disease must also be evaluated. Information must be sought from the following sources by appropriately trained professionals and must be documented using a standard form:

- the donor's next of kin or other person identified as the most likely to be in possession of relevant information
- the medical notes if the donor was admitted to hospital prior to death
- the general practitioner
- the post-mortem (where one is undertaken). If no post-mortem is undertaken, the cause of death of the donor, as ascertained from the medical notes, must be documented in the tissue bank donor record.

A record must be made of how the donor was identified, e.g. toe tag, wristband, etc.

Cadaver donors must be examined at the time of retrieval. The cadaver donor's external appearance should be examined from all aspects. The appearance must be documented with respect to the donor's medical and behavioural history, including the presence of any obvious medical intervention, scars, tattoos, skin or mucosal lesions, jaundice, infection, trauma, or needle tracks.

A note should be made of when the donor's death was recorded and by whom. The estimated time of death must be documented.

All the above information for living and cadaveric donors should be reviewed by the designated medical officer or designee who is familiar with the relevant standards in the field of tissue banking (see Chapter 21).

22.4 Tissue-specific donor considerations

Reference must be made to the JPAC *Donor Selection Guidelines*[1] document for ages and specific donor requirements for different tissues.

22.5 Donor testing

The general principles of microbiological testing that apply to living blood donors (see Chapter 10) will also apply to the testing of tissue donors. In particular, assays should be

validated, approved by United Kingdom Blood Transfusion Services as fit for purpose and compliant with Directive 98/79/EC on '*in vitro* diagnostic medical devices'.[3] When assays have been validated for post-mortem blood samples and have been evaluated and approved by the United Kingdom Blood Transfusion Services, such assays must exclusively be used if no ante-mortem sample is available. Mandatory testing must only be undertaken by Clinical Pathology Accreditation (CPA) accredited laboratories or in Medicines and Healthcare Products Regulatory Agency (MHRA) licensed laboratories. If a third-party laboratory is used to perform any aspect of donor testing, the specific requirements and responsibilities of both parties in achieving them must be defined in a written agreement. Such testing should, as a minimum, be performed in accordance with the guidance in this document.

There should be protocols for assuring the veracity and security of the sample, labelling, and supporting documentation.

The tissue bank should have a documented policy to follow in the case of live or cadaver donors, with repeat reactive screening tests (see Chapter 10). There should be protocols for retesting, if appropriate, confirmatory testing, counselling of donors and contacts and acceptance or rejection of donations. Reports of positive tests should be included in the routine donor surveillance programmes and notified to the appropriate statutory authority.

Rh D testing may be required on donors if the retrieved tissues will contain residual red cells or red cell membranes at the time of implantation.

An archive blood sample must be kept for look-back investigations in the event of an adverse reaction. This must be for a minimum of 11 years after the expiry date of the tissue with the longest storage life. Consideration should be given for an additional blood sample archive for tissues with a long expiry for possible future testing that is not currently available.

When new mandatory tests are introduced consideration should be given to the re-testing of archive samples from the donors of tissue still in issuable stock.

22.6 Testing of living donors

The time from sample acquisition to testing or freezing of the sample should be minimized and must be consistent with test kit manufacturers' recommendations or validated for the purpose.

All blood samples from living donors must be acquired using positive donor identification by an individual trained to ensure the security of the sample and supporting documentation.

A sample taken up to seven days prior to or 24 hours post-donation is considered a current sample and must be tested for the mandatory markers. Due consideration should be given to dilution of the sample (see Section 22.7).

The initial sample must be tested because it enables the timely notification of infected donors and prompt discard of material known to be infectious.

The donation must be quarantined and donors retested for the mandatory markers after a minimum of 180 days from the date of retrieval.

For amnion donation, only a maternal sample taken at the time of donation and at six months post donation requires to be tested for mandatory markers of infection (not a cord blood sample).

A current sample is always preferred but, in exceptional circumstances, when a current donation sample is unavailable, a sample taken up to 30 days prior to the donation, or up to seven days post-donation is acceptable providing a post-quarantine sample is tested and found negative for anti-HBc, in addition to the mandatory markers. A system must be in place to ensure that the pre-quarantine sample reflects the risk status at the time of donation, e.g. no new sexual contact, transfusion of blood or blood products in the intervening period.

When a donation is obtained from a surgical donor who dies before the 180-day sample can be taken (a secondary cadaver), the tissue can be put to clinical use if all other criteria for accepting the donation are met and if Nucleic Acid Tests (NAT) for HIV1 and 2, hepatitis C virus and hepatitis B or for any future mandatory tests required at the time of release, are undertaken on the sample acquired at the time of donation. This would require the routine archiving of a sample appropriate for this type of testing methodology. The cause of death must be ascertained in case there is a relevant contraindication to the use of the tissue including review of information about the post-mortem examination if one was undertaken. If the cause of death cannot be ascertained, the tissue must not be used.

22.7 Testing of cadaver donors

Cadaver donors must be tested for the mandatory markers. If additional testing is employed, it must be fully documented.

An ante-mortem blood sample, up to seven days preceding death, is always preferable to post-mortem samples for testing, but appropriate mechanisms must be in place to ensure:

- the secure identification of samples obtained from hospital laboratories
- where there is doubt about the identity of a blood sample from a tissue donor (inadequate labelling), DNA profiling may be accepted as an accurate method for confirming the identity of the blood sample
- documentation of the date the sample was taken, the name of the individual and laboratory supplying the sample and sample storage conditions.

Where no ante-mortem sample is available, then a post-mortem sample can be used. The anatomical site from which the sample was obtained and the time of sampling must be documented. The sample appearance should be documented. If the sample appears dilute or grossly haemolyzed, a repeat sample preferably from an alternative site should be obtained if possible.

Tissue banks should have a protocol for post-mortem sampling, clearly defining preferred sites for sampling, e.g. cardiac puncture or femoral vessel puncture and avoiding sites close to intravenous lines.

Samples for testing must not be taken more than 24 hours post-mortem and the time from sampling to testing or freezing of the sample should be minimized and must be consistent with test kit manufacturers' recommendations or validated for the purpose.

Where a cadaver donor received ante-mortem transfusions, a pre-transfusion sample should be used for testing, consistent with the paragraph on ante-mortem blood above.

If a pre-transfusion sample is not available, tissue banks must employ an algorithm incorporating the timing, nature and volume of the fluids infused and the donor's own blood volume as well as any blood loss from the intravascular space, to assess any resultant plasma dilution (see JPAC *Donor Selection Guidelines*[1] for sample cadaveric donor intravenous fluid report form). Samples of blood estimated to be more than 50% dilute are not suitable for testing.

Screening and confirmatory tests must be performed as specified in Chapter 10. In the case of repeatably reactive screening tests confirmatory testing is required for counselling purposes.

Repeatably reactive screening tests from post-mortem samples will debar tissues from release unless a superior sample can be obtained, e.g. obtained ante-mortem or closer to the time of death, and this sample is tested and negative results are obtained from a designated diagnostic laboratory. The acquisition of the 'superior' sample must be subject to the same requirements given above.

Where the cadaver donor is less than 18 months of age a maternal sample must be tested as well as an infant sample. Maternal samples are required in the case of older children who have been breast fed within the 12-month period prior to donation.

Nucleic acid testing for mandatory markers should be carried out on both the infant's specimen as well as the mother's specimen in addition to serology testing.

22.8 Counselling

Counselling must be provided (where deemed appropriate) for living donors who, on confirmatory testing, have positive or indeterminate results. In these cases appropriate referral for further medical follow up and assessment must be ensured. Confidentiality must be ensured and the donor's permission sought prior to the counselling of relevant contacts.

For cadaveric donors individual cases must be assessed by the designated medical officer with relevant advice. Relevant contacts of cadaver donors with confirmed positive test results should be confidentially informed of such results if relevant to their health. Appropriate specialist referral should be offered.

22.9 Autologous tissue donation

The designated medical officer should decide the policy in relation to the provision of an autologous service.

Autologous donors should be tested for the mandatory microbiological markers and if positive results are obtained then the tissue should not be stored. Microbiological testing must include bacteriological culture where tissue does not undergo a validated terminal antimicrobial treatment. The medical history may be less relevant than for allogeneic donation of tissues. The rationale for any exceptions must be documented.

Separate storage must be used to avoid inappropriate issue. Autologous tissue must be securely segregated from allogeneic tissue at all stages from collection to issue. A system must be in place to enable the hospital to recognize that the tissue is autologous.

The autologous tissue must be labelled with the donor/recipient name, hospital number and date of birth.

Autologous donations may not be transferred to the allogeneic bank.

References

1. Joint UKBTS/NIBSC Professional Advisory Committee's (JPAC) *Donor Selection Guidelines* available at www.transfusionguidelines.org.uk.

2. Nuffield Council of Bioethics (1995) *Human Tissue: ethical and legal issues* available at www.nuffieldbioethics.org.

3. Directive 98/79/EC of the European Parliament and of the Council 27th October 1998 on '*in vitro* diagnostic medical devices'. *OJ*, L331, 07.12.98, p1.

Chapter 23

Tissue banking: tissue retrieval and processing

23.1 General considerations

Tissue banks should have dedicated processing and storage facilities designed and operated to prevent contamination, cross-contamination, mislabelling and deterioration of tissues.

All processes and equipment which affect the safety or quality of tissues must be validated. Tissue bank non-disposable instruments and other items which come into direct contact with donor tissue during retrieval and processing must be thoroughly washed and sterilised between uses. Where possible, disposable equipment should be used. Where this is impractical or impossible, non-disposable instruments can be used. These must be batch-dedicated to allow tracking through decontamination, sterilization and use. A risk assessment should be performed to determine the period for which instruments are kept before discard. Prompt removal of blood and tissues is an important aspect of decontamination, particularly with regard to CJD. These instruments should be washed and sterilised according to NHS Estates Health Technical Memoranda (HTM) 2010,[1] 2030[2] and 2031.[3]

All purchased materials and solutions which affect the tissue quality and safety must be inspected on receipt to ensure compliance with specification. Local policies must be in place to minimize the risk of tissue contamination by staff during retrieval and processing.

UK Blood Transfusion Services tissue banks may use third parties to perform tissue retrieval, processing steps such as irradiation, tissue evaluation such as bacterial tests, quality control tasks such as environmental monitoring or tissue storage, transport and distribution. Wherever such tasks are performed by or on behalf of a third party, this must be subject to a written agreement between the parties involved. This must specify the processes to be performed, the applicable standards and specifications and the responsibilities of both parties in achieving the desired outcome. The processes should be performed, as a minimum, in accordance with the guidance given in this document.

In the event of a health care worker sustaining an injury such that his/her blood comes into contact with the tissue, the tissue must be discarded.

23.2 Retrieval

Retrieval times and preliminary storage

Tissues should be retrieved as soon after death as possible. If the body has not been refrigerated, procurement of tissues should be completed within 12 hours after death. If

the body has been refrigerated within six hours of death, procurement should preferably start within 24 hours and must be completed within 48 hours of death.

Tissues must be placed at a temperature of 0–10°C within four hours of retrieval.

General considerations for tissue retrieval

Every effort must be made to minimize contamination of tissue during procurement.

The procurement facility must be suitable for procurement of tissues, which may include facilities other than an operating room.

A local sterile field must be created using sterile drapes. An appropriate anti-bacterial skin preparation agent must be used before commencing the retrieval.

All instruments used during the retrieval must be sterile and should be stored on a back table which is covered with a sterile drape. Where possible, disposable equipment should be used.

Staff conducting the retrieval must be appropriately gowned in sterile clothing, and wear sterile gloves and protective masks.

Every effort should be made to minimize the number of people present during cadaveric tissue retrieval and to ensure that a post-mortem is not proceeding during the retrieval.

Where possible the retrieval should precede any post mortem examination of the donor. In cases referred to the Coroner (or the Procurator Fiscal in Scotland), the Coroner's consent must be obtained to enable the retrieval of tissues.

Cadaveric reconstruction

It is integral to the maintenance of the dignity of the donor that the body is cleaned and reconstruction is carefully undertaken. Whenever long bones are removed they must be replaced with appropriate prostheses. All incisions should be neatly sutured.

For similar reasons, skin must not be procured from the neck, arms, face or other areas that may affect funeral viewing.

Every effort should be made to ensure that appropriate advice on the handling of cadaver donors after retrieval should be made available for mortuary and funeral home staff.

Labelling of collections

At the time of collection, the container for each category of tissue, for example skin, bone, or heart valves, must be labelled with the nature of the contained tissue and a barcoded tissue or donor identification (ID) label as appropriate.

The accompanying donation record must be labelled with the same tissue or donor identification number(s), key donor identifiers (name, date of birth, etc.), and the date of collection prior to removal from the procurement site. Bacteriology and blood samples, together with accompanying documentation where relevant, must be labelled according to agreed local procedures such that the results can be linked to the correct donor/tissue whilst still preserving anonymity where required.

A double container system is required for all tissues retrieved. The containers must not be opened until ready for use or further aseptic processing at a facility approved by the tissue bank.

23.3 Transportation conditions

For viable tissue the grafts should be placed into a transport solution with due regard to its effects on the ability of cells to propagate or metabolise, control of buffering capacity, osmolarity, tissue oxygenation, avoidance of external contamination and desiccation. The type, lot, manufacturer and the expiry date of the transport solution shall be documented.

Transportation systems must be validated to show maintenance of the required storage temperature.

23.4 Bacteriostasis and disinfection

Tissue without terminal antimicrobial processing

Tissue must be subjected to one of the following treatments, as soon as possible, within 24 hours of retrieval:

- antibiotic disinfection
- an alternative disinfection method
- deep-frozen storage at –20°C or lower.

In the case of tissue taken from heart-beating donors in the operating theatre at the time of organ retrieval, this period may be extended to 48 hours.

Tissue with terminal antimicrobial processing

Bone from living donors which is refrigerated within four hours of retrieval but not frozen until 24-48 hours after retrieval must be subjected to terminal antimicrobial processing.

Tissue with terminal antimicrobial processing must be subjected to one of the treatments detailed in the above section within 24 hours of retrieval with a maximum of 72 hours following death. A summary of the guidance regarding temperature/time relationships contained in these guidelines is given in Table 23.1.

Positive bacteriology or mycology

It is the responsibility of the designated medical officer or designated microbiologist to develop written policies regarding the selection and conduct of tests for bacterial and fungal contamination and the acceptance criteria for specific tissues.

Where tissues are shown to carry viable bacteria or fungi they may be suitable for clinical use (e.g. skin grafts) depending on microbial types and densities of growth on culture. For other tissues the material may be approved for use providing a validated antimicrobial processing technique is used.

23.5 General guidelines for tissue processing

Processing must not change the physical properties of the tissue so as to make them unacceptable for clinical use. Processing steps must be validated to demonstrate that the final product does not have any clinically significant residual toxicity.

Aseptic processing facilities

Facilities for aseptic processing must comply with the *Rules and Guidance for Pharmaceutical Manufacturers and Distributors 2002*.[4] They must provide separate work areas with defined physical and microbiological parameters. Facilities must have:

- floors, walls and ceilings of non-porous smooth surfaces that are easily sanitized
- temperature control
- air filtered through high-efficiency particulate air (HEPA) filters with appropriate pressure differential between zones, which must be documented
- a documented system for monitoring temperature, air supply conditions, particle numbers and bacterial colony forming units (environmental monitoring)
- a documented system for cleaning and disinfecting rooms and equipment
- documented system for gowning and laundry

Table 23.1 Temperature/time relationships for banked tissues

Retrieval	If the body has not been refrigerated, procurement of tissues should be completed within 12 hours after death. If the body has been refrigerated within six hours of death procurement should preferably start within 24 hours and must be completed within 48 hours of death.
Retrieved tissue	Must be placed at an ambient temperature of 0–10°C within four hours of retrieval.
Bacteriostasis	Freezing tissue to at least –20°C within 24 hours of retrieval (or up to a maximum of 72 hours of death) can be used as a bacteriostatic treatment. Bone from living donors which is not frozen until 24–48 hours after retrieval must be subjected to terminal antimicrobial processing.
Long-term storage	*Frozen tissue* may be stored 1. At –20°C or lower for up to 6 months. 2. At –40°C or lower for up to 3 years. Temporary storage of frozen musculoskeletal tissue between –20°C and –40°C is limited to six months in total. Grafts stored at this temperature must then be transferred to –40°C or colder to give an expiry of up to a maximum of three years from donation. *Cryopreserved tissue* should be stored 1. At –135°C or lower to claim a 10-year expiry for heart valves but for all other cryopreserved tissues this should be three years. 2. At higher temperatures up to –80°C; the same expiry pertains providing it has been validated. *Glycerol preserved tissue* 1. Skin preserved in high concentration (>90%) glycerol may be stored at 0–10°C for up to two years. 2. Amnion preserved in low concentration (50%) glycerol may be stored below –40°C for up to two years.
Transportation and local storage	*Frozen tissues* must be transported and stored locally prior to clinical use, at –20°C or lower in order to have the designated expiry (specified above). *Cryopreserved tissues* may be transported in the vapour phase of liquid nitrogen (<–135°C) or on dry ice (–79°C). If tissues are transported on dry ice they should continue to be stored locally at circa –80°C with a maximum expiry of six months.

- adequate space for staff and storage of sterile garments
- access limited to authorized personnel
- documented system for general staff hygiene practices.

Tissue not destined for terminal microbial processing

Air in critical work areas in which sterile containers/closures or tissue that will not be subjected to further anti-microbial treatment are used shall be of a quality that is

- certified on a quarterly basis
- alternatively, if operational environmental monitoring is in use, at least annual validation

as being of Class A with a Class B background (see Table 23.2 and for comparison Table 23.3).

Wherever possible, representative samples of tissue should be removed and tested for bacterial and fungal contamination using protocols authorised by the designated medical officer or designated microbiologist. Swabs or other validated non-destructive sampling methods should be used where it is impossible to remove tissue without damaging the

Table 23.2 Air classification system for manufacture of sterile medicinal products

Grade	Max. permitted number of particles per m^3			
	At rest		In operation	
	0.5μm	5μm	0.5μm	5μm
A	3,500	0	3,500	0
B	3,500	0	350,000	2,000
C	350,000	2,000	3,500,000	20,000
D	3,500,000	20,000	Not defined	Not defined

Rules and Guidance for Pharmaceutical Manufacturers and Distributors 2002.[4]

Table 23.3 Comparison of British, European and American classifications

EC 'Orange Guide' 2002 Grade	BS 5295 ISO 14644	US 209D 1989 Class
A, B	F 5	100
C	J 7	10,000
D	K 8	100,000
–	L	–

Rules and Guidance for Pharmaceutical Manufacturers and Distributors 2002.[4]

Table 23.4 Microbiological monitoring of controlled work areas

Grade	Air sample cfu/m^3	Settle plates cfu/4hrs	Contact plates cfu/plate	Glove print cfu/glove
A	1	1	1	1
B	10	5	5	5
C	100	50	25	–
D	200	100	50	–

graft. Microbiological inclusion/exclusion criteria should be developed by the designated medical officer or designated microbiologist in accordance with national policy.

Where tissues are processed in batches, procedures must ensure that no cross-contamination between batches can occur. Key process parameters and acceptance limits must be identified and validated. A full record of each process applied to each tissue or batch must be filed in the pool record.

Tissue destined for terminal microbial processing

Work areas in which tissue materials and containers are prepared should have an environment with air quality of at least Grade C (Class 10,000), or better, in the vicinity of exposed tissue.

Terminal anti-microbial processing must follow the filling of the final container. The procurement, processing and filling environment must be of sufficient quality to minimize the microbial contamination of the tissue to ensure that the subsequent antimicrobial processing is effective.

The tissue in its final container must be subjected to a validated procedure utilising an agent such as gamma irradiation or ethylene oxide gas. Ethylene oxide should be avoided unless there is no alternative that provides the required properties for clinical effectiveness. The processing method and dose of the sterilant should be validated as sufficient to bring about at least a six logarithms reduction in a recognised marker resistant organism e.g. *Clostridium sp*. for irradiation.

Physical or chemical indicators must be used according to manufacturer's instructions with each batch to document exposure to the sterilant, either ethylene oxide or gamma irradiation.

Gamma irradiation

Gamma irradiation must be performed in a controlled manner to ensure that all tissue receives at least the minimum specified dose of radiation. This requires the use of standard packaging materials and irradiator load configuration and is usually validated using calibrated dosimeters placed throughout the load. The dose should never be less than 15 KGy, unless pre-irradiation processing has been validated to consistently yield a low microbial bioburden such that there is the required assurance, in accordance with medical device standards, that the dose will result in the tissue being sterile.

Tissue must be irradiated in its final packaging, which must bear a suitable indicator to demonstrate that it has been irradiated. This must be checked before release of the tissue.

If a dose in excess of 25 KGy is required, then consideration must be given to the possible detrimental effect on the biological and physical properties of the tissue.

Many viruses are resistant to irradiation and therefore any claim of viral inactivation must be supported by validation data obtained using appropriate marker viruses.

Ethylene oxide

Ethylene oxide anti-microbial processing procedures must assure the maintenance of the manufacturer's recommended humidity, temperature and ethylene oxide concentration.

A biological spore test indicator (*Bacillus niger* var. *subtilis*) must be included in each ethylene oxide batch and used and interpreted according to the manufacturer's instructions.

Each type of tissue must be tested for ethylene oxide and ethylene chlorohydrin residues. The testing must be repeated each time the process is changed. The residue levels must not exceed the suggested maximum acceptable residue levels as listed in Table 23.5.

Table 23.5 Ethylene oxide and ethylene chlorohydrin residue levels (Residuals parts per million)

Medical device implant	Ethylene oxide (ppm)	Ethylene chlorohydrin (ppm)
Small (< 10 g)	250	250
Medium (10–100 g)	100	100
Large (> 100 g)	25	25

Pooling

Pooling of tissues from different donors is not permitted.

Preservation methods

Where specific attributes of a tissue are claimed the process should be validated to show these attributes are preserved.

Freeze-drying

Where tissues are freeze-dried, a sample of each type of tissue from each freeze-drying run must be analysed for residual moisture content which must be less than 5% (weight/weight) of the dry weight of the graft to allow a three-year expiry at room temperature (15°C–30°C).

Glycerolization

Where tissues are preserved by high concentrations of glycerol the procedure should be validated to demonstrate achievement of the specified glycerol concentration within the tissue or acceptable range within the tissue.

Cryopreservation

Cryopreserved tissue must be stored below –135°C to allow a 10-year expiry for heart valves and three-year expiry for other tissues. For storage at higher temperatures, validation must be performed to demonstrate that the required properties of the graft are maintained for the stated expiry.

Solutions

Rinse solutions, antibiotic mixtures, nutrient media and cryopreservation solutions must be stored at a specified temperature and with a storage period consistent with functional requirements. They must be discarded if not used within 24 hours of opening. Any solutions coming into direct contact with tissues during retrieval or processing must be sterile.

Tissue storage

Refrigeration devices containing tissue shall be suitable for the use intended and procedures for monitoring such devices shall be validated so that tissues are maintained at the required storage temperature. Continuous monitoring and recording of temperature, together with suitable alarm systems, shall be employed on all storage refrigerators, freezers and liquid nitrogen tanks.

Every effort should be made to avoid cross-contamination of material stored in liquid nitrogen vessels. Wherever possible there should be specifically designated pieces of equipment (e.g. nitrogen level rulers, portable thermometers) for each vessel. Where this is not possible (e.g. liquid nitrogen delivery hoses) and the item has to be used for more than one vessel, it should not come into contact with the liquid phase or the sides of the vessel.

Frozen tissue should be double wrapped during storage. The seals and the material employed must be validated for their use at the designated storage temperature and the conditions of use, to demonstrate integrity of the packaging and labelling. This is crucially important for storage with liquid nitrogen owing to the high levels of accumulated microbial contaminants in liquid nitrogen storage vessels (see Section 24.11, 'Storage vessels').

Quarantined and released tissue must be stored in physically segregated, clearly designated locations distinct from each other.

Tissue release

Prior to any tissue being cleared for issue, all relevant records including donor records, processing and storage records, and post-processing quality control test results must have been reviewed, approved and documented as acceptable by the individual(s) responsible according to the relevant local SOPs. Responsibilities for setting policies for exceptional release of tissues resides with the designated medical officer.

Tissue discard

There must be a documented policy for the discard of tissue unsuitable for clinical use. Records should include details of date and method of discard and reason for discard. Tissues for discard should be appropriately handled and disposed of in a manner compliant with local control of infection guidelines.

Labelling and packaging of tissues for issue

Packaging must ensure integrity and maintain sterility of the contents of the final container, and must also comply with current legislation.

The container must be labelled with the graft-specific identification, expiry date and supplying tissue bank, storage instructions and barcoded product description and instruction to see pack insert, as a minimum. In addition, more detailed information should be provided either on the label or package insert or both as follows:

- sizing information
- anti-microbial processing procedure used (if applicable)
- preservative used and its concentration (if applicable)
- special instructions (e.g. 'Do not freeze'), thawing, dilution instructions
- presence of known sensitizing substances
- type and calculated quantity of antibiotics added during processing (if applicable)
- any other potential residual processing agent
- Rh D type (where appropriate)
- a statement that the tissue was prepared from a donor who was non-reactive for current mandatory markers of infection, with the added rider that all biological tissue carries some risk of disease transmission
- results and findings from clinically relevant bacteriological cultures performed on the tissue before final packaging
- storage instructions
- instructions for reconstitution (if appropriate)
- a warning on loss of package integrity
- instructions on dealing with queries, reporting adverse events/reactions and return or disposal of unsuitable or unused tissue
- a statement that tissue use must be authorised by a medical/dental practitioner
- a statement should accompany each tissue product stating that it may not be sterilized after leaving the tissue bank
- a statement should accompany each package stipulating that each package is for single-patient use only
- if the package insert carries graft-specific information it must be labelled with the unique graft-specific identification code
- instructions to the user regarding the need for a documented system for the tracking and follow-up of the fate of the tissue.

