Guidelines for the organization of a blood transfusion service

edited by

W. N. Gibbs
*Health Laboratory Technology and Blood Safety
World Health Organization
Geneva, Switzerland*

A. F. H. Britten
Formerly Head, Blood Programme
League of Red Cross and Red Crescent Societies
Geneva, Switzerland

WORLD HEALTH ORGANIZATION
GENEVA
1992
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Preface

This book updates and replaces Blood transfusion: a guide to the formation and operation of a transfusion service, which was first published by the World Health Organization in 1971, with an addendum in 1978. Its main objective is to facilitate the organization of blood transfusion services in developing countries, and it is therefore intended primarily for those who are entrusted with the task. Technical details are not included, but extensive guidance may be obtained from the selected reading list at the end of the book. Readers are also referred to the manual Management of blood transfusion services (WHO, 1990) for details of some aspects of organizing blood transfusion services, notably personnel matters and cost accounting.

The editors are grateful to all who contributed to this book, both those who wrote the various chapters and those who commented on the material and suggested improvements. However, the editors accept full responsibility for the contents and welcome further comments and suggestions from readers.

Contributors

Dr A. F. H. Britten, Averill Park, New York, NY, USA (formerly, Head, Blood Programme, League of Red Cross and Red Crescent Societies, Geneva, Switzerland).
Miss P. Corcoran, Global Blood Safety Initiative, World Health Organization, Geneva, Switzerland.
Dr D. Farr, Blood Transfusion Centre, Royal Infirmary, Aberdeen, Scotland.
Dr W. N. Gibbs, Chief Medical Officer, Health Laboratory
Dr J. Koistinen, Coordinator, Global Blood Safety Initiative, World Health Organization, Geneva, Switzerland.
Dr J. Leikola, Finnish Red Cross Blood Transfusion Service, Helsinki, Finland.
Dr S. Leong, Director, Hong Kong Red Cross Blood Transfusion Service, Kowloon, Hong Kong.
Dr R. Mackenzie, Department of Laboratory Services, Wellington Hospital, Wellington, New Zealand.
Dr J. A. F. Napier, Welsh Regional Blood Transfusion Centre, Cardiff, Wales.
Dr M. Rustam, Director, Indonesian Red Cross Society, Central Institute for Blood Transfusion, Jakarta, Indonesia.
Dr D. Sondag-Thull, Blood Transfusion Centre, University of Liège, Liège, Belgium.
Dr D. Voak, Regional Transfusion and Immuno-haematology Centre, Cambridge, England.
Professor A. H. Waters, Department of Haematology, St Bartholomew’s Hospital, London, England.
Dr N. de Zoysa, Director, National Blood Transfusion Service, Central Blood Bank, General Hospital, Colombo, Sri Lanka.
Resolution WHA 28.72 of the
Twenty-eighth World Health Assembly,
29 May 1975

UTILIZATION AND SUPPLY OF HUMAN BLOOD AND
BLOOD PRODUCTS

The Twenty-eighth World Health Assembly,
Conscious of the increasing use of blood and blood products;
Having considered the information provided by the Director-
General on utilization and supply of human blood and blood
products;
Bearing in mind resolution XVIII of the XXII International
Conference of the Red Cross;
Noting the extensive and increasing activities of private firms
in trying to establish commercial blood collection and
plasmapheresis projects in developing countries;
Expressing serious concern that such activities may interfere
with efforts to establish efficient national blood transfusion
services based on voluntary nonremunerated donations;
Being aware of the higher risk of transmitting diseases when
blood products have been obtained from paid rather than from
voluntary donors, and of the harmful consequences to the
health of donors of too frequent blood donations (one of the
causes being remuneration),
1. Thanks the Director-General for the actions taken to study
the problems related to commercial plasmapheresis in
developing countries;
2. Urges Member States:
   (1) to promote the development of national blood services
       based on voluntary nonremunerated donation of blood;
(2) to enact effective legislation governing the operation of
blood services and to take other actions necessary to
protect and promote the health of blood donors and of
recipients of blood and blood products;
3. Requests the Director-General:
(1) to increase assistance to Member States in the
development of national blood services based on
voluntary donations, when appropriate in collaboration
with the League of Red Cross Societies;
(2) to assist in establishing cooperation between countries to
secure adequate supply of blood and blood products
based on voluntary donation;
(3) to further study the practice of commercial
plasmapheresis including the health hazards and ethical
implications, particularly in developing countries;
(4) to take steps to develop good manufacturing practices
specifically for blood and blood components in order to
protect the health of both donors and recipients; and
(5) to report to the World Health Assembly on
developments in these matters.
Chapter 1
Organization of a blood transfusion service

IMPORTANCE OF THE BLOOD SUPPLY

The life-saving properties of transfused blood became clear during the Second World War, and thereafter blood transfusion quickly became a routine hospital function. Without blood transfusion, the treatment of severe haemorrhage is difficult or impossible and many surgical procedures cannot be safely attempted. Haematological conditions such as thalassaemia, haemophilia, leukaemia, and aplastic anaemia cannot be treated effectively without support from the blood transfusion service. No general hospital can be effective unless it can perform blood transfusions, and if blood is not available from an outside source, the hospital itself is obliged to undertake the task of blood collection.

The recruitment and selection of blood donors are critical to the success of a blood programme, and every effort must be made to ensure both the safety of the donor and the safety of the transfusion for the recipient. The process of donor selection is reliable only when information provided by donors can be trusted, and experience has shown that this is most likely when there is no material gain from donation. Problems in donor selection are considerably reduced when a blood transfusion service is founded upon the principle of voluntary, unpaid blood donation.

When donors are remunerated, they are often overused by the transfusion service, and there is an increased risk of disease transmission. Recruitment of patients' families as donors ('replacement' or 'family' donation) frequently places undue
pressure upon these families, which may force them to pay professional donors to serve as surrogates. The risk of blood donation and transfusion creating new health hazards necessitates the maintenance of the strictest standards, and rigorous quality control is therefore essential.

Because of the importance of blood transfusion services, and the many problems inherent in their organization, the World Health Assembly adopted resolution WHA28.72 (see page ix) in 1975. This remains the basis of the policy of the World Health Organization as regards blood transfusion services.

The goal

The goal of blood transfusion services is to provide effective blood and blood products, which are as safe as possible, accessible at reasonable cost, and adequate to meet patients' needs.

The amount of blood collected varies greatly from country to country—from more than 100 donations per thousand inhabitants per year to less than 1 donation per thousand. An adequate amount is that which is required to satisfy the needs within a particular health care system. Adequacy, however, depends not only on absolute amounts but also on quality and on the range of products that can be made available. An adequate service should be able to provide at least whole blood, red blood cells, plasma,¹ platelets, and cryoprecipitate.

ORGANIZATION OF BLOOD TRANSFUSION SERVICES

The national blood transfusion service is ultimately the responsibility of the national government. This responsibility may be carried out by government agencies or delegated wholly or in part to other organizations, such as the national Red Cross/Red Crescent Society.

In many countries, funds for health services are restricted, resulting in limited funding for transfusion services. Provision of adequate financing, however, is a prerequisite for satisfactory transfusion services (see Appendix 1 to this chapter, page 12), for which there should be a separate budget,

¹ These guidelines do not deal with the provision of plasma, obtained by plasmapheresis, as source material for plasma fractionation.
generally equivalent to between 0.5% and 1.5% of the total costs of hospitals.

**National blood transfusion committee**

The task of meeting the country’s needs for blood and blood products requires cooperation between the blood transfusion service, health authorities, hospitals, medical associations, the mass media, and the general public. In large countries, there may be considerable regional differences in blood needs, which must also be taken into account. The creation of an effective national transfusion committee, with broad representation from all the above groups, will increase the possibility of cooperation. One of the functions of such a committee should be the formulation of national blood policy, which will include rules and regulations for the import and export of blood and blood products, the collection, processing, storage, transport, and distribution of blood within the country, and the administration of blood and blood products to patients.

**National blood transfusion service**

*Personnel*

The organization and operation of a national blood transfusion service requires both management and medical skills, ideally provided by one medically qualified director with appropriate postgraduate training. A large transfusion service may also require the appointment of a manager (see Appendix 2 to this chapter, page 14). Regardless of the scale of operations, however, the director alone has ultimate responsibility for the operation of the service. Other personnel requirements are outlined in Appendix 2.

*Organizational systems*

The organization of blood transfusion services has evolved differently in different countries, according to their size, climate, history, culture, political structure, and level of economic development. An island nation with a population of, say, 75,000, with only two hospitals, does not need the same organization as an industrialized country with 50 million inhabitants.
A transfusion service may be centralized, regionalized, hospital-based, or some combination of these. Once a system has been established, it is difficult to change. Blood collection may be organized through blood centres, hospitals, or, as in many countries, both. Large countries have found it practical to be organized regionally, with one centre responsible for each region.

There are examples of success in all four systems in developed and developing countries. There are also significant examples of organizational breakdown in some mixed systems, and in some uncoordinated, hospital-based systems. Satisfactory results are most easily achieved with centralized or, in large countries, regionalized systems. Hospital-based systems may be satisfactory if they are centrally regulated and coordinated, particularly in small countries.

*Centralized organization.* In a centralized system, one national blood transfusion centre operates the services for the whole country, with or without satellite regional centres. Finland and Jamaica are examples of countries with this type of system. The advantages of this arrangement are facilitation of planning, and central control of product availability, quality assurance, and problem-solving. In large countries, however, centralization may be cumbersome: in some cases, the needs of hospitals are not satisfied, and hospitals themselves have very little influence on decisions.

*Regionalized organization.* Regionalized organization of a transfusion service depends upon a country being divided into regions that enjoy a considerable degree of autonomy (but that may have different mechanisms for achieving national control and logistic coordination). There may be a strong national transfusion centre with direct control of regional blood collection centres, or a loose national coordinating body that exercises little control. The systems operated in some countries fall between these two extremes.

For large countries, regionalization increases the efficiency of administration by ensuring that planning, control, and problem-solving are coordinated at the regional level, and allows close contact to be maintained with local hospitals. Australia and Zimbabwe are examples of countries with regionalized systems. National coordination is sometimes difficult to achieve, however, and efficiency can be weakened where there are too many centres or regions.
Hospital-based organization. Where organization of the blood transfusion service is hospital-based, as in Denmark and Malaysia for example, each hospital runs its own blood-collection programme, with or without central regulation and logistic coordination. This system utilizes existing institutions and does not require creation of separate blood centres. It may operate satisfactorily when there is adequate central regulation, and is suitable for the smallest countries. When there is no central regulation or logistic coordination, however, community organization of blood donation in hospital-based systems is usually unsatisfactory. Replacement or paid-donor programmes often arise. Regulation, coordination between hospitals, inventory balancing, quality assurance, and rational production planning are difficult or impossible to achieve.

Mixed systems. Some hospitals collect blood independently, and some use the services of blood centres. This encourages institutional independence, competition, and ingenuity, but has the same disadvantages as the uncoordinated, hospital-based system. Competition for blood donors may destroy institutional cooperation and undermine public support for the blood transfusion service. The United States of America has a mixed system.

FUNCTIONS OF NATIONAL BLOOD TRANSFUSION SERVICES

Ultimate responsibility for a national blood transfusion service lies with the national government, even when its operation is delegated wholly or partially to organizations such as the Red Cross or Red Crescent Society. Management and operation of the services should be delegated to suitably qualified professionals.

The scope of the transfusion service can vary widely, depending upon local circumstances and resources. Table 1 outlines the targets for transfusion services at levels of development from basic to sophisticated. The basic requirement for any transfusion service is a quality assurance scheme that is applicable to all of its operations (see Chapter 8), which should include good manufacturing and laboratory practice, with adequate internal quality controls. The amount of blood collected should satisfy national needs, and appropriate storage facilities must be available. The national blood transfusion
Table 1. Evolution of blood transfusion services

<table>
<thead>
<tr>
<th>Stage of development</th>
<th>Products</th>
<th>Screening for transmissible infectious agents</th>
<th>Blood-grouping</th>
<th>Cross-matching</th>
<th>Storage</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Inadequate</td>
<td>Whole blood only</td>
<td>None</td>
<td>ABO</td>
<td>None or slide room temp.</td>
<td>Uncontrolled</td>
<td>—</td>
</tr>
<tr>
<td>2. Basic, adequate</td>
<td>Whole blood (WB) (CPD-A1 preferred) Red cells (RBC)</td>
<td>Syphilis, HBsAg, HIV, and others as determined by national blood policy</td>
<td>ABO, D (tube or microplate)</td>
<td>Major 37°C (AHG)</td>
<td>Controlled</td>
<td>—</td>
</tr>
<tr>
<td>3. Basic, full-range</td>
<td>WB, RBC (0–30%) plasma, platelets, cryoprecipitate</td>
<td>As in 2</td>
<td>As in 2, plus antibody screening (donor)</td>
<td>As in 2, plus antibody screening (patient)</td>
<td>As in 2</td>
<td>Antibody screening and identification Reagent production</td>
</tr>
<tr>
<td>4. Highly productive</td>
<td>As in 3, plus RBC (30–100%) using additive solutions</td>
<td>As in 2</td>
<td>As in 3</td>
<td>As in 3</td>
<td>As in 2</td>
<td>As in 3</td>
</tr>
<tr>
<td>5. Advanced</td>
<td>As in 4, plus frozen RBC, washed RBC, special products, cytapheresis</td>
<td>As in 2</td>
<td>As in 3</td>
<td>As in 3</td>
<td>As in 2</td>
<td>As in 3</td>
</tr>
<tr>
<td>6. Sophisticated</td>
<td>As in 5</td>
<td>As in 2</td>
<td>As in 3</td>
<td>As in 3</td>
<td>As in 2</td>
<td>As in 3, plus automation, computers, plasma fractionation, basic research, etc.</td>
</tr>
</tbody>
</table>

Comments
Stage 1 is unsatisfactory and must be rectified.
Stage 2 is adequate for peripheral hospitals; a transfusion service at this level should progress as soon as possible to Stage 3.
Stage 3 should be regarded as a minimum for referral centres.
Stage 4 or 5 is essential for advanced haematological centres.
Stage 6 is discretionary for countries with adequate resources.
service must also develop the capability to screen for transmissible infectious agents, as determined by the national blood policy.

One of the major responsibilities of a national blood transfusion centre, or of a regional centre in a country in which the transfusion service is organized regionally, is motivation and recruitment of donors (see Chapter 2). The centre is also responsible for blood collection (Chapter 3), testing and screening of donor blood (Chapters 4 and 6), preparation of blood components, and storage and distribution of blood and components to hospitals. A national transfusion centre should organize training, prepare reagents (see Chapter 5), and act as a reference laboratory; it may be involved in more specialized functions such as autologous donation, histocompatibility services, or blood coagulation studies. Most countries are able to prepare at least some of the immunohaematological reagents they require (e.g., anti-A, anti-B). National or regional centres should also be involved in setting policies for transfusion of blood and blood products (Chapter 9).

Detailed arrangements for collection, processing, distribution, and compatibility testing of blood and blood components will depend upon factors such as geography and communications, as well as the availability of trained personnel. There may be collection centres in hospitals, from which all blood is sent to blood centres for processing and then redistributed to hospitals according to their needs. Compatibility testing is then performed at the hospitals (see Chapter 6). On the other hand, some or all of the compatibility testing may be done in the blood centres. Depending upon its size, and its function within the national health service, a hospital may also be involved in some of the functions outlined for blood transfusion centres, such as preparation of blood components, training of personnel, and collecting plasma for fractionation. Blood and blood components are usually transfused in hospitals (see Chapter 9).

**Estimation of the donor requirement**

The driving force for blood collection is usually the need for red cells. Estimates of need may be based on a fixed percentage of the population, but this assumption ignores the disparity that exists in many countries between the size of the population and the number of hospital beds. It is therefore more realistic
to base an estimate of blood needs on the number of acute hospital beds; the figure may vary from 5 to 15 units per bed per year. The lowest ratios apply to hospitals at the first referral level, where blood is required primarily for the management of bleeding as a complication of pregnancy or trauma. The highest ratios apply to hospitals with more specialized needs, e.g. those that have a department of oncology, or that use sophisticated and complex surgical techniques.

All countries should aim for self-sufficiency in blood and blood components. Failure to achieve adequate production can have only two outcomes—shortage or importation.

**Importation of blood products**

Importation of red-cell products has been used as a temporary means of ensuring adequate supplies during emergencies or periods when production capability is being built up; importation of plasma derivatives is also widely practised. However, importation creates two potential dangers: it can introduce transmissible diseases, and it can create a dependence upon external sources of supply, by suppressing the motivation for domestic production. Both are good reasons for controlling this activity most carefully.

**Provision of plasma derivatives**

Many blood transfusion services can prepare blood components, but lack the resources for plasma fractionation. Plasma derivatives such as albumin, immune globulins, factor VIII, and factor IX may thus be unavailable.

Plasma fractionation raises difficult issues for many countries. The principal options are:

1. Full-scale plasma fractionation, with production of the principal plasma derivatives.
2. International purchase of plasma derivatives.
3. Production of plasma for fractionation, the fractionation being performed in another country (contract fractionation).
4. Preparation of essential blood components, e.g. plasma and cryoprecipitate.
5. No decision.
Successful plasma fractionation depends upon the provision of sufficient suitable plasma, adequate financial and professional resources, and a viable market. Many attempts have failed simply because of lack of plasma. The many practical difficulties and high costs of options 1 and 2 have rendered them unworkable in many countries. Option 3 has succeeded among certain industrialized countries and in the Asian and Pacific regions, where several countries (e.g. Indonesia, New Zealand, Papua New Guinea) have utilized the facilities of the Commonwealth Serum Laboratories in Melbourne, Australia, to ensure fractionation of plasma produced within their national blood transfusion services.

Option 4 is practicable if a blood transfusion service is able to produce the essential blood components, in this case plasma and cryoprecipitate, and is an acceptable option for developing countries in which the problems of plasma fractionation have not yet been solved. Table 2 indicates the alternatives to plasma derivatives obtained by fractionation.

The final option, no decision and therefore no derivatives or substitutes, is clearly untenable.

FINANCING A BLOOD TRANSFUSION SERVICE

It is not possible to avoid the high costs of professional staff, blood collection containers, laboratory equipment (see Chapter 7), building facilities, transportation, and utilities. The cost of a blood transfusion service is often a problem, even in industrialized countries. An essential part of the organization and planning phase consists of ensuring adequate financing (see Appendix 3 to this chapter, page 17).

<table>
<thead>
<tr>
<th>Plasma derivative</th>
<th>Alternative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>Dextran, hydroxyethyl starch, gelatin</td>
</tr>
<tr>
<td>Factor VIII</td>
<td>Cryoprecipitate*</td>
</tr>
<tr>
<td>Factor IX</td>
<td>Plasma or cryosupernatant*</td>
</tr>
<tr>
<td>Immunoglobulins</td>
<td>Selected plasma*</td>
</tr>
</tbody>
</table>

* May be frozen or lyophilized.
International funds may be helpful in starting new programmes, and providing capital items and specialized training, but cannot become a source of long-term support. It is generally not possible to pay for an adequate blood service with charitable funds. Government funding, an effective fee-for-service system, or some combination of the two, is therefore necessary if recurrent operating costs are to be met.

CREATING A NEW BLOOD TRANSFUSION SERVICE

Since in many countries there is no structured organization of the blood transfusion service, there is a need to create a number of new services. There is now much international experience of this difficult process, which has provided clear examples of successful efforts and failures and taught some useful lessons.

Successful development projects typically involve long-standing international partnerships, strong local leadership, support by national governments, and sustainable financing. Examples of successful partnerships are Belgium/Rwanda, Australia/Nepal, and Germany/Ecuador.

Projects fail predictably when international support is short-lived, incomplete, or uncoordinated. Providing technology without technical support generally proves useless, and payment of recurrent costs from international sources discourages independence and does not usually lead to sustainable programmes. Lack of government interest and the absence of local leadership are bad signs. Failure to retain key personnel can undermine the most constructive efforts. These observations have led to the establishment of criteria for successful development, which are set out in Appendix 1 to this chapter (page 12).

The procedures to be followed in creating a new transfusion service will depend upon many factors such as existing partnerships, involvement of the national Red Cross or Red Crescent Society, and specific needs. It is therefore impossible to define a specific set of procedures than can be applied in all cases. Nevertheless, the criteria cited above can serve to identify the major steps to be followed and some important pitfalls to be avoided.
CONCLUSIONS

An adequate supply of the five essential blood components—whole blood, red blood cells, plasma, platelets, and cryoprecipitate—is an essential part of a modern health care system.

A blood transfusion service is a complex organization, requiring careful design and management. There are many different ways of achieving this, each suited to specific circumstances. Essential functions of the service are donor recruitment, blood collection, testing of donor blood, component production, distribution of blood products, training, and reference services.

Operating a blood transfusion service is expensive, and adequate financing is a prerequisite for success. Development of a new service is a slow process that requires long-term commitment.
Appendix 1 to Chapter 1

Criteria for successful development of a blood transfusion service

1. The following prerequisites should be met before it can be considered likely that a project will succeed:

   - the need for a project is clear;
   - locally sustainable financing is likely to be forthcoming;
   - there are competent local professionals available to direct the blood transfusion service;
   - the responsible organization is clearly defined;
   - there is government support.

2. Preliminary planning should include:

   - creation of a broadly representative national blood transfusion committee;
   - selection of key personnel;
   - definition of needs;
   - selection of an organizational system;
   - definition of services, organization charts, administration, and financing.

3. Detailed planning of programme operation should consider:

   - donor recruitment;
   - blood collection;
   - laboratory processing;
   - blood distribution and appropriate use;
   - transfusion.

12
4. Project planning (international support) should consider:

- personnel needs;
- capital needs;
- supplies;
- budgeting;
- contracts between the collaborating parties.
Appendix 2 to Chapter 1

Guidelines for personnel needs

Decisions on the staffing of a blood transfusion service must take into account the size of the programme and the specific tasks to be undertaken, and will be influenced by the availability of qualified personnel. The following staffing positions should be considered:

Medical

Qualifications. Medical graduate. Licensed to practise medicine. Specialized training in blood transfusion.

Role. If director, must take overall responsibility for medical/technical aspects of the programme. In large centres, there will be additional medical staff to assist the director (see below).

Numbers. Depend greatly upon services required. In general, doctors are not needed for routine work in donor screening, phlebotomy, or laboratory procedures, but must be available for consultation. Every blood transfusion service must have a doctor constantly available. Major centres (more than 50,000 donors per year) need two or more full-time doctors.

Management

Qualifications. Training and experience in management principles and techniques. May or may not be a medical graduate. Must have special knowledge of the organization of blood transfusion.
Role. Responsibility for internal management and external affairs of the programme.

Number. One.

Donor organizers

Qualifications. Personal attributes: articulate, convincing, hard-working, loyal, and able to handle stress. No professional qualifications required but must be able to understand and accept the scientific principles of a blood transfusion service.

Numbers. In general, one person can handle the responsibility of organizing 5000–10 000 donors per year.

Technologists

Qualifications. Various: for supervisor—technical and management skills; for medical technologist—formal qualification in technology; for technician—some formal training; and for aide—on-the-job training only.

Numbers. Greatly affected by variables such as level of technology and number of tests to be done, component preparation, number of shifts to be covered, and responsibilities outside the laboratory.

Phlebotomists

Qualifications. Qualified as nurses or medical technologists, with specialized training. Personnel with lesser qualifications may work directly under qualified supervision, after appropriate training and demonstration of competence. Will require training in techniques for dealing with the public, and in donor care.

Numbers. Depend upon extent to which performance of other tasks is required (e.g. donor registration, health history, blood-handling, report-writing, canteen work). In general, one phlebotomist may be responsible for 20–40 donations per working day.
Administrators

Sufficient to support and control the financial aspects of the service, human resources, data processing and reporting, planning, building management, utilities, audit, communications, and transport.

Public relations/information personnel

Must not be overlooked, and should be closely involved in day-to-day activities of the transfusion service.

Support personnel

Includes drivers, secretaries, telephonists, janitors (caretakers), laboratory attendants, cleaners.
Appendix 3 to Chapter 1

Operating costs of a blood transfusion service

The simplified example given below provides an approach to calculating the cost of producing a unit of blood. For a more detailed approach, see *Management of blood transfusion services.*

The contribution for salary is based on an average salary of US$7500 per person per year, and assumes that one such person is required for every 500 blood donations each year.

Overhead costs include those related to fuel, telephone, utilities, insurance, audit, legal, building maintenance, equipment maintenance, depreciation, and inflation.

Actual costs will obviously vary considerably in different parts of the world. Suitable values must therefore be substituted for the figures shown in the example.

<table>
<thead>
<tr>
<th>Essential supplies (per unit collected)</th>
<th>US$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collection containers—per donation</td>
<td>2.00</td>
</tr>
<tr>
<td>—per additional component</td>
<td>(2.00)$^2$</td>
</tr>
<tr>
<td>Test kits—HIV</td>
<td>1.00</td>
</tr>
<tr>
<td>—HBsAg</td>
<td>1.00</td>
</tr>
<tr>
<td>Serological reagents</td>
<td>0.75</td>
</tr>
<tr>
<td>Labels, cards, stationery</td>
<td>0.75</td>
</tr>
</tbody>
</table>

Subtotal 5.50 (7.50)$^2$


$^2$ US$2 for each additional component, i.e. total cost of each unit collected rises to US$26.00 if there is one additional component.
Guidelines for the organization of a blood transfusion service

Salary: US$7500 per person per year

\[ \frac{7500}{500} \text{ per person per donation per year} \quad 15.00 \]

Overhead costs (per unit collected): 3.50

Total (per unit collected) 24.00 (26.00)\(^1\)

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\(^1\) US$2 for each additional component, i.e. total cost of each unit collected rises to US$26.00 if there is one additional component.
Chapter 2
Recruitment of blood donors

Early pioneers of blood donor recruitment approached the task from an altruistic point of view; their prime motivation was idealistic—‘the oneness of humanity’. Nevertheless, the notion of voluntary blood donation has not been, and is still not, an easy concept to promote, and recent approaches to recruitment have tended to be more pragmatic. It is to be hoped, however, that the altruistic motives of the early pioneers will not be lost or casually discarded.

As a result of the ethnic origins, religion, and beliefs of its people, each country, irrespective of its stage of development, faces its own unique problems. Motivation of donors may vary not only from one country to another, but also from one region to another within the same country. Generalizations should be avoided, and planning and decisions should be based on realities. Planners should avoid blindly following programmes formulated by and for other countries or regions.

The guiding principles for planners of blood donor recruitment programmes are outlined below. It is assumed that the national government has approved, at the highest level, the establishment of the blood transfusion programme, and that the transfusion service will be based on voluntary, unpaid blood donations. This discussion is restricted to recruitment of donors of whole blood.

PREPARATORY GROUNDWORK

In order to achieve the desired results, it is essential to establish clear objectives and carry out concise studies and cogent
Guidelines for the organization of a blood transfusion service

surveys. In formulating the recruitment programme, the objectives should be:

- to identify the salient factors that will increase the awareness of a given community and its motivation to donate blood voluntarily;
- based on this information, to formulate and plan strategies that will respond to the expectations of potential donors;
- after successful launch of the programme, to build a panel of dependable, loyal, and voluntary blood donors who will also help in the recruitment of other donors.

**Organization**

The area served by the blood transfusion service should be subdivided, taking into account such factors as natural geographical divisions, socioeconomic backgrounds, cultural and religious groupings, population density, and accessibility, together with a knowledge of the location of peer groups and of centres where the surroundings and atmosphere are likely to encourage recruitment. The last two considerations will help to determine the priorities for launching recruitment drives. The guiding principle is to concentrate efforts where the potential donors are most responsive, and where the campaign is thus likely to be most effective.