Distribution

All reasonable efforts must be made to ensure that tissues are sent to qualified individuals/organizations who have accepted responsibility for their proper handling and use.

Where tissue is transported in a refrigerated or frozen condition, adequate safeguards should be taken to ensure that the tissue remains at the designated temperature. Monitoring of temperature should be undertaken wherever practicable but if not, the method should at least have been validated to show that appropriate temperatures are maintained.

Tracking of tissues

User audit trails must be actively encouraged.

Normally tissue should not be accepted back to a tissue bank once it has been accepted by a hospital. However, in exceptional clinical circumstances, tissue may be returned if documented, prospective steps have been taken to ensure that all storage requirements are maintained and that the integrity of the packaging is not compromised.

Mechanisms should be in place to ensure that confidentiality is maximized.

It is the responsibility of the designated medical officer to develop systems for clinical feedback.

Recall and adverse reactions

Guidelines for dealing with complaints and adverse reactions for blood and blood products should also be applied to tissues.

Where the nature of an adverse event may have implications for other recipients of tissues or organs from the same donor, this must be communicated to the relevant clinicians, tissue bank and transplant coordinators as appropriate. It is the responsibility of the designated medical officer to embargo tissue or institute a recall of tissue when indicated.

23.6 Additional guidelines for skeletal tissue retrieval and processing

Procurement of surgically removed bone

A system of documentation must be in place to ensure that theatre staff are clearly informed that a particular patient has or has not consented to bone donation. This may be by enclosing a copy of the consent form in the patient's notes, or some equivalent method.

Where bones are retrieved during surgery by theatre staff on behalf of the tissue bank, these staff must follow a protocol provided by the tissue bank in accordance with third party agreements.

The removed bone should be placed, as quickly as possible, in a sterile container and labelled in a manner to distinguish it from cleared issued bone.

If the donated bone is not destined for terminal antimicrobial processing, it must be cultured for microbial contamination at the time of collection, using a collection and transport system provided by, or approved by, the tissue bank. Bone sampling must be carried out immediately prior to closing the bone container.

Tissue samples for culture should comprise of chips of bone from the cut end of the bone, which should be placed in appropriate transport or culture media. The bone should be finally packaged in a double sterile container.

The bone container, tissue samples and blood samples, if collected at this time, must be clearly labelled with the barcoded donation number and stored cool until collection.

A secure system utilizing barcodes for the identification and linkage of the donation to the donor and samples must be in place.

Documentation must be completed in theatre, detailing the time of bone retrieval and providing the identity of the staff members carrying out the retrieval and labelling.

Alternatively protocols can be put in place to arrange for the hospital blood bank or other appropriate laboratory, to separate serum from the blood samples and to store it and the donation at –20°C or lower, for collection at a later date. Testing should be performed within one month of sampling.

Bone which is not subject to antimicrobial processing can only be released for use if cultures for aerobic and anaerobic bacteria, and fungi are negative.

Where environmental contaminants are detected on surgically retrieved bone, this bone may be further processed and exposed to either gamma irradiation (>1.5 megarads = >15KGy) or ethylene oxide (see Section 23.5).

Procurement of skeletal tissues from cadavers

If iliac crest is to be retrieved, it should be taken last in case the bowel is perforated and stored in a separate container. Where osteochondral allografts are to be retrieved, care should be taken to avoid drying of articular surfaces. It is best to retrieve the joint entirely and to dissect it later in the laboratory.

Processing of skeletal tissues

Cycles of thawing and freezing must be minimized. Skeletal tissues should not be heated above 60°C and tendons and costal cartilage should not be warmed above 30°C.

The maximum storage period for frozen skeletal tissues depends upon the degree of prior processing and the storage temperature. Frozen bone should be stored at temperatures of –40°C or colder with the exception of short term storage (less than six months), which can be at –20°C or lower. Storage at –40°C or lower for up to three years is accepted current practice (see Table 23.1).

Osteochondral allografts, such as proximal or distal femur or femoral hemicondyles, are frozen with cryoprotectant (such as DMSO) on the articular surfaces and cooled following appropriate cryopreservation protocols. Cryopreservation of allografts must begin within 48 hours of procurement. These allografts cannot be exposed to ethylene oxide or gamma irradiation and must therefore be procured and processed aseptically.

23.7 Cardiovascular tissue retrieval and processing

General

This section predominantly relates to the banking of heart valves.

The Human Organ Transplant Act Form A must be completed for all cardiovascular tissue donations and returned to the UK Transplant Authority (NHSBT from October 2005).

Sizing and evaluation of cardiovascular tissue

Aortic and pulmonary valves should be sized at the annulus and the internal diameter recorded in millimetres. The sizing should be performed after the antibiotic decontamination.

The length of the aortic conduit, main pulmonary artery and right and left pulmonary artery remnants should be recorded.

Detailed description of the condition of the valve must be recorded in the donor processing records, which should include a grading system or schematic representation. Under no circumstances should a valve conduit be turned inside out for inspection purposes.

Valve descriptions and evaluation must accompany the allograft distribution and be made available to the surgeon on request.

Heart valves and vessels should be processed using a disinfection process which has been shown to produce decontaminated tissues.

Disinfection time must not exceed that specified in a validated disinfection regime.

Bacteriological testing of tissue

Where tissues are exposed to a decontamination step an assessment of the bacteriological status prior to decontamination must be performed.

Processed tissue must be subjected to bacterial (including *Mycobacterium tuberculosis*) and fungal testing using validated techniques. Each bank should develop a list of exclusion criteria based on type and number of contaminating organisms prior to and following decontamination.

Cryopreservation

Currently accepted optimal procedures involve controlled rate cooling of cardiovascular tissues in the presence of cryoprotectant.

Currently no recommendations can be made for non-cryopreserved valves or other cardiovascular tissues.

Storage and thawing of cardiovascular tissues

For material stored at –135°C or below, if during thawing the tissue is warmed too rapidly between the storage temperature and –100°C, fractures can occur. A validated method of thawing (e.g. on dry ice) must be used to minimize the risk. This must ensure that the valve has reached a temperature above –100°C before thawing in a 37°C water bath.

Material stored at –135°C, which is subsequently transported with solid carbon dioxide (–79°C), should be maintained in a mechanical freezer (at –80°C) if not used immediately. Thereafter, a maximum storage time of six months will pertain.

Distribution

Cryopreserved valves and vessels must be transported either in solid carbon dioxide at –79°C or in a container maintaining a temperature of –135°C or lower. Cardiovascular tissue must not be submerged in liquid nitrogen during transport.

23.8 Skin retrieval and processing

Skin retrieval

Skin sites should be shaved if necessary and treated with an anti-microbial agent such as chlorohexidine.

Samples of skin must be cultured for aerobic and anaerobic bacteria and fungi prior to and following decontamination.

Skin processing

Skin can be processed to provide an acceptable graft in a number of ways. These include cryopreservation, high concentration glycerolization and other methods. The specification for any skin product should clarify the required properties.

References

1. *NHS Estates Health Technical Memoranda (HTM) 2010 Sterilization* www.nhsestates.gov.uk.
2. *NHS Estates Health Technical Memoranda (HTM) 2030 Washer-Disinfectors* www.nhsestates.gov.uk.
3. *NHS Estates Health Technical Memoranda (HTM) 2031 Clean Steam for Sterilization* www.nhsestates.gov.uk.
4. Medicines Control Agency (2002) *Rules and Guidance for Pharmaceutical Manufacturers and Distributors 2002, Sixth Edition*. Norwich: The Stationery Office, ISBN 0-11-322559-8.

Chapter 24

Haemopoietic progenitor cells

Introduction

The advice in these Guidelines is believed to represent acceptable practice at the time of printing. It is policy to revise these Guidelines as new developments occur. However, it may not be possible to do so at the time of such change and the Guidelines should therefore be used with due regard to current acceptable practice.

The guidelines in this chapter apply to haemopoietic progenitor cell (HPC) collection, processing and transplantation activities within the UK Blood Transfusion Services (UKBTS). HPCs are taken to include bone marrow, peripheral blood progenitor cells, and cord blood progenitor cells. The guidelines must be read in conjunction with the other sections of the book including those that apply to quality systems, quality assurance and to testing of donors.

In addition to the relevant general references for tissue banking found in Chapter 21 (in particular Directive 2004/23/EC), the following specialist documents should be referred to:

1. *Standards for Haematopoietic Progenitor Cell Collection, Processing and Transplantation*. Second Edition (2003) from the Joint Accreditation Committee of ISCT-Europe and EBMT. *JACIE Accreditation Manual*. Second Edition 2003 (adapted 2005) available at www.jacie.org.

2. Hurley, C. K. (1999)'Histocompatibility testing guidelines for hematopoietic stem cell transplantation using volunteer donors: report from the World Marrow Donor Association. Quality Assurance and Donor Registries Working Groups of the World Marrow Donor Association'. *Bone Marrow Transplant.* **24**(2), pp119–21.

3. 'Standards for histocompatibility testing' (2003) available at the European Federation for Immunogenetics (EFI) standards available at www.efiweb.org.

4. National Marrow Donor Program (USA) *Standards*, 19th Edition, available at www.marrow.org/NMDP/nmdp_standards.html.

5. World Marrow Donor Association (WMDA) Standards, August 2003, available at www.worldmarrow.org.

6. British Committee for Standards in Haematology (1998) 'Guidelines for the clinical use of blood cell separators'. *Clinical and Laboratory Haematology*, **20,** pp265–78.

7. Department of Health. *Guidance notes on the processing, storage and issue of bone marrow and blood stem cells*, HSG (97)19. 24th March 1997 available at www.dh.gov.uk.

8. British Committee for Standards in Haematology (1995) 'Guidelines on the provision of facilities for the care of adult patients with haematological malignancies' (including leukaemia, lymphoma and severe bone marrow failure). *Clinical and Laboratory Haematology*, **17,** pp3–10.

9. Joint UKBTS/NIBSC Professional Advisory Committee's (JPAC) *Donor Selection Guidelines* available at www.transfusionguidelines.org.uk.

10. NETCORD-FACT *International Standards for Cord Blood Collection, Processing, Testing, Banking, Selection and Release,* Second Edition 2001, available from www.factwebsite.org.

11. Department of Health 'Safety of Blood' leaflet available in the Document Library at www.transfusionguidelines.org.uk.

24.1 Terminology

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

The principles of quality assurance as outlined in Chapter 2 apply.

24.2 Policy and procedure requirements

24.2.1 Each processing facility **must** have a medical director/advisor, appropriately qualified by training and experience. The medical director/advisor will have overall responsibility for the medical activities of the facility. This may include pre-collection evaluation and final approval of donors on the British Bone Marrow Registry (BBMR)/ Welsh Bone Marrow Donor Registry (WBMDR); alternatively this may be delegated to another designated medical practitioner.

24.2.2 Each facility **must** have a laboratory director/manager qualified by training and experience for the scope of activities carried out in the cell processing facility. The laboratory director/manager is responsible for all the administrative operations of the facility including compliance with these guidelines.

24.2.3 The facility may receive donations of HPC collected by bone marrow harvesting or stem cell apheresis from harvest centres. For unrelated donors these centres are approved by the BBMR/WBMDR or other stem cell registries according to their own specifications and are independent of Hospital Transplant Centres. Collections of cord blood HPC are carried out in centres designated by each facility.

BBMR/WBMDR donors are medically assessed and counselled by designated medical practitioners. The medical director of the registry has overall responsibility for this.

The operation of the facility should be monitored by a Steering Group in accordance with Department of Health guidance (see Chapter 21 Ref. 1 and Ref. 2). This group should include representatives of each transplant unit using the service, a representative from a non-transplant hospital, the medical advisor, the laboratory director, a microbiologist/virologist, a quality manager and a representative from the harvesting facility(ies).

24.2.4 Policies and procedures **shall** include all aspects of the operation including donor selection, assessment, consent, microbiological testing, collection, labelling, system of numbering, processing, quality management and improvement, proficiency testing, storage, including alternative storage strategies if the primary storage device fails, transportation, outcome analysis, audits, expiry dates, emergency and safety procedures, equipment and supplies, maintenance and monitoring, cleaning procedures, personnel training, disposal of medical and biohazard waste, release procedures, including criteria for

exceptional release, references, tolerance limits, corrective actions, recall, returns and discard policy.

24.2.5 A policy **must** be in place to deal with any change to the criteria for HPC acceptance/release during the period HPCs are in storage. If appropriate, further information should be sought from the donor or further testing performed on archive samples. If the new criteria cannot be met a decision should be made whether to retain or discard the HPCs and, if retained, whether they should be stored in a separate quarantine vessel. HPCs not meeting current criteria at the time of release should only be issued using a medically authorized concessionary procedure.

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

All clinical and laboratory facilities should conform to the Department of Health Code of Practice. Laboratories **must** participate in appropriate recognized external quality assurance schemes. All test methods **must** be included in the standard operating procedures manual, controlled using validated procedures and the results recorded and reported in an unambiguous format (see Chapter 2).

24.3 Protocols

24.3.1 Documentation of all research protocols performed by the facility **shall** be maintained. This **shall** include copies of research and ethics committee approvals for all relevant procedures and documentation of new drug or device exemption (clinical trials or doctors' and dentists' exemption certificates – CTX, DDX). A record should be maintained of any adverse outcome.

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

24.4 Transmissible disease

The processing facility **shall** ensure that all reasonable measures are taken to receive written notification of confirmed cases of transmissible disease in a recipient, attributable to the transplanted component, so that the appropriate action can be taken. This requirement does not apply to infections previously known to have existed in the donor, such as CMV or EBV.

24.5 Safety requirements

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

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

24.5.3 HPC collections **must** be handled and discarded with precautions that recognize the potential for exposure to infectious agents.

24.6 Donor selection, consent and testing

24.6.1 Bone marrow donors

Unrelated allogeneic bone marrow donors

General principles

- BBMR/WBMDR donors must be unpaid volunteers. Evaluation procedures must be in place to protect the safety of both donor and recipient. The potential for disease transmission from donor to recipient and the risks to the donor from the collection process must be assessed. Medical assessment of fitness to donate must be undertaken by a medical practitioner who is not involved with the transplant procedure.

- For BBMR/WBMDR donors this medical assessment has three stages. When confirmatory typing is undertaken a further limited assessment using the appropriate UKBTS questionnaire (according to the JPAC *Donor Selection Guidelines* (a)) to assess fitness to donate HPCs is undertaken. The willingness of the donor to proceed is also confirmed. At the point of final selection a more detailed assessment is carried out (see below).

- Donor medical history, physical examination and testing must be completed, and the donor found to be suitable and to have given informed written consent (this should include prospective consent for discard after an acceptable period of time has elapsed; see Section 24.18), before conditioning therapy is commenced in the recipient. Relevant results should be made available and reported in writing to the harvest and transplant centre as appropriate. Recommendations for donor follow-up should be documented by the centre assessing the donor. Significant abnormal findings, including markers of infection, must be reported to the potential donor with relevant advice and counselling. Where these do not result in donor deferral and are relevant to the health of the recipient, they must be reported to the transplant centre, with the donor's consent.

- The use of a donor not meeting all criteria shall require careful consideration and documentation of the reason for his/her selection by the assessing physician in consultation with the medical director/advisor at the time of final selection at the donor centre. If there is any increased risk to the donor, this must be fully explained at the time informed consent is sought. Where there is potential risk to the recipient, the transplant physician must also be involved in the decision and must document the rationale for the selection of the donor and obtain informed consent from the recipient (or their parents or legal guardians). Microbiological guidance provided by Microbiological Safety of Blood and Tissues (MSBT)[7] should be followed.

- Where the results of mandatory microbial markers do not test negative and the donor has been accepted, the results must be communicated to the relevant parties. These include the laboratory processing facility and the transplant physician.

Medical history, physical examination and testing

- The donor medical assessment must include:
  - age: criteria for registries may vary, e.g. BBMR donors must be aged 18-55 years
  - previous and current infections and other illnesses, e.g. history of malignancy, systemic autoimmune disease, diseases of unknown aetiology, active systemic infections, and hereditary disease of haemopoiesis, immunity or metabolism
  - if female: pregnancy assessment and test if appropriate
  - history of medication
  - immunization history

- travel history (malaria, *T. cruzi* risk)
- behavioural history (HIV and hepatitis risk)
- transfusion history
- history of body piercing and tattoos in previous 12 months
- history of allergies including latex allergy
- risk factors for prion associated disorders
- any previous problems with general anaesthesia
- any history of neck or back problems
- body mass index.

Policies for donor exclusion are defined by each registry to ensure donor and recipient safety. Reference should be made to the Department of Health 'Safety of Blood' leaflet[11] for lifestyle exclusions and to the JPAC *Donor Selection Guidelines*.[9]

- Donor examination/testing should include:
  - at initial registration: HLA-A, -B, -DR type by a CPA or European Federation of Immunogenetics (EFI) accredited laboratory. As a minimum these antigens should be defined at low/medium resolution level using DNA techniques. ABO group and Rh D type (Chapter 14)
  - malaria antibody testing and testing for *T. cruzi* as indicated
  - sickle test if indicated
  - confirmatory typing (CT): HLA-A, -B, -C types defined at low/medium resolution level using DNA techniques. HLA-DRB1, -DQB1 by molecular techniques to the allelic level
  - after final selection from register: full physical examination by a designated medical practitioner independent of the transplant centre to include blood pressure; height and weight measurements; ABO and RhD type; extended blood group phenotype; red cell antibody screen; full blood count; liver function tests including total protein; coagulation screen; urinalysis; electrolytes; random blood glucose; urea or creatinine; electrocardiogram; chest X-ray (if aged over 45 years or clinical assessment indicates it); pregnancy assessment (if female with childbearing potential); assessment of venous access for potential subsequent leucapheresis. In donors selected for HPC collection, more detailed typing should be undertaken to include molecular typing for HLA-C, -DRB3, -DRB4, -DRB5, and -DQB1
  - mandatory infectious disease testing within a 30-day period prior to collection for all potential donors as given in Chapter 10. In addition, infectious disease markers at the transplant centre's request may include: CMV; HSV; VZV; EBV; toxoplasmosis.

Donor counselling and consent

- Counselling: appropriate information should be given to potential donors at all three times of assessment:
  - recruitment: the donor should be given relevant general literature
  - confirmatory typing: more detailed information about testing and HPC collection procedure should be provided

- at final selection from the register: the entire procedure shall be explained in terms the donor can understand by an appropriately trained designated registered medical practitioner or counsellor and shall include information about: the significant risks of the procedure; tests to be performed to protect the health of the donor and recipient; the policy of informing donors of significant abnormal results; the right to withdraw from the donation up to the point of the recipient's conditioning; the risk of death for the recipient if the donor withdraws after the recipient's conditioning therapy has started; anonymity policy; insurance arrangements; reimbursement of expenses.

- Consent: the donor must be competent to give and have given written consent before conditioning therapy is given to the recipient. Donor consent must be obtained for the collection procedure, testing for markers of infection and for the eventual use of the HPCs for clinical purposes or, if unsuitable or not required, for discard or approved research. This should be in accordance with current UKBTS policies for the non-clinical use of donated material.

Autologous blood

One or more units of autologous blood, according to the weights of both donor and recipient, may be collected in the 28 days prior to bone marrow harvesting but not within four days of the harvest. This practice is currently under review. In exceptional circumstances, if allogeneic blood has to be used, it should be irradiated, CMV negative and matched for Kell and an extended Rh phenotype.

Anonymity

Anonymity should be maintained between BBMR/WBMDR donors and recipients for life.

Donor follow-up

BBMR/WBMDR policies should apply for follow-up.

Related allogeneic bone marrow donors

In general, the guidance given in Section 24.6.1, Bone marrow donors, applies where HPC collections are processed by the HP processing facility. Responsibility for donor care rests with the referring transplant centre or harvest centre performing the harvest on their behalf.

Autologous bone marrow donors

Donor assessment, consent and counselling are not within the scope of these guidelines, however testing of autologous BM donors is included within the scope. Consent must refer to an HPC discard policy (see Section 24.6.1, Donor counselling and consent).

24.6.2 Peripheral blood progenitor cell donors

PBPC may be collected after mobilization with G-CSF, licensed for PBPC mobilization. The choice between PBSC or BM donation will rest finally with the donor.

Unrelated allogeneic peripheral blood progenitor cell donors

Donor assessment, testing and selection

Section 24.6.1, Unrelated allogeneic bone marrow donors (except autologous blood) applies. A donor of PBPC must be found fit for both apheresis and G-CSF administration and also to undergo a limited bone marrow harvest in the event of failure to mobilize PBPC. As donors would generally not have pre-deposited autologous blood in these circumstances, the volume of BM harvested should not exceed that which can be safely taken without transfusion. Donors should have a final assessment to establish their fitness to donate and the safety of their HPCs for the recipient immediately before each collection. A full blood count, including platelet count, shall be performed within 72 hours prior to the first collection and within 24 hours after each apheresis. Policies should be in

place for dealing with high white cell or low platelet counts before and after HPC collection. In the case of multiple collections from the same donor, infectious disease tests in Chapter 10 shall have been performed within 30 days prior to each collection. A system should be in place to ensure that the pre-collection sample reflects the risk status at the time of collection.

Counselling and consent

Section 24.6.1, Donor counselling and consent, applies.

Additionally, specific counselling and consent is required for the use of mobilising agents such as G-CSF. Ordinarily the donor will be offered a choice between bone marrow and PBPC donation (see above). Ordinarily two PBPC collections would be undertaken but the donor should be advised that, in the event of a failed or inadequate collection, it may be necessary to request a third PBPC or a BM collection.

Related allogeneic peripheral blood progenitor cell donors

See Section 24.6.1, Related allogeneic bone marrow donors, and Section 24.6.2, Unrelated allogeneic blood progenitor cell donors.

Autologous peripheral blood progenitor cell donors

See Section 24.6.1, Autologous bone marrow donors.

24.6.3 Second donations

These are requests for further donations, for the same patient, of marrow, peripheral blood or leucocyte infusions from donors who have previously given an HPC donation. Individual assessment of each request is required. This **must** include further medical assessment with appropriate testing, counselling and consent.

After the initial donation the donor should be asked if they are prepared to be approached and asked to donate again for the same patient.Additionally the donor should be asked whether they wish their name to remain on the donor register. Donors **must** not be approached for a donation if they have previously indicated that they are unwilling or unable to donate. The donor may be considered for HPC donation for a second patient after an interval of at least one year.

Donor leucocyte infusions (DLI)

Unrelated DLI

- General principles: these requests **shall** be assessed by the medical director/advisor of the registry assisted by an advisory group in cases where predefined acceptance criteria are not met. Section 24.6.1, General principles, first paragraph above applies. There must be a reasonable expectation of patient benefit from DLI.

- Assessment, testing and selection: potential donors will have previously donated HPC. A designated medical practitioner not involved in the recipient's care **must** assess whether the donor's medical fitness has changed significantly since his/her HPC donation and confirm that the donor is suitable for apheresis with respect to peripheral venous access and weight (see Chapter 6). Depending on the length of time since the previous HPC donation, it will be necessary to undertake either full or limited medical assessment in line with procedures for BM donation (See Section 24.6.1, Confirmatory typing and mandatory infectious disease testing).

- All donors require repeat blood pressure and weight measurements, ABO and RhD type; red cell antibody screen, full blood count, coagulation screen, electrolyte screen, liver function tests and mandatory infectious disease testing (see Chapter 10) as a minimum. If less than 12 months has elapsed since their HPC donation an ECG or CXR would not necessarily be required.

- Donors should have a final assessment to establish their fitness to donate and the safety of their HPCs for the recipient immediately before each collection.
- Counselling and consent: Section 24.6.1, Counselling, on final procedure above applies excluding reference to conditioning therapy in the recipient.

Related DLI

In general guidelines as for unrelated DLI apply.

24.6.4 Allogeneic cord blood (CB) donors

Unrelated CB donors

Donor assessment, testing and selection

Mothers of cord blood donors must be unpaid volunteers. Evaluation procedures must be in place to protect the safety of the potential recipient. The potential for disease transmission from donor to recipient must be assessed in line with blood safety (see Chapter 3). All mandatory microbiology testing of the mother for banking must be complete and documented before the unit is made available for search. Additional tests required at the time of selection must be complete and documented results available for the transplant centre before conditioning therapy is commenced in the recipient.

- Maternal assessment: this must be made by an appropriately trained staff member working to documented protocols as agreed by the medical director/advisor, and should include the following information: serious illness past and present; significant infection past and present; lifestyle/HIV risk; medication; transfusion history; body piercing; travel history; CJD risk factors; family history of inherited disease; health of previous children; pregnancy and delivery history; immediate postnatal health. A personal and family history of the biological father, if available, should also be documented. In the case of surrogate mothers, information from the biological parents as well as the surrogate mother **must** be sought. Donors should be selected in accordance with the JPAC *Donor Selection Guidelines*.[9]
- Infant assessment: A method should be documented for assessing the postnatal health of the infant, by obtaining a medical history, examining relevant medical records or by additional enquiries as relevant.
- Testing: see Section 24.12.

  The cord blood unit and a maternal sample, taken between day 0 and day 7 post-donation, **must** be tested prior to issue for mandatory infectious disease markers (see Section 24.6.1, Bone marrow donors, Mandatory infection disease testing) by an appropriately licensed laboratory. In addition samples **must** be tested for anti-HBc. Tests may be repeated after 180 days. If this is not done a test of higher sensitivity, e.g. PCR or antigen testing may be performed on an initial sample that will have been archived, but this is currently under discussion. The CB unit should be tested by PCR for CMV.

  Markers of infection in the mother and/or cord blood donation **must** be reported confidentially to the mother and appropriate counselling should be offered. Recommendations for follow-up should be documented. A confirmed positive microbiology marker in the sample from either the mother or the CB donation for the tests outlined above, with the exception of CMV, **will** exclude the relevant unit from the bank.

  Testing **must** include HLA-A, -B, -DR type by a CPA or EFI accredited laboratory; ABO and Rh D type (See Section 24.6.1, Donor examination and testing).

  A sample from the donation must be cultured under aerobic and anaerobic conditions to identify bacterial and fungal contamination.

Counselling and consent

Detailed information **must** be provided for mothers of potential CB donors in terms and translations the mother can understand before consent is requested. Explanations **must** include the overall purpose; the possible risks, benefits and alternatives of CB donation. In the case of a surrogate mother informed consent should be sought from both the biological parents and surrogate mother. The consent should cover:

- the reason for collection
- the collection method
- testing of maternal samples and CB for markers of infection relevant to either her or her child's health and to ensure safety for a potential recipient, and that no collection will be performed if the mother should test positive for a microbiology marker
- the policy of informing mothers of significant positive markers of infection and, in exceptional circumstances, consent to inform the mother's or child's physician in the case of a positive microbial markers or other information relevant to the health of the mother or child
- the risk that the donation may not be successful
- in the case of unrelated cord blood donors, that the donation will not be kept for use by the donor family and that there will be anonymity between donor and recipient
- the use of the donation for clinical purposes or, if unsuitable or not required, for validation, quality control or approved research. This should be in accordance with current UKBTS policies for non-clinical use of donated material
- discard policy if the donation is unsuitable or insufficient
- the right to refuse without prejudice.

Written informed maternal consent **is always** required before microbiological testing is undertaken. The timing of consent in relation to CB collection may differ according to the collection method but **must** be obtained prior to or within seven days after delivery of the child. Written informed consent **must not** be sought while the mother is in active labour or under the effects of anaesthesia.

Related CB donors

General principles

The reason for CB collection should be recorded and there should be a reasonable expectation of patient benefit determined by the physician caring for the potential recipient.

Assessment, testing and selection

Section 24.6.4, Unrelated CB donors applies.

Counselling and consent

Details outlined in Section 24.6.4, Counselling and consent apply. In addition consent should cover the risk that the donation may not be suitably matched with the patient and consent for third-party use or discard of cord blood HPCs if this were the case.

24.7 Haemopoietic progenitor cell collection facilities

24.7.1 General

HPC should only be collected in a hospital facility or blood transfusion service apheresis unit with appropriate experience (see Chapter 6, Component donation: apheresis) and which meets the standards required for the Department of Health Code of Practice and

the requirements of JACIE and NETCORD-FACT standards and Directive 2004/23/EC from April 2006.[1,10] Where the laboratory receives a harvest performed by a third party the responsibility and quality standards of the harvest centre should be clearly defined in a service level agreement.

The unit **will** be headed by a physician/director who will have a minimum of 12 months' experience of HPC collection and who has performed or supervised at least 10 collections of each type (BM/PBPC).

The physician/director should be satisfied that a minimum number of procedures per annum of each type undertaken is carried out to ensure the continuing competence of medical, scientific and technical staff.

Progenitor cell assays

The laboratory undertaking progenitor cell assays should be accredited where this is appropriate and must take part in EQA schemes for CD34+ quantitation. Proficiency testing for clonogenic cells where applicable.

There should be a written request from the donor's physician before collection is begun or processing is initiated (except in the case of unrelated cord blood collection).

24.7.2 Bone marrow collection

The physician who is responsible for the harvest must be satisfied that the following are available:

- full physical assessment of donor pre-harvest
- a consultant anaesthetist should take responsibility for the care of the donor during the harvest procedure
- fully trained operating theatre staff
- intensive care and resuscitation facilities on-site
- CPA-accredited blood bank providing 24-hour component support including CMV negative (or leucodepleted) and irradiated blood products
- appropriate arrangements made for post-harvest follow-up of the donor.

24.7.3 Peripheral blood progenitor cell collection

Apheresis to collect HPC will normally be carried out in a hospital facility or blood transfusion service experienced in therapeutic apheresis (see Chapter 6).

Methods used for collection **shall** be aseptic and **shall** use procedures validated to result in acceptable progenitor cell viability and recovery.

Physicians prescribing human growth factors **must** be experienced in their use.

Donors and recipients undergoing progenitor cell mobilization **must** have access to advice and medical supervision 24 hours a day.

The centre should have written procedures for human growth factor administration, apheresis procedures and long-term donor follow-up as appropriate. For procedures performed by third parties the quality framework and responsibilities should be covered by a written agreement.

Venous access

- Peripheral veins should be used for venous access for unrelated donors in all but exceptional circumstances.
- For autologous HPC collections and in exceptional circumstances for unrelated or sibling allogeneic donors, where access via peripheral veins is not feasible and appropriate consent is obtained, central venous catheterization (e.g. via the femoral or other route) may be considered.

- The placing of central catheters should only be undertaken in hospital facilities with access to intensive care and radiology facilities by highly trained staff who regularly perform this procedure.
- UKBTS collection centres **must** ensure that the adequacy of central venous catheterization has been confirmed radiographically, except in the case of femoral lines, where this is not required.

A suitably trained doctor **must** be immediately available on the premises at all times (see Chapter 6).

Records **shall** be kept of any adverse reactions during the apheresis procedure including those that arise when donors are given growth factors by or on behalf of UKBTS staff as part of HPC mobilization procedures (see also Chapter 6).

24.7.4 Cord Blood (CB)

CB will normally be collected in a hospital facility with established collection procedures that will protect the mother and infant. For unrelated CB collection a written agreement, defining responsibilities and expectations, **must** exist between the CB Bank and the obstetric unit of the collection hospital. Delivery practice **must** not be modified in an attempt to increase the volume of cord blood collected.

Methods used **shall** be aseptic and use procedures aimed at maximizing the volume of blood harvested from the placenta with acceptable cell viability, whilst minimizing the risk of contamination.

Ex utero collections **must** be performed in a suitable, clean dedicated area.

A documented procedure **must** be followed for the identification of CB units and for confirming the link with the mother.

24.8 Component definitions

24.8.1 **Unmanipulated components** are HPCs as obtained at the time of collection and not subjected to any form of manipulation.

24.8.2 **Minimally manipulated components** are HPCs that have not been subjected to an ex vivo procedure(s) that selectively removes, enriches, expands or functionally alters progenitor, tumour- or T-cell populations. For the purposes of these guidelines, removal of polymorphonuclear leucocytes, red cells and plasma shall be considered as minimal manipulation.

Bone marrow (BM): HPCs aspirated from the iliac crests, sternum or other bones.

- BM plasma depleted: marrow cells remaining after plasma has been depleted by sedimentation or centrifugation using devices, supplies and techniques validated for the procedure(s).
- BM red cell depleted: marrow cells remaining after depletion of mature erythrocytes by sedimentation, centrifugation, or lysis using devices, supplies and techniques validated for the procedure(s).
- BM buffy coat preparation: marrow cells remaining after depletion of mature erythrocytes and plasma by sedimentation or centrifugation using devices, supplies and techniques validated for the procedure(s).
- BM density-separated cell preparation: marrow cells remaining after depletion of mature erythrocytes, polymorphonuclear leucocytes and plasma by techniques using devices or reagents validated for the separation of cells based on density.
- BM cryopreserved: buffy coat or density-separated marrow cells cryopreserved using devices, supplies and techniques validated for the procedure(s). Section 24.11 applies.

PBPC: HPCs collected from the peripheral blood using an apheresis technique. This yields a buffy coat preparation in which the majority of mature erythrocytes have been depleted. Further depletion of plasma may be undertaken by sedimentation or centrifugation using devices, supplies and techniques validated for the procedure(s).

- PBPC buffy coat preparation: progenitor cells remaining after depletion of the mature erythrocytes and plasma by sedimentation or centrifugation using devices, supplies and techniques validated for the procedure(s).
- PBPC density-separated cell preparation: progenitor cells remaining after depletion of mature erythrocytes, polymorphonuclear leucocytes and plasma by techniques using devices or reagents validated for the separation of cells based on density.
- PBPC cryopreserved: progenitor cells cryopreserved using devices, supplies and techniques validated for the procedure(s). Section 24.11 applies.

Cord blood (CB): HPCs collected from placental and/or umbilical vessels after the umbilical cord is clamped.

- CB buffy coat preparation: progenitor cells remaining after depletion of erythrocytes and plasma by sedimentation or centrifugation using devices, supplies and techniques validated for the procedure(s).
- CB cryopreserved: progenitor cells cryopreserved using devices, supplies and techniques validated for the procedure. Section 24.11 applies.

24.8.3 Manipulated components

These are defined as HPCs that have been subject to a procedure(s) that selectively removes, enriches, expands or functionally alters specific nucleated cell populations. Manipulation of HPCs includes but is not limited to:

- depletion of one or more populations of cells ('purging')
- enrichment of one or more HPC populations ('positive selection')
- expansion in culture of one or more populations of HPCs ('*ex vivo* expansion')
- insertion of one or more genes into one or more populations of HPCs ('genemanipulation').

24.9 Haemopoietic progenitor cell processing standards

24.9.1 Personnel and facilities

The HPC processing facility **shall** have a medical director/advisor (see Section 24.2.1). The medical director/advisor **shall** have responsibility and authority for all medical aspects of the programme including compliance with national and local guidelines as well as ensuring compliance with regulatory requirements.

The laboratory director/manager is responsible for the operational management and technical aspects of the service. There **shall** be adequate numbers of staff whose training and competency to perform the assigned procedures **must** be documented (see Section 24.2.1).

The facilities **must** comply with the Department of Health. *A Code of Practice for Tissue Banks: Providing Tissues of Human Origin for Therapeutic Purposes* (Ref. 1, Chapter 21) and the Medicines Control Agency (2002) *Rules and Guidance for Pharmaceutical Manufacturers and Distributors 2002* (Ref. 18, Chapter 21) and, from April 2006, Directive 2004/23/EC. Open processing as defined, **must** be performed in a facility which is regularly validated as achieving Grade A in the critical work area with a Grade B background.

There must be documented evidence of validated procedures for cleaning, gowning, microbiological environmental monitoring and particle counting **must** to ensure maintenance of the required environmental conditions.

24.9.2 Procedures

Before processing there should be written request from the transplant physician. This is not required for unrelated CB collections.

Processing should be performed according to written procedures and policies. Any deviation from such written procedures shall be documented and reviewed by the medical director/advisor.

The objectives and suitable end points for each procedure will be specified and agreed with the transplant physician.

Following processing, if the agreed end points of processing have not been met then the transplant physician must be notified and this documented.

All procedures, including manipulations of the collection, should be validated to ensure that suitable end points can be ensured.

Upon completion of processing the collection should be packaged and labelled as specified in Section 24.13.

Before material is accepted from a third party including receipt from abroad the laboratory accepting the donation should, wherever possible, ensure that standards equivalent to those in UK guidelines, have been met. HPCs imported from abroad should be accompanied by a trained person or handled by a specialist company.

24.9.3 HPC and DLI processing

Material should be inspected upon receipt and the condition of the product recorded. Where appropriate the HPC donation should be passed through a sterile non-reactive aggregate filter to remove fat, clots or bone spicules that may be present. A closed system **shall** be used wherever practical.

If required, reduction of the volume of plasma and red cells in the collection can be achieved by centrifugation for buffy coat preparations or density separation using devices, supplies or reagents validated for these procedures. All manipulations and transfers should use aseptic techniques. Where there is clinically significant ABO or other anti-donor red cell alloantibody in the recipient, HPCs should be processed, at the request of the transplant unit, to manufacture a product of low haematocrit without significant loss of non-nuclear cells.

T-cell depletion may be performed either by reducing the donation to a mononuclear fraction or buffy coat and then incubating with monoclonal antibodies and a source of complement or by positive selection of CD34 positive cells using immunoaffinity.

Autologous HPC collections may be reduced to either a mononuclear fraction or buffy coat prior to cryopreservation. The collection may be purged, e.g. using monoclonal antibodies, or CD34 cell selected to reduce possible tumour cell contamination before cryopreservation.

Cord blood progenitor cells

These are usually cryopreserved for lengthy periods. Unrelated units may be volume-reduced by the buffy coat, or other method, which has been validated to show adequate HPC recovery and function prior to cryopreservation.

Where *ex vivo* manipulations are required including expansion, these should be performed by validated techniques to ensure adequate recovery and function following these procedures.

24.9.4 Dose requirements

Processing and transplant facilities **shall** agree and validate the adequacy of dose (total nucleated cells, mononuclear cells, CD34 positive cells and/or CFU-GM as appropriate for each source of HPC) required to achieve reliable and sustainable engraftment.

Tests for cell dose and viability should be performed as in Section 24.12.4.

24.10 Liquid storage

Policies **must** be in place for the storage of material not destined for cryopreservation, e.g. bone marrows undergoing red cell depletion and for other HPCs prior to cryopreservation. These should cover:

- labelling
- primary and secondary containers
- storage temperature and duration
- cell concentration (see Section 24.11.2, Cell concentration)
- transport if appropriate.

24.11 Cryopreservation

24.11.1 Archive samples

Aliquots of the HPC component, processed and stored under the same conditions as the HPC component, **must** be available for additional testing as necessary (see Section 24.12.9).

24.11.2 Protocols

Cryopreservation procedures must be included in the standard operating procedures manual of the cell processing laboratory and shall describe the following:

- Preparation of the component for cryopreservation (cryoprotectant and concentration, method of addition with times and temperatures, composition of diluent; final composition of component before freezing).

Cell concentration

Donations with a cell concentration above 200×10^9/L must be diluted to less than 200×10^9/L, preferably with autologous plasma, and placed at 4°C if they are not being processed immediately. The final concentration after addition of the cryoprotectant must be less than 100×10^9/L.

Primary freezing containers

These must be sterile and chemically suitable for the preservation of living cells. Plastic bags are preferred because they provide a high surface area to volume ratio that facilitates the control of rates of change of temperature. It is essential that any container used for this purpose has been documented to tolerate the lowest temperatures that will be encountered during processing and storage. If freezing bags are used, all air must be expelled from the bags.

Secondary freezing containers

A secondary container, 'double bagging', must always be used to prevent cross-contamination between donations and to effectively quarantine the unit. This outer container must be made of material that has been documented to tolerate the lowest temperature that will be encountered during processing and storage. An efficient means of sealing this container must be demonstrated.

Labelling

The freezing containers must be clearly and unambiguously identified using labels that have been validated for use under the required storage conditions. The data on the labels must include the date of collection, a product identifier and a unique identifier of the component as well as a unique identifier of the intended patient, if known (see Section 24.13).

Cryoprotectant

Cryopreservation of the HPC product must be with an established cryoprotectant, e.g. 10% DMSO, used in a validated procedure with defined times and temperatures of exposure to specified concentrations.

Validated diluents such as autologous plasma or HAS may be used.

Established conditions of time and temperature of exposure of the HPC component to the cryoprotectant must be observed. These must be specific to the cryoprotectant system used. Validated storage conditions for the cryoprotectant must be observed.

Method of controlled rate freezing and cooling programme

This can be achieved by using one of two methods:

- an automatic, electronic, controlled-rate system which can be programmed with the desired temperature/time profile
- a passive cooling system, for example using an insulated container placed in a –80°C mechanical freezer.

Only established and validated passive cooling methods may be used. All records of maintenance and service of the cooling systems must be retained (see Section 24.19). Policy should include the endpoint temperature of cooling.

A continuous record of the temperature of a monitoring sample must be made using documented procedures and filed with the records of each freezing process. The unit prepared for freezing must be placed in a specifically designed metal canister to produce a uniform thinness to facilitate heat transfer.

Storage temperature and conditions (gas or liquid phase)

Frozen HPCs should be stored at a sufficiently low temperature to ensure recovery of living cells after the intended preservation period. If indefinite storage is required a temperature below –130°C should be used.

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

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

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

Storage vessels

Storage vessels must be monitored to ensure that they are operating to a predefined specification.

Routine cleaning of storage vessels should not be attempted because of the risk to the viability of stored deposits.

If the above storage conditions are not met and it is discovered that an HPC component contaminated with a transmissible agent has been stored in a vessel the following actions must be taken:

- the vessel must be clearly labelled as containing a high risk donation and no more samples should be added to that vessel
- particular care must be taken to minimize the risk of cross-contamination of other vessels, for example during refilling with liquid nitrogen
- the relevant clinicians must be notified of the possibility of cross-contamination of other deposits in the same vessel in order to decide whether they are prepared to use them or if they wish them to be discarded and the patient reharvested if necessary
- other donations in the same vessel may be released for clinical use at the relevant clinician's discretion and after microbiological advice has been sought. If a harvest is used the patient or legal guardian must be fully informed of the potential risks and appropriate follow-up testing should be performed after consultation with the microbiologist or other appropriate specialist
- if any patient has received material stored in the tank with a contaminated harvest before its high-risk status was known, the medical director/advisor must be informed and consideration given to performing a 'look-back' exercise after discussion with the relevant transplant physician
- when the vessel has been emptied, either by use or discard of all the contained deposits, it and the racking system must be cleaned and disinfected by a technique approved by the microbiology advisor before being brought back into service.

Thawing and preparation for administration

General matters relating to the transport of HPC components are detailed in Section 24.16. The methods used to transport frozen components to the clinic must have been shown to maintain integrity of the component and to provide the temperature specified for storage. Liquid nitrogen dry shippers are suitable. Only components that were stored either partially or completely submerged in liquid nitrogen may be submerged in liquid nitrogen for transport.

The units should be thawed in a manner that has been established as appropriate for the overall preservation technique. Typically this will involve immersion and gentle agitation in a water bath at 37–40°C until the last ice has melted. If the reinfusion has to be delayed the units may then be transferred to ice (<5°C) for short-term storage.

Water baths should be filled with sterile water or isotonic solutions, e.g. normal saline, just before use. After use they should be emptied, cleaned and stored dry.

Precautions to prevent leakage or contamination in the event of compromised container integrity are essential. One approach is to place the whole container in a sterile plastic bag immediately before immersion in the water bath.

Any processing of the thawed sample prior to administration must be according to a protocol that has been established as appropriate for the overall preservation technique. If dilution, centrifugation and/or removal of supernatant fluid with resuspension in fresh media are used the specified conditions of temperature, time and solution composition must be followed.

24.12 Testing of haemopoietic progenitor cell donors and components

24.12.1 Mandatory microbiology tests

Mandatory infectious disease testing (see Chapter 10)

All donors of all bone marrow or peripheral blood progenitor cell collections **must** be screened for the mandatory microbiology tests, within a 30-day period prior to harvest, and the results **must** be available before conditioning therapy in the recipient is initiated. Procedures **must** be in place to ensure that the risk status of the donor has not changed in the time between testing and donation.

Mothers of the cord blood donors **must** be screened for the mandatory microbiology tests on a sample taken on the day of collection to seven days after delivery.

A sample from the cord blood collection should also be screened for the mandatory microbiology tests.

In cases where the results of mandatory microbiology screening tests are positive or status is unknown at the time of processing the harvest, suitable quarantine **must** be arranged for the cryopreserved HPC components and the donations **must** be clearly identifiable.

Additional microbiology tests

Additional tests may be necessary under certain circumstances to increase the safety for susceptible recipients e.g. anti-CMV, anti-malaria, according to local policy.

The cord blood collection should be tested for CMV by PCR prior to issue for transplant.

24.12.2 ABO and Rh D typing

- **must** be performed on blood samples taken from the donor and/or HPC collection.
- **must** be performed on red cells from the cord blood collection.

24.12.3 Volume

The volume of all the HPC components must be determined.

Policies should define the minimum volume of cord blood (excluding anticoagulant) acceptable for banking and how the volume is calculated.

24.12.4 Cell counts

Definition

Total nucleated cell counts: all nucleated cells including nucleated red blood cells.

Mononuclear cells: all nucleated cells excluding granulocytes and nucleated red blood cells.

Total nucleated cell count or mononuclear cell count **must** be performed on all HPC components after collection and after any subsequent processing but before cryopreservation.

Enumeration of CD34 positive cells for HPC components should be undertaken. This may be done on samples taken after processing, before or during cryopreservation and after thawing.

Enumeration of CD3 positive cells is recommended for all DLIs, allogeneic bone marrow and peripheral blood progenitor cell collections.

24.12.5 Clonogenic assays

Clonogenic assays, e.g. CFU-GM may be undertaken as part of a quality programme or when specifically indicated or requested by the transplant physician. Consideration should be given to performing surrogate tests for viability prior to conditioning on a representative archive sample of any cryopreserved HPC components. For cord blood units progenitor cell assays should be assessed on a thawed sample before release of the unit for transplant, if time permits.

24.12.6 Sterility

Bacteriological and fungal screening employing aerobic and anaerobic conditions **must** be performed on the final HPC component after processing and before cryopreservation, unless validation studies demonstrate that bacteriological screening of waste processing material, such as plasma or erythrocytes, are equivalent to screening of the final product.

All positive cultures should be subsequently identified and antibiotic sensitivities performed if the material is to be put to clinical use.

24.12.7 HLA typing

HLA typing should be performed by low/medium resolution DNA techniques by an accredited laboratory (see Section 24.6.1, Donor examination testing).

It is recommended that for cord blood donations a maternal sample is HLA typed to confirm identity.

24.12.8 Manipulated HPC components

For components undergoing manipulation as defined in Section 24.8 the following **shall** be performed:

- For purging or positive selection procedures, a relevant and validated assay, where available, for the target population of cells being purged and/or selected before and after the procedure.

24.12.9 Test samples

Where test samples are removed from an HPC component, the sampling procedure should be designed and validated to ensure the sterility and essential properties of the component are not adversely affected.

Sampling procedures should be designed and validated to ensure adequate identification and handling of all test samples so that they accurately relate to the specific unit of the HPC component being tested, or to its donor, or to the specific recipient, where applicable.

Archival samples **must** be stored for reference and any future testing that may be required. Documentation **must** be kept to ensure security and accurate retrieval of the stored samples when required (see Section 24.14.5).

- For all HPC components aliquots of viable cells **must** be stored (see Section 24.11.1).
- In addition, for CB, aliquots of DNA, cells and plasma from both the CB unit and the mother **must** be stored. This should include suitable material for subsequent NAT-based tests. For other HPC donations an appropriate archive sample of cells and plasma should be retained.
- Storage of samples associated with each donation **must** currently be for 11 years post-expiry date. Samples from transplanted donations **must** be stored indefinitely (this is currently under review).

24.12.10 Test results

The results of mandatory tests must be reported to the cryopreservation facility before HPCs are put into general storage. If any results are outstanding the material should be treated as high risk and put into quarantine until the results are known, those with secondary closure may be accepted. If a donor is proceeding to harvest, the results of mandatory tests and any other results relevant to the donor's health should be reported, with the donor's permission, to the harvest physician. Any results of relevance to the health of the recipient should be reported to the transplant physician. Results should be reported in a timely manner and a copy of all the results must be filed in the HPC donor's file.

Given the unique nature of these components, a positive test result for mandatory microbiological markers or bacteriological screening may not preclude release for use. Local policies must be followed, but policies for release must take MSBT guidance into account.

Each facility should have an agreed policy for the management of test-positive donations.

24.12.11 Review of processing records

The laboratory director/manager or designee **shall** regularly review records pertinent to the HPC components.

A thorough investigation, including the conclusions and follow-up, of any unexplained discrepancy or the failure of a component to meet any of its specifications shall be documented.

24.13 Labels

24.13.1 Labelling operations

Labelling operations **shall** be conducted in a manner adequate to prevent mislabelling of components.

The labelling operation **shall** include the following controls:

- container labels **shall** be held upon receipt from the manufacturer pending review and proofing against a copy approved by the laboratory director/manager to ensure accuracy regarding identity, content and conformity
- stocks of unused labels representing different components **shall** be stored and maintained in a manner to prevent errors. Stocks of obsolete labels **shall** be destroyed
- a system of checks in labelling procedures **shall** be used to prevent errors in translating test results to container labels
- all labelling **shall** be clear and legible and printed using moisture-proof ink
- the labelling system **shall** be validated as reliable for storage under the conditions in use
- procedures must be in place to account for unused labels.

The label provided by the initial processing or collecting facility, and all additions to the label, **shall** be affixed or attached firmly to the container. This label **shall** not be obscured, altered or removed by subsequent facilities.

Components that are subsequently processed may be packaged into new containers with new labels as appropriate. Records to allow tracking of components **must** be maintained.

When the label has been affixed to the container, a sufficient area of the container **shall** remain uncovered to permit inspection of the contents.

24.13.2 Component identification

Each component **shall** be assigned a unique numeric or alphanumeric identifier by which it will be possible to relate any component to its donor, the donor's medical record and to all records describing the handling and final disposition of the component.

Facilities may designate an additional or supplementary unique numeric or alphanumeric identifier to the component. Supplementary identifiers **shall** not obscure the original identifier. No more than one supplementary identifier **shall** be visible on a component container.

24.13.3 Partial label

If the collection or freezing bag is capable of only bearing a partial label, the container **must** show at a minimum the proper name of the component and the unique numeric or alphanumeric identifier of the component. For related or autologous donations the name and/or identifier of the intended recipient, if known, should be included.

At the time of issue for infusion or transfer to another facility, collection bags **must** be accompanied by the full information in Sections 24.13.4 and 24.13.6, and freezing bags

bearing a partial label **must** be accompanied by the full information in Section 24.13.5. Such information **must** be attached securely to the component.

24.13.4 Primary collection container label

This **shall** bear the following information:

- donor's unique numeric or alphanumeric identifier
- recipient's name or unique identifier, if known
- name of the component
- name and hospital identifier of the recipient, if known
- date and time of collection (plus time zone if appropriate)
- date and time of expiry
- volume of the component
- name and volume of anticoagulant and any other additives
- identity of the collection facility or donor registry.

If the space on the label does not allow for a complete label then a partial label as defined in 24.13.3 must be used.