**Communications**

The available means and methods of communication should be listed as follows.

- *National or local mass media*

  National, regional, or subregional advertising increases awareness of the need for blood. Public service announcements on radio and television, and in newspapers, and announcements in general and trade magazines and other influential reading materials should all be utilized.
• **Personalized communications**

Personalized communication is achieved through professional association circulars, club newsletters, school magazines, religious newsheets, or by direct mail.

• **Use of national and local celebrities**

Any celebrities who are genuinely responsive to the cause should be recruited to assist the programme on a voluntary basis.

• **Educational institutions**

Education aimed specifically at the young is the most powerful weapon against the superstition and myths connected with blood donation. It is therefore important to introduce the subject of blood donation into schools as part of the science and civic studies curricula. The young are the potential donors: in this way, they will learn to accept blood donation as a normal activity.

• **Education of the general public**

It is in educating the general public that the support of government and senior civic leaders is required; top-level governmental approval is thus essential to the recruitment programme. In some countries, recruitment publicity has to be cleared through the government. Selective use—if possible, free of charge—of governmental publicity channels should be sought.

• **Recruiters** (see "Personnel", p. 22)

Recruiters are the conduits to the general public and should therefore be informed about scientific and technical advances relating to transfusion medicine. It is good practice to hold monthly meetings for recruiters at which they can express and exchange views about their work, and to provide time during these meetings for technical discussions.

Recruitment publicity material, e.g. posters, television advertisements, "jingles", cartoons, should be prepared and propagated by professionals who have expertise in the
medium being used. Illiteracy among the population should never be an excuse for not involving the media in donor recruitment.

Selecting communication methods

Choosing among the methods of communication involves consideration of their advantages and disadvantages and of the responsiveness of the controllers of the media, and an assessment of their cost-effectiveness.

COSTING AND BUDGETING

Budgets for the recruitment programme should be carefully prepared. The first step in the process is to estimate the number of blood units required annually, on the basis of the population and the number of acute hospital beds (see Chapter 1). It is an advantage to prepare short-term, medium-term, and long-term budgets. The help of people with appropriate expertise (e.g. the organization’s finance committee) should be sought, but the director of the blood transfusion service and the recruitment officers are responsible for the success or failure of the recruitment programme.

PERSONNEL

Because recruitment will involve professionals as well as amateurs, the personnel establishment of the recruitment team should be clearly defined from the start. Recruitment priorities for future developments in the programme should also be planned well in advance: discipline and control will follow naturally.

Professional staff

All the key positions required for donor recruitment should be filled by paid professional recruitment officers working on a full-time or part-time basis. Job descriptions, with details of working hours, including those outside normal working hours, should be written and fully explained to each officer.
Volunteer staff

The importance of the contribution made by voluntary recruitment officers, who are often blood donors themselves, should not be underestimated: their immense enthusiasm and empathy with new donors are invaluable. Ideally, voluntary recruitment officers should be supervised by professional staff. However, control becomes very difficult if a professional staff member has to supervise more than five voluntary recruitment officers.

Selection and accountability

The importance of the work done by recruitment officers and the need for them to exercise tact in what they do, cannot be overemphasized. They are in the “front line” in their dealings with the public, and careful selection is therefore essential for the success of the recruitment programme. The accountability of recruitment officers should be clearly written into their job descriptions, or specified in standing orders for the staff.

Supervision and measurement of work performance

A system for supervising professional donor recruitment officers should be implemented and controlled by the officer in charge of the recruitment programme. Recruitment officers should be required to discuss their work, maintain contact with the officer in charge, and submit written reports on their assigned work.

The need for supervision also applies to the voluntary recruitment officers, but the approach should be more lenient than with the professional staff. The efforts of the voluntary recruitment officers should be channelled directly towards the potential donors, in order to provide information, to convince the undecided, and to enhance the good image and improve the likelihood of success of the recruitment programme. Volunteers will already be convinced of the value of what they do, but must be taught basic communication skills before being sent out into the field, particularly if they are unfamiliar with recruitment work. They will quickly create their own cells of voluntary recruiters, and their work will be spread throughout the community. The information they disseminate by word-of-
mouth will spread much faster than any written communication. If properly motivated and mobilized, volunteers will considerably increase the efficiency of the recruitment programme.

Successful recruitment is a chain reaction: a good recruiter recruits a voluntary blood donor, who recruits a new voluntary blood donor, and so on, until the whole community becomes involved and committed to voluntary donations. Good recruiters also ensure continuity of donations from the people they have recruited.

**DONOR INCENTIVES**

**Policies**

The provision of incentives to blood donors may be misconstrued, and appropriate policies should therefore be established and strictly observed. Decisions should be reached, and recorded, on whether to accept unsolicited gifts from private organizations or individuals, and of what magnitude. The guiding principle is that the incentive should not be a camouflage for remuneration of the donor and that gifts that do not comply with agreed policies for incentives should be rejected. The best approach is thus one of promoting community support and self-help, rather than providing incentives.

Care should also be exercised in seeking sponsorships when a special recruitment drive or seasonal campaign is being conducted: any kind of commercialism, blatant or insidious, should be discreetly avoided.

Donor incentives are useful tools for image-building and sometimes act as reminders for “lapsed donors”. The type of incentive will depend on local factors: simple and attractive tokens of minimal commercial value are generally well received by young donors. Timing and frequency are crucial: too many incentives, provided too frequently, often reduce the effectiveness of this approach.

**SPECIAL RECRUITMENT DRIVES AND SEASONAL CAMPAIGNS**

When a special recruitment drive is planned, it is important to ensure that its objective is clearly defined, and that all staff,
including the blood collection and laboratory personnel, are kept fully informed of the event. All preparatory work should be completed at least one week before the drive is scheduled; a few days then remain that can be devoted to attracting and stimulating donors before the start of the recruitment drive itself.

RETENTION STRATEGIES

Blood collection facilities

Public image-building, and the retention of donors, are more successful if mobile blood collection units and static centres are purpose-designed. "Back-stairs" accommodation should be avoided, no matter how good the location. The facilities should be geared to donors' expectations, comfort, and convenience.

Collection venues

Donors expect cheerful, attractive reception areas at collection centres and venues. There should be adequate seating arrangements and, if a queue forms, the likely waiting time should be indicated. Donors must be comfortable during the blood donation, and any discomfort they experience should be alleviated as soon as possible. Most donors will expect light or liquid refreshments after giving blood, and this fits in well with the period of recuperation required.

Mobile collection centres

Mobile collection centres or "blood buses" are very convenient for many blood donors, and will also attract people to donate for the first time on impulse. The routes and schedules of the blood buses must be planned in advance by the recruiting staff. The efforts outlined elsewhere in this section and in Chapter 3 should concentrate on and be directed towards encouraging first-time donors to become repeat donors.

Staff

Staff responsible for collecting blood should exude confidence and friendliness. The image they present should be one of
Guidelines for the organization of a blood transfusion service

professionalism, care and efficiency, combined with personal warmth.

Donors may be acutely sensitive when they enter the collection centre. They are likely to expect some personal discomfort, or even pain, during the blood donation, and are mildly surprised when little or none is felt. They may also feel self-congratulatory during and after donation, in the realization of a gift freely given. However, if they encounter ill-tempered or uncivil staff, or badly run, disorganized, or dirty collection centres, they are unlikely to return to donate blood again.

It is not part of the recruiters’ work to deal with people who are rejected as donors on medical grounds. They are often tempted to intervene or are sometimes inadvertently drawn into such cases, but they should not be involved in offering medical explanations, which are the responsibility of the medical staff alone.

**Emergencies**

Provided that there is no breach of confidentiality, the general condition of the patients who will receive blood donated in response to emergency appeals may be indicated to donors. Although this does not fully comply with normal policy, it helps to allay suspicions among donors called at unusual hours about whether the emergency was genuine.

**Post-donation strategies**

Simple letters thanking individuals or groups for their donations are important. They show common courtesy in response to a gift, and indicate that donors are not forgotten after the event.

**Special ceremonies**

Annual award ceremonies should be held to acknowledge and congratulate donors who have donated many times. These occasions should be widely publicized, and peer groups and prominent citizens should be invited to address the donors who have given valuable and outstanding service to the community. The recruitment staff should also be invited to attend this
function, which gives them an opportunity to meet the donors in a social setting.

**DONOR RECORDS**

Once a donor recruitment programme has been launched, it is of prime importance to keep proper and effective records of blood donors. The methods available for this range from simple card indexes to computerized systems: the choice for a particular blood programme will be influenced by the number of blood donors, the number and level of training of the staff who will maintain the system, and the financial and technical resources available.

Whichever system is used, security and confidentiality of donor records must be rigorously observed by the personnel who use or have access to them. Donor records are obviously essential for tracing donors when a transfusion has an undesirable and attributable effect on the recipient (e.g. transmission of infection). However, their value in allowing donors to be contacted to encourage them to return for repeat donations is often overlooked. This is an effective method of donor recruitment, which should be exploited more often.

**MONITORING**

A well run donor recruitment programme is never passive. As the behavioural pattern of the community changes, whether suddenly and markedly, as a result—for example—of the epidemic of acquired immunodeficiency syndrome (AIDS), or slowly over decades, the approach to recruitment should adapt accordingly. The need for such flexibility requires the setting up of systems for regular monitoring of the effectiveness of the programme.

The parameters for such monitoring systems are simply defined, and the barometer of success or failure is the increase or decrease in the number of blood units collected. Successful monitoring depends on the early and accurate recognition and detection of these variations, and the promptness of remedial action.
CONCLUSIONS

There are no short cuts to effective recruitment programming. The only way to achieve good results is to approach the task systematically and professionally. The preparatory work must be performed and evaluated, and may often have to be repeated in order to update certain aspects or correct misconceptions. The programme and budget will change in the same uncontrollable and unpredictable ways as do the national and local social, economic, medical, and political conditions. Thus, the work of donor recruitment is essentially progressive and active. The challenge is ever-present.
Chapter 3
Blood collection

DONATION FACILITIES

Since most blood banks rely on voluntary and non-remunerated blood donors, special attention should be paid to the donation premises. A pleasant atmosphere for blood donation will encourage donors to relax and help to eliminate anxieties. The facilities should be attractive, well lit, comfortably ventilated, clean, and as convenient and spacious as possible. Space should also be provided for the blood donors to be under the supervision of donation personnel for 15–30 minutes after the donation, even while having refreshments. The times for donation should be as convenient as possible for the donors.

Mobile sessions for blood donation are more effective if they can be set up in large spaces, such as gymnasiums or cafeterias, rather than inside vehicles, which usually have only a few donor beds. Light, transportable beds are ideal for these sessions, but ordinary tables covered with plastic cloth can well be used as donation beds. Large rooms and comfortable surroundings, as well as space for refreshment and relaxation, will compensate for a certain amount of inconvenience during donation sessions.

As stressed in Chapter 2, the personnel should be interested and friendly, as well as professional and efficient. Local contact people in mobile collection centres can help in the recruitment of donors; their familiarity is important in making donors feel more comfortable and relaxed.

Beds should be arranged so that each attendant can take care of more than one donor at a time: a U-shaped arrangement, for instance, increases staff efficiency and effectiveness.
The blood transfusion service must have a manual of standard operating procedures that covers all aspects of blood collection in detail. This manual serves as a basis for training new personnel, and allows the procedures to be reviewed more easily in case of dispute or litigation. It should contain all local and national regulations pertaining to blood bank operation, and should be available to personnel at all times. Regular updating of the manual is part of the quality assurance of the transfusion service and is the responsibility of the medical director.

**BLOOD DONORS**

**Donor registration**

Information about each blood donor that makes tracing and recall possible should be obtained and recorded each time he or she donates. Records must be retained as required by local regulations, or for at least five years. The following information must be included:

- date of donation;
- name of the blood donor;
- address and telephone number at home and at work;
- sex;
- age and/or date of birth.

The generally accepted lower and upper age limits for blood donors are 18 and 65 years, but there are variations from country to country. It may be useful to have information about ethnic origin, for instance, when donors of rare blood types are needed. Additional information, such as driving licence number, occupation, and time of last meal, may be helpful if there are complications at the time of the donation. Any donors with special characteristics should also be noted, for example those with antibodies, such as anti-D, or those who are seronegative for cytomegalovirus.

The blood group of each donor accepted for donation is determined. Every donor is given a personal card confirming membership of the donor panel of the transfusion centre, on which is recorded his or her name, date of birth, registration number, ABO and Rh groups, and haemoglobin concentration.
or packed cell volume. The name of the blood transfusion centre and date of issue must also be shown on the card. Blood donations can be registered either on the card or on a separate slip, which can be carried in the card-holder: it is often important for the blood donors to be aware of the date and number of the blood donations made. If colour codes for ABO blood groups are used on donor cards and on the labels of the donated blood units, they must be the same as those used by other transfusion centres in the country.

All information on blood donors and on people who have not been accepted as blood donors (including identification, past or present illnesses, medication, and test results) must be kept confidential. The need for confidentiality must be emphasized to all personnel of the transfusion service, bearing in mind that matters concerning patient confidentiality may not be familiar to those who have not received basic medical or nursing education. A special course on this topic is necessary.

**Donor selection for whole-blood collection**

It is essential to determine whether the people volunteering as blood donors are in good health—to protect them against damage to their own health, and to protect recipients from transmission of diseases or from drugs present in blood, which could be detrimental. Only people in good health should be accepted as donors of blood for therapeutic use.

Each donor’s medical history should be evaluated, and the donor accepted, by a person suitably trained to apply accepted guidelines for selection. This person should work under the supervision of a physician. Abnormal findings should be referred to the physician in charge, who will make the final decision on whether blood will be collected. In doubtful cases the donor should not be accepted. A full explanation for the action should be given to the donor and must be registered in the donor file.

In practice, complete medical and physical examination of blood donors is generally not possible, and selection will depend upon the donors’ answers to questions concerning their medical history and general health, combined with inspection of their appearance and laboratory examinations.

Truthful medical histories are more likely to be obtained from voluntary, non-remunerated blood donors. It is
recommended that a preprinted questionnaire be used to obtain the relevant information. The answers must be inspected by a knowledgeable person responsible for the blood donation. Arrangements should be made to ensure that there is reasonable privacy during the interview. The questionnaire should include questions that elicit at least the following information:

1. Previous blood donations. The allowable frequency of standard donations of 450 ml varies from country to country, but must not be greater than one every two months. It is recommended that no more than five donations are allowed annually, regardless of the haemoglobin values of the donor. In general, premenopausal women are bled less frequently than men.

2. Previous rejections from blood donation and reasons for them.

3. Pregnancy and childbirth. Blood donation is not recommended during pregnancy or for at least six months (and preferably one year) after delivery; in any case, the interval must include the lactation period. If the lactation period exceeds one year, blood donation may be started at the discretion of a physician.

4. Surgical procedures, major illnesses, blood transfusions. At least six months should have elapsed since a surgical operation or blood transfusion. Ambiguous answers should be referred to the blood bank physician for further evaluation.

5. Heart, lung, and liver diseases, and endocrine disorders. The type of disease should be defined. Coronary heart disease or rheumatic heart disease, with residual damage, are indications that the donor should not be accepted. People with active pulmonary tuberculosis or active pulmonary disease should not give blood, but previous tuberculosis, which has been successfully treated and is no longer active, need not disqualify a prospective donor. Chronic liver disorders must be evaluated by a physician, but no one with a disease that causes impaired organ function should give blood. Diabetics whose condition is controlled by diet alone and patients who have been hypothyroid but are currently stabilized are acceptable; people with other endocrine disorders (e.g. thyrotoxicosis) are not.
6. Unexplained weight loss and lymphadenopathy. Either of these may be indicative of infection with human immunodeficiency virus (HIV), the causative agent of AIDS. Prospective donors who have any symptoms suggesting AIDS, or who are at increased risk of acquiring HIV infection, should be rejected. Risk factors must be evaluated initially and at each subsequent donation. People who are at increased risk of acquiring HIV infection include: male homosexuals or bisexuals; promiscuous men or women; male or female prostitutes; drug abusers; those who may have been exposed to HIV infection because they have been recipients of blood or blood products; and individuals who have had sexual intercourse with anyone in any of these groups. Information pamphlets and prominently placed posters should be provided so that people who are at increased risk of HIV infection may choose to leave discreetly without starting the screening process.

7. Drugs and medications. Though blood donors are often unable to give details of any diseases they may have, the drugs they are taking may give some indication of their nature. In some cases, donors should be deferred because the drugs in the donated blood may be harmful to recipients. A list of commonly used drugs, with rules for acceptability of donors (as approved by the medical staff of the blood bank), should be available to the staff in the donation centre.

Specific questions should be asked to obtain information about the following drugs:

- **Antibiotics**, which disqualify the donor for two weeks because they may be harmful to a recipient who is allergic to antibiotics.
- **Medication taken continuously for elevated blood pressure**. With the approval of the medical director, a donor taking only a diuretic or a beta-blocker may be acceptable, but rejection is recommended if additional medication is taken regularly.
- **Isotretinoin**, used to treat acne, is a cause for rejection, because the drug may be a teratogen.
- **Non-narcotic pain medication**. This is not a cause for rejection, but donors taking salicylates should not give blood that will be used for preparation of platelet
Guidelines for the organization of a blood transfusion service

concentrates until at least three medication-free days have elapsed.

- Medication taken to prevent or treat hypercholesterolaemia. This is not a cause for rejection.
- Vitamins, over-the-counter bronchodilators, decongestants, and, in most cases, tranquillizers do not disqualify the donor.

8. Hepatitis. A person with a history of jaundice or hepatitis may, at the discretion of the appropriate competent authority, be acceptable as a blood donor provided that an approved test for HBsAg (hepatitis B surface antigen) is negative (see Chapter 4, pages 43–45).

9. Malaria. In areas where malaria is endemic, this disease can be omitted from the questionnaire but it is important in non-endemic areas (see Chapter 4, page 53).

10. Other infectious diseases (see Chapter 4, pages 54–55).

11. Abnormal bleeding tendencies. People with these abnormalities should not be accepted and should be referred for further investigations.

12. Epilepsy, convulsions, and fainting spells. Individuals who have epilepsy or have had convulsions or fainting spells should not donate blood; however, if they are not taking any medication, or have been free from seizures for more than two years, they can be accepted after evaluation by a physician.

13. Cancer. Donors who have cancer should not be accepted. Donation may be resumed when five years have elapsed since the end of treatment and the donor has been declared free of disease.

14. Immunizations. Donors who have received measles, mumps, yellow fever, or oral poliomyelitis vaccine may be accepted for donation, provided that at least two weeks have elapsed since the last immunization. Immunization against German measles (rubella) disqualifies the donor for four weeks after the last injection. People who have received rabies vaccine after an animal bite are excluded until one year after the final injection. Immunization against viral hepatitis B is not a cause for rejection if the donor is otherwise acceptable, unless given for specific exposure risks, in which case the donor would be disqualified for at least six months after the last incident of risk. If hepatitis B immunoglobulin was also administered,
the disqualification should be extended to at least 12 months. Any donor who has received passive immunization using animal serum products must be excluded until four weeks after the final injection.

15. General health of the donor. People with minor ailments, e.g. sore throat, cough, headache, or nausea, may be accepted at the discretion of the physician. However, people who have had teeth extracted or other minor surgery within 72 hours of the proposed donation are not acceptable. In special circumstances, there may be restrictions on donations from individuals who have hereditary red cell abnormalities (e.g. sickle-cell trait, glucose-6-phosphate dehydrogenase deficiency).

**Donor selection for plasma donation**

Plasma donors may be divided into three groups, according to the frequency of their donations:

- those who donate at a frequency comparable to that allowed for whole blood donations;
- those who donate 2–3 times more frequently than donors of whole blood;
- those who donate up to twice weekly.

In general, a more detailed medical history and physical examination, and additional laboratory tests are required for donors in a plasmapheresis programme. The criteria must be particularly stringent for those who donate more frequently than donors of whole blood. Detailed consideration is outside the scope of this publication.

A proper identification system is necessary to avoid bags of concentrated red blood cells becoming confused during their centrifugation and return to the donors. This is the greatest inherent danger in manual plasmapheresis. Each donor may be asked to sign the label of his or her bag and to identify it before the return of the red cells. Donors should also know their blood groups so that these can be checked on the labels of the bags returned to them. In addition, the number on the blood bags and pilot tube may be transferred to the wrist of the donor. Primary responsibility for identification lies always with the donation room personnel; self-identification by donors serves only as an additional control measure.
Physical examination

The health of the donor should be evaluated by the donation room personnel. Physical examination by a physician is not needed, although in some countries the opportunity to be examined may serve as an extra incentive for donors. However, staff may be trained to detect superficial lymphadenopathy, particularly if the prevalence of HIV infection in the community warrants this, referring donors to a physician for further examination if necessary.

The following should be assessed:

1. **Body weight.** Donors of the standard 450 ml of blood should weigh more than 50 kg; those weighing less than 50 kg may donate less than 450 ml. Blood bags designed to hold 300 ml of blood are produced by many manufacturers for this purpose. No more than 13% of a donor’s estimated blood volume should be taken during one blood donation.

2. **Appearance.** Donors should appear generally healthy. Anyone who appears to be under the influence of alcohol or drugs, or to be excessively nervous, should be rejected.

3. **Skin.** Skin lesions at the venepuncture site disqualify a donor. However, minor skin lesions, such as psoriasis or acne, are not a cause for rejection unless they are unusually extensive or present in the venepuncture area.

4. **Pulse, blood pressure, and temperature.** The pulse should be between 50 and 100 per minute, the systolic blood pressure between 90 and 180 mmHg (12.0 and 24.0 kPa), and the diastolic pressure between 50 and 100 mmHg (6.67 and 13.3 kPa). Body temperature, measured orally, should be below 37.5 °C.

5. **Haemoglobin concentration or erythrocyte volume fraction (haematocrit).** The haemoglobin concentration usually recommended is 125 g/litre (7.75 mmol/litre) for female donors and 135 g/litre (8.40 mmol/litre) for male donors. The corresponding figures for erythrocyte volume fraction are 0.38 (38%) for women and 0.41 (41%) for men. However, the values may differ in different countries in accordance with national blood policies. Haemoglobin concentration can be determined photometrically, or by relative density using copper sulfate. The relative density of
blood collection

copper sulfate solution is 1.053 for a haemoglobin concentration of 125 g/litre and 1.055 for 135 g/litre. The copper sulfate solution must be changed daily, or after 25 tests, to avoid false results, and the container must be kept closed to avoid evaporation of the solution. Periodic checking of solutions and procedures is an important part of the quality assurance system. Haemoglobinometers (or photometers used for measuring haemoglobin concentration) must be calibrated daily.

The records of the physical examination and medical history must be initialled or signed by the examiner, and any reason for non-acceptance of a donor must be recorded. The donor should be informed of abnormal findings in the medical history or physical examination.

COLLECTION OF BLOOD

The blood-collecting bag should be inspected for any possible defects. During donation, it should be hung at a low enough level to allow adequate blood flow by gravity. The balance used for weighing the unit of blood must permit accurate determination of the volume collected. If more than one venepuncture is needed, a new donor set must be used for each.

Identification of the blood donor and the blood container at the time of venepuncture is most important. All cards and labels, as well as the identity of the donor, must be checked before the venepuncture and the donor record must be identified with the donor. Identically numbered labels should be immediately attached to the blood collection container, the sample containers, and the donor record. At the end of the donation, all numbers should be rechecked.

Blood should be drawn from a large vein in the forearm. A tourniquet or blood-pressure cuff is applied and the donor is asked to open and close the hand until the selected vein is prominent. If the arm is dirty, it should be washed first with a soap or detergent solution, which is rinsed off with water. The venepuncture site must then be prepared with an antiseptic solution. Examples of recommended antiseptics are: iodine, iodophore complex solution (10% polyvinylpyrrolidone iodine), 0.5% chlorhexidine in 70% alcohol, and alcohol. The antiseptic
solution used must be allowed to dry before the venepuncture is performed.

The anticoagulant of choice is citrate-phosphate-dextrose, supplemented with adenine (CPD-A_1). The expiry date for whole blood collected into this anticoagulant, and for packed red cells with an erythrocyte volume fraction of less than 0.7 (70%) prepared from it, is 35 days. This anticoagulant is also suitable for blood that will be processed into platelet concentrate or cryoprecipitate. Other anticoagulants may be used initially, with later resuspension of the red cells in additive solutions (see also Chapter 9, page 136).

A loose knot can be tied in the blood bag tubing, close to the venepuncture needle. The vein must be punctured immediately after the needle has been uncovered. The donor should open and close the hand, squeezing a rubber ball or similar object every 10–12 seconds during collection. Careful mixing of the blood and anticoagulant by turning the blood bag upside down slowly, once every 30 seconds, is important, especially, at the beginning and end of the donation.

If it takes more than 10 minutes for the bag to be filled, the blood may not be suitable for preparation of platelet concentrate, fresh frozen plasma, or cryoprecipitate. The blood volume being drawn must be monitored using a balance: a unit containing 405–495 ml should weigh 425–520 g, plus the weight of the container and the anticoagulant.

At the end of the donation, the tubing can be clamped by pulling the knot tight. Another clamp should be set between the knot and the needle. The tube is cut between these, the necessary samples are taken, and the needle is removed after the tourniquet has been loosened.

Another knot must be tied in the tubing close to the first one. The blood in the tubing must then be squeezed into the blood bag using a special tube stripper two or three times: this must be done as soon after the blood collection as possible to

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1 A volume of 63 ml of citrate-phosphate-dextrose adenine, formula 1 (CPD-A_1), solution is needed for collection of 450 ml of blood. It contains approximately 18 mmol of sodium. Each 100 ml of CPD-A_1 contains:

- sodium citrate: 2.63 g
- anhydrous dextrose: 2.90 g
- citric acid monohydrate: 327 mg
- sodium acid phosphate: 251 mg
- adenine: 27.5 mg
avoid coagulation of blood in the tubing. The tubing is then tied or heat-sealed into segments of 10–15 cm.

The tourniquet is removed from the arm, pressure is applied to a gauze pad over the venepuncture, and a dressing is then carefully applied to the site. The donor must be told to keep the dressing on during the rest of the day to avoid bleeding.

After the numbers on the container, pilot tubes, and donation record have been checked, the blood is stored at the appropriate temperature. If platelets are not to be removed, the whole blood should be stored immediately at 1–6 °C; if platelets are to be harvested, the blood unit should be kept at room temperature (20–25 °C).

A donor should remain on the bed or in a chair for a few moments under the observation of the staff. He or she may then be allowed to sit up for a few minutes, before proceeding for refreshments, during which time there should ideally be continued observation.

Donors should be thanked for their contribution and encouraged to donate again. Adverse reactions must be recorded in donor files, and any abnormalities detected in subsequent laboratory tests must be reported to donors.

ADVERSE DONOR REACTIONS

Mild reactions to blood donation are quite common but usually harmless. Fainting or vasovagal syndrome may be caused by the sight of blood, especially during the first blood donation. When the reaction is caused by psychological factors, symptoms may include sweating, weakness, dizziness, unconsciousness, convulsions, and even involuntary voiding of faeces or urine. The skin is usually cold and the blood pressure falls. Hyperventilation of an excited donor may result in excess loss of carbon dioxide and possibly hyperventilation tetany.

Routine instructions must be provided to enable the attendant personnel to handle mild donor reactions. Donors experiencing adverse reactions should be removed to an area where they can be attended to in privacy. The physician of the transfusion centre should be called to investigate adverse reactions.