24.13.5 Label at completion of processing

After processing and prior to release to a transplant facility, the label **shall** indicate the information in Section 24.12.4 and in addition:

- ABO and Rh type of the donor
- proper name of the component
- name and volume of any additives, including but not limited to anticoagulant, electrolyte solutions and/or cryoprotectant
- most recent results of laboratory tests of the donor for infectious diseases, if performed, unless the results were forwarded to the transplant facility or are included in the accompanying records
- biohazard label if appropriate – as specified in Section 24.14
- expiry date, when appropriate, including the day, month and year and, if the dating period is 72 hours or less, the hour of expiry (and time zone when applicable)
- method(s) used for HPC manipulation, if appropriate, including but not limited to: purging, positive-selection, *ex vivo* expansion and gene-manipulation
- recommended storage temperature range of component in degrees Celsius
- name and address of the processing facility.

Each container of cells intended for autologous use **shall** be prominently labelled for 'Autologous use only'.

Each container of cells intended for allogeneic or syngeneic use **shall** be prominently labelled 'For use by intended recipient only'.

Components determined to be unsuitable for infusion **shall** be prominently labelled: 'Not for infusion' and the label shall state the reason the unit is considered unsuitable.

If the space on the label does not allow for a complete label then a partial label as defined in 24.13.3 **must** be used.

24.13.6 Labelling prior to transport to an outside facility

The container in which the component is issued **shall** be labelled as in Sections 24.13.4 and 24.13.5.

The following additional information **shall** be securely attached to the primary container of liquid components:

- name and address of the receiving institution
- name and unique identifier of the intended recipient, if known
- name of the collection facility or donor registry and a unique donor identifier
- name of a contact person responsible for the handling and receipt of the component at the receiving institution
- telephone number or numbers through which the contact person may be reached in the event of a delay or emergency
- for cord blood the following information is required:
  - HLA phenotype and technique used for typing
  - number of nucleated cells
  - type of processing
  - any deviations from compliance with the standards
  - a 'Do not irradiate' label.

24.13.7 Labelling prior to issue for infusion

At the time of issue for infusion a tag bearing the following information **shall** be attached to the component container if this information is not already on the primary container label:

- name and unique patient identifier of the intended recipient
- proper component name and the component's unique identifier
- interpretation of any red cell compatibility tests performed.

24.13.8 Issue of components for infusion

Identification

All labelling requirements **must** be met.

Inspection of component prior to infusion

Each component issued for infusion **shall** be inspected by two trained personnel immediately before infusion to verify recipient information and integrity of the component container. The laboratory medical advisor **must** give specific authorization for use when the container is compromised and/or recipient information is not verified.

Return of components after issue

Under exceptional circumstances when components are accepted for return it **must** be shown that the integrity of the component container has not been compromised subsequent to issue and that during this time it has been maintained at the specified temperature range. Such HPC components **must** be considered as non-conforming until they have passed a documented acceptance procedure.

If these conditions have not been met the laboratory medical director/advisor or nominated deputy, e.g. laboratory director/manager, **must** give specific authorization to accept the components for return and, in consultation with the patient's transplant physician, will authorize reissue or discard of the component.

Components to be reissued **must** be inspected and labelled as per Section 24.13.6.

Documentation of the events requiring return, the results of inspection upon return and subsequent action taken to ensure component safety and viability until potential reissue shall be maintained in the laboratory record.

24.14 Conditions for storage of components

24.14.1 Duration

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

24.14.2 Temperature

Liquid storage: This shall be for a period of time and at a temperature specified in a protocol agreed by the laboratory director/manager (see Section 24.10).

Frozen storage: see Section 24.11.2, Storage temperature and conditions.

24.14.3 Alarm systems

Storage devices shall have alarm systems that are continuously active.

Alarm systems shall have audible signals.

If laboratory personnel are not always present in the immediate area of the storage device, a remote alarm device shall be required at a location staffed 24 hours a day. Alternatively an auto-dial facility connecting to an on-call member of staff may be satisfactory.

Alarms shall be set to activate at temperatures or an unsafe level of liquid nitrogen to allow time to salvage components.

There shall be a written procedure to be followed if the storage device fails.

A procedure for notifying laboratory personnel should be in place.

Alarm systems shall be checked periodically for function.

Additional storage devices of appropriate temperature shall be available for component storage if the primary storage device fails.

24.14.4 Security

Materials that may adversely affect HPC components shall not be stored in the same refrigerators or freezers.

24.14.5 Inventory control

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

24.15 Release

Prior to HPCs being cleared for issue, all relevant records, including donor records, processing and storage records, and post-processing quality control tests must have been reviewed, approved and documented as acceptable by the individual(s) responsible according to the relevant local SOPs. Responsibility for setting policies for exceptional release and for issuing products on concession resides with the medical director/advisor.

See also Section 24.2.5 for policy when change is implemented to specifications for donors or testing etc.

24.16 Transportation

For packaging: ensure compliance with the Commission Directives on Technical Aspects expected in 2005/2006.

24.16.1 Transportation of non-cryopreserved components between centres

Where feasible the component should be placed in at least two sealed containers.

The primary component container **shall** meet the following criteria:

- the container should be made of material approved for the storage of human blood cells
- a port that can be entered aseptically should be present
- the primary container should be sealed in a manner that minimizes the risk of cell loss and of microbial contamination.

Secondary container: the primary component container **shall** be placed in a secondary plastic bag and sealed to prevent leakage.

Outer shipping container:

- **shall** be thermally insulated
- should be made of material adequate to withstand leakage of contents, shocks, pressure changes and other conditions incident to ordinary handling in transportation
- should contain adequate non-particulate absorbent material to contain the entire volume of the primary component container.

Labelling

- primary component container: Section 24.13.6 applies
- outer shipping container:
  - **shall** be labelled as 'Bone Marrow/Peripheral Blood Progenitor Cells/Cord Blood for Transplantation' and 'information that the shipment should not be exposed to radiation, heat or freezing'
  - a biohazard label **shall** be applied if tests for mandatory microbiological markers are not negative
  - additional information shall include:
    - name and address of the receiving facility, the name and telephone number of the receiving laboratory and the name of the responsible person at the receiving facility
    - name, address and telephone number of the shipping facility and the name of the person responsible for the shipment
    - description of the contents, including the number of containers and volumes in each.

Temperature

- During transport the component temperature **shall** be maintained at the storage temperature specified by the processing laboratory. Wherever possible the temperature during transit should be logged.
- For components stored at 1–6°C, frozen gel packs with adequate insulation to prevent contact with component primary containers or wet ice may be used.
- Dry ice **shall** not be used for transport of non-cryopreserved components.

- The component **shall** not be frozen or placed near heat.

Method of transport

- The component should be hand-carried by a suitably informed courier in the passenger compartment.
- There **must** be plans for alternative transport in an emergency.
- The components **shall** not be passed through X-ray irradiation devices designed to detect metal objects. If inspection is necessary, the contents of the container shall be inspected by hand. For HPC components shipped by air, the logistics of transportation should be specified in advance.

24.16.2 Cryopreserved components

The component must be labelled as in Section 24.13.6.

Cryopreserved components with an indicated storage temperature below –130°C shall be shipped in a liquid nitrogen 'dry shipper' that contains adequate absorbed liquid nitrogen to maintain temperature at least 48 hours beyond the expected time of arrival at the receiving facility. Users should be aware that dry shippers normally maintain a temperature below –190°C, but once the charge of liquid nitrogen has evaporated the temperature can rise very rapidly. Wherever possible the temperature should be logged during transit.

The receiving facility should verify the temperature upon arrival.

The shipping container **shall** be of appropriate design and construction for transportation of the cryogenic material used.

The shipping container **must** be labelled in accordance with applicable regulations regarding the cryogenic material used and the transportation of biological materials.

24.17 Documentation of infusion and adverse incidents

A component infusion form shall be issued with the product and completed for each component infused. A copy should be returned to the processing laboratory. Any adverse incidents following infusion of an HPC component shall be noted on the form and must, in addition, be communicated verbally to the processing laboratory. All adverse incidents must be reported to the medical director/advisor.

24.18 Disposal of haemopoietic progenitor cells

Ordinarily HPC components should be kept for clinical use for a minimum of between five and ten years.

There **shall** be a written policy for disposal of HPC components.

There **shall** be written documentation of the recipient's death or no further need for any component before it is discarded. Written instructions from the transplant physician should be obtained. Appropriate prospective consent for discard should have been obtained.

The records for discarded components **shall** indicate the component discarded, date of discard and method of disposal.

The medical director/advisor of the processing facility, in consultation with the patient's transplant physician, must approve of component discard and method of disposal. If the patient is still alive their consent for disposal of the components **shall** have been obtained.

The method of disposal and decontamination **shall** meet the UK laws, current codes, rules and regulations for disposal of biohazardous materials.

24.19 Records

24.19.1 General requirements

Records **shall** be made concurrently with each step of the harvesting, processing, testing, cryopreservation, storage, issue and transplant or disposal of each component in such a way that all the steps may be accurately traced from donor to recipient.

Records **shall** be legible and indelible, **must** identify the person or persons responsible for each step and must include dates and times, where appropriate.

Records **must** show test results as well as the interpretation of each result where appropriate.

Records of each step **must** be as detailed as necessary to give a person with relevant experience a clear understanding of each step and **shall** be available for inspection by authorized individuals.

Appropriate records **must** be available from which to determine the lot numbers and manufacturer of supplies and reagents used for the collection and processing of the specific HPC components.

Records of errors, accidents and corrective action regarding collection, processing, storage or infusion **must** be maintained.

All records and communications between the collection, processing and transplant centres **must** be regarded as privileged and confidential. Safeguards to assure this confidentiality **must** be established and followed.

Records **must** be maintained, for a minimum of 30 years, in such a way as to assure their protection and preservation.

24.19.2 Records to be maintained

Records **must** be maintained that include, but are not limited to, the following when applicable:

Donor and patient records

- For BM and PBPC: donor selection, including medical and behavioural history, physical examination and informed consent. Documentation of donor's mandatory microbiology marker screening results.
- For cord blood donors: medical history of the biological mother and the biological father, if available. (Pregnancy, delivery, ethnicity, behavioural history, other family members.) Documentation of donor's mandatory microbiology marker screening results.
- Reasons for permanent and temporary deferral for health reasons.
- Donor adverse reactions, complaints and reports, including results of all investigations and follow-up.

Collection and processing records

- Collection details and data, including lot numbers and expiration dates of reagents and disposals used.
- Component processing, including cell counts (TNC, $CD34^+$ cells, etc.) lot numbers and expiration dates of reagents and disposals used.
- Labelling process including initials of person responsible.
- Results and interpretations of all tests and retests.

Storage and issue record

- Inventory system, detailing location of component and associated aliquots, including number of sub-units and aliquots of components and number and date of sub-units and aliquots issued.
- Distribution and disposal, as appropriate, of HPC components.
- Visual inspection of liquid components during storage and at issue.
- Storage temperature, including initialled temperature record charts.
- Signature of medical director/advisor authorizing release of HPC components in cases where there is a confirmed positive test result for a mandatory microbiology marker or for the bacteriology screening or in any other case of exceptional release where any elements of the collection, processing, testing or storage has not been compliant with these guidelines. In cases where the HPC component is required to be infused prior to completion of the tests, the transplant physician must provide written responsibility for use of the component without the relevant test results.

Compatibility test records

- Results of all compatibility tests.
- Results of all confirmatory testing.

Quality control records

- Maintenance, calibration and standardization of equipment.
- Effectiveness of procedures, equipment and reagents.
- Cleaning and environmental monitoring of HPC collection and processing facilities.
- Proficiency test results.
- Internal and external quality audit reports.

General records

- Personnel employed by the facility, their signature, initials and inclusive dates of employment.
- Personnel training, continued education and competency testing.
- Incidents and errors and corrective action taken.

24.19.3 Adverse reactions

Records **must** be maintained of all reports of complaints of adverse reactions to any HPC component arising as a result of collection or infusion. This is to include acute toxicity associated with the infusion of the component and delayed or failed engraftment that may relate to the component collection or processing procedures.

If it is determined that the HPC component was responsible for the adverse reaction, copies of written reports **must** be forwarded to and maintained by the collection and transplant centres as appropriate.

24.19.4 Engraftment

Records of documentation and review of engraftment data after infusion of the HPC component **must** be maintained and reported to the HPC laboratory.

Chapter 25

Specification for the uniform labelling of blood and blood components

25.1 Introduction

25.1.1 General information

The information contained in this chapter is intended to inform all persons involved in labelling blood and blood components of the specifications for uniform labelling. It is intended to provide users, software developers and printers with an understanding of label design and code formation.

The specification covers labels required by the United Kingdom Blood Transfusion Services (UKBTS) for the labelling of blood donation (collection) packs, satellite packs, associated samples and documentation. It utilises barcodes to encode information in addition to eye-readable symbols.

Blood pack labelling is in a period of transition as the established Codabar system is replaced with the international standard ISBT 128. Currently the UKBTS use ISBT 128 labels for the donation identification number and the blood group code.

Where this document refers to ISBT 128 cross-reference should be made to the ICCBBA Inc. ISBT 128 Standard Technical Specification which gives detailed information on data structures and labelling. This chapter interprets relevant sections of the Technical Specification in the light of UK requirements, and in some cases limits the available options provided in, or contradicts recommendations of, the Technical Specification. In all such cases, this chapter takes precedence.

Full transfer to ISBT 128 barcoding involves converting all the barcodes used on the blood pack to ISBT 128. The timetable for this further change has yet to be determined.

25.1.2 Purpose of the system

The objective is to reduce the dangers of incompatible blood transfusions caused by human error and a central part of the label design is machine-readable coding of essential information.

Each blood donation pack, plus connected satellite packs and associated samples and documentation, must be identified by a *unique* identification number applied at the time of donation. Additionally each pack requires identification by a label showing the ABO group and Rh D type, and a separate label indicating the component type. Such a system will ensure unique identification of every blood component, and secure association between donations and samples.

The purpose of the introduction of ISBT 128 labelling is to comply with international standards, thus facilitating the movement of blood components across national boundaries.

25.1.3 Applicability

All blood pack/sample labels for use in the UKBTS must comply with the specifications in this document.

24.1.4 Referenced documents

1. ISBT 128 *Standard Technical Specification* (Ver 2.0.0 June 2003). ICCBBA Inc (see www.iccbba.com).
2. *Uniform Symbology Specification: Code 128*. (AIM USA) June 1993.

25.2 General specification

25.2.1 The labelling system

The labelling system for blood and blood components comprises the following elements:

a) The base label – the label applied to the blood pack by the manufacturer of the blood pack or harness, bearing the manufacturer's container information and lot number Codabar barcodes, specified cautionary text, and guide marks to assist in the positioning of overstick labels

b) The donation identification number label – a label bearing the ISBT 128 donation identification number barcode. Produced in sets these labels ensure the accurate and unique labelling of all blood packs and samples taken from a single donation event. Allocated at the point of donation, this number is fundamental to the secure audit trail for blood components

c) The blood group label – A label bearing the ISBT 128 nationally defined short form unit identifier barcode, the ISBT 128 blood group barcode, and the Codabar expiry date barcode. Also contains eye-readable information on blood group and expiry. Applied by the Blood Service prior to release into stock

d) The component label – a label bearing the Codabar product barcode, together with component specific information. Applied at the time of component manufacture by the Blood Service.

The labels indicated in b–d above are all affixed onto the base label. The arrangement of labels is shown in Figure 25.2. This diagram is for orientation purposes only. See under the appropriate sections for details of each label content and layout.

All labels must conform to the recommendations set out in the appropriate section of the ISBT 128 Standard Technical Specification unless specifically stated otherwise in this document.

Barcodes specified as ISBT 128 must comply with the Code 128 Bar Code Symbology and Application Specification. Barcodes specified as CODABAR must be built to CODABAR ABC standard with a recommended density of 10 (ten) characters per inch (25 mm).

All labels must be

- self-adhesive using a non-invasive adhesive
- tamper-evident (i.e. removal must deface the label)
- smear-resistant
- resistant to water and humidity

- capable of being affixed readily to paper or other base label material, plastic surfaces, glass (particularly glass tubes of 12 mm diameter) without winging ('winging' is defined as the lifting of a label from the surface to which it is applied. Winging should not exceed 0.1 inch (2.5 mm) as the maximum linear distance of the label not adhering to the tube at any label edge, measured after 24 hrs refrigeration at 4°C)
- capable of withstanding a temperature range of –60°C to +56°C after application to the blood pack. This range may be extended by the ordering authority at the time of order
- capable of being applied without slippage to tubes, etc. subject to temperature variation prior to use, e.g. tubes/packs stored in a vehicle and then used at normal ambient temperature; such equipment being by definition 'damp'
- non-flaking when read using a hand-held light-pen touching the label.

25.2.2 Barcode reading and interpretation

Barcoding is carried out to ensure the accuracy of transmitted information. To gain the maximum benefit from such coding, systems reading and interpreting the codes need to ensure that valid codes have been scanned. The following minimum checks should be carried out by receiving application software:

- ensure that the barcode identifiers (data identifiers in ISBT 128, start/stop sequences in Codabar) are those of the expected code
- ensure that the format (length and character types) of the received data string matches the defined format for the expected code
- ensure that checksums are used to validate correct data transmission (with ISBT 128 ensure that the scanner is performing the internal Code 128 checksum validation)
- ensure that data values are within acceptable ranges.

25.2.3 ISBT 128 barcoding

Code 128 is a high-density alphanumeric barcode symbology which has been adopted by the ISBT for the provision of a worldwide unique numbering and coding system for blood and blood components. ISBT 128 defines the data structures of this system, the supporting reference databases, and application specific information. The specification for concatenation support provided by standard Code 128 has been modified at the request of the ISBT to incorporate the need for spatial, and/or temporal limitation on concatenated reading (refer to ISBT 128 Standard Technical Specification). Barcode readers used for reading concatenated ISBT 128 barcodes must comply with this modification.

The importance of data identifiers in ISBT 128

ISBT 128 barcodes comprise two elements:

- the data identifier characters that identify which ISBT 128 data structure is being transmitted
- the data characters which provide the data values to be interpreted in accordance with the definition of the appropriate structure.

In order to accurately interpret information from an ISBT 128 barcode it is essential that application software carries out the following steps before interpreting the data values:

- analyse the data identifiers to ensure that the barcode entered is of the correct type
- verify that the data length and format match that defined for the barcode type.

Failure to carry out these checks could lead to incorrect assignment of critical information.

The following example, which has occurred in practice, illustrates the point. An ISBT 128 blood group barcode for an A negative unit will read as:

=%0600

Where:

'=%' are the data identifier characters indicating that this is a blood group code

'06' is the code for an A negative.

In the UK, the national donation identifier barcode is also printed on the blood group label. For donation number 061234 this appears as:

&a061234

Where:

'&a' is the data identifier character for the national identifier barcode.

If the data identifier characters are ignored by the application, entry of this second barcode in response to a blood group prompt could cause the system to incorrectly assign an A negative blood group to the unit.

The ISBT 128 Specification allows for two densities of code. This should be taken into account in the choice of barcode density and the selection of reading equipment.

Concatenation

Concatenation requirements for ISBT 128 are laid out in the ISBT 128 Standard Technical Specification and further expanded in ICCBBA Technical Bulletin No. 5. Where concatenated codes are to be read it is essential that the barcode readers used support concatenation of Code 128 barcodes with the defined temporal/spatial limitations of ISBT 128.

In the UK correct blood group labelling will be confirmed by concatenation of the Donation Identification Number with the short form unit identifier (nationally defined – see National ISBT 128 specifications section) and of the donation identification number with the blood group. The relevant data identifiers are specified within the appropriate sections of this document. For further details refer to the ISBT 128 Standard Technical Specification.

National ISBT 128 definitions

National bodies are permitted to allocate nationally defined codes identified by data identifiers of '&' followed by a lower case alpha character. Within the UK this responsibility lies with SACIT.

The following national codes have been assigned:

&a

Defined for the shortened form of a donation number used on demand-printed group labels for concatenated read with the donation number as part of label verification. This code must not be used for any other purpose. The code structure is:

&annnnnn

Where:

&a are the data identifiers

nnnnnn is the six-digit unit serial number from the ISBT 128 donation number definition (see Section 25.3.2).

See also paragraph below.

&b

Defined for the shortened form of a donation number used on demand-printed group labels for concatenated read with the donation number as part of label verification. This code must not be used for any other purpose. The code structure is:

&bnnnnnnk

Where:

&b are the data identifiers

nnnnnn is the six-digit unit serial number from the ISBT 128 donation number definition (see Section 25.3.2)

k is a single-digit iteration number, used to assist in controlling labelling where more than one labelling process takes place (e.g. an additional group label has to be placed over the initial label to display additional testing information such as CMV status).

See also paragraph above.

&d

Defines a sample identification number for patient samples. The data structure is:

&daaaaayynnnnnnff

Where:

&d are the data identifiers

aaaaa is the facility identifier. The first character will always be zero. Values in the range 09900 to 09999 are assigned for use by the English National Blood Service

yy is the nominal year of collection (slippage of one month either side of the end of year is permitted)

nnnnnn is a six-digit sequence number

ff are barcode check characters. These are derived from a two-digit checksum calculated from the data sequence aaaaayynnnnnn as shown in Annex 2.

In the eye-readable form of this number the checksum is represented as a single boxed character.

25.2.4 Codabar ABC barcoding

The ABC symbol

Codabar ABC, a subset of Codabar, is a two-level, seven-bit binary encoding system. The two levels are those of optical reflectance from a dark pattern printed on a light background. Each ABC symbol character is made up of four bars and three spaces for a total of seven bits of information per character. The ABC symbol uses 20 characters as shown below. Each character is stand-alone and is to be decoded individually, independent of adjacent characters. The code patterns in use are bi-directional.

The ABC symbol encodes the following characters:

- 10 numerics {0, 1, 2, 3, 4, 5, 6, 7, 8, 9}
- six control characters {-, $, .,+, :, /}
- four start/stop (or pause) characters {a, b, c, d}.

Control codes

Within Codabar there are alpha characters assigned as start/stop characters. In some instances these are accompanied by a numeric (0–9) thus forming left-hand/right-hand control codes. These are used to identify the type of data encoded between the controls.

The assigned alpha characters are a, b, c, d.

'd' is used as a stand alone start/stop or as a pause code. Decoding must assume stop if no further code commencing with 'd' is encountered within the specified ABC-Codabar concatenation interval.

Where an alpha character is accompanied by a numeric character, the combination will normally constitute the complete left/right-hand control and needs to be treated as such in decoding. Within the UK, however, there are instances where the numeric constituent of the left-hand control has been utilised as part of the data message (see Section 25.5).

Barcode dimensions

The minimum acceptable height for labels in the UK is 6 mm. The standard density of the encoded characters is 10 per inch (0.4 character per mm). Inter character gaps must be a minimum of 0.2 mm to provide adequate resolution between characters. Gap dimensions are not critical as each character is read independently and gaps do not carry information. The barcode is a series of straight parallel lines perpendicular to a base reference line. Individual characters must not be misaligned by more than five degrees from adjacent characters.

A minimum border ('quiet zone') of 2.5 mm must be allowed at each end of the encoded message (but see Section 25.3.1). The border above and below the code is not critical, but will normally include eye-readable information, the printing of which must not touch the code. Maximum depression or embossment of the printed barcode must not exceed 0.05 mm.

Optical parameters

The symbol is insensitive to the light-scattering properties of the substrate, except to the extent to which background reflectance is affected. Background diffuse reflectance is not specified as a separate parameter as it is integral in the definition of contrast (see below). However a background diffuse reflectance of at least 70% (optical density 0.1) in the 500–950 nm range is necessary.

Contrast

Defined as the nominal difference in the diffuse reflectance between the background and the ink film, this should be at least 50% as measured over the 500–950 nm range. Measurements should be averaged over an area equivalent to an 0.008 inch (0.2 mm) diameter circle. A print contrast ratio of at least 90% is recommended.

Voids and ink specks

The missing ink coverage or 'white' spots within the bars or the extraneous dark specks between bars must not exceed 0.002 inch (0.05 mm) diameter, or subtend more than 25% of the area within a 0.004 inch (0.10 mm) diameter circle.

Edge roughness

The maximum area of edge irregularities subtending an 0.004 inch (0.1 mm) diameter circle must not exceed 25% of the area of that circle. The area of irregularity is to be measured with respect to the nominal bar edge.

Ink fill uniformity

Variation in ink film reflectance across the character should not exceed 5% within the same character.

Ink fill-in

Must not expand individual bars within characters to dimensions exceeding the tolerance specified for dimensional parameters ('see Barcode dimensions' above).

25.3 Donation numbers

25.3.1 General structure

The donation identification number ('donation number') plays a critical role in the safety of the blood supply. It provides a unique identification number which cross references blood components and samples taken at the time of donation.

An example set of identification numbers is shown in Figure 25.1. Barcode density information is provided in the ISBT 128 Standard Technical Specification. The structure of the donation identification number is described further below.

Figure 25.1 ISBT 128 donation number set

Production control

Requirements for pre-printed labels

Donation identification number labels must be generated in primary sets under strictly controlled conditions which ensure that all the labels in a set bear the same number, and that each set is unique. It is the responsibility of the manufacturer of the label sets to undertake appropriate quality control measures to ensure these conditions are met.

The required number of individual labels comprising a set, the configuration of the labels, and the commencing number for the print run, must be defined by the ordering authority at the time of order.

Quality control of sequential print must be organised to obviate the possibility of duplication within a print run, and also to avoid the misplacement of the various cutting devices which would cause any set to contain two different numbers.

Any unusable numbers or missing numbers *must not be replaced*.

Any roll/pad containing an incomplete sequence for any reason must have the discrepancy marked at the beginning of the roll/pad, or the manufacturer must supply a separate list of missing numbers. The total permissible missing numbers must not exceed 1% of the quantity ordered. Each roll/pad should not contain more than six missing numbers per 200 sets.

Requirements for demand-printed labels

Additional donation identification labels may be demand-printed at the point of use.

Where demand-printing is used to generate additional labels for an existing set, the label must only be generated in direct response to the electronic input of a number from the original set.

Table 25.1 Centre codes and names

Codabar Alpha Code	ISBT128 Centre ID	Codabar Centre ID	Name
N	G0967	a070001001b	NBS – Newcastle
C	G0912	a070002001b	NBS – Leeds
D	G0923	a070003001b	NBS – Sheffield
G	n/a	a070004001b	NBS – Cambridge
W	G0724	a070005001b	NBS – Colindale
J	G0735	a070006001b	NBS – Brentwood
P	G0746	a070007001b	NBS – Tooting
S	G0547	a070009001b	NBS – Southampton
F	n/a	a070010001b	NBS – Oxford
T	G0525	a070011001b	NBS – Bristol
H	G0536	a070012001b	NBS – Birmingham
K	n/a	a070013001b	NBS – Liverpool
M	G0956	a070014001b	NBS – Manchester
L	n/a	a070014101b	NBS – Lancaster
	G0010		NBS – Import Centre 1
	G0020		NBS – Import Centre 2
U	G1517	a072015001b	Welsh Blood Service
Y	n/a	a071020001b	SNBTS – Aberdeen
Q	n/a	a071021001b	SNBTS – Dundee
E	n/a	a071022001b	SNBTS – Edinburgh
X	n/a	a071023001b	SNBTS – Carluke (Glasgow)
I	n/a	a071024001b	SNBTS – Inverness
	G1016	n/a	SNBTS
R	G1618	a073030001b	Belfast
D		a073031001b	Dublin
C		a073031101b	Cork
Z	G0315	a070040001b	Army Blood Supply Depot
A	n/a	n/a	Scottish National Plasma Fractionation Centre
B	n/a	n/a	Bio Products Laboratory, Elstree
b	G0337	a070013101b	Isle of Man (Nobles Hospital)
a	G0326	a074000001b	Jersey
	G2200		United Bristol Healthcare NHS Trust
	G2201		Royal Marsden Hospital NHS Trust
	G2202		Nottingham City Hospital NHS Trust
	G2203		Barking, Havering and Redbridge Hospitals NHS Trust
	G2204		Salford Royal Hospitals NHS Trust
	G2205		Barts and the London NHS Trust
	G2206		Aspen Healthcare Ltd. (Holly House Hospital)
	G2207		Whiston Hospital, Prescot

Table 25.1 Centre codes and names – *continued*

Codabar Alpha Code	ISBT128 Centre ID	Codabar Centre ID	Name
Tissue banks			
	G1700		NBS Tissue Bank London and SE
	G1701		NBS Tissue Bank Midlands and SW
	G1702		NBS Tissue Bank North
	G1703		SNBTS – Tissues
	G1704		SNBTS – Tissue Samples
	G1801		NBS – Stem Cell Services
Test sites			
	G0913	a070015001b	NBS Test Site 1
	G0914	a070016001b	NBS Test Site 2
	G0915		NBS Test Site 3 (Imports)
	G0916		SNBTS Test Site (Tissues)
	G0111		WBS Test Site 1
	G0116		SNBTS Test Site 1
	G0117		SNBTS Test Site 2
	G0118		SNBTS Test Site 3

Where demand-printing is used to generate new label sets, there must be controls to prevent number duplication.

Label colour: the labels must be printed black on a white background. Where required by the ordering authority, part of an order may incorporate a coloured stripe (usually to assist in the identification of new {first-time} donors). A colour must be selected which will not interfere with the efficiency of any barcode reader in decoding essential information.

25.3.2 ISBT 128 barcode and text

Labels will contain an eye-readable representation of the donation identification number as described below, the ISBT 128 barcode, and optionally the name of the collection facility. The barcodes should be of standard density for labels to be applied to blood packs, but of high density for numbers to be applied to sample tubes.

The format of the ISBT 128 barcoded number must be:

=αCCCCYYNNNNNNFF

where:

= α are the data identifiers (α takes values A–Z, 1–9), but note that α is also the first character of the data string)

CCCC are the second to fifth characters of the facility identification code (i.e. the code in full is made up of αCCCC) (see Table 25.1)

YY is a two-digit year of collection identifier

NNNNNN is a six-digit unit serial number.

FF are flag characters. Where the donation number is to be applied to a blood collection pack the flag characters must be the barcode check characters derived from ISO 7064 modulo 37,2 checksum. Where the number is to be applied to samples or documentation alternative flag characters as defined in the ISBT 128 Standard Technical Specification may be used.

The content of the eye-readable section of the ISBT 128 number must be:

XCCCCYYNNNNNNK

Where:

XCCCC is the country and blood centre identifier (see Table 25.1)

YY is a two-digit year of collection identifier

NNNNNN is a six-digit unit serial number

K is an ISO 7064 modulo 37,2 check character.

This identifier must be presented in a 4, 3, 3, 3, 1 format with the check character boxed viz.:

XCCC CYY NNN NNN [K]

It is strongly recommended that all characters are of equal size and weight. The font used should be selected to clearly distinguish between similar alpha/numerics (e.g. 0 and O, 1 and I), and should be as large as possible within the constraints of label size.

Where keyboard entry of donation number is used, the full number and check character should be entered, and application software should verify the string format and check character value. Use of pre-programmed 'hot keys' is not an acceptable alternative.

Calculation of checksums and the corresponding check characters for the ISBT 128 numbers is described in Annex 2.

25.4 Blood group labels

25.4.1 General specification

These labels are required for the purpose of blood group identification on the blood collection and satellite packs. The blood group can fall into one of eight classifications as shown below. Alternative labels, for use in special circumstances, are also described.

The blood group label is part of a complete overstick label and must be attached to the blood collection pack and/or satellite pack in the appropriate place immediately adjacent to the donation number. This is to allow a continuous straight line read of the combined labels.

An illustration of this labelling alignment is shown in Figure 25.2.

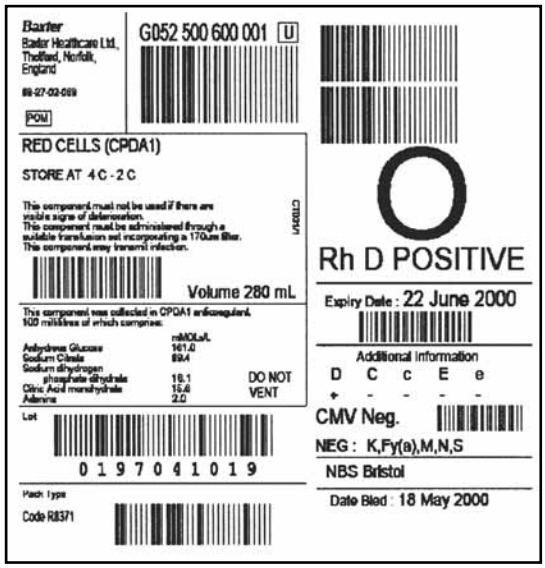

Figure 25.2 Example of label alignment

Label colour

All labels must be produced in black and white. All characters must be solid black on white except for Rh D negatives where the ABO character must be in outline, and the 'Rh D NEGATIVE' must be in solid white on a black background.

Printing

Group labels must be demand printed at the point of labelling. The label must be generated in response to the electronic entry of a donation number, and once affixed to the blood pack, must be verified by the concatenated electronic entry of the codes from the donation number label and the group label. Group labels must only be generated for units which have been fully tested and are suitable for transfusion.

Information content

The label design is illustrated in Figure 25.3. The content is described from the top down.

Group label verification number

This must be printed at the top left-hand side of the label in barcode format only. It must be an ISBT 128 number, complying with one of the national definitions (&a or &b; see Section 25.2.3) described above. The distance of the barcode from the left-hand edge of the label must not be less than 2.5 mm or more than 4 mm. The barcode must be between 7 mm and 10 mm high.

Expiry day/month

The day and month of expiry may be included in the top right hand corner of the label. Where present this must be in a DD/MM format.

Blood group

The blood group barcode must be positioned below the group label verification number barcode separated by a gap of between 1 mm and 5 mm. The left-hand edges of the codes must be aligned. The blood group barcode must be between 14 mm and 18 mm high.

Figure 25.3 Sample blood group label

The format of the ISBT 128 group code is:

=%ggre

Where:

=% are the data identifiers

gg designates the ABO and Rh D blood group and may also specify donation use limitations (see Tables 25.2a to 25.2c)

r not used in UK – set to 0

e not used in UK – set to 0

The values of gg for standard donations (without donation use limitations) are indicated in Table 25.2a.

Table 25.2a Blood group classifications

Text	gg	Text	gg
O Rh D positive	51	O Rh D negative	95
A Rh D positive	62	A Rh D negative	06
B Rh D positive	73	B Rh D negative	17
AB Rh D positive	84	AB Rh D negative	28

For donations using these group codes, the eye-readable blood group must be presented in two parts. The ABO group must be printed immediately below the group barcode. The characters must be solid black for Rh D positives, and outline for Rh D negatives.

The Rh D status must be indicated immediately below the group barcode and eye-readable ABO. The text must be 'Rh D POSITIVE' in solid black characters, or 'Rh D NEGATIVE' in solid white characters enclosed in a black rectangular background.

The values of gg used where donation use limitations apply is indicated in Table 25.2b.

Expiry date

The expiry date must be presented in eye-readable and barcode formats. The eye-readable text must be printed with characters of no less than 3 mm height. The content must comprise the day number, the month represented by its first three characters, and the four-digit year (e.g. 1 FEB 2001).

Currently the expiry date is coded using a Codabar barcode. The barcode must have a start code of 'a2' and a stop code of '4a'. The data content must be the last three digits of the year, and a three-digit Julian day number, thus 1 Feb 2001 would be represented by '001032'.

See also Section 25.7 for future changes to this barcode.

Additional information (standard donations)

Additional information may appear immediately below the expiry date in an area no less than 10 mm and no more than 25 mm high. The data content of this section is at the discretion of the labelling authority, but is available for providing additional information on phenotypes, CMV status, etc. Some UK Blood Services use a Codabar barcode of a8738a to indicate CMV-negative.

Collection facility identification

The identification of the collecting facility may be indicated in eye-readable format below the additional information section of the group label. Alternatively, this information may be printed as part of the donation identification number (see Section 25.3). The text content will be identified by the relevant national service and may comprise one or two lines of text.

Table 25.2b Blood group and donation use limitations

gg	Blood group	Limitation of use
47	O Rh D Positive	Directed donation only
48	O Rh D Positive	Emergency use only
50	O Rh D Positive	Directed donation, crossover permitted
52	O Rh D Positive	Autologous donation, crossover permitted
53	O Rh D Positive	Autologous use only
58	A Rh D Positive	Directed donation only
59	A Rh D Positive	Emergency use only
61	A Rh D Positive	Directed donation, crossover permitted
63	A Rh D Positive	Autologous donation, crossover permitted
64	A Rh D Positive	Autologous use only
69	B Rh D Positive	Directed donation only
70	B Rh D Positive	Emergency use only
72	B Rh D Positive	Directed donation, crossover permitted
74	B Rh D Positive	Autologous donation, crossover permitted
75	B Rh D Positive	Autologous use only
80	AB Rh D Positive	Directed donation only
81	AB Rh D Positive	Emergency use only
83	AB Rh D Positive	Directed donation, crossover permitted
85	AB Rh D Positive	Autologous donation, crossover permitted
86	AB Rh D Positive	Autologous use only
91	O Rh D Negative	Directed donation only
92	O Rh D Negative	Emergency use only
94	O Rh D Negative	Directed donation, crossover permitted
96	O Rh D Negative	Autologous donation, crossover permitted
97	O Rh D Negative	Autologous use only
02	A Rh D Negative	Directed donation only
03	A Rh D Negative	Emergency use only
05	A Rh D Negative	Directed donation, crossover permitted
07	A Rh D Negative	Autologous donation, crossover permitted
08	A Rh D Negative	Autologous use only
13	B Rh D Negative	Directed donation only
14	B Rh D Negative	Emergency use only
16	B Rh D Negative	Directed donation, crossover permitted
18	B Rh D Negative	Autologous donation, crossover permitted
19	B Rh D Negative	Autologous use only
24	AB Rh D Negative	Directed donation only
25	AB Rh D Negative	Emergency use only
27	AB Rh D Negative	Directed donation, crossover permitted
29	AB Rh D Negative	Autologous donation, crossover permitted
30	AB Rh D Negative	Autologous use only

The eye-readable text associated with these codes is illustrated in Table 25.2c using the O Rh D positive example.

Table 25.2c Blood group and donation use label text

gg	Label text
47	DIRECTED USE ONLY O Rh D POSITIVE
48	EMERGENCY USE ONLY O Rh D POSITIVE
50	DIRECTED (Eligible for Crossover) O Rh D POSITIVE
52	AUTOLOGOUS (Eligible for Crossover) O Rh D POSITIVE
53	AUTOLOGOUS USE ONLY O Rh D POSITIVE

Date bled

This must be printed in eye-readable form only at the bottom of the label. The characters must be no less than 3 mm high. The format must be day number, first three characters of the month name, and four-digit year, e.g. 1 JAN 2001. For components pooled from a number of source donations the date bled shall be the collection date of the oldest unit in the pool.

Label design for units with use limitations

The blood group label design for units labelled with use limitations is shown in Figure 25.4. The lower section, used for additional information on standard labels, is used to indicate recipient information. It is important to recognize that this information is for identification of the recipient for which the donation is intended, but does not replace the need for crossmatch labelling or documentation.

25.4.2 Alternative labels

There are six status labels defined for use in the 'blood group label' location. The specification for these labels is divided into two sections, one for essential information which must be present on the label as specified, and one for optional information which may be added if desired.

All labels are to be demand printed black on white.

The labels covered by this section of the specification are:

- HOLD label for use on donations where testing information is outstanding
- NOT FOR TRANSFUSION label for use on units which are microbiology negative but not suitable for transfusion
- RED CELLS NOT FOR CLINICAL USE label for use on donations which are microbiology-negative but where the red cell component is unsuitable for transfusion
- BIOHAZARD label for use on donations found to be microbiology-positive
- NOT TESTED label for use on donations unsuitable for transfusion for which microbiology testing has not been carried out or is incomplete
- EMERGENCY USE ONLY label for use on donations which are to be issued for transfusion prior to completion of all mandatory testing (see Section 7.10).

Figure 25.4 Label with use limitations

'HOLD' label specification

Essential information

ISBT 128 barcode: ISBT 128 group code where gg = 'Mq'. Positioned to allow concatenated read with an adjacent donation number

Text: The word 'HOLD' in upper case letters of minimum height 6mm

Text: The words 'FURTHER INVESTIGATION REQUIRED' in upper-case letters of minimum height 3 mm.

Optional information

Text: The word 'Reason:' followed by a free-format message giving the reason for hold

Text: Identification text of the testing centre

Text: The words 'Date Bled:' followed by the date bled.

'NOT FOR TRANSFUSION' label specification

Essential information

ISBT 128 barcode: ISBT 128 group code where gg = 'Md'. Positioned to allow concatenated read with an adjacent donation number

Text: The words 'NOT FOR TRANSFUSION' in upper-case letters of minimum height 4 mm.

Optional information

Text: The word 'Reason:' followed by a free-format message

Text: The words 'Blood Group' followed by the ABO/Rh D type if known

Text: Identification text of the testing centre

Text: The words 'Date Bled:' followed by the date bled.

'RED CELLS NOT FOR CLINICAL USE' label specification

Essential information

ISBT 128 barcode: ISBT 128 group code where gg = 'Mf'. Positioned to allow concatenated read with an adjacent donation number

Text: The words 'PLASMA USE ONLY' in upper-case letters of minimum height 2 mm

Text: The words 'RED CELLS NOT FOR CLINICAL USE' in upper-case letters of minimum height 4 mm.

Optional information

Text: The word 'Reason:' followed by a free-format message

Text: The words 'Blood Group' followed by the ABO/Rh D type

Text: Identification text of the testing centre

Text: The words 'Date Bled:' followed by the date bled.

'BIOHAZARD' label specification

Essential information

ISBT 128 barcode: ISBT 128 group code where gg = 'Mb'. Positioned to allow concatenated read with an adjacent donation number

Text: The word 'BIOHAZARD' in upper-case letters of minimum height 4 mm

Text: The words 'HIGH RISK' in upper-case letters of minimum height 6 mm

Symbol: Biohazard warning symbol of minimum height 20 mm

Text: The words 'INACTIVATE BEFORE DISPOSAL' in upper-case letters of minimum height 2 mm.

Optional information

Text: Identification text of the testing centre

Text: The words 'Date Bled:' followed by the date bled.

'NOT TESTED' label specification

Essential information

ISBT 128 barcode: No comparable code defined

Text: The words 'NOT TESTED' in upper-case letters of minimum height 6 mm

Text: The words 'NOT FOR ISSUE' in upper-case letters of minimum height 6 mm.

Optional information

Text: Identification text of the testing centre

Text: The words 'Date Bled:' followed by the date bled.

'USE IN EMERGENCY ONLY' label specification

Essential information

ISBT 128 barcode: Under ISBT 128 it is necessary to include the historic ABO/Rh D type within the barcode. This is achieved by using the modified ISBT 128 group code, where gg is as defined in Table 25.3. Positioned to allow concatenated read with an adjacent donation number

Text: The words 'BLOOD GROUP NOT CONFIRMED, USE IN EMERGENCY ONLY' in upper-case letters of minimum height 4 mm

Text: The words 'UNCONFIRMED BLOOD GROUP:' followed by the unconfirmed ABO/Rh D type if known

Text: The words 'DATE BLED:' followed by the date of collection

Text: The words 'EXPIRY DATE:' followed by the expiry date. Minimum text height 2 mm.

Optional information

Text: Identification text of the testing centre

Text: Free-format additional status information such as 'Mandatory Microbiology Tests Negative'.

25.5 Component labels

25.5.1 General description

These labels are for use on blood collection packs and/or satellite packs. Each label will display a component description printed in bold text, a Codabar barcode and additional information. All information is printed in black on a white background. These labels may be pre-printed or produced using a demand printed system where the information is transferred electronically from a host system.

See also Section 25.7 for future changes to this label.

25.5.2 Label dimensions

Two sets of label dimensions are defined:

a) 55 mm × 55 mm

b) 55 mm × 35 mm.

The barcode height will be 10 mm and the code will be positioned at the bottom left of the label. The 'quiet zone' must again be maintained. The smaller size b) is intended for use with all labels where the component retains its original anticoagulant which will be printed on the base label. The larger size a) is to be used whenever the anticoagulant is modified and must be respecified.

Examples of component labels are shown in Figure 25.5.

25.5.3 Component barcodes

See Section 25.7. for future changes to this label.

All components have an individual barcode. The barcode is comprised of three main elements:

- start code 'a0'
- a five-character code to uniquely denote the component
- a stop code '3b'.

Table 25.3 Historic blood group classifications (only for use on 'Use in emergency only' labels)

Text	gg	Text	gg
O Rh D positive	48	O Rh D negative	92
A Rh D positive	59	A Rh D negative	03
B Rh D positive	70	B Rh D negative	14
AB Rh D positive	81	AB Rh D negative	25

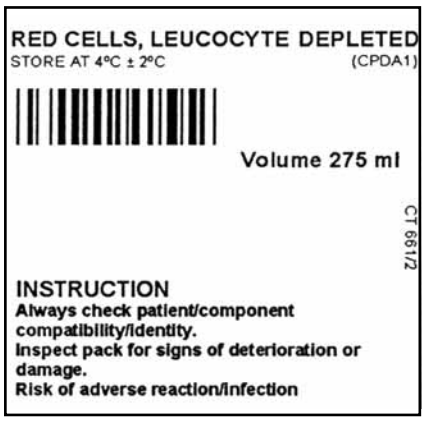

Figure 25.5 Sample component label

25.5.4 Component code reference table

The current component code reference table is held and managed by SACIT. The table includes:

- the text defining the component. Where possible this text is the same as that defined in Chapter 8 of these guidelines
- the start code for the component 'a0'
- the barcode for the component, e.g. 04260
- the stop code for the component '3b'.

25.5.5 Allocation of new component codes

In the event of a Blood Service requiring a code for a component that will be issued on a regular basis, the following steps must be carried out:

- a request form for a new component code must be completed and sent to JPAC Standing Advisory Committee on Blood Components (SACBC). This committee will determine if the component is a 'new' one or is simply a 'variant' on an established one and hence would not require a new code
- the text to describe the component and any additional information should be approved by SACBC
- SACBC will refer the request to SACIT
- SACIT will assign a bar code for the new component
- the component will be added to the component code reference table.

25.6 Manufacturers' base labels

For base labels applied to blood collection packs for use in the UK and their associated satellites, it is required that the label applied must accommodate such overstick labels as may be applied by the Transfusion Service for the identification of the donation and blood group or additional information labels as in use from time to time.

Complete details of the size, content and layout of all base labels are contained in Chapter 28.

All pack manufacturers now place Codabar bar coded information on each pack. Two classes of data are recorded, namely:

Pack type

This label defines the type of pack e.g whole blood, platelet etc. The code is defined as follows:

cppppppk7b

Where:

c is the start code

pppppp is the manufacturer's list/catalogue number

k is the Mod 11 check digit

7b is the stop code.

Pack batch/lot number

This number describes the batch/lot number for each individual pack. The format is:

b1XXDDDDDDDC6b

Where:

b1 is the start code

XX is the manufacturer's code (see Table 25.4)

DDDDDDD is the batch/lot number

C is a modulus 11 check digit based on XXDDDDDDD

6b is the stop code.

Table 25.4 Manufacturers' codes

Manufacturer	Code
Baxter Healthcare	01
TUTA Laboratories	02
Biotest UK	03
Haemonetics	04
NPBI	05
Terumo	06
Gambro BTC – US	07
Maco Pharma	08
PALL	09
ASAHI Medical Gmb H	10
Gambro BTC – UK	11
Kawasumi Laboratories Inc	12
Charter Medical	13
Baxter Healthcare – La Chatre	14
Baxter Healthcare – Haina	15
Baxter Healthcare – Mountain Home	16

25.7 Future developments in labelling

In order to achieve full ISBT 128 labelling compliance a number of further changes are required to blood pack labelling. This section identifies the changes that will be required, but at the time of publication a timetable for implementing these changes was not available.

25.7.1 Component labels

The existing Codabar component label will be replaced with the ISBT 128 product code (blood components). The component label is a 50 mm × 50 mm label which is affixed to the lower left quadrant of the base label. The template for the label is indicated in Figure 25.6.

The barcode is the appropriate code taken from the ISBT 128 product code (blood components) database with format:

=<aooootds

Where:

=< are the data identifiers

a has value 'E' for blood components

oooo is the four-digit sequence which together with the previous character forms the product code

t is the donation type identifier set to 0 in the UK

d is the first split identifier (A–Z) or 0 if not specified

s is the second split identifier (a–z) or 0 if not specified.

The first two lines of text contain the name of the product e.g. 'FRESH FROZEN PLASMA'. Lines three and four contain storage information e.g. 'Store at 4°C ± 2°C'. The fifth to seventh lines contain attribute descriptions such as 'Irradiated', 'Pack 1 Split 1'. The volume field will contain either the actual or nominal pack volume. Immediately below the barcode will be the unique reference number of the label which will correspond to the eye-readable barcode without the data identifier characters.

Codes for components prepared by the UK Blood Services will be a subset of the ISBT 128 product code (blood components) database. The list of codes approved for use by the UK Blood Services, together with the corresponding UK text approved by the Standing Advisory Committee on Blood Components (SACBC), will be maintained by SACIT. It should however be noted that blood components imported from other countries using ISBT 128 may utilize any of the product codes identified in the ICCBBA database, or may use nationally assigned codes.

New components will have codes assigned as required by SACIT in liaison with SACBC and ICCBBA. Requests for new codes should be made in writing or by e-mail to the Chair of the SACIT.

In the UK the sixth character of the product code (identified as *t* in the ISBT 128 Standard Technical Specification) may be set to 1 to indicate 'autologous use only'. In all other cases this character is set to zero.

25.7.2 Expiry date barcode

The current Codabar expiry date will be replaced with the ISBT 128 expiry date and time barcode. This has the structure:

&>cyyjjjhhmm

Figure 25.6 Component label template

Where:

&> are the data identifiers

c designates the century (e.g. 0 for 2000-2099, 1 for 2100–2199)

yy designate the year in the century

jjj is the Julian date (the number of the day in the year, e.g. 022 is 22 JAN)

hh specify the hour (00–23)

mm specify minutes (00–59).

Where it is not necessary to indicate an expiry time hh mm will be set to 23 59. Thus expiry at the end of 22 January 2001 would code as 0010222359.

The expiry date must be presented in eye-readable and barcode formats. When the default 23:59 is used it is *not necessary* to show the time in the text, since a midnight expiration is assumed. The eye-readable text must be printed with characters of no less than 3 mm height. The content must comprise the day number, the month represented by its first three characters, and the four digit year (e.g. 1 FEB 2001). Where a non-default time is specified in the barcode this should follow the year and be expressed using the 24-hour clock (e.g. 1 FEB 2001 18:00).

The use of the date format DD MMM YYYY avoids problems which may arise due to national differences in the order of the elements of numerically expressed dates. The accepted month abbreviations are JAN; FEB; MAR; APR; MAY; JUN; JUL; AUG; SEP; OCT; NOV; DEC.

25.7.3 Label layout changes

A number of other layout changes are required to make the labels more closely aligned with the international standard:

- the donation number eye-readable text will appear *below* the barcode instead of above
- label widths will be standardised at 50 mm, currently the component label is 55 mm and the group label 45 mm
- the data characters for the expiry date and blood group will be printed immediately below the barcode in a small font.