A donor who faints must be placed so that his or her legs are above head level. Tight clothing should be loosened, and an adequate airway must be guaranteed. Cold compresses can be
applied to the donor’s forehead or to the back of the neck. Blood pressure, pulse, and respiration must be checked periodically. Inhalation of aromatic spirits of ammonia often helps, and a glass of fruit juice or cup of coffee may be beneficial.

Donors who become nauseated or who vomit should be made as comfortable as possible and instructed to breathe slowly and deeply. A cold compress on the forehead is again helpful, and aspiration of vomit can be avoided by turning the head to the side. Water should be provided for rinsing the mouth.

If convulsions develop because of hyperventilation, the donor should be made to breathe into a paper bag, which brings prompt relief. If the donor has true convulsions, however, a physician should be called immediately. A small object must be placed between the teeth to prevent biting of the tongue, and the donor should be held on the chair or bed if possible. An adequate airway must be guaranteed.

If a haematoma develops during the donation, the tourniquet should be removed immediately from the donor’s arm and three or four sterile gauzes should be held quite firmly over the haematoma for 10 min with the donor’s arm elevated above heart level. Alternatively, ice can be applied to the area for 5 min under pressure. If an arterial puncture is suspected, the needle should be withdrawn immediately and firm pressure applied for 10 min; a dressing should then be applied. The radial pulse must be checked; if it is not palpable, a physician should be consulted.

Serious cardiac attacks as a reaction to blood donation are extremely rare. In the case of an attack, a physician must be called immediately. Cardiopulmonary resuscitation must be started immediately if there is cardiac arrest, and continued until emergency aid arrives.

The nature and treatment of all donor reactions must be recorded on the donor record, including a note about the future acceptability of the donor.

CONCLUSIONS

Blood collection is the most important of all the functions of a transfusion service, and its organization must be given proper attention. It has been widely accepted that the highest levels of
quality and safety are achieved when the supply is based on voluntary, non-remunerated donations. The donation must be made a pleasant and rewarding experience for the blood donor.

Donors should be in good health to reduce the risk, to both themselves and blood recipients, of untoward effects. Every blood centre should have detailed guidelines for the evaluation of donor eligibility, which should be readily available to the donation room personnel. If plasma donations are performed, special guidelines should be available. Instructions are needed for the physical examination of donors and for laboratory tests, as well as detailed instructions for the collection of blood.

Although most of the adverse donor reactions experienced in the donation room are fairly mild and require no special treatment, instructions for handling both these and the more severe reactions must be available.
Chapter 4
Screening for transmissible diseases

Traditionally, blood transfusion laboratories have dealt predominantly with serological procedures. Grouping, cross-matching, and screening tests for irregular antibodies still remain the cornerstones of safe transfusion, but preventing transmission of infectious diseases has become more important recently.

Development has been rapid. Almost a decade elapsed between the discovery of the hepatitis B virus as a cause of transfusion-transmitted hepatitis and the widespread application of blood donor screening. In the case of human immunodeficiency virus (HIV) this delay was reduced to 18 months. New screening tests, sometimes of controversial value, have recently been introduced to prevent the transmission of diseases such as non-A non-B hepatitis and human T-cell lymphotropic virus type I (HTLV-I) infection. Recognition of the possibility of infectious complications of transfusion has had a profound effect on clinical practice: whole blood is transfused less frequently, and there is greater use of products such as albumin, whose safety can be assured by appropriate treatment.

Reducing the risk of transmission of infection involves more than the performance of screening tests. Knowledge of the early clinical features of an infection helps in identifying those individuals who might pass on disease in a blood donation. This provides the basis for formulating appropriate questions and performing physical examinations when necessary. Infections detected in this way would disqualify potential donors.

Epidemiological studies provide information about endemic areas and the characteristics of people whose blood is most
likely to be infective. They are therefore helpful for formulating national policy for the clinical assessment of donors, and for deciding which screening tests are necessary and when. Donors should be well informed about the infectious diseases that are endemic in their own country and about the major clinical features associated with blood-transmitted diseases. They are more likely to exclude themselves voluntarily if they have this information and if blood donation is unpaid.

Screening for transmissible diseases identifies not only potentially infective blood that must not be transfused, but also blood containing particular antibodies, which may be useful source material for specific immunoglobulin preparations.

**HEPATITIS**

Viral hepatitis B and non-A non-B hepatitis are important hazards of blood transfusion. Transmission of hepatitis A virus by transfusion is very rare, however, and this possibility is therefore ignored in transfusion practice.

**Viral hepatitis B**

A complete virus particle with infective capacity is called a virion. The hepatitis B virus virion (Dane particle) is 42 nm in diameter and consists of a nucleocapsid surrounded by an envelope. Complete virions occur in blood only occasionally, whereas the appearance of empty, tubular envelopes is a typical feature of the chronic carrier state. The virus has three main antigens:

- hepatitis B surface antigen (HBsAg) in the envelope;
- hepatitis B core antigen (HBeAg) in the capsid;
- hepatitis B e antigen (HBeAg), related to capsid, and soluble in blood in the presence of virions.

Antibodies may be formed against each of the main antigens. Anti-HBe usually precedes anti-HBs, and is more transient. Infection with hepatitis B virus (HBV) sometimes also results in formation of anti-HBe.

The most reliable method for preventing transmission of viral hepatitis B is the screening of blood donors for the presence of
HBsAg. The sensitivity and specificity of the laboratory methods based on enzyme immunoassay (EIA), enzyme-linked immunosorbent assay (ELISA) or radioimmunoassay (RIA) are satisfactory. Reverse passive haemagglutination (RPHA), which is simple to perform and requires almost no specialized equipment, is suitable for use in field conditions, but its sensitivity is 10–100 times less than that of EIA or RIA techniques. However, the better sensitivity of the EIA and RIA techniques results in an increase in detection rate of only 10% at most. All blood units should be shown to be HBsAg-negative before transfusion.

In exceptional cases, HBV has been transmitted by blood despite negative results in HBsAg screening, and attempts have therefore been made to increase the sensitivity of standard EIA and RIA techniques in order to identify at least some of these hazardous blood units. Amplification of enzymatic indicator systems has been successful, but there is no marked increase in sensitivity compared with standard assays. Time-resolved fluoroimmunoassay offers some theoretical advantages, but is not yet suitable for large-scale routine application.

The prevalence of HBV carriers varies considerably in different populations: in northern Europe it is as low as 0.1%, whereas in some parts of tropical Africa and Asia it reaches 20%. Among voluntary blood donors, who are mainly repeat donors, the prevalence is much lower, since carriers are excluded from the donor pool. The carrier state lasts for many years and possibly for a lifetime.

Choice of an assay for HBsAg screening should be influenced by the following considerations:

- Sensitivity should equal or exceed that of RPHA tests.
- Specificity is usually not a major problem, but positive results should be confirmed by neutralization or other assays.
- The speed of the test procedure and the possibility of automation are important factors, especially when large numbers of blood samples from donors are to be tested.
- Cost may ultimately be the deciding factor. In some countries, human labour and maintenance of equipment are the most important cost elements.

In addition to HBsAg assay, screening for anti-HBc and anti-HBs may be used for selected donors. Anti-HBc suggests a
recent HBV infection, particularly if IgM antibodies can be demonstrated. People who have high-titre IgG anti-HBc and who are anti-HBs negative, may transmit HBV infection. However, anti-HBc alone cannot be used to screen for viral hepatitis B; its use as a surrogate test for non-A non-B hepatitis is discussed below.

Anti-HBs is formed relatively late after HBV infection. Screening may be performed to identify suitable plasma for immunoglobulin preparations. There is no evidence that blood with anti-HBs would be more hazardous than blood with no hepatitis B markers, and presence of this antibody is ignored in transfusion practice.

HBeAg or anti-HBe may or may not be detectable in people who are HBsAg-positive. HBeAg-positivity indicates relatively high infectivity, whereas detection of anti-HBe indicates relatively low infectivity.

**Delta agent**

Delta agent can replicate only in HBV-infected hosts. It consists of viral RNA coated with HBsAg, produced by HBV—its "helper" virus. Delta infection is endemic in countries of the Mediterranean and southern Pacific among people with viral hepatitis B. It can be transmitted by blood products, and it may transform asymptomatic, or mild chronic, HBV infection into severe disease. Since it cannot replicate without HBV, careful screening of blood donors for HBsAg is the best means of preventing its transmission.

**Non-A non-B hepatitis**

In countries where all donations are routinely screened for HBsAg, most cases of post-transfusion hepatitis are caused by one or more viral agents, called non-A non-B hepatitis viruses. Until recently, diagnosis of non-A non-B post-transfusion hepatitis was based on the exclusion of non-viral causes and hepatotropic viral agents (such as hepatitis B virus and cytomegalovirus). It has recently been shown that, in most cases of non-A non-B post-transfusion hepatitis, there is
serological evidence of infection with hepatitis C virus (HCV), a single-stranded RNA virus.

The incidence of non-A non-B post-transfusion hepatitis among recipients of blood components, as indicated by increased serum transaminase levels 5–12 weeks after transfusion, is 2–19%, depending on the donor population and the number of units transfused. In most cases, this evidence of hepatic dysfunction is the only indication of infection, which is otherwise mild and subclinical and would not be identified without follow-up studies. However, abnormal liver function persists in between 8% and 68% of cases, depending on the donor and patient population; often there is a histological picture of mild, chronic, active hepatitis. Biopsies show histological changes indicative of cirrhosis in 10–20% of patients in whom the infection takes a chronic course. Deaths from liver failure or hepatocellular carcinoma have been associated with chronic non-A non-B post-transfusion hepatitis.

Some countries have introduced "surrogate tests" to identify donors at risk of transmitting non-A non-B hepatitis. These tests included determination of serum alanine aminotransferase activity and detection of antibodies to hepatitis B core antigen (anti-HBc). Introduction of testing for serum alanine aminotransferase seemed logical in that it might reveal donors with subclinical, otherwise unidentified, hepatitis. However, increased activity of this enzyme is not specific to non-A non-B carriers: it may also be demonstrated, for example, in obesity or after the consumption of alcohol.

Correlation of anti-HBc with non-A non-B post-transfusion hepatitis was an unexpected observation in some large studies carried out in the United States of America. Studies in other parts of the world, however, seem to indicate that this correlation is dependent largely on the type of donor population and on geographical location. For example, the use of anti-HBc as a surrogate test for non-A non-B hepatitis is inappropriate in HBV endemic areas.

The correlation between the surrogate markers and the incidence of non-A non-B post-transfusion hepatitis remains unconfirmed in some countries. Moreover, there is not necessarily a correlation in some individual cases. Thus, although patients with post-transfusion hepatitis have received blood from donors with at least one positive surrogate marker more often than other patients, the majority of positive donors do not transmit hepatitis. In as many as 50% of cases of post-
transfusion hepatitis, none of the implicated donors has positive surrogate markers.

**Viral hepatitis C**

The development of anti-HCV assays has reduced, if not eliminated, the need for surrogate testing. HCV is a 10-kilobase, single-stranded RNA virus, included in the family Flaviviridae because of its genomic structure. The prevalence of anti-HCV in patients with chronic non-A non-B post-transfusion hepatitis is 60–80%, and 60–90% of haemophiliac patients treated with commercial clotting-factor concentrate are anti-HCV seropositive. In developed countries, almost 90% of cases of transfusion-associated hepatitis are due to HCV; the figure is lower in most developing countries because a varying but larger proportion of cases are due to HBV. Epidemiological data indicate an association between anti-HCV seropositivity and hepatocellular carcinoma, but a causative relationship has not been established.

The detection of antibody in solid-phase immunoassays to the antigen c100-3 has been the basis for first-generation tests. This antigen is the product of the non-structural region of the genome. Antibody is usually first detected 10–15 weeks after exposure, but the delay in detection may be as long as 12 months in some people. Virus replication and viraemia are demonstrable during this “window” period, so that the blood of some seronegative people may be infectious.

Second-generation assays have now been developed which are more sensitive than the first-generation assays and are based on antigens detecting antibodies that appear earlier. These tests increase the proportion of viral hepatitis C cases that can be identified, and shorten the window period.

In low-risk populations, e.g. some blood donor groups, the proportion of false-positive reactions varies between 30% and 50%. Supplementary tests—neutralization assays and recombinant immunoblot assays—have been developed to overcome this problem.

**Conclusion**

Immunoassays for anti-HCV have been introduced in several countries for testing blood donors. Results of supplementary
tests should be known before donors are informed of reactive immunoassays. These tests are not yet widely available, and their use will increase costs, but they are essential to improving the safety of blood and blood products. The need to develop cheaper reagents with greater specificity and sensitivity, and to devise means of minimizing the cost of their use, is urgent.

It should be remembered that careful donor selection alone will considerably reduce the risk of transmission of infectious agents by blood and blood products. It has been shown, for example, that paid donors transmit hepatitis more frequently than voluntary, unpaid donors. Every effort must therefore be made to use only unpaid donors. Careful investigation of the medical history is also important in reducing the number of donors who have had clinical hepatitis, who have a history of transmitting infectious agents, or for whom there are associated risk factors such as intravenous drug abuse.

**ACQUIRED IMMUNODEFICIENCY SYNDROME**

Acquired immunodeficiency syndrome (AIDS) is a relatively recent addition to the infectious hazards of blood transfusion. Although in most cases the virus is transmitted by sexual intercourse, transfused blood may also be a source of infection. Screening for antibodies to the causative agent, human immunodeficiency virus (HIV), is now standard practice in modern blood banking.

**Risk of AIDS**

The first indication that AIDS could be a hazard of blood transfusion became apparent in December 1982, when the Centers for Disease Control in the USA reported a possible case in a 20-month-old infant. Since then, the link between blood transfusion and AIDS has become firmly established.

Before the introduction of screening of donor plasma for anti-HIV, and heat-treatment of certain plasma products, coagulation factor VIII concentrates effectively spread the infection to patients with haemophilia. In patients who used large-pool, commercial concentrate, especially that derived from the USA, the prevalence of anti-HIV varies between 70% and 90%. The prevalence is much lower in countries where the AIDS epidemic started later and where haemophiliacs were
treated predominantly or exclusively with domestic preparations.

Infection of the recipient is apparently almost invariable following transfusion of a blood unit contaminated with HIV. Whether or not the infection develops into full-blown AIDS is determined by factors that are largely unknown. The proportion of all haemophiliacs developing clinical AIDS is apparently lower than for infected male homosexuals. In contrast, a relatively high proportion of haemophiliac children infected with HIV by blood transfusion seem to develop AIDS quickly. The eventual risk for the development of AIDS by people infected with HIV through blood products is unpredictable at present.

**HIV screening**

HIV causes a persistent infection. Anti-HIV is demonstrable in most infected people 6–12 weeks after infection, but antibody formation may be delayed for as long as one year. The presence of the antibody indicates the presence of the virus; thus a positive antibody test implies infectivity. It seems that transfusion of a blood unit from a donor whose screening test is positive invariably results in transmission of HIV infection.

In transfusion practice, screening tests are used to detect healthy carriers. The tests are normally enzyme immunoassays, using disrupted viruses or a mixture of viral antigens, and based on either direct or competitive principles. The antigen is fixed on a solid phase, usually plastic beads or the wells of a microtiter plate. In the direct assay, the serum to be tested is incubated with the antigen, and, after appropriate washings, presence of the antigen is shown by an enzyme-labelled antiglobulin. In the competitive assay, a labelled anti-HIV antibody is mixed with the reaction components; if anti-HIV is also present in the sample, an inhibition of the labelled anti-HIV is observed. Some assays use recombinant antigen from the two types of virus, HIV-1 and HIV-2, in a single test.

Both types of assay are widely used. The direct assay is easier to interpret, but the competitive method gives fewer false-positive results. The choice of assay depends on the number of tests to be done daily, the technical expertise and equipment available, and the costs involved.

There has been recent interest in the development of particle agglutination tests and rapid tests, because they are technically
less demanding than the enzyme immunoassays and do not require the use of costly equipment. Some of these tests have been fully evaluated.¹

The presence of HIV has important implications for the transfusion routine and for the donor, and each initially positive result must therefore be confirmed. Recommended procedure is to repeat the test, preferably using another type of screening method. If the repeat is also positive, a supplementary test is done, the most widely used being the Western blot assay.

The antibody response associated with HIV infection is directed against a variety of viral proteins—gp160, gp120 and gp41 (encoded by the env gene), p55, p24 and p17 (encoded by the gag gene), and p66, p51 and p32 (encoded by the pol gene). In the Western blot assay, these antigenic proteins are separated and transferred to a nitrocellulose strip, and the serum with anti-HIV to be analysed is allowed to react with the different antigens. Criteria for interpretation have been proposed.² Correct recognition of relevant reaction patterns requires considerable expertise, but Western blot has retained its value as a reference method. Enzyme immunoassays with antigens synthesized by molecular bioengineering, and particulate agglutination tests can also be used to confirm initially positive results, although experience of their use in routine conditions is still limited.

In primary HIV infection, the antibodies that are detected earliest are specific for p24 and gp160. These antigens should therefore be included in screening kits. It should be noted, however, that very early infection cannot be detected with an antibody assay, since the immune response takes time to develop (see below).

An important adjunct to HIV testing and detection is the provision of counselling services. Although individual pretest

¹ Reports on operational characteristics of commercially available assays to determine antibodies to HIV-1 and/or HIV-2 in human sera: unpublished WHO documents WHO/GPA/BMR/89.4, WHO/GPA/BMR/90.1, WHO/GPA/RES/DIA/91.1, and WHO/GPA/RES/DIA/91.6, available on request from Global Programme on AIDS, World Health Organization, 1211 Geneva 27, Switzerland.

counselling may not be possible in transfusion practice, all prospective donors must be clearly informed that their blood will be tested for infectious agents, including HIV. It will not usually be possible for the transfusion service itself to provide counselling for HIV-positive individuals, but arrangements must be made for this elsewhere and must maintain patient confidentiality.

Blood is not released for transfusion if the donor’s test result is positive, even if only in the first screening (see above). However, donors are informed that they are positive only when the result has been confirmed.

**HIV variants**

A different but partially cross-reacting form of HIV, known as HIV-2, is prevalent in West Africa. So far only a small number of infected people have been identified outside the endemic areas. Many of the present screening assays for HIV can detect some, but not all, HIV-2 infections.

**Antigen testing**

In the primary HIV infection, viraemia precedes the development of antibodies. Ideally, therefore, blood donors should be screened for both the antigen and the antibody in order to increase the chances of detecting the infection in the early phases. Existing viral antigen capture assays, however, cannot identify all viraemic sera. Antigen assays are therefore not generally used for screening purposes in blood transfusion services, although they have been on trial in some centres. Virus culture, or provirus isolation in lymphocytes after amplification, increases the chances of detection, but neither technique is suitable for screening.

**“Look-back” programmes**

The exceptionally long incubation time of AIDS (at least 5 years on average and possibly more than 10 years in some cases) makes management of the epidemic very difficult. Although measures to increase the safety of blood transfusion do not substantially affect the spread of the infection in the industrialized world (in more than 95% of adults with AIDS,
the disease was contracted by means other than transfusion),
the ethics of medical treatment require that as many cases as
possible should be traced.
“Look-back” programmes designed for this purpose have
two principal objectives:

- Determining whether a donor whose seropositivity was
detected during screening by the transfusion service had
given blood before and, if so, what happened to the
recipient(s).
- Asking seropositive people whether they have donated blood
and, if so, tracing the recipients(s).

AIDS was first recognized in the USA in 1981, the causative
agent was identified in 1984, and tests for anti-HIV became
available in mid-1985. Thus, during a period of 5–8 years,
blood may well have been donated by people whose infection
went unrecognized.

**SYPHILIS AND YAWS**

Fresh blood components may transmit syphilis, but experience
in industrialized countries indicates that, at present, the risk of
this remains very small. Three factors contribute to this low
risk: the spirochaetes die within 3–4 days in stored blood; most
patients who need fresh blood components also receive
antibiotic therapy because of their clinical condition; and
antibody screening of blood donors for syphilis excludes some
of the potentially infective blood units.

Serological screening of blood donors, however, does not
totally preclude transmission of syphilis. Early in its course, the
disease is contagious despite negative serological tests.
Furthermore, the screening methods that are generally used in
blood transfusion services are based on the cardiolipin reaction;
this is less specific than the treponemal antibody determination,
and may produce false-negative results, especially in the early
phases of the disease. Nevertheless, the practice of screening all
units of blood, which was started before the Second World
War, has been continued in most countries. Flocculation tests,
such as Venereal Disease Research Laboratory and rapid
plasma reagin assays, are simple, inexpensive, and widely used.
In some countries, preference has been given to specific tests
such as the *Treponema pallidum* haemagglutination assay.

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Doubts have been expressed about the need to continue to screen all blood units for syphilis, because the probability that this will prevent serious transfusion complications has diminished. However, public health authorities are reluctant to give up this form of syphilis case-finding. Because the system is in place and functioning and because the cost—both in terms of money and donor loss—is small, serological testing for syphilis will probably continue.

The considerations outlined for syphilis apply as well to yaws. Treponema pertenue, the causative agent, is morphologically and immunologically identical to Treponema pallidum and, in some countries, is more prevalent. Appropriate questioning and brief clinical examination of donors will aid the detection of affected individuals, and the serological tests for syphilis will be positive.

MALARIA

The organisms that cause malaria in humans (Plasmodium vivax, P. ovale, P. malariae, and P. falciparum) infect red blood cells, and thus transfusion of cellular components may transmit the disease. In non-endemic areas there has been growing concern about this hazard because of the increasing numbers of people who travel to endemic areas, but the number of reported cases does not substantiate this fear.

Nevertheless, it is important in non-endemic areas to exclude donors who have returned from endemic areas. A prospective donor who has lived in an endemic area, and who has been receiving chemoprophylaxis, is not accepted until at least 3 years after leaving the endemic area. Donors who have had malaria are not accepted for at least 3 years following successful treatment of the disease. People who have visited an endemic area are not accepted for at least 6 months after their return. Antibody screening tests are sometimes helpful, but are not universally applicable because they depend upon the availability of the appropriate antigens. Tests based on fluorescent antibody conjugates are the most widely used.

In countries where malaria is endemic, it is not practicable to reject donors who have had a previous history of malaria infection or to screen the blood for malaria. Recipients are given chloroquine or, in areas where the parasite is resistant to chloroquine, sulfadoxine/pyrimethamine or mefloquine.
CHAGAS DISEASE

Chagas disease (American trypanosomiasis) is common in Central and South America, particularly in rural areas. The causative agent, Trypanosoma cruzi, can be transmitted by blood. In affected regions, screening for trypanosomal antibodies may be performed and seropositive donors rejected. However, in areas where the seroprevalence is very high, it is more practical to mix the blood with gentian violet (dilution 1:4000) for 24 hours before transfusion. Acute disease caused by transfusion requires treatment.

African trypanosomiasis seems to be less of a problem for transfusion practice.

CYTOMEGALOVIRUS

Transmission of human (beta) herpesvirus 5 (cytomegalovirus; CMV) by transfusion of blood or blood products containing white cells is of increasing importance in patients with severely impaired immunity who require supportive therapy. There are two groups of immunocompromised individuals in whom CMV infection can have very serious consequences: premature infants and recipients of transplants.

CMV infection occurs in immunologically healthy people as a mild or totally asymptomatic disease. The virus is not eliminated after the primary infection, but persists in a latent form in blood leukocytes. As with HIV infection, detection of antibodies indicates the presence of the virus; anti-CMV assays, rather than tests for viral antigens, are therefore used to screen blood donors for potential infectivity.

Because of the generally mild nature of the disease and the high prevalence of positive donors (over 50% in most European and North American populations), screening of all donors is not feasible. However, blood transfusion services should be able to provide CMV-negative blood to the special patient categories mentioned above. Registers should therefore be established of CMV-negative donors who can be called on if the need arises.

The screening can be carried out by EIA, or by latex-agglutination or complement-fixation techniques. Complement fixation has been used as a reference method, but is not ideal for mass screening. Latex-agglutination tests can be done
rapidly, and have the further advantages of technical simplicity and modest requirements for equipment.

OTHER DISEASES

Human T-cell lymphotropic virus type I (HTLV-I) has been shown to be associated with adult T-cell leukaemia/lymphoma and various neurological disorders. HTLV-I infection can be transmitted by blood, which has caused concern in those parts of the world where the virus is prevalent, notably in some countries in the Western Pacific and the Caribbean. Surveys conducted in Europe and North America indicate that HTLV-I infection is very rare.

Donor screening should be considered in those countries where HTLV-I is endemic. The prevalence of the infection, its estimated clinical significance, and the effect on the national or local blood supply of the exclusion of seropositive individuals from donor pools are important factors in reaching a decision. Preliminary epidemiological studies are therefore necessary. ELISA techniques are used for screening for HTLV-I antibodies, and Western blot, immunofluorescent, or radioimmune precipitation techniques are used for confirmatory testing.

There are case reports of the transmission of other infectious diseases. The most suitable approach to preventing their spread is to reject as donors people who have lived in, or visited, areas endemic for a particular disease, until three months after their arrival in the non-endemic area. A history of diseases such as dengue fever, Rift Valley fever, sandfly fever, schistosomiasis, West Nile fever, leptospirosis, yellow fever, amoebic dysentery, or any form of encephalitis does not permanently debar an individual from donation after the disease has been cured. Microfilariae (*Wuchereria bancrofti*) are not transmitted by blood transfusion.

CONCLUSIONS

The risk of transmitting an infectious disease is associated with every transfusion. While it is true that appropriate testing reduces this risk, no transmissible disease is always detectable and, indeed, there are probably some that have not yet been recognized.
For these reasons, if blood or blood products must be transfused, the greatest assurance of safety for recipients comes from unpaid and voluntary blood donation, combined with self-exclusion of prospective blood donors who are aware that their blood may represent some risk.

The decision about which screening tests should be used will depend on the prevalence of any particular disease (determined through epidemiological studies) and the consequences of the disease itself.
Chapter 5
Production of laboratory reagents

The most important reagents required by a blood transfusion service are those used in the routine grouping and cross-matching of blood, and in screening for and identifying antibodies. Although these reagents are expensive to purchase commercially, countries that can ill afford the foreign currency exchange continue to import them.

One objective of a transfusion service should be to prepare as many of these reagents as possible at national or regional level, depending on the organizational structure of the service (see Chapter 1), and to distribute them to blood banks within the service and to other users within the country. Arrangements should be made to recover the cost of production.

When the starting material for preparation of the reagents is of human origin, it must be free from infectious agents such as hepatitis B virus and human immunodeficiency virus. Donors must therefore be screened (see below) before serological tests are performed to assess the suitability of their plasma or serum for the preparation of reagents. The final product must be tested for specificity, avidity, and freedom from unexpected antibodies, and for cold agglutinins and rouleaux-forming properties.

Blood-grouping reagents can be prepared from human plasma or serum. Preparation from serum is easier, but use of plasma avoids the wastage of the red cell concentrate, which may be transfused to a patient or—in plasmapheresis—returned to the donor. Plasmapheresis has the added advantage of allowing plasma to be harvested from the donor more frequently than would be possible with whole blood donation.

Some blood-grouping reagents can also be prepared inexpensively from non-human or non-immune sources, such as
plant seeds or animals. The most widely used plant-derived reagent is anti-A\textsubscript{1}, obtained from the seeds of *Dolichos biflorus*. Anti-A from the albumin gland of the snails *Helix pomatia* and *H. aspersa* is an example of a reagent obtained from animals. Reagents prepared from non-immune sources are known as lectins.

**ABO BLOOD-GROUPING REAGENTS**\textsuperscript{1}

Anti-A, anti-B, and anti-A,B can be prepared from: “natural” high-titre human plasma, from plasma of artificially immunized donors, from animal sources (anti-A only), or from mouse hybridomas (preparation of monoclonal reagents by the *in vitro* culture of IgM-secreting mouse hybridomas). Only one of the three reagents can be prepared from animal sources and the equipment and technical skill required for the production of monoclonal reagents are not widely available. Most blood transfusion services will therefore prepare these reagents from the plasma of donors who may or may not have been artificially immunized.

Reagents produced from the plasma of artificially immunized donors are superior to those produced from “natural” high-titre plasma, because their titre is usually significantly higher and they show better avidity. Moreover, because they are immune reagents, they have a higher thermal range than reagents produced from “natural” high-titre plasma. The latter are usually cold agglutinins, which may react at lower room temperatures and may therefore be unsuitable for use in laboratories where, because of climatic conditions or artificial heating, the temperature is relatively high. In some countries, however, reagents prepared from people who have not been artificially immunized are satisfactory, and it is therefore worth assessing the titres in the local donor population before starting an immunization programme.

\textsuperscript{1} For details see: 
*Quality control in blood transfusion services*. Strasbourg, Council of Europe, 1986.
Selection of volunteers for immunization and plasmapheresis

The starting material is obtained from men or post-menopausal women by plasmapheresis. Women in the reproductive age group are not included because of the increased risk of ABO haemolytic disease of the newborn inherent in the procedure. The criteria given in Chapter 3 (pages 31–37) for donor selection apply, except that the conditions indicated for reducing the risk of transmission of malaria are not relevant.

Immunization and plasmapheresis

Informed consent of the donor, and approval of the national health authorities that govern the blood transfusion service must be obtained. The reasons for this are both ethical and legal, even though reactions to the procedures are rare.

Immunization is carried out by intramuscular injection of blood-group-specific substances A, B, or AB (depending on the blood group of the donor), which may be of human or animal origin. Substances of animal origin are available commercially, while those of human origin can be prepared from the saliva of secretors. One injection is given initially, and only one booster is recommended when the antibody levels decrease, because repeated injections may cause anaphylactic reactions. Although adverse reactions occur more commonly with animal than with human preparations, the immune response with the former is superior to that with the latter. If human material is to be used, donors must be screened for hepatitis B surface antigen (HBsAg), anti-HIV, anti-HCV, the antibody to hepatitis B core antigen (anti-HBe), and alanine aminotransferase. The material is collected and frozen for at least 6 months; it is used only if all the initial tests, and a repeat anti-HIV test on another sample collected at the end of that period, are negative.

The first plasmapheresis should be performed between 2 and 3 weeks after immunization, when the antibody response is usually at a maximum. The interval between subsequent plasma donations, the maximum volume of plasma to be drawn at each donation, and the total number of donations per year should be in accordance with the national blood policy or regulations or, in countries where these do not exist, as decided by the director of the transfusion service.
The method of choice for harvesting plasma from the donor is an apheresis system. However, in countries that cannot afford the high cost of the disposable and capital equipment, a manual plasmapheresis method may be used; the double blood-bags necessary do not incur any additional expenditure, but the method is more tedious for the donor and for the transfusion service.

Individual immune-plasma donations are tested for antibody levels, cold agglutinins, unwanted antibodies, and rouleaux-forming properties. Units of plasma that do not conform to the required specifications (see Chapter 8, pages 113–117) are excluded. The selected group-specific units of plasma are pooled (about 2.5 litres per pool), and are defibrinated using bovine thrombin (2000–3000 NIH units per litre of plasma) or calcium chloride (1.3 g/litre of plasma). The thrombin method is better and quicker but, where funds are limited, the use of calcium chloride is acceptable.

Decalcification is necessary only if calcium chloride was used for defibrination. The methods available include: addition of sodium oxalate (2 g/litre of serum); use of a cation exchange resin; and dialysis using a tubular membrane with the serum immersed in sodium chloride solution (9 g/litre). Sodium oxalate is the method of choice for developing countries, because of its low cost and simplicity. The dialysis method is cumbersome and time-consuming, and the cation exchange resin method is expensive. However, when the oxalate method is used, turbidity of the reagent occurs after prolonged storage. This can be overcome by storing the reagent in bulk and refiltering it just before bottling for use.

Complement is inactivated, sodium azide is added, and dyes are added to anti-A and anti-B for colour coding. The final product is tested for potency (titre and avidity) against reference samples of reagent using the recommended test cells, and for unwanted antibodies using a commercial or a laboratory screening panel of red cells (see Chapter 8, page 124). Avidity is enhanced by the addition of sodium chloride, when necessary.

The reagent is clarified and sterilized by filtration through suitable membrane filters. The pools are put into sterile 500-ml bottles, which are labelled and stored frozen (see below); when required, pools are thawed and aliquoted into clear, colourless, sterile bottles of capacity 5 or 10 ml. Alternatively, pools may be aliquoted into these small vials as soon as they are prepared,
and then stored frozen or at 2–8°C (see below). Storing in bulk is preferable when calcium chloride is used for defibrination, because the turbidity is removed by refiltration before the pools are dispensed into small vials. Immediate aliquoting is preferable when thrombin is used and refiltration is unnecessary.

Antibody levels are maintained for at least 24 months when the reagent is stored at −20°C or below, and for at least 12 months when the reagent is stored at 2–8°C. Appropriate expiry dates must be written on the vials.

**Preparation of anti-A from snails**

Anti-A can be prepared from the albumin gland of the snails *Helix pomatia* and *Helix aspersa*; *H. pomatia* yields the more potent reagent. Because the anti-A harvested from snails has a very high titre, as much as 2.5 litres of potent reagent can be prepared from one snail. For unknown reasons, the reaction of this reagent with $A_2B$ cells is significantly weaker than with other test cells ($A_1$, $A_2$, and $A_1B$); fortunately, reactivity with $A_2B$ cells may be easily enhanced by adding enzyme when the reagent is being diluted. As this is an inexpensive method of preparing anti-A, it is appropriate for use in countries where these species of snail are found.

**Anti-$A_1$ reagent**

Anti-$A_1$ reagent is necessary for the routine distinction of $A_1$ cells from $A_2$, but it is very expensive to purchase commercially. It can be produced by the absorption of anti-A from the serum of a group B donor (which has anti-A + anti-A$_1$), using $A_2$ cells. This leaves anti-A$_1$ which is referred to as "absorbed anti-A (anti-A$_1$)".

Anti-A$_1$ lectin may also be produced from the seeds of *Dolichos biflorus*, a plant grown in tropical and subtropical countries; both seeds and plants can be purchased inexpensively. The reagent prepared from the seeds is potent and stable. After the soaked seeds have been ground in a mortar or liquidizer, the reagent is extracted using sodium chloride, with centrifugation and prolonged cold precipitation (4 °C for 3 months). Alternatively, 0.31 mol/litre (23 g/litre)
glycine and 0.31 mol/litre (18 g/litre) sodium chloride may be used. In this case, the filtered extract is further diluted with the same solution of glycine, if necessary. This is followed by heating at 56°C for 30 min, freezing overnight, thawing, and cold precipitation at 4°C for 3 weeks.

The reactivity with A₂ cells, which occurs in higher dilutions of the extract obtained by either of these methods, can be overcome by further dilution of the extract or by absorption using A₂ cells.

ANTI-D

Anti-D may be obtained from the plasma of rhesus-negative donors who have been immunized following pregnancy with an Rh-positive fetus or after an Rh-positive blood transfusion. This is the simplest and best method, provided that the cooperation of the donor can be obtained. In some cases these donors are restimulated intentionally because the antibody concentration is below acceptable levels; this is the next best method, because immunization has already occurred and only booster injections are necessary.

Intentional immunization of Rh-negative donors for the specific purpose of producing the reagent is a third method, more often adopted by commercial manufacturers of the reagent, although it is also used by blood transfusion services. It places a greater moral obligation on the transfusion service to ensure the availability of group-compatible Rh-negative blood if the donor requires a blood transfusion.

A fourth method, the production of monoclonal anti-D, is as yet beyond the capacity of most transfusion services.

Criteria for the selection of volunteers for primary immunization, or for restimulation if they are already immunized, are the same as those described for the production of ABO reagents. Informed consent of the donor, and approval from the appropriate national health authorities must be obtained. In contrast to the production of ABO reagents, where purified substances (usually of animal origin) are used for immunization, anti-D is produced by immunization with washed human red cells. It is therefore essential to ensure that the red cells are taken from an individual who is free from diseases that can be transmitted by blood. The red cell donor is first assessed by clinical history and physical examination, and samples of his or her blood are rigorously screened for HBsAg,
anti-HCV, anti-HBc, alanine aminotransferase, anti-HIV, and malaria parasites. The cells must also be stored frozen (in glycerol) for 6 months, and then washed cells are used for immunization only if all the initial tests and the anti-HIV test repeated on the donor at the end of the period are negative. The red cells selected for immunization must be of as close a genotype as possible to that of the person to be immunized, except for the D antigen. This reduces the risk of formation of Rh antibodies other than anti-D, which would contaminate the final product. For example, if the prospective plasma donor (i.e. the person to be immunized) has a genotype dce/dce the cells used for immunization must be of the genotype Dce/Dce or Dce/dce and also be ABO group-compatible with the plasma donor. It is desirable to use red cells from the same individual for repeated injections to the same plasma donor.

ANTI-HUMAN GLOBULIN

Anti-human globulin can be produced only in a reference laboratory where skilled staff and facilities for keeping animals are available. Although several types of animal (sheep, goats, horses, and chickens) have been used to produce the reagent, the most commonly used is the rabbit.

Most of the red cell allo-antibodies are IgG and do not require complement for reaction. Anti-IgG is therefore an essential component of the anti-human globulin reagent. The presence of anti-complement antibodies in the reagent is also necessary, because some antibodies (e.g. those of the anti-Kell and anti-Kidd specificities) are detected only by anti-complement antibodies and not by the routine anti-IgG techniques employed in most laboratories. An added advantage of including anti-complement antibodies in the anti-human globulin reagent is that certain antibodies, such as anti-Jk$, which show weak reactions when tested separately with anti-IgG or anti-complement reagents, give strong reactions when these reagents are used in combination. The anti-complement antibodies recommended for inclusion in the anti-human globulin reagent are anti-C3c and anti-C3d, or anti-C3c and anti-C3g, because such combinations increase the strength of the reaction. Anti-C3g is superior to anti-C3d for use with anti-C3c, because it causes fewer false-positive reactions, but it is at present available only as a monoclonal antibody and only one potent reagent has so far been developed. Although there is still
a problem of false-positive reactions with monoclonal anti-C3d, anti-C3g, and even with anti-C3c, there is a monoclonal anti-C3d, BRIC-8, which makes a satisfactory reagent when mixed with polyclonal anti-IgG without anti-C3c.

Anti-IgG is relatively easy to produce, whereas production of anti-C3c and anti-C3d is more difficult and time-consuming. The best approach in many cases is to make anti-IgG, and purchase a small amount of anti-C3d (e.g. BRIC-8, which has only to be diluted to a final dilution of 1:320 to make the anti-human globulin reagent).

**RED CELL PANELS** (see also Chapter 6, page 87)

The preparation of red cell panels for screening and identification of antibodies can be undertaken at the national level, and both sets of panels made available to blood banks within the national transfusion service. Alternatively, the screening panel can be distributed and positive samples sent to the national centre for identification.

About 20 or more group O blood samples will have to be grouped to achieve an antigen profile capable of detecting and identifying most of the common unexpected antibodies that may be found in a population. The antigens in the following blood-group systems must be included as a minimum:

<table>
<thead>
<tr>
<th>Blood-group system</th>
<th>Antigens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhesus</td>
<td>D, C, E, c, e</td>
</tr>
<tr>
<td>Kell</td>
<td>K k</td>
</tr>
<tr>
<td>Duffy</td>
<td>Fy\textsuperscript{a}, Fy\textsuperscript{b}</td>
</tr>
<tr>
<td>Kidd</td>
<td>Jk\textsuperscript{a}, Jk\textsuperscript{b}</td>
</tr>
<tr>
<td>Xg (sex-linked)</td>
<td>Xg\textsuperscript{a}</td>
</tr>
<tr>
<td>Lewis</td>
<td>Le\textsuperscript{a}, Le\textsuperscript{b}</td>
</tr>
<tr>
<td>MNS</td>
<td>M, N, S, s</td>
</tr>
<tr>
<td>P</td>
<td>P\textsubscript{1}</td>
</tr>
</tbody>
</table>

If potent reagents and the necessary expertise are available, the grouping should be done at a reference laboratory within the country concerned. When this is not possible, the panel may be sent abroad to a laboratory where such facilities are available.

The cells selected for the screening panel (usually two samples) must cover all the antigens in the eight blood-group

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systems mentioned above. The identification panel (usually 10 samples) must also be selected so that the antibodies corresponding to all of these antigens can be identified by the profile. Samples of blood for the panels may be obtained fresh from fully-grouped staff of the blood bank or from blood donors at regular intervals of 3–4 weeks, or from fully-grouped units of donor blood that are kept frozen in small aliquots, in quantities needed for the number of sets of screening and identification panels, also prepared at intervals of 3–4 weeks. The red cells are frozen at or below $-20^\circ$C in glycerol, and thawed when needed, followed by dialysis in buffered saline.

Cell panels prepared by the blood transfusion service can be used for routine laboratory procedures. Commercial panels, which are expensive, should be reserved for circumstances when a broader spectrum of antigens is needed to make up for any that are lacking in the laboratory panel.

**NON-IMMUNOHAEMATOLOGICAL REAGENTS USED IN BLOOD GROUP SEROLOGY**

**Normal ionic-strength saline (NISS) (0.154 mol/litre; 9 g/litre)**

To produce normal ionic-strength saline (NISS), 180 g of sodium chloride (NaCl) are weighed accurately and dissolved in 20 litres of distilled water (90 g of NaCl and 10 litres of distilled water may be used if a smaller volume is required). The solution is mixed well with a paddle stirrer and dispensed into 1-litre or 2-litre containers.

Lysis of red cells will occur if the concentration is incorrect. The pH must be between 5.5 and 8.0: if the pH is below 5.3, elution of antibodies from the red cells will take place during washing for the antiglobulin test, and cause false-negative results.

**Phosphate buffered saline (PBS)**

The following quantities of chemicals are weighed accurately:

- $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$: 137.0 g
- $\text{KH}_2\text{PO}_4$: 40.8 g
- NaN$_3$: 2.0 g
- NaCl: 126.0 g
Each chemical is dissolved (one at a time) in the order mentioned while mixing, in 10 litres of distilled water. This is then made up to 20 litres with distilled water.

The pH should be between 7.09 and 7.14. Many workers prefer to use PBS instead of NISS, because it is better for suspension of red cells for use over periods of 12–24 hours and for storage at 4°C. It also reduces the slow haemolysis observed with red cells.

**Low ionic-strength saline (0.03 mol/litre)**

To prepare 1 litre of low ionic-strength saline (LISS), 18 g of glycine are first dissolved in 500 ml of distilled water. The pH is adjusted to 6.7 by drop-wise addition of 1.0 mol/litre sodium hydroxide solution; 20 ml of phosphate buffer (0.15 mol/litre, pH 6.7) are then added. The phosphate buffer is prepared by adding 0.15 mol/litre sodium dihydrogen phosphate (NaH₂PO₄·2H₂O) to 25 ml of 0.15 mol/litre disodium hydrogen phosphate (Na₂HPO₄) until a pH of 6.7 is achieved. 1.79 g of sodium chloride dissolved in 100 ml of distilled water is added to the solution, which is then made up to 1 litre with distilled water, mixed thoroughly, and dispensed in 100-ml amounts. It is sterile-filtered for storage at 4°C, or stored at −20°C to prevent bacterial growth.

LISS is widely used in blood-group serology because it increases 2- to 4-fold the rate of uptake of antibody of many different specificities compared with NISS.

**CONCLUSIONS**

When a national blood transfusion service is planned, it is important to provide for the production of as many of the essential immunohaematological and non-immunohaematological reagents as possible, and to have staff trained for the purpose. The reagents should be prepared on a small scale at central level initially; production can then be stepped up to fulfil the requirements of regional centres, as and when the service expands. This will not only ensure availability of the reagents, but will also lead to a saving in foreign currency exchange.
Chapter 6

Blood group serology

Blood-grouping and compatibility testing are among the basic investigations that any transfusion service must be able to undertake. Screening for, and identification of, antibodies are not listed among the basic requirements (see Table 1, page 6) but a transfusion service must develop the capability to undertake these procedures at an early stage of its development. This chapter outlines the advantages and disadvantages of various methods, special precautions to be taken, and pitfalls to be avoided, and describes practical applications. Details of methods are not included: these may be found in the selected reading list at the end of this book.

A population survey should be carried out during the early stages of developing a blood transfusion serology service in order to determine the prevalence of red cell antigens, haemolysins, and antibodies, and to identify rare blood groups. This information can be used to predict the needs for reagents, and the most suitable techniques, and the antigens that should be included in red cell panels, for antibody screening and identification.

**GENERAL PRINCIPLES**

Thorough practical training, as well as theoretical knowledge, is necessary for performing blood group serological tests. This training must also emphasize the care needed to reduce the risk of clerical errors, as well as the technical skills required for carrying out the procedures. Clerical error can be a major cause of injury or death as a result of blood transfusion.
Fig. 1. Specimen work protocol for ABO- and Rh(D)-grouping using IgM anti-D reagent

Note: The design of the work protocol should be the same as that of the rack. A similar protocol may be used for the microplate technique.

<table>
<thead>
<tr>
<th>Block no. 1</th>
<th>Test: Cell group (Anti-A, anti-B, anti-D)</th>
<th>Temperature</th>
<th>Control cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Identification no.</td>
<td>Patient or donor red cells</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Anti-A</td>
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<tr>
<td>Anti-B</td>
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<td></td>
<td></td>
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<tr>
<td>Auto-control</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Saline reagent*</td>
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<td></td>
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<tr>
<td>anti-D</td>
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<th></th>
<th></th>
<th>Time set up</th>
<th>Time read</th>
</tr>
</thead>
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<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>A</td>
<td>B</td>
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<tr>
<td>Controls</td>
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</tbody>
</table>
**Block no. 2**  
*Test: Serum group*  
Patient or donor sera  
*Temperature*  
Control cells

<table>
<thead>
<tr>
<th>Known cells</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>A</th>
<th>B</th>
<th>O</th>
<th>Time set up</th>
<th>Time read</th>
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</tbody>
</table>

*For example, monoclonal IgM anti-D. See Fig. 2 for work protocol for enhanced IgG anti-D reagents.*
Accurate documentation

Written work protocols should be used for all tests. Ideally, there should also be a double-check system, whereby cell typing and serum typing are undertaken separately by different workers, who then check each other’s results according to a “call-back” procedure. If discrepancies are identified, the tests concerned are crossed through on the protocol, indicating that they must be repeated.

In reality this double-check system will not always be practicable (for instance, when only one trained worker is available to perform all of the tests). A work protocol, such as the one outlined in Fig. 1, reduces the risk of clerical error.

Reliable ABO- and Rh(D)-grouping of donors’ and patients’ blood samples

Anti-A and/or anti-B normally occur in the absence of the corresponding antigens on red cells. ABO-incompatible transfusion reactions may be fatal, especially if group A or B blood is given to a group O patient with high-titre anti-A or anti-B.

The Rh(D) antigen is highly immunogenic. It is particularly important to avoid immunization of Rh-negative women who have not yet reached the menopause, because of the likelihood of rhesus haemolytic disease of the newborn in subsequent Rh(D)-positive babies. Rh(D)-grouping is often omitted in countries where the prevalence of Rh(D)-negative individuals is very low, but this decision should be made as part of the national blood policy. Appropriate controls must always be included when ABO- and Rh(D)-grouping tests are performed.

Compatibility tests

Compatibility of donors’ red cells with recipients’ sera is one of the requirements for safe blood transfusion. Cross-matching in test-tubes, with strict adherence to work protocols, is therefore necessary, particularly when antibody screening is not possible.

Selection of blood for patients with irregular antibodies

Irregular antibodies are usually first detected during cross-matching, during investigation of problems in ABO- or Rh(D)-
grouping, or in patients with acquired haemolytic anaemia or
haemolytic disease of the newborn. If time allows, the
specificity of an irregular antibody should be determined, to
facilitate the selection of blood containing red cells that lack
the corresponding antigen. If this cannot be done locally, the
patient's serum and red cells should be sent to a reference
laboratory.

It is sometimes impossible to determine the specificity of an
irregular antibody, either because of the lack of a red cell panel
or because there are emergency requirements for transfusion. In
such a case, serum must be cross-matched with several units of
the same ABO and Rh(D) group as the patient, to select
compatible blood.

**Transfusion reactions**

Thorough investigation of transfusion reactions is important,
both to determine their causes and to prevent their recurrence.
A system for the identification, reporting, and investigation of
all transfusion reactions should be established.

**BLOOD SAMPLES**

Clotted 10-ml blood samples are best for manual ABO- and
Rh(D)-grouping tests, but blood treated with an anticoagulant
can also be used. Serum is essential for compatibility (cross-
match) tests. All samples must be carefully and clearly labelled.

On receipt in the laboratory, each sample is centrifuged (e.g.
2000g for 3 minutes). Any serum that is not used, and that is
likely to be needed again, should be stored at or below −20°C.
Red cell samples should be retained at 4°C for a few days for
reference purposes.

**ABO-GROUPING TECHNIQUES**

ABO-grouping should be performed according to the
instructions provided by the manufacturers of the typing sera.
Slides or tiles, tubes, or microplates may be used.

**Slide or tile tests**

Slide tests are used by some transfusion services for preliminary
ABO-grouping at the time of blood collection (e.g. during
mobile sessions or in satellite collection centres). The grouping is repeated later using the tube or microplate method. Slide or tile tests may be used for emergency ABO-grouping if centrifuges are not available for spin-tube tests, but they are unreliable for serum grouping with low-titre anti-A and anti-B, and are not recommended for routine use. When slide tests are used, controls cannot be set up and examined simultaneously with the test samples themselves.

Microscope slides are used for slide tests. Any clean, non-porous surface may be used for tile tests, but opaque glass or ceramic tiles or white plastic tiles are preferred.

**Tube tests**

The work racks are filled with tubes as shown in the work protocol in Fig. 1.

*Spin-tube tests*

Spin-tube tests may be performed in 10 mm × 75 mm or 12 mm × 75 mm glass or plastic tubes. Plastic tubes are cheaper than glass but are not reusable. Glass tubes must be thoroughly washed and dried in an oven before reuse. Immediate spin tests may be used in emergencies, but for routine tests samples are usually incubated at room temperature for 15 minutes before centrifugation. The force and duration of centrifugation in spin-tube tests depend on the available equipment.

*Sedimentation-tube tests*

Sedimentation-tube tests may be performed in 10 mm × 75 mm or 12 mm × 75 mm tubes as above, or in smaller glass precipitation tubes (7 mm × 50 mm), which are cheaper. With good quality reagents the method is reliable and suitable for ABO-grouping of large batches of samples (40 or more). Samples should be incubated at room temperature for between 30 and 90 minutes.

*Reagents*

If very potent anti-A and anti-B (e.g. monoclonal antibodies) are used, it is not necessary to use anti-A,B reagents (group O
serum or equivalent monoclonal blends). However, if reagents of lower potency (e.g. polyclonal antibodies from donors) are used, the use of anti-A,B becomes essential, or weaker A or B subgroups will be missed.

The use of coloured reagents (blue for anti-A, yellow for anti-B) enables the technician to confirm that a particular reagent has been added to the test. The reagents should also contain ethylenediaminetetraacetate (EDTA; 0.1 mol/litre, pH 7.1–7.3): EDTA inhibits complement and therefore prevents haemolysis by potent haemolysins when fresh serum is used.

**Red cell suspensions**

Red cell suspensions should be 2–3% in saline (0.154 mol/litre NaCl; 9 g/litre) and may be made directly from the sample without washing the red cells. The addition of EDTA (0.1 mol/litre, pH 7.1–7.3) to the saline used for preparing the group A and group B red cell suspensions for serum grouping will prevent haemolysis due to high-titre anti-A and anti-B in the presence of complement (fresh serum).

**Sequence of work for efficient batch testing**

Racks of tubes are prepared according to the work protocol (Fig. 1), and the identity of the samples is recorded in sequence (e.g. from left to right). The samples in racks are always kept in the same sequence, and never in random arrangement or loose on the bench, because this may lead to clerical errors.

Errors in handling the samples are avoided by:

- Checking the sample number against the number on the protocol.
- Handling the sample once only to dispense serum and red cells.
- Working always in the same sequence (e.g. left to right).
- Replacing each sample, immediately after use, in the rack, in sequence. It is advisable to leave the first row of a rack empty. This allows each tested sample to be replaced in the preceding row, thus automatically marking the place in the sequence of the last sample tested; it is an essential safeguard against sequence errors where staff are likely to be interrupted in the course of their serological work. Control cells (A, B, and O) are dispensed last and read first, to
ensure that they work in the shortest incubation time of the samples tested in each batch.

Reading the tests

The supernatant in each tube is first examined for haemolysis, which indicates a positive test. (If all tests are haemolysed, a faulty batch of saline is to be suspected.) Each test is then read for agglutination.

Microplate tests

Microplate tests are performed in rigid polystyrene plates consisting of 96 U-shaped wells. The plates may be reused if they are thoroughly washed and dried first.

Microplates can be labelled so that the group for each patient tested can be seen in its entirety, which reduces the risk of clerical error. Smaller quantities of reagents and sample are used than for the tube tests, and plates can be preloaded and stored ready for use. Controls can be included in each plate.

The protocol shown in Fig. 1 can be applied to microplates. The procedure is the same as for sedimentation-tube tests (or spin-tube tests if a microplate centrifuge is used). The tests may be read automatically using a microplate reader, or by eye using a mirror or light-box after gently resuspending the cell buttons in the wells (manually, or mechanically using a shaker).

Rh(D)-GROUPING TECHNIQUES

High-titre monoclonal IgM anti-D reagents have recently been developed which work equally well at room temperature or at 37°C. They may be used in slide, tile, tube (spin-tube or sedimentation-tube), or microplate methods.

IgG anti-D reagents, however, cannot be used in saline tests unless they are chemically treated to increase agglutinating ability (see below), or potentiating substances, such as albumin or protease enzymes, are used to bring about agglutination. The reagents usually require incubation at 37°C and must be used and controlled as described in manufacturers' instructions.
Sequence of work for efficient batch testing

Batch testing is performed at the same time as ABO-grouping to minimize clerical errors through multiple handling of the samples. Work protocols are shown in Fig. 1 (IgM anti-D reagent) and Fig. 2 (IgG anti-D reagent).

Working in sequence for ABO- and Rh(D)-grouping is simplified by arranging the racks, or microplates, in straight lines to minimize operator movement and error. The Rh(D)-grouping controls should be dispensed last and read first to ensure that they work in the shortest incubation time compared with the test samples. The positive control should be an O-positive cell (R₁r or R₁R₂ phenotypes are the best choice if they are available) and the negative control is an O Rh(D)-negative cell.

The ABO autocontrol also serves as the Rh(D) autocontrol when IgM monoclonal reagents are used in a saline test. However, when IgG anti-D reagents are used, the diluent control is performed at 37°C. A positive autocontrol or diluent control test may indicate that the patient’s red cells are sensitized with in vivo bound antibody. If so, they will be agglutinated by the enhancing agent, such as albumin, even if the patient is Rh(D)-negative. High-titre IgM anti-D reagents can be used for grouping in such cases, if the saline autotest is negative. However, in some cases of haemolytic disease of the newborn, the baby’s red cells may be so heavily coated with IgG anti-D that they give false-negative results due to the blocking of IgM anti-D uptake by the sensitizing IgG anti-D.