A sample layout for a fully modified label is shown in Figure 25.7.

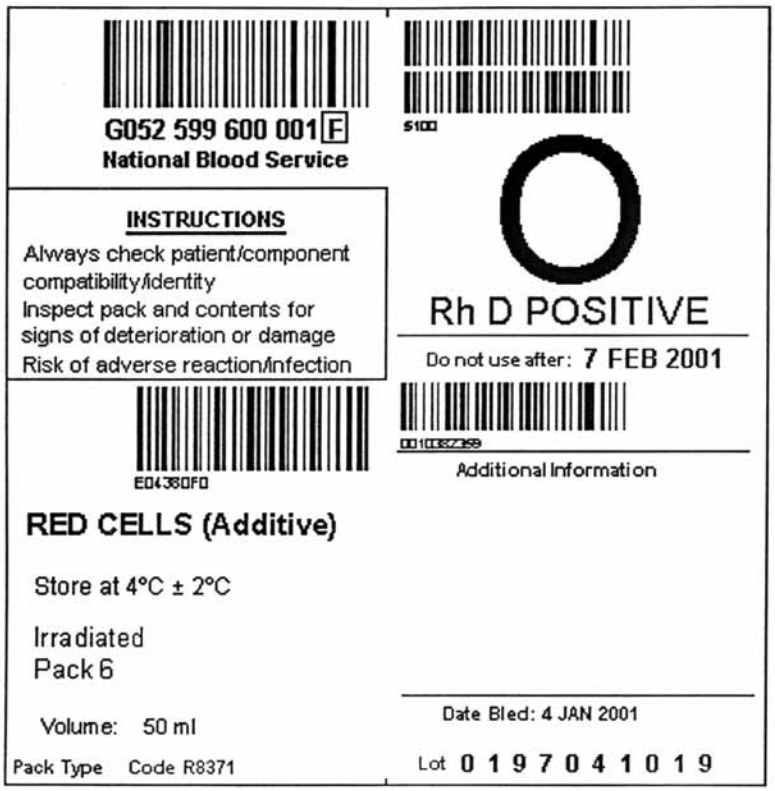

Figure 25.7 Future label layout sample

Chapter 26

Uniform labelling of human tissue products using ISBT 128

26.1 Introduction

ISBT 128 is an internationally defined coding system for the barcoding of information on blood components, progenitor cells and tissue. Designed by the International Society of Blood Transfusion (ISBT) working party on automation and data processing, the responsibility for the worldwide management and distribution of the ISBT 128 Technical Specification and associated databases now resides with the International Council for Commonality in Blood Banking Automation Inc. (ICCBBA).

A specification for the use of the coding system for the description of tissue products has been agreed by ICCBBA (ISBT 128 Product Code Database (Tissues), V.1.0.0., January 2002).

This chapter details the manner in which internationally agreed ISBT 128 tissue donation identification numbers, blood group codes and product description codes will be incorporated into tissue labelling systems in the UK where ISBT 128 is in use. The document cross-references the UK database of tissue product description label text which is maintained by the UKBTS/NIBSC Standing Advisory Committee on Tissues and Stem Cells. The labelling of stem cell donations and products is outside the scope of this document.

The ISBT 128 Technical Specification is provided to companies and Blood Transfusion Services that have registered with ICCBBA, or may be purchased directly from ICCBBA Inc (www.iccbba.com).

The ISBT 128 Technical Specification: summary

The ISBT 128 Technical Specification

- describes the standard layout for a blood group label
- defines the data identifiers for barcodes used in the blood bank environment
- defines the data structures that carry information, i.e. how a particular barcode will be recognized by a reader, how many characters there are, and whether the characters are letters, numbers or both
- includes tables that define how complex barcodes should be translated, such as ABO/Rh blood groups

- defines technical details for the barcode itself, such as the width of the narrowest bars and the minimum height of bars
- describes the variation made in Code 128 to support specialised concatenation
- identifies the authority of ICCBBA, acting for the ISBT, to define other databases, particularly the product code database
- designates national groups as responsible for the definition of other tables that will have more limited use.

26.2 The labelling system

The labelling system for retrieved tissues and tissue products comprises the following elements:

- base label: the label applied to the retrieved tissue container following tissue retrieval and/or to the final container following tissue processing. It includes guide marks (preferably corner marks to prevent interference with barcode reading) to assist in the positioning of overstick labels. Retrieved tissue may be from living donors (retrieved during surgery) or from cadaveric donors (retrieved after death). It is noted that in the majority of cases, tissue is transferred during processing to a secondary/final container. In these circumstances a new base label is applied to the final container
- donation identification number label: a label bearing the ISBT 128 donation number barcode. Produced in sets, these labels ensure the accurate and unique labelling of all tissue donations and samples. Allocated at the point of donation, this number is fundamental to the secure audit trail for tissues. Where a retrieved tissue is processed without pooling or is issued unprocessed, the original donation number barcode is used to identify it to the point of implantation. This label will bear the title of the Blood Transfusion Service supplying the tissue, unless this is included on another label
- batch/pool identification number label: a label bearing an ISBT 128 donation number barcode. These labels are demand-printed when different tissues from one donor or tissues from more than one donor are pooled for processing. They ensure the accurate and unique identification of tissues once they have been pooled through to the individual resulting tissue grafts/units each of which bears the same identification number. Ideally, the number sequence used for batch/pool identification numbers should be different from donation number sequences and should be easily identifiable as batches/pools. This label will bear the title of the Blood Transfusion Service supplying the tissue, unless this is included on another label
- product label: a label bearing the ISBT 128 tissue product barcode, together with tissue product information, applied at the time of tissue retrieval and final tissue product manufacture. Where individual tissue units have been produced from a pool of tissues (from one or more donors) the product barcode can be used to individually identify up to 999 splits from the pool. This label can include unit-specific information
- tissue status label: a label indicating the status of a particular product in barcoded and eye-readable form. This is equivalent to the blood group label in blood banking. The following status labels can be applied:
  - Fit for clinical use (Rh D not specified)
  - In quarantine – not yet cleared for clinical use
  - Rh D POS – fit for clinical use
  - Rh D NEG – fit for clinical use
  - Must be sterilized
  - For in vitro R & D only
  - Biohazardous
  - Discard

- See outer container for product status (for cryopreserved products)
- Autologous donation - fit for clinical use
- Autologous donation - in quarantine

The tissue status label will also bear the nationally defined short form unit identifier and other donation specific information, e.g. date of donation or retrieval site. This label will be applied by the Blood Transfusion Service prior to release into stock, allocation for R & D or discard, except for the 'See outer container' label which will be applied to the base label before the product is cryopreserved. These labels are positioned to allow concatenation between the unit identifier on the base label and the short form identifier on the status label

- expiry date label: a label indicating the date by which the tissue must be processed (if in quarantine), issued (if in issuable stock) or used (if dispatched for clinical use). Different expiry date labels may be overstuck on products at different times. For example, some banks shorten the shelf life of products once they are issued from a bank due to concerns relating to appropriate long-term storage and control in hospitals.

The labels indicated above are all affixed onto a base label, except in the case of cryopreserved products where two status labels may be used, one on the product container itself (applied before cryopreservation), 'See outer container for product status' and one on the outer container giving the product status. In this case, a new base label, product label and expiry label should all be attached to the outer container. The arrangement of labels depends on the product and container type. Two options are shown in Figures 26.1 and 26.2; each would require a different base label. These diagrams are for orientation purposes only: see under the appropriate sections for details of each label content and layout.

The four basic quadrant labels may be printed as combination labels; for example, the donation number label and the product description label may be printed as a single vertical strip label and affixed to cover the left-hand half of a square base label. Similarly, expiry date information may be printed on a status label so that the two right-hand quadrants are printed as a single strip.

26.3 General requirements

Label quality

Labels used for tissue and sample labelling must be:

- self-adhesive using a non-invasive adhesive
- tamper-evident (i.e. removal must deface the label)
- smear-resistant and non-fading
- resistant to water and humidity
- capable of being affixed readily to paper or other base label material, plastic surfaces, glass (particularly glass tubes of 12 mm diameter) without winging ('winging' is defined as the lifting of a label from the surface to which it is applied. Winging should not exceed 2.5 mm as the maximum linear distance of the label not adhering to the tube at any label edge, measured after 24 hours' refrigeration at 4°C)
- capable of withstanding a temperature range of –80°C to +56°C after application to the tissue container. This range may be extended by the ordering authority at the time of order. This condition must extend to the printed text which must not deteriorate due to thermal conditions. Where products are stored at lower temperatures, labels will be sealed into plastic pockets on the container

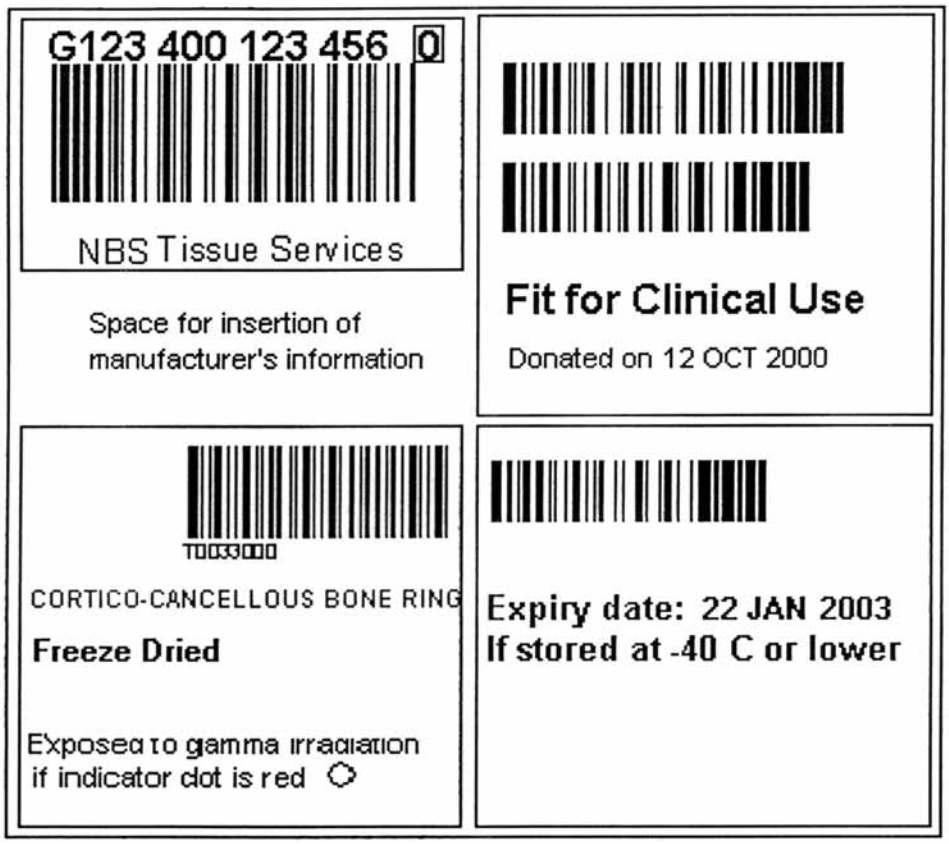

Figure 26.1 Label positioning: option 1 (example; see cautionary note in text)

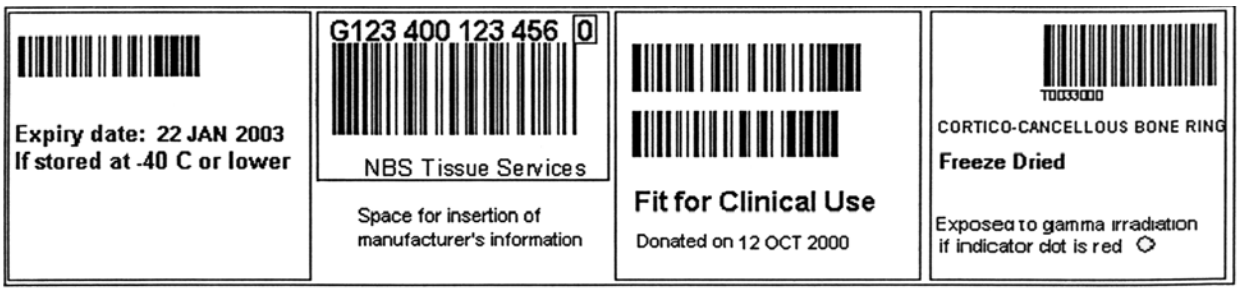

Figure 26.2 Label positioning: option 2 (example; not to scale)

- capable of being applied without slippage to tubes etc. subject to temperature variation prior to use, for example, tubes/packs stored in a vehicle and then used at normal ambient temperature, such equipment being by definition 'damp'
- non-flaking when read using a hand-held light-pen touching the label.

Printing requirements: eye-readable information

The ISBT 128 Technical Specification indicates that 'Every bar code, with the exception of the donation identification number, should be accompanied by an exact eye-readable representation of the data characters in the bar code'. In the UK this requirement is *not* being implemented for the short form unit identifier, and is currently optional for the blood group/status code and expiry data code.

Requirements for each type of label are defined in the appropriate sections.

Printing requirements for barcodes

All barcodes will be printed as specified in the ISBT 128 Technical Specification.

Concatenation

Concatenation requirements for ISBT 128 are laid out in the ISBT 128 Technical Specification and further expanded in ICCBBA Technical Bulletin No. 5. Where concatenated codes are to be read it is essential that the barcode readers used support concatenation of Code 128 barcodes with the defined temporal/spatial limitations of ISBT 128.

In the UK release status labelling will be confirmed by concatenation of the donation identification number with the short form unit identifier (nationally defined – see Section 26.10) and donation ID. The relevant data identifiers are specified within the appropriate sections of this document. For further details refer to the ISBT 128 Technical Specification.

26.4 The base label

The base label dimensions are to be at least 110 mm wide × 104 mm high on all tissue containers/labels where the square format is used. Where the horizontal format is used they will be at least 220 mm wide × 52 mm high.

It is noted that where tissues are stored in very small containers, the base label may be attached to a secondary container. In these circumstances, the inner container will be labelled with the donation number barcode to provide a link to the label on the outer packaging.

The guide marks on Figures 26.3 and 26.4 are required to assist positioning of later labels.

Square format

The top left-hand quadrant will be used for the application of a donation identification number label. The space below this on the left-hand side of the label between 25 mm and 40 mm from the top edge must be left blank. This space may be pre-printed, for certain donations, by the container supplying company with lot/batch number of the container. This text must be visible at all times.

The bottom left-hand quadrant will be overstuck with a product description label.

The top right-hand quadrant will have pre-printed text indicating that the donation is in quarantine, as follows:

IN QUARANTINE

Not yet released for clinical use

The top right-hand quadrant will be overstuck with a status label once its status has been determined (for cryopreserved products, this quadrant can be overstuck with a 'See outer container for product status' label). The status label can then be applied to a secondary container.

The bottom right-hand quadrant will be overstuck with an expiry date label which relates to expiry date prior to issue or to expiry date following issue.

Horizontal format

The same four quadrants will be used but the order in this case will be as shown in Figure 26.4.

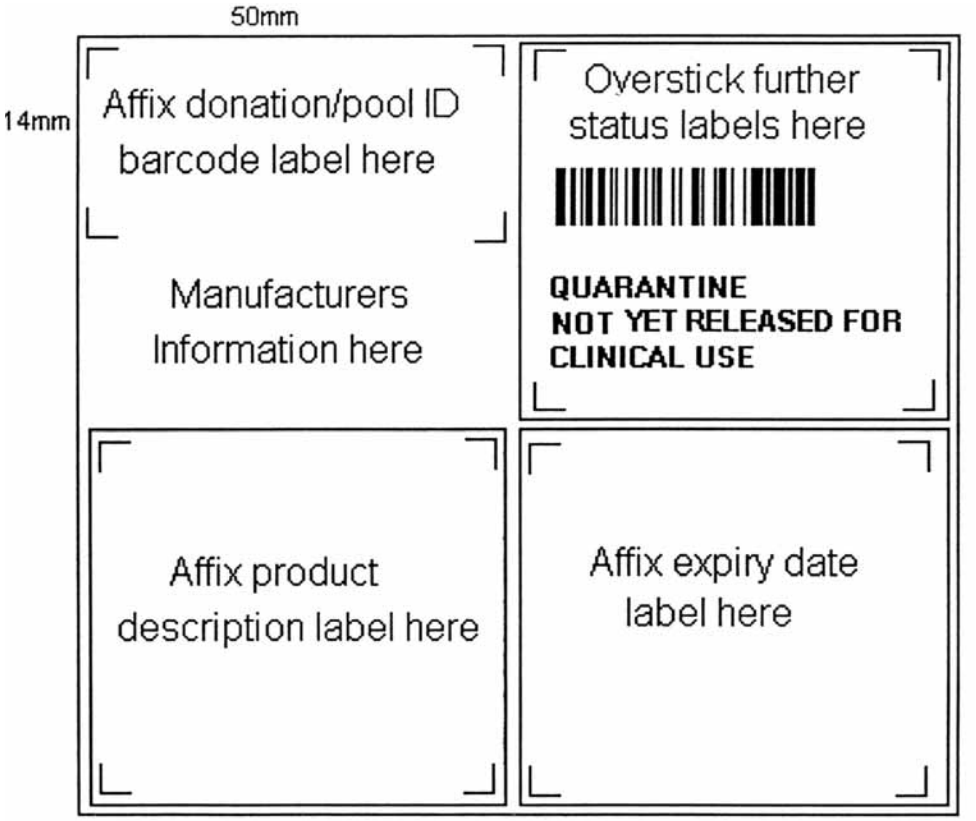

Figure 26.3 Base label design: square format

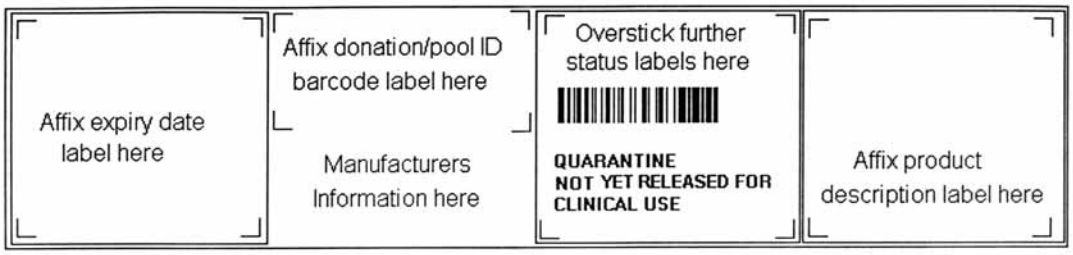

Figure 26.4 Base label design: horizontal format (not to scale)

26.5 Donation and pool identification number labels

The donation identification number plays a critical role in the safety of the tissue supply. It provides a unique identification number which cross-references tissue donations and products and samples taken at the time of donation. Where tissue products are not further processed, they are issued with the same donation identification number label.

Donation identification number labels must be generated in sets under strictly controlled conditions which ensure that all the labels in a set bear the same number, and that each set is unique. It is the responsibility of the manufacturer of the label sets to undertake appropriate quality control measures to ensure these conditions are met.

Pool identification number labels are generated on demand when donations are processed together, whether from one or more donors.

As tissues are usually transferred from their original containers to secondary and tertiary containers during processing, it is a requirement that donation and pool identification numbers can be printed on demand.

An example set of identification numbers is shown in Figure 26.5. Barcode density information is provided in the ISBT 128 Technical Specification. The structure of the donation identification number is described further below.

Figure 26.5 Donation identification number label set

For identification number labels to be applied to tissue products, the dimensions of the label are as indicated in Figure 26.6. As with all barcodes, there should be a minimum 'quiet zone' of 2.5 mm between the edge of the label and the start of the barcode. This label is affixed to the top left-hand section of the base label.

The donation identification number complies with the structure defined in the ISBT 128 Technical Specification. The Country/Collection Facility Identification Codes defined for tissue banking use in the UK are shown in Table 25.1 (the issue of new codes is controlled by ICCBBA as part of the registration process. Any site within the UK requiring a new code must apply to SACIT in the first instance).

The collection year characters (6 and 7) should correspond to the last two digits of the year in which collection took place. In practice, this cannot be readily achieved using pre-printed labels without considerable wastage. Within the UK it is therefore permissible to allow a maximum variation of one month either way, i.e., it is permissible to use the previous year's collection year characters up to 31 January in the current year, and to use donation numbers with the following year's collection year characters from 1 December in the current year.

All UK donation identification numbers must use the flag characters 'ff' as data transmission check characters in the range 60-96 (Type 3 flag as defined in the Technical Specification). The algorithm for calculating check characters is included in Appendix VII. However, designers of software systems will need to take into account that units imported from countries outside the UK may well use these flag characters for other purposes as permitted in the Technical Specification (Type 1 and 2 flags).

Figure 26.6 Donation number label dimensions

The eye-readable format of the donation identification number in the UK comprises the data characters excluding the flag characters, followed by the manual entry check character. The layout differs from that in the ISBT 128 Technical Specification in that all characters of the number must be of equal size and weight. Printing of the six-digit unit serial number in larger or bold characters is not permitted. Software manufacturers should ensure that only the eye-readable format is presented in screen displays.

The number should be displayed with the characters grouped in a 4, 3, 3, 3, 1 arrangement. It is recommended that the check character be enclosed in a box where this is possible. The check character set uses the characters I and 1, 0 and O and the font selected should be one which allows easy differentiation between these characters.

G151 798 123 456 **4**

Where the donation identification number has to be recorded manually, form designs that assist accurate recording, such as the use of boxes to encourage correct character grouping, are recommended. An example is given in Figure 26.7.

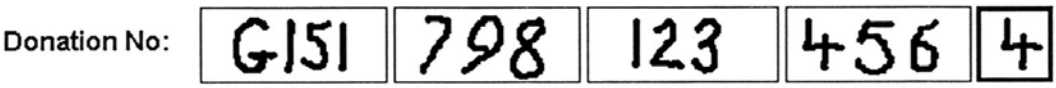

Figure 26.7 Form boxes designed to enable accurate recording

The full eye-readable donation identification number, including check character, must be recorded and entered in all cases. The use of pre-programmed shortcut keys ('hot' keys) or pre-printing of part of a number is not acceptable.

26.6 The product label

The product label (50–55 mm wide × 50–52 mm high) is affixed to the left lower quadrant of the square base label or the left-hand side of the horizontal base label, if printed as a single quadrant (it may be printed as a combined label with another quadrant). A start product label is attached to all base labels at the time of tissue retrieval. If the tissue remains in the same container until the time of issue for implantation it is issued with this product label. If it is subjected to further processing while remaining in its original container (e.g. gamma irradiation) the start product label is overstuck with the appropriate final product label before application of a status label. If it is transferred to another container following processing, a new base label is attached and the appropriate final product label attached to the lower half of the base label following processing. The template for the product label is shown in Figure 26.8.

Figure 26.8 Tissue product label template

Codes for tissues and tissue products used in the UK are taken from the international Human Tissue Code Database (ICCBBA, 2002), together with the corresponding UK text approved by the Standing Advisory Committees on Tissues and Stem Cells (SACTSC) and the Standing Advisory Committee on Information Technology (SACIT).

New tissue donations and tissue products will have codes assigned as required by SACIT in liaison with SACTSC and ICCBBA. Requests for new codes should be made in writing or by email to the Chair of SACIT.

Updates of the Human Tissue Code Database that occur between publications can be obtained from SACIT.

The first two lines of text contain the tissue component class and modifier, for example:

CORTICO-CANCELLOUS BONE RING

Freeze-dried

Lines three to seven describe various attributes, where relevant, though one of these can be used to provide further product description information (see Section 26.8). It is not necessary to include the unit of issue attribute details. Space will be available next to the method of sterilization attribute where exposure 'dots' can be applied. The significance of the dot colour need not be detailed on the label but can be explained in the package insert. The volume/dimension field will contain either the actual or nominal tissue volume or other relevant dimensions (e.g. length, depth etc.). Immediately under the product barcode will be the unique reference number of the label which will correspond to the eye-readable barcode without the data identifier characters.

An example of a product label is given in Figure 26.9.

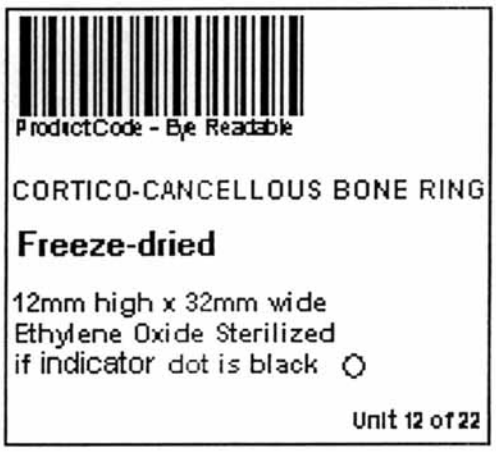

Figure 26.9 Product label (example)

26.7 The tissue status label

The tissue status label is a demand printed label (50–55 mm wide × 50–52 mm high). The overall layout of the label is shown in Figure 26.10. This label can include graft-specific information such as date and site of donation.

An example of a status label is given in Figure 26.11.

The elements of the label content from top to bottom are as follows.

Short form unit identifier

The tissue status label is unique to a specific donation. To ensure that it is affixed to the correct container a barcode, the nationally defined short form unit identifier, is printed on the label. This is used by the Blood Transfusion Service in a concatenated read with the donation identification number at the time of labelling.

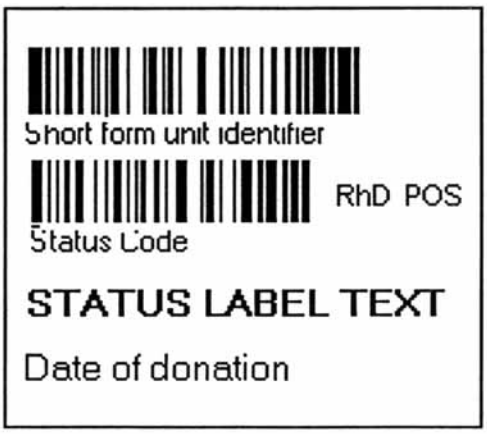

Figure 26.10 Tissue release status label

Figure 26.11 Status label (example)

The structure of the short form unit identifier is defined in Section 26.10. Either the '&a' or '&b' versions of the code can be used.