In donor blood-grouping, negative tests should be confirmed by a second reagent or two-phase test (see below).

Rh(D)-grouping problems due to weak D (D⁻) and D variants

Expression of the Rh(D) antigen complex is variable. In a small proportion of people, the red cells have relatively little Rh(D) antigen and may fail to react with IgM anti-D monoclonal reagents, chemically modified IgG anti-D reagents, and albumin-potentiated reagents. However, they may react with selected IgG anti-D reagents in enzyme or antiglobulin tests. These are called weak D groups (low grade D⁻). They are not considered to be of major clinical significance in transfusion
Fig. 2. Specimen work protocol for Rh(D)-grouping using enhanced IgG anti-D reagents

Temperature: 37°C

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<th>Identification no.</th>
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<th>OD− (rr)</th>
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Date

Technician
work, because they are not thought to be immunogenic. In addition to weak D groups, there is also a very low incidence of D variants that express some epitopes strongly, but that do not express other epitopes of this complex mosaic antigen.

The failure of a saline anti-D reagent to detect an Rh(D) variant on a patient’s red cells, resulting in that patient being classified as Rh(D)-negative, is acceptable, because such individuals may produce anti-D to the missing epitopes of D. In donor grouping, the position is different: it is essential for the variants to be detected and labelled as Rh(D)-positive, since they may be immunogenic. All negative Rh(D) donor tests should therefore be confirmed by a second phase or test. This may be achieved by converting the negative saline test to an antiglobulin phase with IgM monoclonal anti-D reagents, blended with IgG anti-D for this purpose, or by performing a second test with a potentiated (e.g. albumin-potentiated) anti-D known to detect Rh(D) variants.

COMPATIBILITY TESTS

Immediately a blood sample is received, laboratory staff should confirm that it is appropriately labelled and that the information on the sample and on the request form is identical. The patient’s relevant history (e.g. transfusion, pregnancy, medication) must also be noted; this should be stated on the request form.

Great care must be taken to identify and label correctly any serum separated from the patient’s original blood sample. The serum should be stored at −20°C, if possible, or kept at 4°C for use within 3 days. A new blood sample is required for cross-matching if the patient has been transfused since the date of collection of the current serum sample, and if that transfusion was given more than 3 days previously, in order to detect antibodies that may have been produced more recently.

Only the “major” cross-match between the patient’s serum and the donor red cells is required. The function of the cross-match is to prevent incompatibility in vivo. This is most serious when there is ABO incompatibility and, in particular, when A or B cells are transfused into a group O patient with a high titre anti-A or anti-B. Incompatibility is usually detected by agglutination, but can also be demonstrated by haemolysis.

The actual procedure followed will depend on the clinical circumstances. In emergencies, sensitivity may have to be
sacrificed in order to provide blood quickly for the patient. Two serious errors must be avoided: the issue of ABO-incompatible blood because of inadequate and rushed procedures, and failure to supply blood before the patient suffers irreversible shock.

After determination of the patient’s ABO and Rh(D) groups, donor blood of the same ABO and Rh(D) groups is selected. Correct Rh(D) group is particularly important for all premenopausal female patients. The patient’s serum is cross-matched against the donor’s red cells to confirm donor–recipient compatibility, using a work protocol such as that shown in Fig. 3. Standard saline (normal or low ionic-strength solution) may be used.

**Standard test (see Fig. 3a)**

In the standard test, one volume of the donor’s cells (3% suspension in normal ionic-strength saline) is added to four volumes of the patient’s serum. As with the work protocol for ABO- and Rh(D)-grouping, the addition of reagents and samples to the tubes follows a defined sequence. The tubes containing the donor’s red cells are labelled in sequence (1, 2, 3, 4, etc.), but the corresponding donor pack numbers are recorded on the protocol.

The contents of the tubes are mixed, incubated at 37°C for 45 minutes, centrifuged, and read for agglutination. If agglutinates are read by pipette transfer technique, separate tubes must be used for agglutination and for the antiglobulin test. A positive result (agglutination and/or haemolysis) indicates incompatibility and the corresponding donor unit must not be issued for transfusion to the patient whose blood was cross-matched.

If the test is negative (no agglutination or haemolysis), the red cells in each tube are washed four times with clean saline and the anti-human globulin (AHG) test is performed (“immediate spin” AHG test). If the AHG test is negative, the donation is compatible and may be labelled for issue. Cross-match labels are prepared and the identity of the patient and donor packs is checked with the protocols. The ABO- and Rh(D)-grouping results are also checked against those of the pack label. (See Chapter 8, pages 118–121, for discussion of quality control of the AHG test.)
**Low ionic-strength tests** (See Fig. 3b)

The principle of low ionic-strength tests is the same as that of the standard test. However, two volumes of donor’s cells (1.5–2% suspension in 0.03 mol/litre low ionic-strength saline) are added to two volumes of the patient’s serum (see also Chapter 8).

**Urgent compatibility tests**

For urgent compatibility tests, the protocols outlined above are used. The patient’s ABO group and Rh(D) group are determined using rapid slide or immediate spin-tube tests. Laboratories that use low ionic-strength tests for routine work are well placed for the performance of urgent compatibility tests with full sensitivity after short (15-minute) incubation. However, it is not recommended that laboratories that use normal ionic-strength tests routinely should change to low ionic-strength tests for emergency techniques. A shorter incubation time of 15 minutes by the standard test system is recommended, although it is acknowledged that some weaker incompatibilities may be missed.

**Emergency blood issue, with no time for a full cross-match test**

In an emergency, EDTA samples and clotted samples should be obtained from patients before administration of intravenous colloids such as dextran and hydroxyethyl starch. Patients should be ABO- and Rh(D)-grouped by rapid techniques. Group-compatible blood can be issued after ABO-incompatibility is excluded by a rapid (2–5 minutes) saline, spin-tube cross-match using two volumes of patient’s serum to one volume of 3% donor red cells. Routine compatibility tests must be set up in any case, and appropriate steps should be taken if incompatibility is detected at this stage.

If this procedure is followed, it should seldom be necessary to resort to the use of group O Rh(D)-negative blood. Should this need arise, only units that have previously been group-checked should be issued.

When massive transfusions are given (that is, when the number of units transfused in any 24-hour period exceeds the
Fig. 3. Specimen work protocol for compatibility tests

Cross-match tests—spin tube

(a) Standard 45-minute test: 1 tube—2 phase
4 volumes (drops) patient serum: 1 volume 3% donor's red cells in normal ionic-strength saline (NISS)

Block no. 1

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<th>Donor unit numbers</th>
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Red cell suspension, 3% in saline, washed twice

Time set up

- Weak D
  - AB/S*

Time read

AHG CONTROLS

*Group AB serum

Sheet No.  
Date  
Technician
AHG test—indirect antiglobulin test

(b) Low ionic-strength solution (LISS)—15-minute test—1 tube—2 phase
2 volumes (drops) patient’s serum: 2 volumes 1.5–2.0% donor’s\(^a\) red cells in low ionic-strength saline (LISS)

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Red cell suspension, 1.5–2.0% in LISS

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<th>Phase 2: AHG test</th>
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\(^a\) Donor’s cells must be washed twice in saline (NISS) before they are suspended in LISS.

\(^b\) Group AB serum

**Blood group serology**

18
recipient’s blood volume), “compatibility testing” may be reduced to checking the ABO and Rh(D) groups of the transfused units. After the emergency has been dealt with, retrospective cross-matching should be undertaken with the pretransfusion sample. Compatible blood should be selected, in case further transfusion is necessary; ideally, any antibodies that are found should be identified.

Donor units that have not been tested, or not fully tested against the patient’s serum, should be clearly labelled “Selected for patient.......... but not cross-matched”.

Transfusion of infants

Before blood transfusion of an infant, the ABO and Rh(D) groups of both mother and infant must be determined. A direct AHG test is performed on the infant’s red cells and, if this test is negative, blood of the same ABO and Rh(D) group as the infant is cross-matched using the mother’s serum (if ABO-compatible) or the infant’s serum. This is to exclude the possibility that there may be incompatible antibody from the mother in the infant’s serum, in the absence of an antigen on the infant’s red cells to that antibody. Infants less than 4 months old do not make red cell allo-antibodies, even after multiple transfusions.

It is important to use maternal serum for compatibility testing if the infant has haemolytic disease of the newborn, or if an incompatible cross-match occurs. This may dictate the use of group O blood. If the infant is not group O, care should be taken to ensure that donor units have low-titre anti-A and anti-B. If ABO haemolytic disease of the newborn is suspected, group O blood of low-titre anti-A and anti-B should be used. It is good practice to use packed O cells resuspended in one-third volume of AB plasma (or A plasma or B plasma as appropriate), especially for exchange transfusion, as the A or B substance helps to neutralize any anti-A or anti-B antibodies.

Selection of blood for patients with irregular antibodies

If incompatible units are found, the first step should be to ensure that there has been no confusion of units through
clerical error, that the units are ABO-compatible with the patient’s sample, and that the correct patient’s sample is being used. The cross-match should be repeated with the same and additional units. An autocontrol is included because a positive autotest may be the cause of the reactions. If the antibody is directed against an antigen that is of low or moderate frequency, it should be possible to obtain sufficient compatible blood to meet the needs of most cases. In small blood banks, with few units available, it is advisable to screen relatives of the patient to try to find more compatible donors; this is particularly important if the antibody reacts with high-frequency antigens, or if there is a mixture of antibodies.

This simple procedure of selecting by cross-match screening usually meets the clinical need for the patient. However, the procedure of antibody identification is very important, since it provides experience in problem-solving that is essential for the development of the service.

**INVESTIGATION OF HAEMOLYTIC TRANSFUSION REACTIONS**

The commonest causes of severe haemolytic transfusion reactions are ABO incompatibility or infected blood. The former usually results from clerical errors, when samples are from the wrong patient, or blood compatible for one patient is given to another. Moreover, clerical errors involving one patient may involve others cross-matched at the same time. It is therefore essential to check the samples, donations, and documentation of all cross-matches done at that time. The occurrence of clerical errors should be notified to all staff, so that they may learn from mistakes, and the clerical and documentation system should be examined with a view to improving it.

If infected blood proves to be the cause of transfusion reactions, a thorough investigation of all procedures involved in the collection, processing, storage, and transport of the blood or blood products is essential, and must include the daily routine cleaning of all equipment and centrifuges used in the transfusion centre. If reactions to infected blood occur more than once in a 6-month period, an outside expert should be called in to investigate the cause.
**Serological tests**

Specimens required for serological investigation of transfusion reactions are as follows:

- Pretransfusion serum and red cells of the patient.
- Clotted samples obtained from the patient immediately and about 24 hours after transfusion. The later sample is necessary because irregular antibodies may not be detectable immediately after an incompatible transfusion.
- The remains of all the donor units, and the recipient sets.
- Urine from the patient for the first 24 hours after the reaction. In the case of intravascular haemolysis this will be dark owing to the presence of methaemoglobin.

The ABO- and Rh(D)-grouping of the patient’s samples and the donor units are repeated. Direct antiglobulin tests are performed on the patient’s pre- and post-transfusion washed red cells. The cross-match tests of donor’s red cells with patient’s serum are repeated using pre- and post-transfusion samples. Donor’s plasma and the patient’s pre- and post-transfusion samples of serum are screened for irregular antibodies. If the donor is group O and the patient group A or B, the anti-A and anti-B titres in the donor’s plasma are determined. Donors with high titres (>128) are “dangerous group O donors”.

**Bacteriological tests**

Donor blood packs should be bacteriologically tested by Gram staining and smear test examination, and by culturing the blood at 4°C, 20°C, and 32°C.

**Biochemical tests**

The patient’s post-transfusion serum should be inspected for haemolysis, and tested for bilirubin, free haemoglobin, and haemoglobin derivatives. The results should then be compared with those obtained on the pretransfusion samples.
HAEMOLYTIC DISEASE OF THE NEWBORN

Tests before delivery

In countries that lack the resources to perform antibody screening tests on all pregnant women, priority should be given to testing Rh(D)-negative women, or those who may have been previously immunized by transfusion or during a pregnancy. In such cases, the ABO and Rh(D) group should be determined and the serum screened against at least three sets of cells by saline and spin-tube AHG tests. The cells should be group O and include the antigens, C, c, D, E, e, M, N, S, s, K, k, Le\textsuperscript{a}, Le\textsuperscript{b}, Fy\textsuperscript{a}, Fy\textsuperscript{b}, Jk\textsuperscript{a}, Jk\textsuperscript{b}. At least one set should have the stronger D antigen combination R\textsubscript{2}, or be CDe/CD\textit{e} phenotype.

In the absence of screening cells, a saline and AHG test of the mother’s serum against the father’s red cells is a second choice. This will reveal whether the father has an antigen to the antibody detected. Anti-A or anti-B is neutralized if there is ABO incompatibility. There are obvious limitations to this approach in cases of disputed paternity, or in women who have been sensitized through transfusion.

The specificities of the antibodies are determined. It should be remembered that only IgG antibodies cross the placenta. Determining the specificities of antibodies will enable the obstetrician to monitor the baby’s progress and give the blood bank time to find compatible blood if the mother or the baby needs transfusion.

Tests at delivery

The most economical approach to testing at the time of delivery is to determine the ABO and Rh(D) group of the mother’s and the baby’s blood samples, and then to perform a direct antiglobulin test on the red cells of any baby who is jaundiced and/or anaemic. If the direct AHG test is negative, a serological incompatibility is excluded, except for the possibility of ABO haemolytic disease of the newborn (see below).

However, if the direct AHG test is positive, the mother’s serum must be tested to identify the antibody. If the baby’s direct AHG test is positive, and there are no antibodies in the mother’s serum, serological incompatibility is excluded, unless
it is due to ABO haemolytic disease of the newborn. Weakly positive results of direct AHG tests can occur on cold-stored clotted samples and are of no clinical significance.

In order to ensure that the cell panel has not failed to detect an antibody due to the absence of the corresponding antigen, the mother’s serum is tested against the baby’s red cells by the AHG test to exclude serological incompatibility. In cases of ABO incompatibility, it will be necessary to neutralize anti-A or anti-B.

For transfusion of infants in cases of haemolytic disease, reference should be made to the earlier section on compatibility testing for infants (see page 82).

ABO haemolytic disease of the newborn

ABO haemolytic disease of the newborn occurs when high titres of IgG anti-A or anti-B overcome protective A and B substances in the baby’s plasma and destroy the red cells on which the A or B antigens are rather weakly developed. It is therefore confined almost entirely to infants of group O mothers whose titres of IgG anti-A or anti-B are about 16 times higher than in group B or group A mothers. ABO haemolytic disease of the newborn is difficult to diagnose: special techniques are necessary to demonstrate the IgG anti-A or anti-B, and the direct AHG test in affected infants may be negative. Moreover, positive AHG tests may be weak.

The best diagnostic test is to attempt to detect anti-A or anti-B in a heat eluate of red cells from the baby’s cord blood sample with adult A, and B cells, using the AHG technique. Anti-A or anti-B would be demonstrable in most cases of ABO haemolytic disease. Tests with group O cells and the last-wash control should be negative.

A simpler test is to demonstrate anti-A or anti-B in the serum of the cord sample by the AHG technique with adult A, and B cells. If the baby is group A, the critical test is with the A cells, which will be positive in ABO haemolytic disease; conversely, if the baby is group B, the critical test is with group B cells. A strong reaction with group B cells will always occur with cells from a group A baby, and a strong reaction with A cells is expected with cells from a group B baby, but these reactions are of no significance because the anti-B or anti-A, respectively, is not involved in the pathogenesis of the disorder.
The test with group O cells should be negative: if it is positive, it indicates the presence of a further antibody as a possible contributory cause of the disease, especially if the direct antiglobulin test is strongly positive.

Serological tests may be negative if the blood sample from the baby is not taken until about 2 or 3 days after delivery. This is because the incompatible anti-A or anti-B is removed during the destruction of the red cells.

**IDENTIFICATION OF IRREGULAR ANTIBODIES**

Any laboratory that undertakes the identification of antibodies must have a suitable panel of red cells, fully grouped for as many antigens as possible. The red cells are arranged in selected combinations of antigens that enable the most commonly occurring antibodies to be identified from the pattern of positive and negative tests. A cell panel is made up of at least 8 samples of group O cells (to avoid anti-A or anti-B reactions), but typed A₁, A₂, and B cells are also usually available. A commercial or other reliable cell panel may be used initially. Later, when the laboratory has sufficient experience, a panel can be prepared through cell-grouping of the laboratory staff, confirmed by a reference laboratory. Grouping sera are, however, very expensive.

The combination of antigens must cover the Rh genotypes: CDₑₑ/CDₑₑ, CDₑₑ/cdₑₑ, Cdₑₑ/cdₑₑ, cDₑₑ/cDₑₑ, cdₑₑ/cdₑₑ, and cdₑₑ/cdₑₑ. A Cₖₚ-positive cell is also useful. Other antigens are K on a rr cell; Fy(a+b−) and Jk(a+b+) or Jk(a+b−) on separate rr cells; and combinations of MM, MN, NN, S+, S−, P₁, P₂, Le(a+b−), Le(a−b+), and Le(a−b−) arranged on 8–10 samples of group O red cells. To improve the chances of detecting antibodies, the antigen combinations of a panel should reflect variations in their prevalence in different ethnic groups in the local population.

**Techniques for antibody identification**

The antibody-containing serum is tested against the panel of red cells by, at least, saline and AHG tests at 37°C, because some antibodies behave differently in these tests. The use of washed cells treated with papain or bromelin is useful, as this test is very sensitive—equal to or better than the spin-tube
AHG test for Rh antibodies. In addition, the enzyme treatment destroys M, N, S, and Fy<sup>a</sup> antigens, and this helps in the identification of these antibody specificities, especially in mixtures of antibodies.

Antibodies of the IgM class work in saline tests. When these are found, the use of saline tests at 20–23°C may increase the strength of the reaction, and save time in the identification of antibodies that are optimally active in cold conditions, e.g. anti-P<sub>1</sub>, anti-Le<sup>a</sup>, anti-Le<sup>b</sup>, or anti-HI. In tropical countries, with ambient temperatures higher than 28°C, cold antibodies are less readily detected. It is unlikely that cold antibodies will interfere in ABO-grouping or cross-matching at these temperatures.

Antibodies of the IgG class are not usually active in saline tests. Exceptions are anti-A, anti-B, anti-M, and anti-N antibodies in cases where the reactive red cells have a very high antigen density. IgG antibodies are best detected by potentiated tests (albumin, enzyme, or indirect antiglobulin), and the spin-tube indirect antiglobulin test is the most important for the detection of clinically significant antibodies. Albumin tests are not as sensitive as indirect antiglobulin tests and need not be used. If they are used, however, the long-incubation, albumin-replacement techniques are preferable to the albumin suspended-cell techniques with short incubation times, because they are more sensitive.

The serum to be investigated should also be tested against the cells of the patient (autotest). If the autotest is positive, the possibility of an auto-antibody, of non-specific panagglutination, or of rouleaux formation must be considered. If an auto-antibody is present, a positive direct antiglobulin test on the patient’s cells, together with clinical and haematological findings, will indicate autoimmune haemolytic anaemia.

Identification of antibody specificity should be followed by typing the patient’s red cells to show that they lack the antigen for that antibody. It is advisable to have the results confirmed by sending samples of serum, and red cells in acid citrate-dextrose (or, preferably, Alsever’s solution), to a reference laboratory.
Chapter 7

Equipment and consumables

This chapter provides guidance on the selection of some of the basic equipment and consumables required for operating a blood transfusion service.¹

BLOOD COLLECTION

Plastic blood-collection packs have several advantages over glass bottles and are therefore recommended. They have blood-taking sets attached, with identically numbered segments that serve as sources of red cells for cross-matching purposes. They are pre-labelled, disposable, and easy to store. Each is a closed system, and no air-venting system is necessary during blood taking or administration. Because of the direct connection between the bag and the blood-taking set, the risk of contamination is very small. Plastic blood packs are available with up to four satellite packs attached, and this makes them ideal for the preparation of blood components.

STORAGE AND TRANSPORT OF BLOOD AND BLOOD PRODUCTS

Refrigerators

Whole blood and packed red cells should be stored at between 2°C and 6°C. At higher temperatures the risk of bacterial

¹ A comprehensive list of equipment and consumables is provided in Essential consumables and equipment (unpublished WHO document WHO/LAB/89.8), available on request from Health Laboratory Technology and Blood Safety, World Health Organization, 1211 Geneva 27, Switzerland.
proliferation is increased. At temperatures below 0°C the blood may freeze and will be haemolysed: transfusion of haemolysed blood may cause severe illness or death.

Blood may be stored for up to 5 weeks if collected into citrate-phosphate-dextrose supplemented with adenine (CPD-A₅) (see Chapter 3, page 38). Alternatively, the red cells may be suspended in an additive solution within 24 hours of collection (see Chapter 9, page 136).

Ideally, blood should be stored in a specially designed blood-bank refrigerator. The cylindrical type with rotating shelves has proved satisfactory, but standard cabinet types that give good access to blood stocks are available. If the refrigerator has adequate shelving, the blood may be conveniently arranged so that all units of the same group are on one shelf. The units should be arranged so that the oldest blood is closest to hand and is used first. Separate shelves should be reserved for cross-matched blood if a special blood-bank refrigerator is not available for this purpose, and the bottom of the refrigerator is convenient for holding units of blood products that are being stored for longer periods.

The blood-bank refrigerator must be kept under constant supervision by the officer in charge of the blood bank and should not be used for the storage of food or of laboratory specimens other than those being used in the blood bank.

To be suitable for blood storage a refrigerator should include the following features:

- The cabinet should be fan-cooled, and the fan should operate when all doors are closed. There should be no freezing compartment.
- There should be a continuous temperature-recording device, preferably clockwork but otherwise electrical.
- There should be an alarm system that is independent of the main electrical power supply. The alarm should operate if there is a power failure, or if the cabinet temperature rises above 6°C or falls below 2°C, and should give visual and audible signals in a location that is permanently staffed, such as the telephone switchboard room of a hospital.

There should be a locking device on the thermostat to prevent unauthorized or inadvertent alteration of the setting that determines the cabinet temperature.

If the alarm is designed to respond to the air temperature within the refrigerator, it should be set for a temperature
range of 1–7°C. The probes of the alarm system may be placed in 50 ml of liquid in a suitable container in order to decrease the sensitivity of the system. This will prevent the alarm from being activated by frequent opening of the cabinet door during busy periods.

A blackboard or white-board should be kept near the blood-bank refrigerator, on which the current stock of blood is recorded. The record should be amended every time units of blood are added or withdrawn.

A domestic-type refrigerator should preferably not be used for the storage of blood because its temperature is difficult to regulate with sufficient accuracy. If no alternative is available, however, the following requirements must be met:

- The refrigerator must be reserved solely for the storage of blood and blood products.
- Under normal service conditions the refrigerator must be capable of holding the stored blood between 2°C and 6°C.
- A maximum/minimum thermometer must be kept permanently inside the cabinet in a water-filled bottle and the temperature must be recorded twice daily. Any reading outside the 2–6°C range must be reported immediately, and steps must be taken to rectify the problem. The officer in charge must examine the temperature records at least daily. The thermometer should always be situated in the flow of cold air leaving the heat exchanger.
- Unless the refrigerator is fitted with an automatic defrosting mechanism, it must be defrosted at regular and frequent intervals to maintain a constant temperature.
- The freezing compartment should be locked so that blood cannot be placed in it by accident. The blood must be stored as far away from the freezing compartment as possible and should never touch the wall of the freezing compartment.
- Blood that is not suitable for use must be clearly labelled as such and must never be stored in the same compartment as blood intended for transfusion.

In addition to the blood-bank refrigerator, a domestic-type refrigerator is required for storing blood-group reagents in use, and samples of sera and blood awaiting cross-matching or other investigation.
Freezers

Fresh frozen plasma, cryoprecipitate, and cryosupernatant must be stored at $-20^\circ\text{C}$ or below, as soon as possible after preparation. Depending on local conditions, the blood bank will probably be required to hold 2–3 months' supply of these products. Stocks should be rotated so that the oldest product is used first. To maintain maximum quality, all stock should be used within 12 months and preferably sooner.

A freezer that operates at $-20^\circ\text{C}$ is the basic requirement, but one capable of operating at $-30^\circ\text{C}$ is better (but considerably more expensive). Both upright and chest-type models are available. Upright models, which are provided with separate smaller interior compartments to minimize temperature fluctuation, are more convenient for storage and retrieval. Temperature fluctuations are less likely with the chest-type models.

The freezer should be provided with a temperature monitor and alarm as described for the refrigerator.

BASIC EQUIPMENT FOR THE PREPARATION OF BLOOD COMPONENTS

The following basic items, together with multiple blood bags, are required for the production of cells, plasma (including fresh frozen plasma), cryoprecipitate and platelets.

Refrigerated centrifuge (see page 96)

Balance

A two-pan balance is required for balancing loaded buckets before placing them on the centrifuge rotor. Rubber cap-liners in two or three different sizes are useful to add to the lighter bucket to bring it to the balance point.

Plasma expressor

The plasma expressor consists of a hinged, spring-loaded flap mounted on a solid base, on which the centrifuged blood bag is placed. The flap applies pressure to aid the flow of plasma to
the transfer pack. Reliance on gravity or the use of hand pressure is a less satisfactory alternative.

**Tube sealing**

The connecting tubing on blood bags must be sealed and cut after separation and transfer of plasma to the transfer pack. The red cell pack also requires “seals” at intervals along the integral donor bleeding-tube to provide samples for cross-matching purposes.

Special electrical equipment is available to “weld” the sides of the tube together. This is ideal, providing clean joints at which segments of tubing may be separated by cutting with scissors or simply pulled apart. However, the equipment is expensive. A portable model with rechargeable batteries is preferable.

Knots or metal clips are cheaper alternatives. No equipment or expense is necessary to tie a knot in the tube and pull it tight. The loop in the tubing (for the knot) should be made before processing begins, and then simply pulled tight at the appropriate time. Metal clips can be crimped over the folded donor tubing using special pliers. Knots are preferable, however, because clips sometimes become detached.

**Platelet agitator**

It is recommended that platelets be stored at between 20°C and 24°C with constant agitation. Elliptical or circular rotators and flat-bed agitators are available. The type of agitator selected must take account of the type of plastic used for storing the platelets.

**BASIC EQUIPMENT FOR LABORATORY METHODS ASSOCIATED WITH BLOOD TRANSFUSION**

**Microscope**

Microscopes are used in the blood-bank laboratory for detecting microscopic agglutination. A good quality monocular instrument is satisfactory for blood-bank work, but the binocular type is preferable where prolonged microscopy is
undertaken (e.g. in general laboratory work). Users of the microscope should understand the mechanical and optical principles of its operation, and know how to maintain it.

A microscope for use in the blood bank should be fitted with a simple mechanical stage and a triple revolving turret with 10× and 40× objectives. (A 100× objective may be required if the microscope is also to be used for other laboratory work, but is otherwise unnecessary).

The instrument may also be fitted with a condenser for effective light control. For general blood-bank work a simple microscope lamp is suitable; its base should be of sufficient size and weight to ensure stability. The lamp housing must have good ventilation to prevent overheating, and should be mounted on a stand that permits vertical and horizontal adjustment. A more sophisticated (and expensive) lamp may provide for the use of coloured filters and be fitted with a condenser and an iris diaphragm. A focusing mechanism may also be incorporated for providing Köhler illumination, and a rheostat is useful to give control over light intensity. In areas without a reliable electrical supply, however, daylight illumination is adequate for microscopes used in the blood-bank laboratory.

**Water-baths, heating blocks and incubators**

Constant-temperature water-baths (at 37°C and 56°C) are required for many of the routine procedures in the blood bank. Alternatively, heating blocks may be used. These are available in a variety of sizes to accommodate tubes of various dimensions; however, they are more expensive than water-baths and less versatile for accommodating containers of unusual shape or size. If the ambient temperature exceeds 30°C, a 20°C incubator may be necessary for procedures that must be carried out at "room temperature".

Rapid-heating baths with wide temperature ranges and mechanical stirrers are also available, and allow any temperature up to 90°C to be obtained readily. Most water-baths are heated by electricity and controlled by stem-type, bimetallic thermostats.

If they are properly maintained, water-baths give little trouble. They should be cleaned regularly and refilled with
distilled or deionized water. A suitable container filled with water and kept in a 37°C incubator provides a convenient substitute for a 37°C water-bath.

Centrifuges

There are two main types of centrifuge: angle head and horizontal head. In angle-head centrifuges, the containers holding the material being centrifuged are maintained in a fixed position. Angle-head centrifuges can reach a higher rotational speed than horizontal-head centrifuges, and the relative centrifugal force is therefore greater. However, sediments are deposited on the sides of containers and are easily disturbed when centrifugation stops and the containers are removed. Horizontal-type centrifuges are preferable for use in blood banks, particularly for preparation of blood components.

There are several different options for the selection of heads and other accessories when a centrifuge is ordered. The precise choice will depend on the purposes for which the centrifuge will be used. However, all centrifuges must be satisfactorily shielded, and the cups must be provided with covers to reduce the risk of aerosols developing during centrifugation. Provision of built-in timers and tachometers is an advantage and is mandatory in the larger models that are used for preparation of blood components.

The centrifuge must be kept in proper mechanical condition by lubrication of the bearings according to the manufacturer’s instructions, and periodical inspection of the brush, with replacement when necessary. It must be properly balanced during operation: this is facilitated by marking the accessories, which are sold in sets, with distinctive colours to help operators preparing the centrifuge for a run. The bowl must be kept clean and dry, and spillages and breakages must be dealt with as indicated in Chapter 8 (page 130). The actual speed of rotation must be checked periodically using a tachometer,1 and the accuracy of the timer should also be verified periodically. Operating instructions should be kept immediately to hand.

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1 Relative centrifugal force (RCF) is calculated using the formula: 
\[ RCF \, (g) = 1.118 \times 10^{-2} \times r \times N^2 \]
where \( r \) is the radius (to the bottom of the container from the centre of the axis) in centimetres, and \( N \) is the rotational speed in revolutions per minute.
Clinical centrifuge

The clinical centrifuge is a small, bench-top model with a multi-place head to accommodate tubes holding up to 30 ml. This centrifuge is commonly used with 75 mm x 10 mm or 75 mm x 12 mm tubes for separating serum, washing red cells, and for numerous other tasks in red-cell serology and antibody identification.

Floor-mounted centrifuge

Larger, floor-mounted centrifuges that will centrifuge volumes of up to 2000 ml are commonly used for separating plasma or packing red cells, although a refrigerated centrifuge is preferable. These larger centrifuges usually have built-in tachometers.

Serology centrifuge

The serology centrifuge is a bench model that has been modified for easy, quick, and thorough washing of red cells during red-cell serology testing. It usually has a 12-place, lift-off, semi-swing-out head, and will accommodate standard serology tubes. An elastic band can be slipped around groups of tubes to hold them in position between the slots of the centrifuge head when it is removed and inverted to discard the saline.

Refrigerated centrifuge

A refrigerated centrifuge is used in the blood bank for the preparation of fresh frozen plasma, red cell concentrates, platelets, and cryoprecipitate. A compressor located in the base of the equipment can maintain the temperature of the blood below room temperature during centrifugation.

Pasteur pipettes

Pasteur pipettes are available commercially, but can also be prepared in the laboratory using soda-glass tubing. The tubing (external diameter 95 mm) is cut into 20-cm lengths. The centre of each tube is heated in a flame with continuous rotation; the central softened portion is drawn out into capillary tubing.
about 25 cm long, which is then cut. The stock of Pasteur pipettes should include some with a wider bore for transferring samples to slides for reading ("reading Pasteur pipettes").

In blood-bank work, Pasteur pipettes are usually reused after rinsing in water and saline; they can be kept on the laboratory bench in saline wash-pots. Disposable Pasteur pipettes are also available.

Rubber teats of the best possible quality should be purchased for use with Pasteur pipettes. Cheap, thin-walled teats do not give adequate suction and deteriorate quickly in tropical climates.

**Serology tubes**

Serology tubes are a basic item for a blood-bank laboratory. The most commonly used sizes are 75 mm × 10 mm and 75 mm × 12 mm. Plastic disposable and glass reusable tubes are available. However, plastic tubes have a tendency to float in the water-bath and, in addition, proteins may adhere to the walls, causing interference in some tests.

**Racks**

Plastic or metal racks are used in water-baths during cross-matching procedures; glass containers of an appropriate size to support the tubes will suffice if racks are not available. Wooden grouping-blocks have also been used, but are not recommended because they cannot be adequately cleaned or autoclaved and may present a health hazard.

**Grease pencils and glass-marking pens**

Grease pencils and glass-marking pens are used for identification of tubes and slides. The type chosen should ensure that marks are not rubbed off during the technical procedures, but will be erased when the glassware is washed.

**Glazed or opal glass tiles**

Glazed or opal glass tiles are used in the tile method of blood-grouping. They can be obtained plain (in which case the
divisions are marked with a grease pencil or marking pen before each use) or permanently ruled. Opal glass tiles are preferred, and a convenient size is 15 cm × 30 cm.

**Microscope slides**

Microscope slides must be of good quality, particularly if they are to be recycled. After use they should be soaked in hypochlorite or other virus-inactivating agent, washed thoroughly in detergent, rinsed and placed in methanol. After removal from the methanol, they are dried and stored.

**Vacuum pump (water Venturi type)**

The vacuum pump may be made of metal or glass and is connected to a water tap as a source of suction. It is used most frequently for removing saline after washing cells, and during the washing and drying of pipettes.

**Equipment for screening for infectious disease**

Most laboratories use commercially prepared kits for screening donor sera for infectious diseases. The package insert accompanying the kit will contain a complete list of the required equipment, which may include micropipettes, incubators, microtitration plates or strips, plate washers and readers, shakers or rotators, and microscopes.

**Water stills and deionizers**

Water required for the preparation of anticoagulant solutions for blood-collection bottles, or for the preparation of solutions or equipment for intravenous use, must be both chemically pure and pyrogen-free. The water used in the blood-bank laboratory for the preparation of reagents must be chemically pure. Dissolved gases and salts may be removed by distillation, deionization, or reverse osmosis, or by a combination of these methods.

A reliable still must be used for preparation of pyrogen-free water by distillation. It is designed to prevent carry-over of impurities by splashing, allowing only steam to pass to the
condenser. Suitable containers must be set aside and used only for the collection of the distillate, in order to prevent contamination by impurities. The numerous models of still available will deliver distilled water at rates ranging from 2 to 75 litres/hour. They may be heated by gas or electricity, and incorporate an automatic water-feed that maintains the volume of boiling water.

Chemically pure water may be obtained by deionization or reverse osmosis. These techniques produce water free from ions, but do not remove all non-electrolyte contaminants, so that the water is not pyrogen-free. There may also be some extraction of organic impurities in the deionization process. Water obtained by this process is usually chemically purer than that obtained by distillation.

**Laboratory glassware**

Only glassware made to withstand mechanical and thermal shock (e.g. borosilicate glass) should be considered for use in the laboratory.

The squat form of beaker, with a spout, is the most convenient type; graduated beakers are not necessary for general use. Beakers are often supplied in sets or "nests" of assorted sizes. Useful sizes to hold in stock are 10 ml, 30 ml, 100 ml, 500 ml, and 1 litre.

Reagent bottles should be of the cylindrical type with narrow necks, made of glass, and fitted with ground-glass or polyethylene stoppers. Those of 500-ml capacity are most useful and can be used for holding stock reagents.

Glass or polystyrene measuring cylinders are essential items of equipment; useful sizes are 50 ml, 100 ml, 500 ml, 1 litre, and 2 litres.

Volumetric flasks are required for the preparation of reagents.

Pipettes are used to measure liquid volumes. There should be a small stock of class A volumetric pipettes of capacity 1 ml, 2 ml, 5 ml, and 10 ml. Volumetric pipettes are calibrated "to deliver" (TD) or "to contain" (TC). A small amount of fluid remains in the TD pipettes after free delivery. This is not added to the liquid that has already been delivered, unless there is an indication that the pipette is calibrated for blowing-out this residuum. After delivery of its contents, a TC pipette must be washed out in the liquid that has already been delivered.
Guidelines for the organization of a blood transfusion service

Straight, graduated pipettes are satisfactory for most routine purposes if a high degree of accuracy is not essential. They are graduated along the stem and allow variable amounts of liquid to be delivered.

Mouth pipetting is prohibited, and the use of mechanical pipetting aids is therefore mandatory. Several varieties are available, and the selection of suitable devices will depend on such variables as: the volume and characteristics of the liquid to be manipulated; the ease of use of the device; the type of pipette being used (or whether pipette tips are used); and the ease with which the device can be cleaned and sterilized.

CLEANING OF GLASSWARE

The glassware used in a blood-bank laboratory must be cleaned thoroughly. Cell-serum mixtures must not be allowed to dry on glass. Immediately after the completion of technical procedures, dirty tubes and slides should be placed in water containing a suitable disinfectant. Buckets kept under the work bench are convenient for this purpose. Serology tubes can be bundled together with a rubber band after use, then placed in the bucket. This not only saves time, but is also a useful safety feature: sorting individual glass tubes may be dangerous, because many are damaged and may cause injury.

Detergents must be selected carefully, and choice may well be dictated by the nature of the water available. For safety reasons, it is strongly recommended that all glassware used in the blood-bank area and contaminated with biological material be placed in a hypochlorite soak (see page 106) immediately after use. Hypochlorite solution is a most effective means of getting rid of protein residues. If this practice is followed, only a small amount of a good general-purpose detergent will be required during the washing process to remove grease and inorganic material, and clean glassware of a high standard will result.

Whichever detergent is used, however, it is absolutely essential that no trace of it should remain on the glassware. It has been shown that as little as one part in 5000 of some detergents and disinfectants can cause haemolysis of red cells.

HAEMOGLOBIN MEASUREMENT

Screening for anaemia is an important part of the assessment of blood donors. Copper sulfate solutions may be used for this
purpose (see Chapter 3, pages 36–37) but the haemoglobin concentration may also be determined photometrically. In this case, a haemiglobincyanide method is recommended, using a photometer or a haemoglobinometer.

**STOCK CONTROL**

Replacement stocks of consumable supplies, including glassware, should be kept in a properly organized storage space. The simplest method for stock control is a stock-card system. Each commodity has a stock-card indicating the amount of reagent, or number of items, to be held, and the level at which reordering should take place. The cards must be updated each time items are removed from the storage area. This system should be used for all consumable supplies.

**INFUSION OF BLOOD AND BLOOD PRODUCTS**

The infusion sets that were widely used until the early 1960s have been almost completely replaced by disposable plastic sets. The former were recycled by the transfusion services in many countries, but this proved less economical than using disposable sets. Moreover, pyrogenic reactions, caused by products of bacterial contamination, often complicated transfusion of blood and blood products when the recycled sets were used. It was difficult to avoid contamination by pyrogens unless freshly distilled water was used for rinsing. Pyrogens are removed from disposable sets during their manufacture.

The risk of transmission to donors and recipients of infection with, for instance, HBV or HIV increases when recycled sets are used, particularly when there are inadequate facilities for screening, cleaning, or sterilization.

For these reasons, the recycling of both infusion and collection equipment is unacceptable.

Though no longer recommended for blood transfusion, glass bottles are still used in many places, and disposable administration sets for use with them are readily available. Each set contains an air inlet tube and a giving set. The air inlet consists of a needle and a length of plastic tubing with a small plug of cotton wool or paper to act as a sterile filter. This type of air-inlet is satisfactory, provided that the level of the
blood in the bottle is below the air filter and that the blood is not allowed to run back into the air-inlet tube. An alternative air-inlet assembly consists of a large needle (11 gauge), about 20–22 cm long, with a piece of plastic tubing and an attached air filter. The needle is inserted so that the tip is above the level of the blood when the bottle is inverted.

Air-inlet assemblies are not required for the giving sets used with plastic packs. The pack simply collapses as the fluid runs out.

Giving sets are supplied individually in sterile, pyrogen-free packets. Each consists of a plastic blood pack (or bottle) attachment, and a filter chamber leading directly into the drip chamber. Plastic tubing leads from this to the needle attachment.

It is claimed that superior filtering of blood or blood products is achieved with these sets, and that the danger of trapping air during the setting up of the apparatus, and when changing from one bottle or pack to the next, is eliminated. The standard set has either a single chamber combining filter and drip, or a double filter-drip chamber, is fitted with a drip flow-regulator, and can be used with or without a hand pump for applying positive pressure. Special paediatric and volumetric disposable blood administration sets are also available. A sufficient stock of disposable intravenous equipment should be held in case of late delivery of new supplies. Storage should be in a cool, dry place.

**UTILITIES**

The primary requirement for operation of the service is space. A blood bank in a district hospital, for instance, should have at least enough space for a blood donor waiting and bleeding area, blood-bank refrigeration and laboratory facilities, refreshment and care of donors after donation, operation of a donor call-up and records system, and storage. There must be adequate supplies of consumables and replacement parts for equipment. Unless sufficient storage space is available and good stock control is maintained, clutter will result and valuable working space will be lost. If possible, space should also be provided for general work activities, such as the preparation of donor recruitment materials. A separate building is sometimes necessary (e.g. blood transfusion centre), the size
and complexity of which will vary with its functions and the number and range of activities, it undertakes.

Additional requirements are reliable supplies of clean water and electricity, and effective communications. A considerable volume of water is required for domestic and sanitation purposes, and an adequate supply of distilled or deionized water for laboratory use. In some areas where a blood transfusion centre or blood bank is to be set up, water may be scarce or contaminated and require treatment before use. If running water is not available, water may be circulated by means of gravity flow from large plastic containers, through plastic tubing.

A mains electrical supply is desirable, but some of the techniques required may be carried out (e.g. at district hospital level) using battery-operated or gas-operated equipment. The possibility of solar energy as the source of power should also be explored. An emergency generator is essential in areas where the public electrical services are unreliable, and voltage stabilizers may also be necessary. In these areas it is also advisable to keep a stock of freezing pads (small, heavy-duty, plastic bags filled with water will do) in the deep freeze at all times. Provided that they are not allowed to come into direct contact with units of blood (with the risk of causing red-cell haemolysis), these pads can be used to prepare chilled storage containers in the event of power failure and temporary loss of refrigeration. Cardboard boxes heavily lined with newspaper make adequate containers for this purpose, if polystyrene boxes are not available.

Communication between blood transfusion centres, satellite collection centres, hospitals (including hospital blood banks), and blood donors considerably influences the efficiency of the transfusion service. Planning for the operation of a transfusion service must therefore take into account the likely availability of adequate telecommunication and transport services.

**WASTE DISPOSAL**

Blood transfusion services generate a variety of waste materials during blood donations, laboratory procedures, and the preparation of blood components. This waste is of two kinds:

- *Waste that is not biological in nature*, not contaminated, and not potentially infective for those who handle it. This waste
can therefore be treated like normal domestic refuse, disposed of daily. Waste-bins should contain plastic or heavy paper bags in which general and non-infective waste is collected; the bags should be stapled or tape-sealed, and disposed of by routine rubbish collection.

- *Waste that is potentially infective* consists of blood or blood fractions, plus the disposable equipment used in donor blood collection, preparation, and storage. It must undergo a decontamination process before being released for final disposal. Safety precautions are also important among the staff at greatest risk, i.e. those involved in bleeding donors, in blood-testing procedures, or in the preparation of blood components, and among those people within and outside the service who handle this waste material before its final disposal.

Care should be taken that no leakage occurs when waste materials are being transported within the donor bleeding or blood-bank area. This is best done by discarding all waste into containers lined with strong plastic bags. The bags must not be overfilled. Ideally, the sealed bags should be put into multi-walled paper sacks that are also sealed before they are removed.

Decontamination may be achieved by physical means (e.g. autoclaving) or with chemicals (disinfectants).

**Decontamination by autoclaving**

The autoclave is the quickest, cheapest, and most effective way of decontaminating blood-bank waste. High-temperature steam is produced by boiling water under pressure, and the steam rapidly penetrates bulky materials. A simple downward displacement or laboratory autoclave is all that is required for sterilizing waste material from a blood bank. It may be of the vertical or horizontal type and should be large enough to deal with a day’s accumulation of waste material. High-vacuum autoclaves operate on a shorter time-cycle, but they are expensive and have refinements that are not necessary for the simple decontamination process.

The autoclave consists essentially of a heavy bronzed-copper boiler encased in a sheet-metal jacket. A heavy gun-metal lid
can be clamped to the boiler by means of bolts and wing-nuts to produce airtightness. The pressure gauge, blow-off valve cock, and screw, spring-loaded, safety valve are mounted on the lid in the vertical type and on top of the boiler in the horizontal model.

Depending on the type, an autoclave may be used with mains electricity, gas, or primus stove. The horizontal type is usually provided with steam from an external source.

Proper handling and maintenance and regular testing of the autoclave are necessary in order to ensure that decontamination is consistently effective. One method of testing is to use Browne's sterilizing control tubes; these contain an indicator liquid that changes from red to green if the autoclaving temperature and time are correct. Various types of heat-sensitive tape are also available, on which a coloured pattern appears when the correct temperature is reached inside the autoclave chamber.

Waste to be decontaminated is placed in shallow containers; these containers must be waterproof to eliminate leakage of infected material and contamination of the work area before the contents are treated. Tall containers do not allow adequate steam penetration. If plastic bags are used, they must be left unsealed.

The manufacturer's operating instructions must be followed, particularly as regards the volume of water to be used and the method of securing the lid. The autoclave must not be overloaded: there should be room for the steam to circulate freely, so that it displaces the air that would otherwise depress the temperature. The safety valve is adjusted to the required pressure and temperature. For decontamination of blood-bank waste, values are 1.1 kg/cm² (15 lb/in²) and 121°C, respectively, for 30 minutes after the steam has displaced all of the air from the autoclave. Ideally, a thermocouple should be used periodically to determine the time taken for the centre of a full load to reach 121°C. This time should then be added to the exposure time at 121°C, to obtain the time required for the full cycle. In addition, heat-sensitive tape (see above) should be placed on the containers of material to be decontaminated during each run.

At the end of the cycle, the lid of the autoclave is opened after the pressure gauge registers zero, and the decontaminated materials are removed after the autoclave has been allowed to cool. To prevent leakage and contain odours, the contents
should be transferred into large plastic bags for transport to the final disposal point.

**Decontamination by means of disinfectants**

If an autoclave is not available, blood-bank waste may be decontaminated by chemical means, i.e. by disinfectants. This method is slower than autoclaving, requires space for containers and disinfectants, and is more costly.

Decontamination with disinfectants is carried out in buckets or other suitable large containers with lids. A wide range of chemical disinfectants is available. The two most suited to the needs of the blood bank are hypochlorites and glutaraldehyde, but ethanol and isopropanol also have limited usefulness.

Chemical disinfectants must be used at the correct dilution. Once diluted, they must not be kept beyond their useful life, which varies from a few hours to a week or more, depending on the disinfectant. Disinfectants will work effectively on a limited quantity of material and must not be overloaded. The articles to be decontaminated must be in contact with the disinfectant and not protected by a coating of air bubbles or films of grease and protein. All disinfectants are inactivated to some extent by protein, rubber, plastics, hard water, and detergents.

Hypochlorite solution is the disinfectant of choice for use in the blood bank. It is cheap, effective against viruses and bacteria, and can be used for decontaminating blood-bank waste, and for pipettes, discard jars, and work surfaces. The alkali it contains readily dissolves organic matter. Hypochlorite is used for soaking discarded serology tubes and slides. Plastic buckets with lids are suitable discard containers; hypochlorite corrodes metal and metal buckets should therefore not be used. Little detergent is required for subsequent washing, but thorough rinsing of all glassware that has been decontaminated in a hypochlorite solution is essential. The solution has a strong odour and its bleaching effect will ruin clothing.

Hypochlorite solution should be used at a concentration of 10 000 ppm available chlorine, with a holding (exposure) time of at least 30 minutes. Working dilutions must be made up freshly each day, as hypochlorite is unstable in solution. For this reason, granular calcium hypochlorite is preferred to liquid sodium hypochlorite or ammonium hypochlorite concentrates.
The dilution factor required to produce a solution with 10 000 ppm free chlorine (1% hypochlorite) depends on the concentration of the stock; for laboratory-grade sodium hypochlorite liquid concentrates, this is nominally 15–16%; for domestic products, such as chlorine bleaches, it is nominally 3–5%; and for granular hypochlorite it is 65%. Gloves must be worn when working with stock hypochlorites.

Glutaraldehyde may be used as an alternative to hypochlorite solutions; it is a liquid disinfectant used at 2% strength. It has a wide range of action and requires a holding time of at least 30 minutes.

Glutaraldehyde must be used within 24 hours of activation. It is highly irritating to the eyes and mucous membranes, and gloves must be worn to avoid sensitization of the skin.

Ethanol and isopropanol are used at a concentration of 70% v/v and require a holding time of at least 30 minutes. However, alcohol is not a suitable alternative to hypochlorite or glutaraldehyde and has limited use as a disinfectant in the blood bank. Alcohol at 70% v/v is an effective skin sterilizer prior to veneupuncture, but must be used with caution near electrical appliances or naked flames.

**Disposal of needles, sharp instruments, and non-reusable equipment**

Disposable materials make up much of the waste from blood transfusion services. The safe disposal of needles from blood collection facilities requires special attention to prevent accidental needle-stick injury and infection.

After the blood bag is sealed, the donor tube with the attached needle is cut off and dropped into a suitable autoclavable container with a lid. After autoclaving, the needles and attached tubes are transferred into containers for transport and final disposal; large tins with tightly fitting lids are suitable for this purpose.

A more convenient method, however, is to use 1-litre plastic containers with screw tops. These are disposable, will withstand autoclaving, and are readily obtainable. If needles are dropped into containers of this sort immediately on withdrawal from blood donors, transfer to another container for final disposal after the autoclaving procedure is unnecessary. The screw tops must be removed before autoclaving and replaced before disposal.
If an autoclave is not available and decontamination is carried out by chemical means, the materials are soaked in hypochlorite solution of the recommended strength for 24 hours and are then bagged for final disposal. Needles and any other sharp objects must be placed in tins or strong plastic containers.

**Disposal of waste by incineration**

Incinerators rarely consume all combustible material. Microorganisms may be carried up an incinerator chimney by draught before being killed by heat, and may then be recovered from the smoke at the top of the chimney. Live organisms may also be recovered from the ash pit of an incinerator, if too much material has been piled into the combustion chamber. In district hospitals and blood banks, open fire (bonfire) disposal is frequently practised, with much the same results. If open burning is used for blood-bank waste before decontamination, the waste should be placed in metal cans, for example kerosene tins or oil drums. The cans should then be placed in the centre of a fierce fire, where the temperature must be high enough to kill all organisms and none can escape in smoke or air draughts.

**Disposal of waste by burial**

Disposal of waste by burial in refuse tips is a method commonly employed in large hospitals. It is essential, however, that all blood-bank waste that is potentially infective be sterilized, preferably by autoclaving, before being released as general refuse and transported to a tip area.

**Decontamination of instruments, reusable glass, and plastic ware**

Disposable materials should be used wherever possible, but *must* be discarded after use. It is likely, however, that reusable instruments, glass, and plastic ware will continue to be employed for a variety of purposes.

Glass and reusable plastic ware should be placed in hypochlorite solution in covered containers immediately after
use, and left for the recommended soak time. Metal instruments should not be placed in hypochlorite solution because of its corrosive effect: they may be autoclaved or soaked in 2% glutaraldehyde before preparation for reuse.

Many chemical disinfectants are inhibited by organic material. Equipment contaminated with blood or derivatives must be cleaned before sterilization or disinfection, using cold water and detergent. Personnel doing this must wear suitable protective clothing, including heavy-duty gloves.

The handling of any potentially contaminated blood-bank linen should also be minimized, and linen should be decontaminated before laundering. Some transfusion services use dissolving bags to contain the linen, which then goes through a hot wash (60–70°C) with ordinary bleach concentrate.

CONCLUSIONS

Many advances have taken place in blood transfusion technology over recent years, and with them have come greatly increased technical sophistication and cost. However, the techniques and equipment required to establish and operate an effective blood transfusion service can still remain reasonably simple.

The choice of equipment for a new or existing service should reflect real needs, financial and human resources, and local climatic conditions. The concept of appropriate technology must always be borne in mind.
Chapter 8

Quality assurance and biosafety

In the context of blood transfusion practice, "quality assurance" is the term used for all of the measures, from recruitment of the donor to transfusion of the blood or blood product, to ensure that products are of the quality required for their intended use, and that laboratory results are reliable. This ensures that the patient receives a positive, defined advantage from a particular product, that donor, patient, and staff are not harmed, and that the activities are cost-effective. Some activities are entirely within the control of the blood transfusion service, while others (e.g. collection of blood samples, administration of blood) may be outside its immediate control, although even these should meet the requirements set by the service. The emphasis in this chapter is on quality assurance related to laboratory aspects; however, quality assurance of blood donor recruitment and blood collection (Chapters 3 and 4), and of transfusion of blood and blood products (Chapter 9) is equally important.

Quality assurance entails defining, controlling, and documenting every aspect of a process or procedure in such a way that compliance with determined standards can be predicted. Quality control is therefore an integral part of quality assurance. It consists of a set of procedures undertaken by the staff of the transfusion service for continued evaluation of the quality of the work. The procedures include the sampling, testing, documenting, and release of the product when specified standards have been met. Quality control procedures are prospective in nature.

In the context of blood transfusion services, quality assurance also includes good manufacturing practice. The activities of the transfusion service involve mainly the
preparation of whole blood or blood components for transfusion, and very little manufacturing actually takes place. However, because these products are intended for intravenous administration, the appropriate standards are those applicable to the pharmaceutical industry.

Quality assurance in the transfusion service also includes end-product testing (analysis of products at the end of preparation) and retrospective assessment of the quality of work in the service (for instance, through an external quality assessment scheme). These procedures may alert staff to the need for corrective action in some areas of the service, but they are complementary to, and cannot replace, the other aspects of quality assurance.

PRINCIPLES OF QUALITY ASSURANCE IN BLOOD TRANSFUSION

For safe transfusion practice, quality assurance requires a correctly organized scheme of management, with clearly identified levels of individual responsibility, properly trained staff, and adequately designed and equipped laboratories.

A department with specific responsibility for quality control should be created and, depending on the scale of the operation, may consist of one senior staff member, or of several staff headed by a senior staff member. The department must be independent of all other sections or departments in the transfusion service. The supervising staff member must be directly responsible to the director of the service and will have the authority to reject products that do not meet the specifications, and to take any necessary corrective action.