Barcode indicating product status and the statement of product status

The statement of product status (which appears below the barcode) will be one of those listed in Table 26.1.

The barcode giving the product status is taken from the ISBT 128 Technical Specification.

Donation-specific information

Specific information which applies to the donation may be included on this label. This includes the Rh D blood group and the date of donation.

The Rh D blood group is only relevant where red cells remain in the final tissue product and where the product is supplied for female recipients of childbearing age. Below the short form unit identifier is printed the Rh D blood group barcode for certain products. The text relating to Rh D type will be printed to the right of the status barcode. Where the Rh D type is indicated, products will always be fit for clinical use.

Within the UK the blood group data structure will reflect that given in the ISBT 128 Technical Specification:

=%ggre

Where:

'gg' is the ABO/Rh/status code. The default ABO/Rh codes or the special message codes from the ISBT 128 Technical Specification will be used as indicated.

Table 26.1 Statements of product status

Statement	Status code
Fit for clinical use (Rh D not defined)	T3
In quarantine	
Not yet available for clinical use	Mq
Rh D Pos	
Fit for clinical use	T1
Rh D Neg	
Fit for clinical use	T2
Must be sterilised	T6
Biohazardous	Mb
Discard	Md
For *in vitro* R & D only	Mr
See outer container for product status	T5
Autologous use	
Fit for clinical use	Ma
Autologous use	
In quarantine	T4

An Rh D positive unit will code as:

=%T100

The barcode and text content of these labels is described in Section 26.8.

Date of donation can also be included on this label following the conventions described for expiry dates. This information does not need to be barcoded; it is represented in text only and must comprise the day number, the month represented by its first three characters, and the four-digit year (e.g. 1 FEB 2002).

26.8 Status label definitions

For each of the special message codes indicated in the status label section an associated status label is defined. The specification for these labels is divided into two sections, one for essential information which must be present on the label as specified, and one for optional information which may be added if desired. All labels are to be demand-printed black on white.

T3 'FIT FOR CLINICAL USE' (Rh D not specified)

Essential information

Barcode: ISBT 128 Fit for clinical use code where gg = 'T3'. Positioned to allow concatenated read with an adjacent donation number

Text: The words 'FIT FOR CLINICAL USE' in upper-case letters of minimum height 4 mm.

Mq 'IN QUARANTINE – NOT YET AVAILABLE FOR CLINICAL USE'

Essential information

Barcode: ISBT 128 group code where gg = 'Mq'. Positioned to allow concatenated read with an adjacent donation number

Text: The words 'IN QUARANTINE' in upper-case letters of minimum height 6 mm

Text: The words 'NOT YET AVAILABLE FOR CLINICAL USE' in upper-case letters of minimum height 3 mm.

Optional information

Text: The word 'Reason:' followed by a free-format message giving the reason for hold.

T1 'RH D POS – FIT FOR CLINICAL USE'

Essential information

Barcode: ISBT 128 group code where gg = 'T1'. Positioned to allow concatenated read with an adjacent donation number

Text: The words 'Rh D POS' in upper-case letters of minimum height 6 mm to appear to the right of the status barcode

Text: The words 'FIT FOR CLINICAL USE' in upper-case letters of minimum height 3 mm.

T2 'Rh D NEG – FIT FOR CLINICAL USE'

Essential information

Barcode: ISBT 128 group code where gg = 'T2'. Positioned to allow concatenated read with an adjacent donation number

Text: The words 'Rh D NEG' in upper-case letters (except the 'h') of minimum height 6 mm to appear to the right of the status barcode

Text: The words 'FIT FOR CLINICAL USE' in upper-case letters of minimum height 3 mm.

T6 'MUST BE STERILIZED'

Essential information

Barcode: ISBT 128 group code where gg = 'T6'. Positioned to allow concatenated read with an adjacent donation number

Text: The words 'MUST BE STERILIZED' in upper-case letters of minimum height 3 mm

Optional information

Text: The word 'Reason:' followed by a free-format message.

Mb 'BIOHAZARDOUS'

Essential information

Barcode: ISBT 128 group code where gg = 'Mb'. Positioned to allow concatenated read with an adjacent donation number

Text: The word 'BIOHAZARD' in upper-case letters of minimum height 4 mm

Text: The words 'HIGH RISK' in upper-case letters of minimum height 6 mm

Symbol: Biohazard warning symbol of minimum height 20 mm.

Md 'DISCARD'

Essential information

Barcode: ISBT 128 group code where gg = 'Md'. Positioned to allow concatenated read with an adjacent donation number

Text: The words 'DISCARD' in upper-case letters of minimum height 4 mm

Optional information

Text: The word 'Reason:' followed by a free-format message.

Mr 'FOR IN VITRO R & D ONLY'

Essential information

Barcode: ISBT 128 R & D code where gg = 'Mr'. Positioned to allow concatenated read with adjacent donation number

Text: The words 'FOR IN VITRO R & D ONLY' in upper-case letters of minimum height 3 mm.

T5 'SEE OUTER CONTAINER FOR PRODUCT STATUS'

Essential information

Barcode: ISBT 128 R&D code where gg = 'T5'. Positioned to allow concatenated read with adjacent donation number

Text: The words 'SEE OUTER CONTAINER FOR PRODUCT STATUS' in upper-case letters of minimum height 3 mm.

Ma 'AUTOLOGOUS COLLECTION (FIT FOR CLINICAL USE)'

Essential information

Barcode: ISBT 128 group code where gg = 'Ma'. Positioned to allow concatenated read with an adjacent donation number

Text: The words 'FOR AUTOLOGOUS USE ONLY' to appear in letters of minimum height 4 mm

Text: The words 'FIT FOR CLINICAL USE' to appear in letters of minimum height of 4 mm.

T4 'AUTOLOGOUS COLLECTION (IN QUARANTINE)'

Essential information

Barcode: ISBT 128 group code where gg = 'T4'. Positioned to allow concatenated read with an adjacent donation number

Text: The words 'FOR AUTOLOGOUS USE ONLY' to appear in letters of minimum height 4 mm

Text: The words 'IN QUARANTINE' to appear in letters of minimum height 4 mm.

26.9 The expiry date label

An expiry date label will be applied to base labels at the time of tissue retrieval and whenever another base label is used. A final expiry date label may be applied at the time of issue if the bank follows a policy of shortening the shelf life at the time of issue.

The use of a barcoded version of expiry date is optional. If it is to be used it should have the following structure:

= > cy yj jj

where 'c' designates the century (e.g., 9 for 1999; 0 for 2000); 'yy' designates the year, and 'jjj' is the Julian date (i.e., the number of the day in the year, e.g., 022 is 22 JAN).

The expiry date must be presented in eye-readable format. Additional text will follow each expiry date and will be specific for each product, e.g.:

22 JAN 2003 if stored at –40°C or lower

The eye-readable text must be printed with characters of no less than 3 mm height. The content must comprise the day number, the month represented by its first three characters, and the four-digit year (e.g. 1 FEB 2002).

The use of the date format DD MMM YYYY avoids problems which may arise due to national differences in the order of the elements of numerically expressed dates. The accepted month abbreviations are JAN; FEB; MAR; APR; MAY; JUN; JUL; AUG; SEP; OCT; NOV; DEC.

The expiry date label should also include the following text:

See package insert for further information

Unit-specific product information such as product weight may also be included on the expiry date label, e.g.:

84g

Where the expiry date label is printed as a quadrant label on its own it should also have the short form donation number barcode identifier. This is not necessary where the label is printed as part of a status label (already including this identifier). An example of an expiry date label is shown in Figure 26.12.

Figure 26.12 Expiry date label (example)

26.10 National ISBT 128 definitions

National bodies are permitted to allocate nationally defined codes commencing with '&' and a lower-case alpha character. Within the UK this responsibility lies with SACIT.

The following national codes have been assigned which will be applied in tissue labelling.

'&a': short form unit identifier (Type 1)

Defined for the shortened form of a donation number used on demand-printed status labels for concatenated read with the donation number as part of label verification or on expiry date labels which are demand-printed independently of the status label. This code must not be used for any other purpose.

The code structure is:

&annnnnn

Where:

'nnnnnn' is the six-digit unit serial number from the ISBT 128 donation number definition.

'&b': short form unit identifier (Type 2)

Defined for the shortened form of a donation number used on demand-printed group labels for concatenated read with the donation number as part of label verification. This code must not be used for any other purpose. The code structure is:

&bnnnnnnk

Where:

'nnnnnn' is the six-digit unit serial number from the ISBT 128 donation number definition

'k' is a single-digit iteration number used to assist in controlling labelling where more than one labelling process takes place (for example, an additional group label has to be placed over the initial label to display additional testing information such as CMV status).

Table 26.2 Glossary: uniform labelling of human tissue products

ICCBBA	International Council for Commonality in Blood Banking Automation Inc.
ISBT	International Society of Blood Transfusion
SACTSC	Standing Advisory Committee on Tissues and Stem Cells
SACIT	Standing Advisory Committee on Information Technology
UKBTS	United Kingdom Blood Transfusion Services
UKCS	The United Kingdom Consensus Standard for ISBT128
Check digits	The barcoded check digits are machine-readable. They are calculated using the ISO 7064 Mod 37, 2 algorithm
Check character	The check character is eye-readable and is determined by cross-mapping the check sum against the ISO 7064 check values. It is always a single character.

References

Report of the Committee for Commonality in Blood Banking Automation (CCBBA) July 1977

ISBT 128 Technical Specification

Uniform Symbology Specification: Code 128. (AIM USA) June 1993

ICCBBA Technical Bulletin 5

Chapter 27

Standards for electronic data interchange within the UK Blood Transfusion Services

27.1 Introduction

UK Blood Transfusion Service (UKBTS) and hospital blood bank computer systems have developed to provide sophisticated control of information on donors, blood components and patients, with secure methods of information transfer utilising barcodes and electronic data capture. However, secure information transfer between the transfusion centres and their customer hospitals has been limited to the barcoded information incorporated on the blood packs, and is of restricted scope.

Interest in developing electronic data transfer has been stimulated recently, with the emphasis on the electronic transfer of ordering and dispatch information. The Joint UKBTS/NIBSC Professional Advisory Committee (JPAC) Standing Advisory Committee on IT recognised the desirability of developing UK-wide standards for data transfer at an early stage in this process.

This document describes a standard for messages used in communication between blood services and their customers. Each message comprises a standard envelope and a message content. The envelope specifies the overall structure of UKBTS messages and identifies the specific message content included inside the envelope. The message content will comply with one of the message protocols defined in this document. Each message protocol defines the content and format of a specific type of data transaction.

The standard does not address the delivery mechanism, or any surrounding envelopes. Thus, it provides a standard which is relevant to delivery mechanisms as diverse as e-mail messages, web page downloads, ftp transfers, or ASCII text files.

At the same time it retains a standard presentation of messages which readily identifies them as belonging to the UKBTS set, and allows a general process to identify the type of message received, the source and the destination.

In the future it is hoped that ordering and dispatch information will be incorporated into HL7 message structures, and this will provide a future migration path for electronic messaging. However, the HL7 Blood Banking Special Interest Group have not started work in this area yet, and so it is likely to be some years before an HL7 message is available. In the meantime the messages defined in this document should be used.

27.2 Control of message structures

The standard is controlled by the JPAC Standing Advisory Committee on Information Technology (SACIT). All messages utilising the UKBTS envelope must comply with an approved message structure.

Proposals for new messages, or amendment to existing messages, should be submitted in the first instance to the chairman of SACIT. These will be reviewed by the electronic data interchange (EDI) group and if approved will be incorporated into the standard. Whilst the objective is to obtain standards applied throughout the UK, the two-level structure does allow the flexibility of defining different structures at the message protocol level where essential.

27.3 General protocol

The general protocol defines the general character of the overall message, and elements which are common to both the envelope and the message content. The message uses standard ASCII characters throughout, and lines are terminated with the carriage return (ASCII 13) character. Fields are all fixed width and left-justified. Leading zeros for numeric fields are used only where explicitly indicated.

The following are standard components of every line transmitted:

The line number

A sequential number defining the line in the file, which is located in character positions 1 to 5 of every line. The header line will always have a line number of 00001.

The checksum

The checksum immediately precedes the carriage return terminator of each line. The checksum is calculated by taking the sum of the ASCII value of all characters in the line, and then determining the modulus 97 remainder which becomes the check sum.

27.4 Envelope definition

The envelope definition defines the content of the first and last lines of the file/transmission (see Table 27.1).

The first or header line contains an identifier specifying that this is a message complying with a UKBTS specification, the date and time generated, the source and destination of the message, and the protocol number which identifies the relevant protocol to which the message conforms.

Source and destination identifiers for the Blood Services will be the ISBT 128 collection facility identification code. It is anticipated that hospital blood banks will use the identifier assigned by their local Blood Service.

The terminator line contains a record count indicating the total number of message lines excluding the header and terminator lines, and a standard message terminator message.

27.5 Message protocols

Table 27.2 indicates the protocols defined to date. The message protocols contain a range of data defined as either mandatory or optional. The mandatory fields give essential information and must contain valid data. The optional fields give the flexibility to build in a wide range of additional information, but if not required are left as blank (space character) fields.

27.6 Protocol 000001 – Blood component dispatch information

Two data line structures are defined within this protocol. The first is a single line containing administrative information (order no., dispatch no.), and the second is a multiple occurrence line with an entry for each item on the dispatch. To distinguish

Table 27.1 Envelope definition

Header line					
Field	**Length**	**Description**	**Format**	**Mandatory?**	**Notes**
1	5	Line number	NNNNN	Y	Always 00001 for header
2	10	Fixed Text	'UKBTSSTART'	Y	
3	8	Date	YYYYMMDD	Y	
4	4	Time	HHMM	Y	
5	6	Protocol Number	NNNNNN	Y	Allocated by UKSACIT
6	6	Source ID	XXXXXX	Y	
7	6	Destination ID	XXXXXX	Y	
8	2	Check sum	NN	Y	
9	1	Terminator	Carriage return	Y	
Footer Line					
1	5	Line number	NNNNN	Y	
2	9	Fixed text	'UKBTSSTOP'	Y	
3	5	No of records	NNNNN	Y	
4	2	Check sum	NN	Y	
5	1	Terminator	Carriage return	Y	

Table 27.2 Message protocol numbers

Protocol number	**Title**	**Description**
000001	Blood component dispatch information	Defines the message used to transfer information on blood component issues
000002	Blood derivative dispatch information	Defines the message used to transfer information on blood derivative issues
000003	Reagent dispatch information	Defines the message used to transfer information on reagent issues
000004	Blood component dispatch Acknowledgement	Defines the message used to transfer information on blood components received
000005	Reagent component fate information	Defines the message used to transfer information on the fate of blood components received

between the two line types, a line type indicator is included as the first field following the line number (see Tables 27.3 to 27.6).

27.7 Protocol 000002 – blood derivative dispatch information

Two data line structures are defined within this protocol. The first is a single line containing administrative information (order no., dispatch no.), and the second is a multiple occurrence line with an entry for each item on the dispatch. To distinguish between the two line types, a line type indicator is included as the first field following the line number (see Tables 27.7 and 27.8).

27.8 Protocol 000003 – reagent dispatch information

Two data line structures are defined within this protocol. The first is a single line containing administrative information (order no., dispatch no.), and the second is a

Table 27.3 Message protocol 000001: blood component dispatch information: administration line

Field	Length	Description	Format	Mandatory?	Notes
1	5	Line number	NNNNN	Y	
2	1	Line type	N	Y	='1'
3	12	Order no	C(12)	Y	
4	12	Dispatch no	C(12)	Y	
5	8	Date	YYYYMMDD	Y	
6	4	Time	HHMM	N	
7	2	Checksum	NN	Y	
8	1	Terminator	Carriage return	Y	

Table 27.4 Message protocol 000001: blood component dispatch information: dispatch line

Field	Length	Description	Format	Mandatory?	Notes
1	5	Line number	NNNNN	Y	
2	1	Line type	N	Y	='2'
3	15	Unit identifier	C(15)	Y	ISBT 128 donation identification number (data characters with check character, e.g. 'G151797123456L '
4	9	Product code	C(9)	Y	Component code (either a full 9-character codabar code (including start and stop characters), or an 8-character ISBT 128 product code excluding the data identifier characters)
5	2	Group ABO	C(2)	Y	'A', 'B', 'O' or 'AB'
6	1	Group Rh D	C(1)	Y	'+' or '–'
7	8	Date bled	YYYYMMDD	N	
8	8	Date of expiry	YYYYMMDD	Y	
9	4	Time of expiry	HHMM	N	
10	30	Red cell phenotypes	C(30)	N	Position indicates antigen (see below), content '+' or '–' for confirmed (tested this time) results, 'P' or 'N' for unconfirmed (historic) results
11	1	HLA flag	C(1)	N	'Y': indicates that HLA information is included either in the comment field or on separate documentation Space: no information
12	1	CMV	C(1)	N	'+': positive '–': negative Space: unknown
13	1	Irradiated	C(1)	N	'Y': yes 'N' or space: no 'P': info in product code
14	10	Platelet specific phenotype	C(10)	N	Position indicates antigen, content (see Table 27.6) '+': positive result '–' : negative result

Table 27.4 Message protocol 000001: blood component dispatch: information dispatch line – *continued*

Field	Length	Description	Format	Mandatory?	Notes
15	1	IgA	C(1)	N	'Y': indicates that IgA information is included either in the comment field or on separate documentation Space: no information
16	1	H T haemolysin	C(1)	N	'Y': present 'N': not present Space: untested
17	1	Neonatal	C(1)	N	'Y': suitable for neonatal use 'N': unsuitable Space: untested 'P': info in product code
18	1	Filtered	C(1)	N	No longer used
19	3	Volume	NNN	N	mL
20	10	Pack lot no	C(10)	N	
21	1	Methylene blue	C(1)	N	No longer used
22	1	Clinical use	C(1)	Y	'Y': suitable for clinical use 'N': unsuitable for clinical use
23	1	Issue type	C(1)	Y	'R': routine issue 'S': selected, unmatched 'X': crossmatched 'G': autologous
24	10	Cost code/price	C(10)	N	
25	2	Added value code	C(2)	N	
26	30	Comment	Free text	N	
27	2	Checksum	NN	Y	
28	1	Terminator	Carriage return	Y	

multiple occurrence line with an entry for each item on the dispatch. To distinguish between the two line types, a line type indicator is included as the first field following the line number (see Tables 27.9 and 27.10).

27.9 Protocol 000004 – blood component dispatch acknowledgement

Two data line structures are defined within this protocol. The first is a single line containing administrative information (order no, dispatch no), and the second is a multiple occurrence line with an entry for each item on the dispatch. To distinguish between the two line types, a line type indicator is included as the first field following the line number (see Tables 27.11 and 27.12).

27.10 Protocol 000005 – blood component fate information

One data line structure is currently defined within this protocol (see Tables 27.13 and 27.14). The data line is a multiple occurrence line with an entry for each item in the message. The data line has a line type indicator in common with the previous protocols to allow for additional line types to be created if required. It is expected that this message will be generated daily and will include information on all units that are:

- free for use
- allocated to patient (either directly or notionally)

- marked as transfused or wasted in the period from the date the report was last gathered (minus five days) up until the present date.

This message will be used for all products with the exception of certain batched products (such as anti-D). Some batched products are excluded as each dose may not be allocated a unique unit number (platelet pools are not excluded).

Table 27.5 Message protocol 000001: blood component dispatch information. Field 10: red cell phenotype field – antigen codes

Character position in field	Antigen
1	C
2	c
3	E
4	e
5	C^w
6	M
7	N
8	S
9	s
10	K
11	k
12	Le^a
13	Le^b
14	Fy^a
15	Fy^b
16	Jk^a
17	Jk^b
18	P_1
19	A_1
20	Lu^a
21	Lu^b
22	Kp^a
23	Kp^b
24	Unassigned
25	Unassigned
26	Unassigned
27	Unassigned
28	Unassigned
29	Unassigned
30	Unassigned

Table 27.6 Message protocol 000001: blood component dispatch information. Field 14: platelet-specific phenotype

Character position in field	Antigen
1	HPA-1a
2	HPA-1b
3	HPA-3a
4	HPA-3b
5	HPA-5a
6	HPA-5b
7	Unassigned
8	Unassigned
9	Unassigned
10	Unassigned

Table 27.7 Message protocol 000002: blood derivative dispatch information: administration line

Field	Length	Description	Format	Mandatory?	Notes
1	5	Line number	NNNNN	Y	
2	1	Line type	N	Y	='1'
3	12	Order no	C(12)	Y	
4	12	Dispatch no	C(12)	Y	
5	8	Date	YYYYMMDD	Y	
6	4	Time	HHMM	N	
7	2	Checksum	NN	Y	
8	1	Terminator	Carriage return	Y	

Table 27.8 Message protocol 000002: blood derivative dispatch information: dispatch line

Field	Length	Description	Format	Mandatory?	Notes
1	5	Line number	NNNNN	Y	
2	1	Line type	N	Y	='2'
3	15	Batch number	C(15)	Y	
4	15	Product code	C(15)	Y	Unique pharmaceutical product identification code
5	40	Product description	C(40)	N	Free-format text
6	30	Manufacturer's name	C(30)	N	Free-format text
7	8	Expiry date	YYYYMMDD	Y	
8	4	No of vials/bottles	N(4)	Y	
9	10	Cost code/price	C(10)	N	
10	5	Actual dosage value	N(5)	N	Decimal values permitted
11	5	Actual dosage units	C(5)	N	Free-format text
12	30	Comment	C(30)	N	Free-format text
13	2	Checksum	NN	Y	
14	1	Terminator	Carriage return	Y	

Table 27.9 Message protocol 000003: reagent dispatch information: administration line

Field	Length	Description	Format	Mandatory?	Notes
1	5	Line number	NNNNN	Y	
2	1	Line type	N	Y	='1'
3	12	Order no	C(12)	Y	
4	12	Dispatch no	C(12)	Y	
5	8	Date	YYYYMMDD	Y	
6	4	Time	HHMM	N	
7	2	Checksum	NN	Y	
8	1	Terminator	Carriage return	Y	

Table 27.10 Message protocol 000003: reagent dispatch information: dispatch line

Field	Length	Description	Format	Mandatory?	Notes
1	5	Line number	NNNNN	Y	
2	1	Line type	N	Y	='2'
3	15	Batch number	C(15)	Y	
4	15	Product code	C(15)	Y	Unique reagent identification code
5	40	Product description	C(40)	N	Free-format text
6	30	Manufacturer's name	C(30)	N	Free-format text
7	8	Expiry date	YYYYMMDD	Y	
8	4	No of vials/bottles	N(4)	Y	
9	10	Cost code/price	C(10)	N	
10	30	Comment	C(30)	N	Free-format text
11	2	Checksum	NN	Y	
12	1	Terminator	Carriage return	Y	

Table 27.11 Message protocol 000004: blood component dispatch acknowledgement: administration line

Field	Length	Description	Format	Mandatory?	Notes
1	5	Line number	NNNNN	Y	
2	1	Line type	N	Y	='1'
3	12	Order no	C(12)	N	
4	12	Dispatch no	C(12)	Y	From associated dispatch information message
5	8	Date of acknowledgement	YYYYMMDD	Y	
6	4	Time of acknowledgement	HHMM	N	
7	2	Checksum	NN	Y	
8	1	Terminator	Carriage return	Y	

Table 27.12 Message protocol 000004: blood component dispatch acknowledgement: dispatch line

Field	Length	Description	Format	Mandatory?	Notes
1	5	Line number	NNNNN	Y	
2	1	Line type	N	Y	='2'
3	15	Unit identifier	C(15)	Y	ISBT 128 donation identification number (data characters with check character, e.g. 'G151797123456L '
4	9	Product code	C(9)	Y	Component code (either a full 9-character codabar code (including start and stop characters), or an 8-character ISBT 128 product code excluding the data identifier characters)
5	1	Received	C(1)	Y	'Y' or 'N'
6	2	Checksum	NN	Y	
7	1	Terminator	Carriage return	Y	

Table 27.13 Message protocol 000005: blood component fate information: data line

Field	Length	Description	Format	Mandatory?	Notes
1	5	Line number	NNNNN	Y	
2	1	Line type	N	Y	='1'
3	15	Unit number	C(15)	Y	ISBT 128 donation identification number (data characters with check character, e.g. 'G151797123456L '
4	9	Product code	C(9)	Y	Component code (either a full nine-character codabar code (including start and stop characters), or an eight-character ISBT 128 product code excluding the data identifier characters)
5	2	Group ABO	C(2)	Y	'A','B','O' or 'AB'
6	1	Group Rh D	C(1)	Y	'+' or '-'
7	1	Status	C(1)	Y	F = free A = allocated T = transfused W = wasted C = confirmed transfusion NB: confirmed transfusion refers to transfusions that have been positively confirmed by electronic means
8	5	Wasted classification code	C(5)	N	Only used if 'Status' (Field 7) is marked as wasted (W) The wasted classification codes are maintained by the Blood Stocks Management Scheme (see Table 27.14)
9	8	Date used/wasted	YYYYMMDD	N	Only if marked as wasted (W) or transfused (T or C) Presumptive YYYYMMDD should be included if exact date not known
10	4	Time used/wasted	HHMM	N	Only if marked as wasted (W) or transfused (T or C) Optional
11	3	Patient age	NNN	N	Only if marked as transfused (T or C) Optional. Age in number of full years
12	1	Patient gender	C(1)	N	Only if marked as transfused (T or C) Optional. M = Male F = Female
13	10	Blank field	C(10)	N	Area reserved for future use
14	2	Checksum	NN	Y	
15	1	Terminator	Carriage return	Y	

Table 27.14 Message protocol 000005: blood component fate information. Field 8: wasted classification code

Product super-group	Code	Full name	Code usage	Date started	Date stopped
RED CELL	TIMEX	Time expiry	The expiry date on the unit has passed	01 Apr 2001	N/A
RED CELL	OTCOL	Out of temperature control inside laboratory	Unit has been left out of temperature range for longer than 30 minutes on the wards, in theatres or in any other non-laboratory location	01 Apr 2001	N/A
RED CELL	OTCIL	Out of temperature control inside laboratory	Unit has been left out of temperature range for longer than 30 mins in the laboratory	01 Apr 2001	31 Mar 2003
RED CELL	FFAIL	Fridge failure	The unit has been discarded as a direct result of a fridge failure	01 Apr 2003	N/A
RED CELL	MISCN	Miscellaneous	Any other reason the unit is wasted that is not covered by other codes	01 Apr 2001	N/A
PLATELET	MORNU	Medically ordered not used	Platelets ordered for medical procedure but not used	01 Apr 2003	N/A
PLATELET	SORNU	Surgically order not used	Platelets ordered for surgical procedure but not used	01 Apr 2003	N/A
PLATELET	STMEX	Stock time expired	If a stock of platelets is held, the expiry date on the unit has passed	01 Apr 2003	N/A
PLATELET	WOSOL	Wasted outside of laboratory	Unit has been left out of temperature range for longer than 30 minutes outside the laboratory	01 Apr 2003	N/A
PLATELET	WIMPT	Wasted import	Unit imported with patient but not used	01 Apr 2003	N/A
PLATELET	MISCN	Miscellaneous	Any other reason the unit is wasted that is not covered by other codes	01 Apr 2003	N/A

Note: these codes are managed by the Blood Stocks Management Scheme. For further information visit http://www.bloodstocks.co.uk.

Chapter 28

Specification for blood pack base labels

Introduction

This chapter defines the requirements of the United Kingdom Blood Transfusion Services for the layout and information content of the blood pack base label affixed to the blood pack by the pack manufacturer.

This specification applies to the base labels provided on all blood component packs in a given assembly (in this context, a blood component is a component as described in Chapter 8).

References to linear dimensions in this document are in millimetres.

In addition to these labelling requirements, all blood packs must satisfy the validation criteria of Chapter 9.

28.1 Specification

The layout of the blood pack base label is shown in Figure 28.1. Labels should be within ±5% of the specified dimensions. Numbers on the diagram in italics are for reference purposes only and should not be reproduced on the label.

Where volumes are to be specified on the base label they must be given in millilitres.

The manufacturer's logo may be printed in a colour of their choice. All other printing to be black on white. Labels, adhesive and ink must comply with the requirements of the International Organization for Standardization Standard ISO 3826-1:2003, Plastics collapsible containers for human blood and blood components - Part 1: Conventional containers.

Area 1 is available to the manufacturer to print their logo, name and address, and CE mark.

The following text must be printed in Area 4:

- on all packs the words:
  - 'Do not re-use this container'
  - 'Do not vent'
  - or the symbols shown in Figure 28.1.
- on all packs the words ISO 3826-1:2003
- on primary blood collection packs the formulation and volume of anticoagulant
- on additive packs the formulation and volume of the additive solution.

Figure 28.1 Symbols used on blood packs: 'Do not reuse this container' (left); 'Do not vent' (right)

The following text must be printed in Area 3 (subsequently covered when the Blood Transfusion Service applies a component label):

- the maximum volume of blood/component that is to be collected into the container
- the statement 'Do not use if there is any visible sign of deterioration'
- the statement 'The contents and/or fluid pathways of this pack are sterile and pyrogen-free' or 'The contents and/or fluid pathways of this pack are sterile and non-pyrogenic'
- the storage temperature range for unused packs
- where a pack is specifically intended for the storage of a particular component, the identity of that component, e.g. 'Suitable for the storage of platelets'
- the statement 'Do not use after DD/MM/YY' where DD is the day number, MM the month number and YY the year. For clarity, the expiry date will be midnight on the date shown. It is permissible to use only MM/YY; in this instance the expiry date will be midnight on the last day of the month/year shown
- if appropriate, the words 'Use within × days of opening the immediate overwrap' where × is the number of days validated by the manufacturer.

In Area 5 the pack batch or lot number must be printed in both eye-readable and barcoded formats. The specification for the lot/batch number is given in Chapter 25.

In Area 6 the pack type (list/catalogue) number must be printed in eye-readable and barcoded formats. The specification for the pack type number is given in Chapter 25.

Labelling of the overwrapping shall comply with ISO 3826-1:2003.

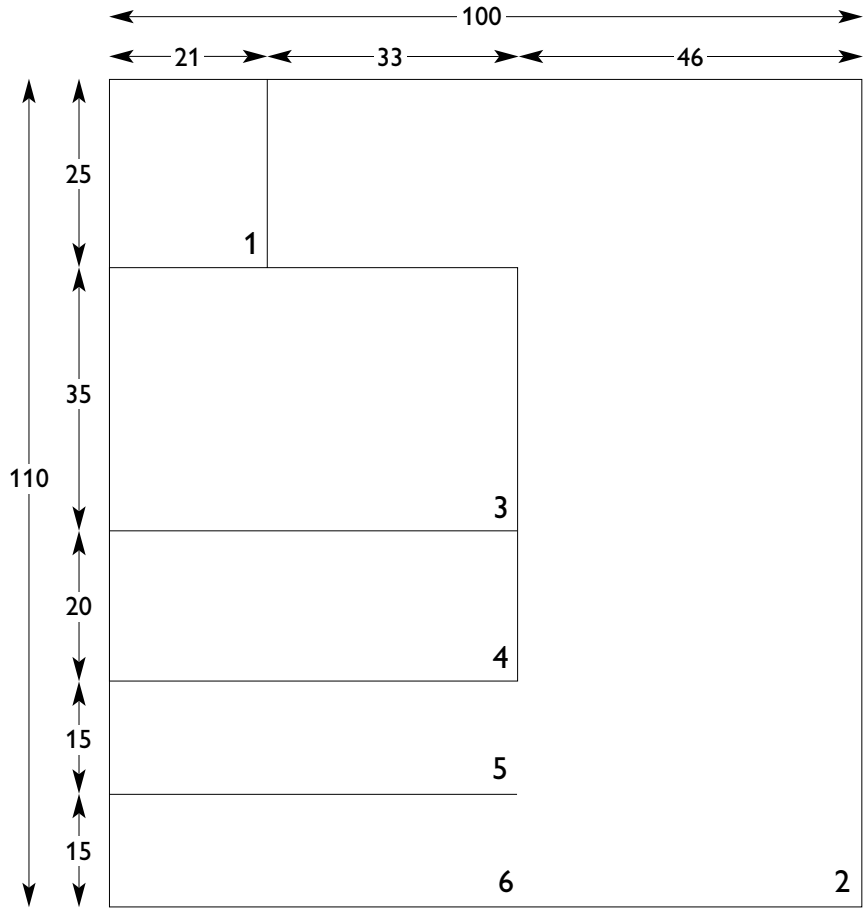

Figure 28.2 Base label layout: dimensions in millimetres

Annex 1

Standards available at the National Institute for Biological Standards and Control (NIBSC)

The following details some of the various standards and reference materials available from NIBSC relevant to transfusion medicine. A catalogue is available from NIBSC on request and on the website www.nibsc.ac.uk.

Table A1.1 Serological, virological and other preparations

Serological	**Status**	**Code**
Anti-Human Platelet Antigen-1a minimum potency reagent	IRR	93/710
Anti-Human Platelet Antigen-5b minimum potency reagent	IRR	99/666
Anti-Human Platelet Antigen-1a	NR	03/152
Intended for quantitation of anti-HPA-1a in arbitrary units		
Anti-A minimum potency for blood grouping reagents*†	B	88/722
Anti-B minimum potency for blood grouping reagents*†	B	88/724
Anti-D minimum potency for blood grouping reagents	IS	99/836
Intended to be used as the reference preparation for minimum acceptable potency of anti-D blood grouping reagents		
Anti-D for quantitation of anti-D in plasma/serum (Anti-D (Rho) antibodies, human)	B	73/515
Intended to be used in the assay of plasma/serum anti-D levels by automated haemagglutination		
Anti-c for quantitation of anti-c in plasma/serum	B	84/628
Intended to be used in the assay of plasma/serum anti-c levels by automated haemagglutination		
Anti-D for operator proficiency*	B	95/784
Intended to be used to assure the efficacy of red cell washing prior to the addition of an anti-globulin reagent, and to assess operator variability in the detection of weak, macroscopic agglutination in the spin-tube anti-globulin test		
Anti-human globulin	ICSH/ISBT	96/666
Papain preparation for use with anti-D preparation 91/562*	ICSH/ISBT	92/658
Anti-D for use with papain preparation 92/658*	ICSH/ISBT	91/562
Intended to check the efficacy of enzyme preparations		
Anti-D for quantitation of anti-D immunoglobulin (Anti-D immunoglobulin, human)	IS	01/572
Intended for use in anti-D potency assays of anti-D immunoglobulin products		
Anti-D immunoglobulin control*	NR	99/728
Intended for use as a control in anti-D potency assays of anti-D immunoglobulin products to monitor assay consistency		

Table A1.1 Serological, virological and other preparations – *continued*

Serological	**Status**	**Code**
Biotinylated Brad-5 (Bio-Brad-5)*	NR	99/698
Intended for use in the competitive enzyme-linked immunoassay of potency of anti-D immunoglobulin products		
Anti-HLA-A2*†	B	90/692
Anti-B lymphocyte antibody*†	B	92/556
Rabbit complement used in HLA Class I and Class II serology*†	B	02/314
Normal human AB serum*	B	02/282
For use as a negative control for flow cytometry		
In preparation		
International standards for minimum potency of anti-A (03/188) and anti-B (03/164) blood grouping reagents.		
Anti-bw4 and bw6 pooled human serum for use as a weak positive control for flow cytometry		
Virological		
Anti-measles serum	IS	66/202
Anti-parvovirus B19 serum (IgG), human	IS	01/602
Anti-poliovirus serum, types 1, 2 and 3	IS	66/202
Anti-rubella serum	B	67/182
Anti-varicella zoster	B	90/690
Hepatitis B surface antigen (HBsAg) (subtype adw2, genotype A)	IS	00/588
Hepatitis A virus RNA IS 00/560		
Hepatitis C virus RNA	IS	96/798
HCV genotype panel NR 02/202		
Hepatitis B virus DNA	IS	97/746
HIV-1 p24 antigen	IRR	90/636
HIV RNA for NAT IS 97/656		
HIV RNA genotype reference panel IRR 01/466		
Low-level control materials for HBsAg, anti-HCV and anti-HIV*	B	
Other		
See order form Appendix 3 (on our website) for details of materials currently available		
Parvovirus B19 DNA	IS	99/800
NAT working reagent for HAV RNA*	NR	01/488
NAT working reagent for HBV DNA*	NR	98/780
NAT working reagent for HCV RNA*	NR	02/264
NAT working reagent for HIV-1 PWS-1 (medium copy number)*	NR	99/634
NAT working reagent for HIV-1 PWS-2 (high copy number)*	NR	99/636
NAT working reagent for B19 DNA*	NR	99/736
NAT working reagent for multiplex (HCV, HBV, HBV, HIV, B19)*	NR	99/732
Endotoxin	IS	94/580
Tetanus antitoxin	NR	76/589

Key
IRR – International Reference Reagent
IS – International Standard
ICSH/ISBT – International Committee for Standardization in Haematology/International Society of Blood Transfusion
B – British Standard/Reference Reagent
NR – NIBSC Reagent
* Working Standards supplied as multiples of 5.
† available until December 2005

Table A1.2 Coagulation preparations – WHO International Standards

Factor	Plasma	Concentrate	Purified	Code
ATIII	✔			93/768
ATIII		✔		96/520
Fibrinogen	✔			98/612
Fibrinogen		✔		98/614
II	✔			99/826
II		✔		98/590
IX	✔			98/826
IX		✔		96/854
IXa			✔	97/562
Protein C	✔			86/622
Protein S	✔			93/590
Thrombin			✔	01/580
VII	✔			99/826
VIIa			✔	89/688
VII		✔		97/592
VIII	✔			02/150
VIII		✔		99/678
VWF	✔			99/826
VWF		✔		00/514
X	✔			99/826
X		✔		98/590
XIII	✔			02/206

Table A1.3 Coagulation preparations – British Standards

Preparation	Code
Blood Coagulation Factor VIII, Concentrate, Human (12th BS)	02/122
Blood Coagulation Factors II, IX, X, Concentrate, Human (4th BS)	96/794

How to order

NIBSC does not charge for the reference materials themselves, however, there is a handling charge to part-cover administration and storage costs. The current handling charge (from 1 April 2004) is GB £50 per ampoule/vial. NIBSC periodically adjusts the handling charge. Any changes to the handling charge are detailed on our website.

Those preparations identified as 'working standards' are supplied as multiples of five with a current handling charge of GB £50 per five ampoules/vials. CE marked working preparations are GB £13 per ampoule/vial.

This handling charge covers the shipping cost of preparations that can be dispatched by (air) mail.

You can place an order by:

Mail: NIBSC, PO Box 1193, Blanche Lane, South Mimms, Potters Bar, Herts, EN6 3QH, United Kingdom.

Fax: +44 (0) 1707 646977

E-mail: standards@nibsc.ac.uk

Telephone: +44 (0) 1707 646399, with 24 hour answer-phone (For enquiries only).

Orders should include the following information:

- your full dispatch address and invoice address (if different)
- your purchase order number
- for EU customers, your VAT number or VAT exemption certificate
- for each item ordered, the NIBSC code number, preparation name and number of ampoules/vials required
- for preparations that are to be sent by courier/air freight, we must have a contact name along with both contact telephone and fax numbers
- your e-mail address or fax number. We will acknowledge receipt of your order. We would prefer to contact you by e-mail.

In addition, orders for infectious materials must be ordered on an NIBSC order form for infectious materials. These forms are available from our website www.nibsc.ac.uk.

Annex 2

ISBT 128 check character calculation

ISBT 128 donation numbers utilize check characters based on the ISO 7064 Mod 37, 2 algorithm. This Annex shows how to calculate the check character for a given number. The calculation is based on the donation number string excluding the leading '=' symbol and the flag characters.

The steps in the process are as follows:

1. For each character in the string determine its check value as required by ISO 7064 (see Table A2.1).
2. For each character determine its weighted check value by multiplying the check value from (1) by the nth power of 2 where n is the position of the character from the right-hand end of the string.
3. Sum the weighted check values from (2).
4. Find the modulus 37 value of the sum from (3).
5. Subtract the value obtained in (4) from 38.
6. Find the modulus 37 value of the result of (5). This is the 37,2 check sum.

The calculated check sum is used to generate both the barcode check characters used in the flag positions of the ISBT 128 barcode and the eye-readable check character. The barcode check characters are determined by adding 60 to the check sum. The eye-readable check character is determined by cross-referencing the checksum to Table A2.1.

Table A2.1 Mapping from characters to ISO 7064 check values

Char	0	1	2	3	4	5	6	7	8	9	A	B	C	D
Value	0	1	2	3	4	5	6	7	8	9	10	11	12	13
Char	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
Value	14	15	16	17	18	19	20	21	22	23	24	25	26	27
Char	S	T	U	V	W	X	Y	Z	*					
Value	28	29	30	31	32	33	34	35	36					

Table A2.2 Example of displayed numbers

Donation number G123 498 654 321				
Position from right (n)	**2^n**	**Character**	**ISO7064 value (step 1)**	**Weighted value (step 2)**
13	8192	G	16	131072
12	4096	1	1	4096
11	2048	2	2	4096
10	1024	3	3	3072
9	512	4	4	2048
8	256	9	9	2304
7	128	8	8	1024
6	64	6	6	384
5	32	5	5	160
4	16	4	4	64
3	8	3	3	24
2	4	2	2	8
1	2	1	1	2
Step 3		Sum of weighted values		148354
Step 4		Sum mod 37		21
Step 5		Subtract from 38		17
Step 6		Mod 37		17
ISO 37,2 check sum =				17
ISBT128 barcode check characters =				77
ISBT128 eye-readable check =				H

Definitions

These definitions are taken from The Blood Safety and Quality Regulations 2005 unless stated otherwise.

Additive solution means a solution specifically formulated to maintain beneficial properties of cellular components during storage.

Allogeneic donation means blood and blood components collected from an individual and intended for transfusion to another individual, for use in medical devices or as starting material or raw material for manufacturing into medicinal products.

Apheresis means a method of obtaining one or more blood components by machine processing of whole blood in which the residual components of the blood are returned to the donor during or at the end of the process.

Autologous donation means blood and blood components collected from an individual and intended solely for subsequent autologous transfusion or other human application to that same individual.

Autologous transfusion means a transfusion in which the donor and the recipient are the same person and in which pre-deposited blood or blood components are used.

Blood means whole human blood collected from a donor and processed either for transfusion or for further manufacturing.

Blood component means a therapeutic constituent of human blood (red cells, white cells, platelets and plasma) that can be prepared by various methods.

Blood component release means a process which enables a blood component to be released from a quarantine status by the use of systems and procedures to ensure that the finished product meets its release specification.

Blood establishment shall mean any structure or body that is responsible for any aspect of the collection and testing of human blood or blood components, whatever their intended purpose, and their processing, storage, and distribution when intended for transfusion. This does not include hospital blood banks. (EU Directive 2002/98/EC definition).

The four UK Blood Services / Blood Transfusion Services (National Blood Service, Scottish National Blood Transfusion Service, Northern Ireland Blood Transfusion Service and the Welsh Blood Service) are blood establishments.

Blood product means any therapeutic product derived from human blood or plasma.

Buffy coat means a blood component prepared by centrifugation of a unit of whole blood, and which contains a considerable proportion of the leucocytes and platelets.

Commission means the European Commission.

Cryoprecipitate means a plasma component prepared from plasma, fresh-frozen, by freeze-thaw precipitation of proteins and subsequent concentration and re-suspension of the precipitated proteins in a small volume of the plasma.

Cryopreservation means prolongation of the storage life of blood components by freezing.

Deferral means suspension of the eligibility of an individual to donate blood or blood components, such suspension being either permanent or temporary.

Distribution means the act of delivery of blood and blood components to other blood establishments, hospital blood banks and manufacturers of blood products, other than the issuing of blood or blood components for transfusion.

Doctor means a registered medical practitioner.

Donor carer means a person who has passed both the written and practical examinations of the National Blood Authority, the Scottish National Blood Transfusion Service, the Northern Ireland Blood Transfusion Service or the Welsh Blood Service in the care of blood donors and who holds a current certificate of competence, awarded by that body, in the care of blood donors.

Emerging infectious disease means a newly recognised, clinically distinct infectious disease, or a known disease whose reported incidence within the past two decades is increasing in a given place or among a specific population (Health Protection Agency definition).

Facilities means hospitals, clinics, manufacturers, and biomedical research institutions to which blood or blood components may be delivered (Commission Directives on haemovigilance/traceability).

Granulocytes, apheresis means a concentrated suspension of granulocytes obtained by apheresis.

Health service hospital has the same meaning as in section 128 of the National Health Service Act 1977.

Haemovigilance means a set of organised surveillance procedures relating to serious adverse or unexpected events or reactions in donors or recipients, and the epidemiological follow-up of donors.

Hospital means a health service hospital or an independent hospital.

Hospital blood bank means any unit within a hospital which stores and distributes, and may perform compatibility tests on, blood and blood components exclusively for use within hospital facilities, including hospital based transfusion activities.

Imputability means the likelihood that a serious adverse reaction in a recipient can be attributed to the blood or blood component transfused or that a serious adverse reaction in a donor can be attributed to the donation process (Commission Directives on haemovigilance/traceability).

Independent hospital has the same meaning as in Section 2 of the Care Standards Act 2000.

Inspection means formal and objective control to identify problems in accordance with standards adopted to assess compliance with these Regulations.

Inspector means a person appointed by the Secretary of State to carry out inspections pursuant to regulation 15(10).

Nurse means a registered nurse or registered midwife.

Person responsible for management of a hospital blood bank means:

(a) in the case of hospital blood bank located in a hospital managed by a health service body, that body, and

(b) in the case of an independent hospital, the registered person.

Plasma means the liquid portion of the blood in which the cells are suspended. Plasma may be separated from the cellular portion of a whole blood collection for therapeutic use as fresh-frozen plasma or further processed to cryoprecipitate and cryoprecipitate-depleted plasma for transfusion. It may be used for the manufacture of medicinal products derived from human blood and human plasma or used in the preparation of pooled platelets, or pooled, leucocyte-depleted platelets. It may also be used for resuspension of red cell preparations for exchange transfusion or perinatal transfusion.

Plasma, cryoprecipitate-depleted for transfusion means a plasma component prepared from a unit of plasma, fresh-frozen. It comprises the residual portion after the cryoprecipitate has been removed.

Plasma, fresh-frozen means the supernatant plasma separated from a whole blood donation or plasma collected by apheresis, frozen and stored.

Platelets, apheresis means a concentrated suspension of blood platelets obtained by apheresis.

Platelets, apheresis, leucocyte-depleted means a concentrated suspension of blood platelets, obtained by apheresis, and from which leucocytes are removed.

Platelets, recovered, pooled means a concentrated suspension of blood platelets, obtained by processing of whole blood units and pooling the platelets from the units during or after separation.

Platelets, recovered, pooled, leucocyte-depleted means a concentrated suspension of blood platelets, obtained by processing of whole blood units and pooling the platelets from the units during or after separation, and from which leucocytes are removed.

Platelets, recovered, single unit means a concentrated suspension of blood platelets, obtained by processing of a single unit of whole blood.

Platelets, recovered, single unit, leucocyte-depleted means a concentrated suspension of blood platelets, obtained by processing of a single whole blood unit from which leucocytes are removed.

Qualified health professional means:

(a) a doctor

(b) a nurse or

(c) a donor carer.

Quality assurance means all the activities from blood collection to distribution made with the object of ensuring that blood and blood components are of the quality required for their intended use (Commission Directive on Quality System).

Red cells means the red cells from a single whole blood donation, with a large proportion of the plasma from the donation removed.

Red cells, apheresis means the red cells from an apheresis red cell donation.

Red cells, buffy coat removed means the red cells from a single whole blood donation, with a large proportion of the plasma from the donation removed. The buffy coat, containing a large proportion of the platelets and leucocytes in the donated unit, is removed.

Red cells, buffy coat removed, in additive solution means the red cells from a single whole blood donation, with a large proportion of the plasma from the donation removed. The buffy coat, containing a large proportion of the platelets and leucocytes in the donated unit, is removed. A nutrient or preservative solution is added.

Red cells in additive solution means the red cells from a single whole blood donation, with a large proportion of the plasma from the donation removed. A nutrient or preservative solution is added.

Red cells, leucocyte-depleted means the red cells from a single whole blood donation, with a large proportion of the plasma from the donation removed, and from which leucocytes are removed.

Red cells, leucocyte-depleted, in additive solution means the red cells from a single whole blood donation, with a large proportion of the plasma from the donation removed, and from which leucocytes are removed. A nutrient or preservative solution is added.

Reporting establishment means the blood establishment, the hospital blood bank or facilities where the transfusion takes place that reports serious adverse reactions and/or serious adverse events to the competent authority (Commission Directives on haemovigilance/traceability).

Reporting year means the period of twelve months ending on 31 March.

Responsible person in relation to a blood establishment means the person who has been designated pursuant to Regulation 6 as the responsible person for that blood establishment.

Serious adverse event means any untoward occurrence associated with the collection, testing, processing, storage and distribution, of blood or blood components that might lead to death or life-threatening, disabling or incapacitating conditions for patients or which results in, or prolongs, hospitalization or morbidity.

Serious adverse reaction means an unintended response in a donor or in a patient associated with the collection or transfusion of blood or blood components that is fatal, life-threatening, disabling, or which results in or prolongs hospitalization or morbidity.

Site means any premises at which a blood establishment carries out any of the activities listed in regulation 3(2), but shall not include any premises not owned or managed by the blood establishment at which blood is collected, or any mobile blood collection unit.

Statistical process control means a method of quality control of a product or a process that relies on a system of analysis of an adequate sample size without the need to measure every product of the process.

Traceability means the ability to trace each individual unit of blood or blood component derived thereof from the donor to its final destination, whether this is a recipient, a manufacturer of medicinal products or disposal, and vice versa (Commission Directives on haemovigilance/traceability).

Tissue establishment means a tissue bank or a unit of a hospital or another body where activities of processing, preservation, storage or distribution of human tissues and cells are undertaken. It may also be responsible for procurement or testing of tissues and cells (Directive 2004/23/EC).

Validation means the establishment of documented and objective evidence that the particular requirements for a specific intended use can be consistently fulfilled.

Washed means a process of removing plasma or storage medium from cellular products by centrifugation, decanting of the supernatant liquid from the cells and addition of an isotonic suspension fluid, which in turn is generally removed and replaced following further centrifugation of the suspension. The centrifugation, decanting, replacing process may be repeated several times.

Whole blood means a single blood donation.

Explanation of terms used in the Seventh Edition

The terms 'Blood Service'/'Blood Transfusion Service'/'Transfusion Service' refer to blood establishments. The terms 'Blood Centre'/'Blood Transfusion Centre'/'Transfusion Centre' refer to the sites from which the services of the blood establishments are delivered.

The reason for such diversity is that in 2005 the four blood establishments in the United Kingdom use slightly different titles.

Index

Names of blood components are in **bold** type. Page numbers relating to tabulated information are also in **bold**.