The main areas requiring attention in a quality assurance programme include:

- good manufacturing practice;
- clerical procedures;
- provision of standard operating procedures, including documentation;
- specifications for, and quality control of, reagents, techniques and equipment;
- quality control of technical performance;
- collection, storage and transportation of samples and blood donations;
- arrangements for administration of blood;
• a reporting system for errors and adverse reactions;
• educational, research and development programmes;
• the introduction of automation and computing (wherever possible).

GOOD MANUFACTURING PRACTICE

The design and construction of blood transfusion premises are important aspects of good manufacturing practice. Accidents are often caused by overcrowding and there must therefore be adequate space for work and for circulation of staff; movement through entry and exit doors must be unobstructed. Furnishings, fittings, and floor materials must be carefully selected to reduce the risk of accidents and to avoid hazards such as fire. Lighting must be adequate throughout the premises, and balanced lighting may be necessary for areas in which tests such as titrations with visual colour end-points are carried out. Attention should also be given to ventilation, with fume cupboards provided where necessary. Adequate power and water supplies, facilities for waste disposal (see Chapter 7), and strict adherence to standards of sanitation are essential. The premises should also be designed to prevent the entry of animals and insects.

Good manufacturing practice also includes a comprehensive system of organization and management (administrative and supervisory), and specifies many aspects of training, equipment, reagents, techniques, and documentation.

CLERICAL PROCEDURES

Safe blood transfusion requires that patients and blood samples be accurately identified at all stages, from collection of a patient’s blood sample to administration of a transfusion.

Clerical procedures must be established for laboratory work and strictly followed. Request forms for blood or blood products should give a patient’s full name, hospital number, date of birth, sex, and relevant serological history (e.g. previous transfusions or pregnancies), and the name of the hospital ward or outpatient department in which he or she will be treated. Unlabelled samples must never be accepted.

Clerical errors in the laboratory can be avoided if staff members who perform the tests meticulously check
identification details on the samples with those on the relevant request forms. When blood or blood products are being issued, these details must also be checked with the compatibility labels affixed to the donor units.

**STANDARD OPERATING PROCEDURES, INCLUDING DOCUMENTATION**

Every routine activity must be carried out according to clearly written instructions authorized by the departmental head. These standard operating procedures should be revised as often as necessary, and the date of each revision should be clearly recorded. Obsolete operating procedures must be removed from circulation.

Each standard operating procedure should include:

- the title, and a brief explanation of the purpose of the procedure;
- a brief description of the scientific principles involved;
- specifications for reagents and equipment;
- details of the method, and examples of work protocols;
- the reporting procedure for results, and the action to be taken if problems occur;
- specific quality-control procedures;
- specifications and training requirements for staff allowed to perform the procedure;
- relevant health and safety aspects.

Efficient documentation requires the appropriate design of forms and labels, a sound system of record-keeping, and written specifications for starting (e.g. whole blood) and intermediate (e.g. plasma) materials, finished products, and sampling programmes. Standard operating procedures will also include clear instructions on the documentation required for procedures and products.

**SPECIFICATION AND QUALITY CONTROL OF REAGENTS**

**General principles**

All reagents derived from human blood must be obtained from individuals who have been tested and found negative for
HBsAg, HCV and HIV antibody (see Chapter 5), and must be labelled with a statement confirming this. However, no known test offers complete assurance that such products will not transmit infection, and all samples must therefore be handled with care.

International reference preparations have been established for ABO and Rh(D) reagents by WHO and the United States Food and Drug Administration (FDA). These preparations are not to be used as working materials in routine work, but are provided to national authorities for standardization of national reference preparations. They may also be provided to individual workers, particularly in countries that do not have national authorities.

The anti-human globulin reference preparations established by the Joint Anti-human Globulin Working Party of the International Society for Blood Transfusion (ISBT) and the International Council for Standardization in Haematology (ICSHP) are designed to be working preparations, not minimum specification reagents. The specifications for all of these reference preparations are expressed in functional terms rather than in mass units or concentrations (e.g. μg/ml).

All blood-grouping and anti-human globulin reagents should contain a preservative to inhibit bacterial and fungal growth. If coloured dyes are included in reagents, they should follow the codes laid down by WHO and FDA: blue for anti-A, yellow for anti-B, and green for anti-human globulin. However, coloured dyes are valuable solely for confirming that appropriate reagents have been used in the tests: specificity must be identified by the label, not by the colour. Reagents should be used according to the manufacturer’s instructions, and must be restandardized if they are to be used in alternative techniques or in a diluted form (e.g. for ABO- or Rh(D)-grouping in microplates or automated systems).

Each reagent must be clearly labelled with its specificity, batch number, expiry date, and storage temperature; instructions for use should be enclosed with each container. Reagents must be of good quality and free of turbidity or other evidence of deterioration.

A new reagent must be compared with the reagent currently in use and with a reference preparation; it should not be introduced into routine work until internal serological assessments have confirmed that it is satisfactory. If a laboratory is unable to evaluate the specification fully, it
should perform parallel testing (of 30–50 blood samples, for example) to demonstrate that the new reagent is at least as good as the previous one. Saline diluents for titration studies should contain 1–30% bovine albumin, to avoid incorrect assessment due to adhesion of apparent agglutinates to the surface of the glass tube.

Routine quality control of antibody reagents is based on the use of positive and negative controls with each batch of tests, in order to show that the reagents are both potent and specific. However, the scarcity of some red-cell phenotypes often means that routine quality control tests cannot guarantee that a given reagent will detect weak variants of the antigen concerned.

When the results of serological reactions are being recorded, agglutination must be graded according to clump size (intensity), and titrations are scored.

**ABO reagents**

*Specifications*

The reliability of ABO reagents can be tested by determining the speed and clump size (intensity) of agglutination, and by titrating against selected examples of readily available red-cell phenotypes. All reagents must be shown to be free of false reactions by 5-minute slide techniques, standard 15–20-minute spin-tube and 1.5-hour tube sedimentation techniques.

*Routine quality control of ABO typing*

Each batch of tests should include A, B, and O cells as controls in order to confirm the specificity of the reagents. Unless high-quality anti-A and anti-B are used (e.g. superior examples of monoclonal reagents), test and control cells should also be tested with anti-A,B. The sera of donors or patients being grouped should be tested with A, B, and O cells (serum or “reverse” grouping). There should also be an autoagglutinin control, consisting of the serum and cells of the person being grouped.

Confident identification of an ABO group can be made only if cell and serum grouping are in complete agreement, and the auto control test is negative. Blood samples from infants less than 6 weeks old should be “cell grouped” twice, as the lack of allo-antibodies means that the group cannot be checked by reverse grouping.
For extra security, the reading of cell and serum reactions should be separated, the results of each being recorded "blind" by different individuals, to avoid the possibility of any previous result influencing the interpretation. Discrepancies must be investigated and conclusive results obtained, in order to confirm that reagents do not contain contaminating antibodies, and to eliminate the possibility of unexpected antibodies or red-cell variants in a test sample.

Quality control of diluted reagents

Considerable economy can be achieved by diluting high quality anti-A and anti-B reagents for use in blood-grouping by automated and microplate techniques. However, responsibility for the reliability and stability of the reagents is thereby transferred to the user. Reagents should be diluted only at the time of use, and should not be stored for long periods at 4°C without added preservative (1 g/litre sodium azide) or stabilizer (bovine serum albumin, 10–20 g/litre). Excessive dilution may result in poor sensitivity and failure to detect weaker antigens.

Rh(D) reagents

Specification

The specifications for anti-Rh(D) reagents are more complex than for ABO reagents because of the greater variety of types of reagent, methods of use, and necessary control procedures. The reagents are tested using the recommended technique(s)—saline, potentiated, albumin displacement, enzyme, or anti-human globulin tests for D⁺—against a cell panel including up to 10 R,r samples, and several examples of high- and low-grade weak D (D⁺) and D variant cells, if available. The presence of any contaminating anti-C or anti-E must be excluded by tests with group O r'r', r"r" (or r'r and r"r if homozygous phenotypes are not available), and several examples of group O rr, group A rr, and group B rr cells. Reagents should also be evaluated against the reagents in routine use.

Saline D-grouping methods are simpler to perform than methods requiring enhancing agents (e.g. enzymes or albumin). High-titre monoclonal IgM "saline" anti-D reagents are suitable even for rapid slide or tile and emergency spin-tube techniques. If used with low protein (<7% albumin) and no
potentiators, these methods require no diluent controls and often give better results than mercaptan-treated IgG anti-D, or polyclonal IgM anti-D reagents. However, like conventional IgM or mercaptan-expanded IgG anti-D, they are unreliable for the detection of weak D (D\textsuperscript{u}), and some D variant phenotypes.

Potentiated or high-protein anti-D reagents (e.g. those requiring 200 g/litre albumin) are provided with a diluent control, which must be used to demonstrate that the diluent itself is not causing agglutination of test cells sensitized in vivo.

**Routine Rh(D)-grouping and quality control**

Two distinct anti-D reagents—not two different batches of the same anti-D—must be used. Economies can be made by using the second potentiated anti-D to check only Rh(D)-negative donor bloods. Positive (group O R\textsubscript{1}r) and negative (O rr) controls must be included with each batch of tests.

Each test sample must give a negative “auto” test (own cells and own serum) by the technique in use. If specified by the manufacturer, the auto test should also be performed with the addition of the diluent control. Auto controls are necessary to minimize false-positive results caused by auto agglutination or by the presence of in vivo sensitized cells.

Discrepancies in results should always be followed up and analysed. They may point to a potentially dangerous reagent defect, but they are usually due to examples of weak D (D\textsuperscript{u}) phenotypes. However, there is no clinical need to detect D\textsuperscript{u} in patients: transfusion of Rh(D)-negative blood to D\textsuperscript{u} recipients is at worst slightly wasteful, and prophylactic anti-D is not harmful to women during pregnancy. Use of an indirect antiglobulin test for Rh(D)-grouping is dangerous: patients with positive direct antiglobulin tests have been wrongly recorded as Rh-positive in such tests.

Stronger grades of D\textsuperscript{u} and D variant bloods are potentially immunogenic, and should therefore be detected when grouping blood donors. Anti-D reagents for this purpose must be assessed for their ability to detect weak D types, and many laboratories still investigate D\textsuperscript{u} status manually by an antiglobulin test. Selected IgG anti-D papain reagents are probably also adequate, since the low-grade D\textsuperscript{u} that they may fail to detect is not regarded as immunogenic.
Anti-human globulin reagents

Polyspecific anti-human globulin reagents have anti-complement as well as anti-IgG activity. It is usual for the anti-complement to be mainly anti-C3c for detection of in vivo bound complement, with a smaller amount of anti-C3d for detection of in vitro bound complement. Most of these reagents contain anti-IgG prepared in rabbits, and the anti-complement may be conventional rabbit serum or monoclonal anti-C3c and anti-C3d (or just suitable IgM anti-C3d) antibodies. The very complex nature of anti-human globulin tests results in considerable variations in titre between different laboratories. It is essential that these tests are performed in parallel with at least one reference reagent.

False-positive tests and anti-C3d complement levels

Small amounts of C3d occur on normal red cells, and this gives rise to false-positive reactions when anti-human globulin reagents with excessive levels of anti-C3d are used. However, reagents cannot be assessed reliably from reactions with washed red cells alone. Incubation of red cells with fresh serum at 37°C may cause a further increase in the C3d uptake, and this is the critical test for false-positive reactions with any anti-human globulin. An anti-human globulin that gives macroscopic false-positives by this procedure should be rejected.

Some transfusion services may be unable to evaluate anti-human globulin reagents comprehensively. Nevertheless, they should always complete the following evaluations:

- Demonstrate that the reagent does not give false-positive reactions in simulated cross-match tests using fresh ABO-compatible sera and red cells from citrated donor-pack segments.
- Compare the test anti-human globulin with the current reagent, using a selection of weak antibodies.
- Perform sensitive check-board tests as follows. Make doubling dilutions of a weak IgG anti-D (0.8 IU/ml) designed for anti-human globulin control tests, from undiluted to 1:16, and prepare sensitized R1r cells with each dilution. Each of the sensitized cell preparations is washed and tested with dilutions of anti-human globulin, from undiluted to 1:8. The reagent should not show prozones by immediate spin tests, with two volumes of anti-human
globulin per test. The test reagent should be at least as potent as that in current use.

It is also important that anti-human globulin reagents detect clinically significant antibodies that bind complement but that may react only weakly in anti-IgG tests. The most important is anti-Jkα. These antibodies may be barely detectable with anti-IgG against heterozygous (e.g. Jka+b+) cells, but are readily detected with a polyspecific anti-human globulin. Enhanced reactions are often seen with complement-binding antibodies when a polyspecific reagent is compared with an anti-IgG reagent.

**Routine anti-human globulin tests and quality control**

The indirect antiglobulin test is the most important test for determining compatibility. Antibodies reacting in this procedure are generally assumed to be clinically significant unless proved otherwise. It is to this procedure, therefore, that maximum effort should be directed. Of the reasons for substandard performance, the two most frequent are:

- failure of cell-washing procedures to remove serum proteins adequately, so that the antiglobulin reagent is rendered ineffective;
- false-negative reactions, sometimes recorded if there is vigorous agitation of the suspended cells during the reading of results, causing weak or even moderately strong reactions to be destroyed.

These problems are exacerbated by the use of strongly sensitized control cells.

**Recommended procedures for the indirect antiglobulin test**

Red cells are sensitized efficiently by using four volumes of normal ionic-strength saline (NISS) to one volume of a 3% suspension of washed cells in NISS, and then incubating the mixture at 37°C for at least 45 minutes. Alternatively, if low ionic-strength saline (LISS) is used, two volumes of serum are incubated with two volumes of a 1.5% suspension of cells in 0.03 mol/litre LISS, and the mixture is incubated for at least 15 minutes. If commercial low ionic-strength additive solutions are used, the manufacturer’s instructions should be followed.
The test cells are washed at least three and preferably four times after incubation, with a minimum of 3 ml of saline per wash. As much supernatant fluid as possible is removed at the end of each washing cycle, and it is then important that the cell button be resuspended and the red cells mixed by vigorous injection of fresh saline.

Note: Saline of low pH—below 5.5—can cause antibodies to be eluted from the red cells.

After the last wash, two volumes of polyspecific anti-human globulin are added to the cell button which has been shaken from the bottom of the tube, and the tube is spun immediately after thorough mixing. Use of a coloured (green) reagent shows that anti-human globulin has been added to the tube. The tube requires gentle handling after centrifugation: the cell button is removed from the bottom of the tube by gentle tipping, or shaking and rolling. Reactions are read macroscopically, or microscopically after transfer of the cell suspension by pipette on to a microscope slide.

Quality control of each batch of tests should be by separate internal controls consisting of:

(a) an IgG anti-D, diluted to give only +/+ reactions with R_{1,1} cells, used as a positive control to show that the anti-human globulin is potent and the saline wash fluid is not contaminated with serum;
(b) an inert (group AB) serum, with the same R_{1,1} cells as used in (a), as a negative control.

Control of all apparently negative anti-human globulin tests, by the addition of sensitized cells, is widely recommended to demonstrate that the reagent has not been neutralized as a result of incomplete removal of free serum protein. However, the principles of this procedure are poorly understood. Cells that are very weakly sensitized are not suitable for this purpose, as the non-sensitized cells in negative tests weaken the reaction even further, and can give negative results even when the antiglobulin is fully active. The widespread practice of using strongly sensitized cells unfortunately gives a false sense of security. These cells will react strongly even with partially neutralized antiglobulin, even though this reduced level of reagent will be unable to detect sensitized cells that would normally give weak agglutination with fully active antiglobulin. The most useful strength of sensitized cells should give an
intermediate ( ++/+ ++ + ) reaction, and will give a reduced ( +/+ ++ ) reaction in the presence of non-sensitized cells in a negative antiglobulin test.

**Quality control of anti-human globulin tests when automated cell-washing centrifuges are used**

Automated cell-washing centrifuges have undoubtedly reduced the work involved in antiglobulin tests. However, their use requires considerable care and attention to detail: the presence of residual serum resulting from imperfect washing in some machines has been a major cause of false-negative results.

New machines should be accepted only after installation by the manufacturer's representative, and demonstration of washing efficiency in at least three loads of replicate tests with weakly sensitized cells. The centrifuges should also conform to all relevant safety standards.

These machines are designed to wash sensitized cells completely free of serum, without cell loss or contamination with protein from elsewhere, and they must never be used for preparing washed cell suspensions directly from whole blood.

**Special problems of anti-human globulin tests in microplates**

Anti-human globulin reagents standardized for use in spin-tube tests contain very high levels of anti-IgG; when weakly sensitized cells are tested, this may lead to false-negative reactions, due to excess anti-IgG. For this reason, reagents must be diluted approximately 1:4 to make an optimum reagent for microplate work. However, the diluted anti-complement component will then no longer be adequate for detection of red-cell-bound complement. Moreover, the wash volume in microplate wells is only about 0.32 ml (i.e. about one-tenth of that in tubes measuring 10 × 75 mm or 12 × 75 mm). Thus, there is a serious danger that the diluted antiglobulin may be neutralized by residual protein in microplate tests.

The specifications of antiglobulin reagents for use in microplates need to be fully defined. Quality-control programmes should be instituted to ensure that there are no prozones with weakly sensitized cells, and that potency of anti-C3c/C3d is adequate for detection of both in vivo and in vitro bound complement.
Bovine serum albumin

Bovine serum albumin is still widely used in blood group serology, usually:

- at a concentration of 200 g/litre (sometimes 300 g/litre) as an enhancer of agglutination; and
- at concentrations of 10–70 g/litre as a stabilizer in other reagents, especially those to be stored at 4°C.

Many early claims that albumin increased antibody uptake and improved sensitivity of the anti-human globulin test were actually demonstrations of the potentiating effect of the low ionic-strength saline in which the albumin was dissolved. Albumin-potentiated agglutination tests, especially rapid (15-minute) or albumin suspension techniques, are less sensitive than either enzyme tests for Rh antibodies, or anti-human globulin tests for antibodies of other specificities. Albumin tests, although widely used, are therefore unnecessary for compatibility testing and wasteful of resources.

The situation is different, however, for Rh-grouping with potent standardized anti-D reagents, and albumin displacement tests using 200 g/litre solutions are very efficient. Recent developments by commercial manufacturers have involved the blending of albumin with other additives, or the use of specially formulated albumin solutions (with a high polymer content). These are claimed to have greater sensitivity than “standard” 200 g/litre albumin.

Specification for bovine serum albumin as a 200 g/litre solution

The albumin should give a titre of 32–64 with an in-house reference IgG anti-D and a pool of four R₁r cells, and its performance should be equivalent to that of the previous batch of albumin by the technique in routine use. It should not give false-positive reactions, or cause haemolysis during incubation with inert sera and red cells.

The quality control of albumin tests depends, in the first instance, on selecting a good quality albumin reagent. Adequate performance must be demonstrated in routine Rh-grouping by including both positive and negative controls, as well as auto and diluent controls.
Normal ionic-strength saline

For use in blood group serology, saline should have a concentration of 0.154 mol/litre (9 g/litre). Saline is freshly prepared on each occasion and separate 0.1-ml aliquots are mixed with 0.1 ml of 5% red cells suspended in saline of the previous batch. The tubes are centrifuged after being left at room temperature for 10 minutes and the supernatant is examined for traces of haemolysis. If haemolysis is noted, but is absent in a control test using red cells in the current batch of saline, the batch must be rejected.

The pH of aliquots is tested and should be between 5.5 and 8.0. Saline with a pH below 5.5 may cause elution of certain antibodies from red cells during the washing phase of anti-human globulin tests.

Phosphate-buffered saline

Phosphate-buffered saline, with a pH of 7.1 (0.045 mol/litre), is better than normal ionic-strength saline for suspension of red cells for use over periods of 12–24 hours and for storage at 4°C; it also decreases the haemolysis observed with recovered, frozen red cells.

Low ionic-strength saline

Low ionic-strength saline (LISS) is widely used in blood group serology. For many antibody specificities it increases the rate of antibody uptake 2–4-fold, compared with normal ionic-strength saline.

Quality control

Conductance should be 3.6–3.7 siemens and pH should be 6.7. Using LISS solution, a weak IgG anti-D (0.25 IU/ml), such as is used for an anti-human globulin control in a 5-minute indirect antiglobulin test, should give a + / ++ reaction with R_1r red cells by the routine LISS anti-human globulin test. This examination should be carried out in parallel with tests using the previous batch of LISS.
Routine procedure with LISS—practical points

For routine work, LISS should be used at ambient temperature: cold LISS increases unwanted cold-antibody reactions. Red cells should be washed twice in normal saline to free them of serum before LISS is added to them. Traces of residual serum in 0.03 mol/litre LISS will result in non-specific uptake of autologous serum complement on to the red cells.

Equal volumes of serum and LISS-suspended 1.5% red cells (2 volumes each) are mixed thoroughly before incubation.

Red cells for antibody detection and identification

Commercial red cell panels are obtained from accredited donors and the cells are suspended in various types of solution, with additives to minimize bacterial growth and maximize shelf life (usually 21–28 days). The antigen grouping of red cell panels prepared in-house should be carried out twice, using different samples of blood from each donor to ensure accuracy. These panels are best obtained from regular donors, or from members of staff, to ensure maximum benefit from the detailed grouping which is expensive and time-consuming. Specifications are indicated in Chapter 5, page 64, and Chapter 6, page 87.

Frozen red cells are best stored below —65°C, although they can be stored at —20°C for up to 6 months. Recovered, frozen red cells should be suspended in phosphate-buffered saline (pH 7.1) and are usable for up to 12 hours when stored at 4°C. They lyse slowly, however, and may be virtually useless after 24 hours.

Discrepant results with control red cells and known antibody reagents are often the first indication of inaccuracies in cell grouping. It is important, therefore, to repeat the grouping, and resolve any discrepancies, before the cells are used for antibody identification.

QUALITY CONTROL OF TECHNIQUES

Techniques that have proved to be reliable, and the reagents used, are monitored by the use of positive and negative control procedures. However, routine quality control of tests in saline at 37°C, anti-human globulin tests, and albumin and enzyme techniques used for detection of irregular blood group
antibodies is more difficult. It is impossible to ensure that each test is capable of detecting weak antibodies of all specificities. Fortunately, it is possible to ensure that only high-quality reagents are used, and that they work sufficiently well to detect chosen examples of weak antibodies. For example, a weak IgG anti-D (0.25 IU/ml) used as a positive control with each batch of antiglobulin tests is far superior to the widespread practice of adding strongly sensitized red cells to all negative antiglobulin tests (see page 120).

Most of the antibodies to red cell antigens that cause clinically significant incompatibilities can be detected by currently available techniques. The objective of quality assurance is to ensure a consistently high standard of performance for those techniques that detect antibodies of potential pathological significance, and not to waste resources on procedures of limited clinical relevance.

QUALITY CONTROL OF EQUIPMENT

Selection and evaluation

Equipment specifications should meet, or exceed, mandatory technical, electrical engineering, and health and safety standards. Guidance is sometimes available from officially sponsored evaluations.

Installation procedures should be formalized. Installation begins with the assistance of commercial installation staff, frequently in conjunction with hospital engineering departments, to ensure compliance with electrical safety standards.

Once the equipment has been installed and calibrated according to the supplier’s specifications, it is commissioned by confirming that its performance meets required standards. It may then be introduced into routine work. At this stage, careful supervision of the results of quality control procedures should disclose any shortcomings in performance.

Expensive laboratory equipment may be sold together with equally expensive maintenance contracts, which may amount annually to 8–10% of the original purchase price. It is a wise precaution to investigate whether local suppliers can provide the spare parts and expertise necessary to maintain equipment.
Routine quality control of equipment

The performance of laboratory equipment must be monitored at predetermined intervals; the results must be recorded and adjustments made if necessary. A programme of preventive maintenance, including cleaning and recalibration, and replacement as necessary, is mandatory. This programme should be planned, in conjunction with hospital maintenance engineers or outside contract specialists, in order to minimize disruption of services. Full logs should be kept for all equipment and should include documentation of this programme, and of repair work and recommissioning.

QUALITY CONTROL OF PERFORMANCE

External quality assessment schemes (proficiency trials)

External quality assessment schemes involve simulated clinical samples being distributed to laboratories internationally, nationally; or regionally, in order to examine the range of competence for a given laboratory activity. It is usually intended that the clinical samples—of composition unknown to the participating laboratories—are subjected to normal analytical procedures. Regrettably, it is generally impracticable to disguise these samples among the routine clinical specimens; consequently, their handling necessarily follows a somewhat artificial route, which may at times give a misleading indication of the true level of performance. There may be a temptation to pass these samples to senior staff for evaluation, but this may frustrate the intention of identifying areas where improvement is needed: it is important for materials used in proficiency evaluation to be examined by a cross-section of staff, so as to reflect the actual level of laboratory performance. A further disadvantage lies in analysis of the results being retrospective, and therefore providing no assessment of performance at the time of the assay.

The procedures most frequently examined have been ABO- and Rh(D)-grouping, antibody screening, and simulated cross-match testing. They have sometimes indicated problems that needed investigation, and for which corrective action had to be taken. In some cases, they have also helped to identify intrinsically faulty techniques. These procedures thus play a
useful part in quality assurance, provided that processing of the samples is similar to that of routine samples.

**Blind replicate tests**

Some of the disadvantages inherent in external quality assessment schemes can be overcome by assessing each worker regularly with “blind” replicate tests, identifying those workers with technical problems, and giving immediate tuition if necessary. Follow-up blind replicate tests should demonstrate improvement and restore the confidence of the worker.

**QUALITY CONTROL OF TESTS FOR TRANSMISSIBLE INFECTIOUS AGENTS**

The risk of transmission of disease by transfusion is reduced as far as possible by screening all blood donations for markers for the main transmissible agents. Internal quality control is provided by including positive and negative controls for the markers with each batch of tests. Other applicable aspects of quality assurance outlined elsewhere (for instance in standard operating procedure manuals, specifications of equipment, reagents, and other materials) are also important, and are supplemented by participation in external quality assessment schemes.

**QUALITY CONTROL OF BLOOD COMPONENT PREPARATION**

It is essential that each product complies with the relevant product specification, which should in turn conform to appropriate legal requirements or other internationally recognized standards. All product specifications depend in the first instance on the quality of the starting material and, therefore, on the specifications for selecting the donor, the type(s) of container and anticoagulant used, the volume of blood withdrawn at any one time, the duration of venesection, and the minimum interval between donations (see Chapter 3). These specifications will also indicate the minimum requirement for serological testing and screening for infectious diseases (see also Chapters 3, 4 and 6), storage and transport conditions, and the procedures involved in preparation of the component and its administration to the patient (Chapter 9). The quality
control department will be responsible for ensuring that all of
the conditions are met, at all stages, by sampling, testing, and
documentation, with validation of the end-product.

Blood components must be sterile, and are usually prepared
in closed systems, but the testing of a product beyond simple
determination of weight is destructive because the system must
be opened for sampling. It is usual, therefore, to monitor the
quality of production by testing a proportion of each product
during processing and at the end of production. Threshold
levels, at which action becomes essential, should be established,
and the action to be taken should be defined and documented.

BIOSAFETY IN THE TRANSFUSION LABORATORY

The quality of techniques used in transfusion laboratories must
be monitored to ensure not only technical accuracy but also the
safety of laboratory staff. Workers in blood transfusion
laboratories are constantly at risk of infection from the blood
that they handle every day, and it is therefore essential that
safe working practices are instituted and maintained. All
samples and donations must always be handled carefully to
minimize exposure of skin and mucous membranes.

All laboratory work involving potential contact with blood
should take place where there is minimum interruption from
outside staff, blood donors or patients; microbiological tests
(e.g. those for HBsAg and antibody to HIV) must be
performed in a separate room. When such tests are being
undertaken, or when blood components are being prepared,
staff should wear thin rubber gloves for hand protection, and
must wash their hands thoroughly before leaving the
laboratory. All work surfaces and apparatus should be
thoroughly cleaned after every work session using a suitable
disinfectant. Although the positive control sera provided
in manufacturers’ kits have been treated (usually with
β-propiolactone) to render them non-infective, the efficacy of
this treatment can never be guaranteed, and control sera should
always be handled as if infective.

For disinfection of viral contamination, phenolic or other
antibacterial disinfectants are unsuitable, and a solution of
sodium hypochlorite to give 1000 parts per million (0.1%
available chlorine) of free chlorine is the disinfectant of choice
for general use, that is, cleaning work benches, specimen
containers and gloves. If this chemical is not readily available,
a 1% dilution of ordinary domestic bleach is a suitable alternative. For disinfecting blood spillages and heavily soiled equipment, a solution of 10 times this strength should be used (see Chapter 7, page 106). However, hypochlorites are corrosive, and a 2–3% solution of glutaraldehyde should be used for disinfection of metal objects or equipment such as centrifuges. If glutaraldehyde is not available, a formaldehyde solution of similar strength may be used, although it is an irritant and has an unpleasant smell. Neither glutaraldehyde nor formaldehyde readily penetrates organic materials, and these chemicals are therefore unsuitable for disinfecting spillages.

Decontamination methods for needles and for disposable items (e.g. microtitre pipette tips, tissues, and plastic tubes used for microbiological testing or for grouping) are described in Chapter 7, page 107.

The essential safety rules are simple. Laboratories and blood donor areas must be kept scrupulously clean and free of blood stains. Protective coats must be worn in the laboratory, but must be removed before leaving the laboratory. General safety precautions should include the following:

- There must be no eating, drinking, smoking, or application of cosmetics in the laboratory. Mouth pipetting is forbidden.
- Any break in the skin—cut, puncture wound or skin eruption—must be kept covered, either with a waterproof dressing or, if on the hands, a rubber glove (or both).
- Since the greatest risk of infection is through puncture wounds sustained in the laboratory, all such wounds, however trivial, must immediately be made to bleed freely under a running tap to wash out any infected material. They must be reported to the supervisor of the laboratory immediately, so that any appropriate prophylactic treatment can be given.
- The splashing of blood, serum, or plasma in the face is equally serious, and an eye-bath should always be available to wash out any material that splashes into the eye. If the nose, lips, and mouth are involved, they must also be washed out with copious amounts of water. The incident must then be reported to the supervisor of the laboratory immediately, so that any appropriate prophylactic treatment can be given.
- Whenever anyone is exposed to blood through a cut or from splashes in the face, the source of the blood should be
identified. Results of HBsAg and anti-HIV screening tests will indicate the risk. For example, if the blood concerned is HBsAg-positive, this is an absolute indication for administration of prophylactic anti-HBV immunoglobulin, unless the person at risk is known to be immune.

- If it is suspected that a tube has broken or a bag has leaked during centrifugation, the centrifuge motor must be switched off and the lid must not be opened for 30 minutes. If the leakage or breakage is discovered only on opening the centrifuge, the lid must be closed again immediately and kept closed for at least 30 minutes. Glass debris should be picked up with forceps, and broken tubes, glass fragments, buckets and the rotor should be placed in gluteraldehyde solution (see above) overnight. General-purpose, utility gloves must be worn throughout this procedure. The centrifuge should be wiped out with the disinfectant and allowed to dry, and the rotor and buckets should also be swabbed with disinfectant, washed with water, and dried before being re-installed.

- Special care must be taken in wash-up areas, because the risk of contact with blood-stained, broken glass is especially high. Appropriate training of staff who work in these areas to avoid infection is mandatory. Disposable equipment must not be reused.

STORAGE AND TRANSPORTATION OF BLOOD AND BLOOD COMPONENTS

Whenever blood or a blood component is issued, it must be inspected carefully for evidence of haemolysis in the plasma or discoloration of red cells. Blood and components should be transported at a temperature as near as possible to that recommended for the particular product, and, on receipt, should be transferred immediately to storage under the recommended conditions. Products containing red cells must be kept between +2°C and +10°C, and platelet products between +22°C and +24°C. Frozen products should be transported in the frozen state and transfused immediately after thawing; if there is any delay in transfusion, they must be kept below +4°C.

Blood and components must be delivered as soon as possible. This applies equally to delivery of freshly donated blood to the transfusion centre, since delays at this stage may result in loss of activity of labile components such as coagulation factor VIII.
Blood should be cooled to 4°C as soon as possible after collection, and transported at this temperature, unless it is intended for platelet production, when it should not be cooled below 20°C until after separation of these cells.

The temperature of each container should be checked on receipt, to ensure that the contents have not departed from the temperature limits outlined above. Frozen components should be checked to ensure that they are still in the frozen state.

**ADMINISTRATION OF BLOOD AT THE BEDSIDE**
(see also Chapter 9)

Blood or blood products leaving the laboratory must be accompanied by full documentation and be handled only by responsible personnel, from the point of issue to receipt in the ward or theatre. Before transfusing the blood, the doctor or nurse must ensure that the ABO group and Rh(D) group match those of the patient, and that the personal identification details and case-record number on the compatibility label agree with those of the patient.

For maximum safety, the patient (if conscious) should be asked to confirm his or her name, address, and date of birth. In the case of unconscious patients, the wrist band or case-notes provide the most reliable identification details.

**REPORTING SYSTEM FOR ERRORS**

Any errors that occur must be documented and reported in full for discussion and action by a hospital transfusion committee (see Chapter 9). In this way it is possible to learn from mistakes and prevent their recurrence.

**EDUCATIONAL PROGRAMMES**

Adequate staff training is important to ensure good working practices; the initial requirement is for formal educational qualifications. Compatibility testing should be performed only by accredited staff. Basic training should be supplemented by formal “in-house” training in each laboratory procedure.

Patient safety depends greatly on accuracy in the areas of serology and microbiology, but also on more general working practices in the transfusion laboratory, which must be designed to eliminate clerical errors and inadvertent confusion of
samples. The person with ultimate responsibility for the transfusion laboratory should liaise with clinical staff to ensure compliance with agreed procedures for specimen collection and blood administration. Within the laboratory, there should be similar discipline regarding adherence to operational instructions; supervision should be entrusted only to senior staff members with at least five years’ experience in blood transfusion work.

Research and development work is an essential part of educational programmes, and helps to stimulate interest in improvement of techniques and procedures (e.g. evaluation of new reagents and techniques, and the introduction of computerization).

AUTOMATION AND COMPUTERIZATION

Automation and computerization can help substantially in minimizing errors in the laboratory. Although they are expensive, automated blood-grouping machines offer reliable sample identification, objective reading of reaction patterns, and interpretation of reactions by consistent and logical criteria. Since the results they yield are uninfluenced by operator bias, their use will prevent at least some of the errors to which laboratories are prone. Blood groups can readily be compared with those previously recorded for the same donor or patient. Reviewing discrepant results from samples allegedly from the same patient can provide valuable information regarding the reliability of identification procedures.

Use of computers in hospital blood banks should substantially improve the standard of laboratory operation and, by enforcing a degree of discipline in the laboratory environment, can form part of the quality assurance programme. Another valuable application is monitoring of expiry dates of reagents, and of temperatures and reliability of malfunction warnings in incubators and refrigerators.

CONCLUSIONS

Good blood transfusion practice requires a series of quality control procedures, each designed to identify deficiencies in particular processes. The quality assurance programme is more than a passive monitoring activity: to be successful, it requires positive, purposeful activity.
Chapter 9
Clinical uses of blood and blood products

INTRODUCTION

Blood and blood products should be prescribed only if less hazardous therapy has proved or is likely to be ineffective and if the benefits of transfusion outweigh the risks. Thus, although this chapter focuses on the indications for using whole blood and blood products, it starts with a brief discussion on the use of plasma-volume expanders in patients suffering acute blood loss. It also outlines the complications of administering blood and blood products, and the principles of their prevention.

PLASMA-VOLUME EXPANDERS

Restoration of blood volume is more important than replacement of red cells in the management of previously healthy patients who have suffered an acute loss of less than 25% of their blood volume. Crystalloids (e.g. saline and Ringer's lactate), synthetic colloids (e.g. gelatin, dextran, and hydroxyethyl starch), and albumin can be used effectively for restoring the blood volume.

Because of their small molecular size, crystalloids diffuse rapidly into the interstitial spaces. Their effect is therefore short-lived and the volume administered must be two to three times the volume of blood lost. For this reason, the administration of colloids should be considered if the estimated blood loss exceeds 15% of the total blood volume.
Colloids are of larger molecular size than crystalloids, remain in the circulation longer, and exert appreciable oncotic pressure. They are therefore more effective than crystalloids for restoring and maintaining plasma volume and, though more expensive, should be used when the hypovolaemia is moderately severe (see above).

The most widely used synthetic colloids are the gelatins (e.g. succinylated gelatin, urea-linked gelatin), dextran 70, and hydroxyethyl starch. The gelatins and dextran are less expensive than hydroxyethyl starch, and gelatins have fewer side-effects than dextran. For example, gelatins do not interfere with haemostatic mechanisms, and present less risk of circulatory overload than other synthetic colloids. Anaphylactic reactions have been reported in association with all synthetic colloids, but these are rare. Difficulties in blood-grouping or cross-matching caused by red cell aggregation are avoided by taking the blood samples before colloids are transfused.

Albumin is an effective plasma substitute, and is pasteurized during its preparation, so that the risk of transmitting HIV, hepatitis B virus, non-A non-B hepatitis and other blood-borne infectious agents is eliminated. Unfortunately, its high cost inhibits its use in many countries.

Crystalloids and colloids have been used effectively in the management of patients with acute blood loss. In many cases, blood transfusion can be avoided, thus reducing the risks of blood transfusion. Moreover, it is often difficult to obtain blood, particularly in emergencies, and the procurement and processing of blood is complex and expensive.

Further information on plasma substitutes is provided in the WHO unpublished document *Use of plasma substitutes and plasma in developing countries* included in the list of selected reading at the end of this book.

**INDICATIONS FOR THE USE OF BLOOD AND BLOOD PRODUCTS**

Ideally, a patient should receive only the blood component(s) actually required for therapy. Unfortunately, many countries cannot achieve this ideal because of lack of resources, and in these circumstances whole blood is issued for all—or nearly all—patients.
A blood bank that has a refrigerated centrifuge will be able to prepare red cells, plasma, and platelets. Even if a refrigerated centrifuge is not available, it is possible to separate red cells from plasma by sedimentation in the refrigerator in which the blood is stored. Preparation of cryoprecipitate is also possible, if the blood bank has an ultra-low-temperature freezer, or if solid carbon dioxide ("dry ice") is available.

Plastic bags offer several advantages over glass bottles for preparation of blood components. Closed multi-bag systems (double, triple or quadruple packs), although more expensive than single packs, are preferable because they reduce the risk of bacterial contamination during preparation of components. Obviously, each transfusion service will have to make these decisions on the basis of careful analysis of its needs and the resources available. Equipment and materials required for preparing blood components are outlined in Chapter 7 and quality assurance is discussed in Chapter 8.

Only a few countries have the plasma-fractionation facilities required for the preparation of albumin and plasma protein fraction, coagulation factor concentrates such as factor VIII and factor IX complex, immunoglobulins, and other products such as antithrombin III and enzymes. Consequently, many countries import these products, but some obtain them by sending plasma to another country for processing (see Chapter 1).

Indications for the use of whole blood, red cells, plasma, platelets, cryoprecipitate, factor VIII concentrate, factor IX complex, albumin and immunoglobulins are outlined in this section.

**Whole blood**

As discussed earlier, restoration of blood volume is usually more important than red cell replacement for patients suffering acute blood loss. Crystalloids and/or colloids can be used in these circumstances, thus reducing the need for transfusion of whole blood. However, red cells will also be required if anoxia develops or is likely to develop, and transfusion of whole blood then becomes appropriate. The risk of anoxia increases considerably when blood loss exceeds 25% of the total blood volume, particularly when the haemorrhage has not been controlled. Obstetric complications and severe trauma are the commonest causes of acute haemorrhage in many countries.
Red cell concentrates (packed red cells; "packed cells")

Red cells are obtained from whole blood by centrifugation or sedimentation. If a closed multi-bag system is used for preparation, the shelf-life of the red cells will be determined by the anticoagulant used for collecting the blood (see page 38). However, if a single-pack unit is used for the blood collection, its sterility will be breached during preparation of the red cells, which must therefore be transfused within 24 hours.

Some multi-bag systems contain additive solutions that provide effective nutrition for the red cells, prolonging their shelf-life to 7 weeks. The most widely available of these additives contain saline, adenine, dextrose, and mannitol. They are added within 24 hours of collection to red cell concentrates obtained by centrifuging blood collected into a citrate anticoagulant, such as citrate-phosphate-dextrose. Such red cell suspensions are easier to administer than cell concentrates. Moreover, the use of additive solutions increases the availability of plasma for fractionation. However, the cost of the systems containing additive solutions has inhibited their use in some countries.

Where no refrigerated centrifuge is available, red cells may be prepared by allowing them to sediment in the refrigerator, and then removing the plasma. If this is not possible, and only red cells are indicated for the patient, the cells are allowed to sediment during the transfusion, which is stopped when the red cell–plasma interface reaches the outlet.

Red cells are indicated for correction of hypoxia in patients with chronic anaemia (e.g. chronic haemolytic anaemia, aplastic anaemia, and anaemia secondary to chronic infections, malignancy, or chronic inflammatory disorders). It is important, however, to treat the patient and not the haemoglobin concentration: most patients adapt well to chronic anaemia and need transfusion only when the hypoxia becomes severe enough to cause symptoms. Thus, patients with chronic haemolytic anaemia, such as sickle cell anaemia, would generally not require transfusion in the steady state, but only in aplastic crises or splenic sequestration crises when the haemoglobin level falls abruptly. Furthermore, red cell transfusions should generally not be considered when treatment of the underlying cause will correct or significantly attenuate the anaemia. For example, transfusions are usually not
indicated in patients with nutritional anaemias, because these conditions will respond to the appropriate haematinic.

The blood volume of patients with chronic anaemia is normal or increased. Whole blood is contraindicated in such patients because the plasma, which is not required, increases the volume of fluid transfused and consequently the risk of circulatory overload. Red cells must be transfused slowly (e.g. 1 ml/kg per hour) to reduce this risk, and in some cases it may also be necessary to administer diuretics.

Red cells suspended in one-third of their volume of plasma ("plasma-reduced red cells") and less than 5 days old are used for neonatal exchange transfusion. This decreases the risk of hyperkalaemia and of the increased oxygen affinity associated with the decreased concentration of 2,3-diphosphoglycerate in "older" blood. Red cells for patients with advanced renal or hepatic disease should have been stored for less than 5 days, and should preferably be suspended in additive solutions where these are available.

Leukocyte-poor red blood cells, containing at least 70% of the original red cells but less than 70% of the original white cells, are indicated for patients who have severe or repeated febrile reactions to leukocyte antibodies. These cells may be prepared by a variety of centrifugation methods, by the use of microaggregate filters, or by freezing and deglycerolizing red cells. The centrifugation methods include inverted centrifugation, double centrifugation, and centrifugation of saline-diluted red cells, and are feasible in any blood bank equipped with a refrigerated centrifuge. More efficient removal of leukocytes is accomplished through washing of the cells, but this process is more time-consuming.

The in vitro effectiveness of whole blood or red cells is assessed by measuring the change in the haemoglobin concentration or packed-cell volume after transfusion. For each unit of whole blood or red cells infused, the haemoglobin level should rise by 1 g/dl.

**Platelets**

Platelets are best administered as platelet concentrate, because of the large volume of platelet-rich plasma that would be necessary in order to achieve the same effect. They are prepared by centrifuging whole blood using a light spin,
decanting the supernatant platelet-rich plasma and centrifuging this using a heavy spin. Most of the supernatant platelet-poor plasma is expressed, leaving 50–70 ml for resuspension of the platelet button. Resuspension is delayed for 2 hours to reduce the risk of platelet aggregation.

The blood from which platelet concentrates are to be prepared is maintained at 22 ± 2 °C. Separation of platelet-rich plasma from the red cells must be performed within 6 hours of collecting the blood, and both centrifugations must be carried out at 22 ± 2 °C (not at 4 °C).

Platelets may be stored for up to 72 hours at 22 °C, or for up to 7 days if the plastics approved for this purpose are used, and must be gently agitated during this time. They maintain their mobility and function better at 22 °C than at 4 °C. ABO-matching of platelets is not required, but most of the donor plasma must be removed if the platelet concentrate contains ABO antibodies incompatible with the recipient’s red cells. Rh(D)-negative recipients, particularly female patients who have not yet reached the menopause, should receive platelets obtained from Rh(D)-negative donors. This rule may have to be disregarded in a life-threatening emergency, in which case Rh(D)-immunoglobulin must be given to prevent immunization.

Platelets may be indicated for the management of patients who have thrombocytopenia or qualitative platelet disorders. In managing thrombocytopenia, it is important to treat the patient and not the platelet count. Platelet therapy is clearly indicated in severe thrombocytopenia when life-threatening haemorrhage occurs or is likely to occur, particularly when the condition is due to poor production of thrombocytes (e.g. in acute leukaemia or following cytotoxic chemotherapy). In these circumstances the patient’s platelet count is usually less than 20 × 10⁹/litre, but there is some variability in the risk of haemorrhage, presumably related to platelet function. The requirement for platelets is usually increased in patients who are also febrile and in those who are septicaemic. Platelet transfusions are of limited and transient value, and therefore generally contraindicated, in patients in whom the thrombocytopenia is due to increased destruction (e.g. in immune thrombocytopenia and disseminated intravascular coagulation).

The in vivo effectiveness of platelet concentrates can be assessed by observing the effect on bleeding and/or measuring
the change in the platelet count after transfusion. An increment of between $5 \times 10^9$ and $10 \times 10^9$ per litre might be expected in an adult for each blood unit from which the platelet concentrate was obtained. In practice, the increment is usually smaller, particularly in patients who are febrile, who are suffering from infections, splenomegaly, or disseminated intravascular coagulation, or who are actively bleeding.

Patients become refractory to repeated platelet infusions because of the formation of allo-antibodies that impair platelet survival. It is important to bear this in mind when platelet transfusions are being considered, for example, in patients with aplastic anaemia.

**Plasma and plasma fractions**

Platelet-poor plasma is obtained from whole blood, or during the preparation of platelet concentrate from platelet-rich plasma, by centrifugation using a heavy spin. Platelet-poor plasma has been used as a plasma-volume expander in patients with acute haemorrhage and in patients with burns. Because of the dangers involved in using blood products, treatment with albumin or synthetic colloids is preferable.

Fresh frozen plasma or cryosupernatant (see below) may be required when specific coagulation factor concentrates are not available or are inappropriate (e.g. in the management of patients with multiple clotting-factor deficiencies). However, cryosupernatant cannot be used for replacement of labile clotting factor, such as factor V or factor VIII.

**Cryoprecipitate**

Cryoprecipitate is prepared by rapidly freezing plasma, within 6 hours of collecting the blood, in a $-60^\circ$C mechanical refrigerator or an ethanol–dry ice bath. It is then thawed slowly at 2–4°C and the precipitate is collected by centrifugation. Most of the supernatant plasma (cryosupernatant) is then removed, leaving about 10–15 ml in which the cryoprecipitate is dissolved.

Cryoprecipitate is rich in factor VIII and fibrinogen, and can be used for replacement therapy in patients with haemophilia A (including those with major bleeding or requiring major surgery), with von Willebrand's disease, and with disseminated intravascular coagulation.
The high-molecular-weight components of factor VIII present in cryoprecipitate are essential for effective replacement therapy in von Willebrand's disease: some preparations of factor VIII concentrate do not contain these components. Cryoprecipitate is a valuable resource for a developing country, since it is easily and relatively cheaply produced if the necessary equipment is available, and has a shelf-life of up to 1 year when stored at or below −30°C.

Factor VIII concentrates

Factor VIII concentrates are prepared by fractionation of plasma. Some of the advantages and disadvantages, compared with cryoprecipitate, are outlined in Table 3.

Prothrombin complex

Prothrombin complex concentrates are rich in factors II, VII, IX, and X, and may therefore be useful during the management of patients deficient in one or more of these factors. Factor IX concentrates are also available.

Table 3. Advantages and disadvantages of cryoprecipitate and factor VIII concentrate

<table>
<thead>
<tr>
<th>Product</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cryoprecipitate</td>
<td>Simple to prepare</td>
<td>Unpredictable dosage</td>
</tr>
<tr>
<td></td>
<td>Relatively low cost</td>
<td>Inconsistent yield</td>
</tr>
<tr>
<td></td>
<td>Can be prepared in most countries</td>
<td>Inconvenient for home use or travel</td>
</tr>
<tr>
<td></td>
<td>Almost unlimited supply is possible</td>
<td>Allergic reactions are common</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Viral inactivation procedures are not widely available</td>
</tr>
<tr>
<td>Factor VIII concentrate</td>
<td>Convenient for use, including at home and during travel</td>
<td>High cost</td>
</tr>
<tr>
<td></td>
<td>Dosage can be accurately determined</td>
<td>Limited supply</td>
</tr>
<tr>
<td></td>
<td>Can be made almost totally safe by:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>– viral inactivation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>– high purification</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Side-effects are rare</td>
<td></td>
</tr>
</tbody>
</table>

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Albumin and plasma protein fraction

Albumin and plasma protein fraction are most widely available at concentrations from 4 g/dl to 25 g/dl. The chief indications for their use are in the management of patients with hypovolaemia caused by burns or haemorrhage. Their cost severely limits their use in many countries, and synthetic colloids are a reasonable alternative for the management of haemorrhage. Cryosupernatant plasma may also be used but, unlike albumin, carries the risk of transmission of infectious agents such as HIV, hepatitis B virus, and non-A non-B hepatitis viruses.

Albumin solutions must be administered with care. The more concentrated solution, in particular, should be given to dehydrated patients only if other fluids are administered at the same time.

Immunoglobulin preparations

Immunoglobulin preparations may be derived from plasma pools obtained from normal adults. These are composed of IgG and are indicated as replacement therapy in patients with primary hypogammaglobulinaemia. The availability during the last decade of immunoglobulin preparations that can be given intravenously without causing anaphylactic reactions has made management of these patients much easier. Pooled preparations have also been used for conferring passive immunity against measles and viral hepatitis A. Immunoglobulin preparations containing IgA are contraindicated in IgA-deficient patients who have antibodies against IgA.

More recently, it has been shown that intravenously administered immunoglobulins are effective in the management of patients with autoimmune thrombocytopenia and autoimmune haemolytic anaemia. However, the indications for this treatment relative to those for established therapeutic regimens (e.g. corticosteroids) have not been clearly defined.

Recent reports of hepatitis following intravenous administration of immunoglobulins prompted a reassessment of their safety. It is now generally accepted that viral hepatitis B, non-A non-B hepatitis, and HIV are not transmitted by these preparations when procedures recommended by WHO for their production are followed.

Immunoglobulin preparations are also prepared from plasma obtained by plasmapheresis of recently immunized volunteers or convalescent patients. These include Rh-immunoglobulin,
and preparations used for passive immunization against tetanus, viral hepatitis B, rabies, and zoster.

Rh-immunoglobulin is used to prevent sensitization of Rh(D)-negative individuals exposed to Rh(D)-positive blood. It is therefore indicated when a Rh(D)-negative woman has a Rh(D)-positive baby, or following abortions or ectopic pregnancies in Rh(D)-negative women. It also may be indicated in Rh(D)-negative individuals who receive Rh(D)-positive blood.

Hepatitis B immunoglobulin is recommended for individuals who have been exposed to hepatitis B virus through needle-sticks with HBsAg-positive materials (e.g. plasma or blood), or through oral, ophthalmic, or sexual exposure to hepatitis B virus, and for neonates born to HBsAg-positive mothers. Hepatitis B vaccine is also indicated in some of these situations.

Passive prophylaxis against tetanus is recommended for non-immunized individuals, or for those whose immune status is unknown, following injury or animal bites. The administration of tetanus toxoid is also indicated. In addition, tetanus immunoglobulin is used in established cases of tetanus, together with antibiotics and appropriate management of wounds.

Rabies immunoprophylaxis is indicated following animal bites when the risk of rabies cannot be excluded. It is also recommended following exposure to proven or suspected rabid animals, even if the person exposed has not been bitten.

There is primary indication for varicella-zoster immunoglobulin following exposure of susceptible immunocompromised individuals to human (alpha) herpesvirus 3 (varicella zoster). Typical patients include babies whose mothers developed varicella up to 5 days after delivery, immunosuppressed children or adults, and pregnant women.

**ADMINISTRATION OF BLOOD PRODUCTS**

Doctors, nurses and technicians have a special responsibility to ensure that samples of blood sent for grouping and cross-matching are fully and correctly labelled, and that recipients are properly identified before transfusions are started. In many cases, red-cell transfusion reactions are caused by simple clerical errors.

Blood and blood components must be carefully examined before being transfused, in order to detect abnormalities, haemolysis, infection, or evidence that the bag has been
breached, which would make them unsuitable for transfusion. In most instances, whole blood or red cell concentrates may be administered direct from the refrigerator. When large volumes are given rapidly, warming becomes necessary, but only of the blood flowing through the administration set: the entire unit should not be warmed.

Blood should be drawn from the bank only when required, and should be stored only in refrigerators in which the temperature is carefully controlled (see Chapter 7, pages 89–91).

It is dangerous to withdraw blood from the bank and later return it, unused, for further storage: it may not have been stored satisfactorily, in the interim.

Blood and components must be transfused through standard blood filters (pore size 170–260 μm) designed to retain blood clots and other debris. In general, blood should not be transfused through infusion sets used for other fluids. Some of these fluids (e.g. 5% dextrose in water) may cause haemolysis and others, such as Ringer's lactate, may cause clotting because they contain calcium ions. Sodium chloride (9.0 g/litre; 0.15 mol/litre) may, however, be used for flushing infusion sets containing blood.

Whole blood or red cell concentrates should be administered slowly, at least during the first 30 minutes, and patients should be closely monitored for the earliest signs of transfusion reactions. Measurements of pulse, temperature, blood pressure, and respiratory rate should be recorded before the start of the transfusion. These measurements should be repeated 10 minutes and 30 minutes after the start and every 2 hours thereafter throughout the transfusion. The volume and rate of transfusion are then adjusted according to the patient's blood volume, to prevent circulatory overload. Some blood components (e.g. cryoprecipitate, factor VIII concentrate, platelets), however, must be given rapidly.

The starting time of administration and volume of each blood component administered are recorded. Pulse, temperature, blood pressure, and respiratory rate are recorded every 2 hours throughout the transfusion. The patient is observed carefully, for at least 1 hour after the transfusion, for possible ill-effects, and appropriate investigations (e.g. haemoglobin level, platelet count) are carried out to assess the effectiveness of the component transfused. It is also good practice to try to ascertain whether there are any long-term undesirable effects, such as transmission of infectious agents.
If a haemolytic transfusion reaction is suspected, the transfusion must be stopped immediately. A fluid-balance chart is started, and the colour of all urine samples is noted. The samples to be collected, and the investigations to be carried out are outlined in Chapter 8 (pages 83–84). The laboratory must always be notified whenever a transfusion reaction of any kind has occurred, and all such reactions must be fully investigated to determine the causes.

HAZARDS OF BLOOD TRANSFUSION

The hazards of blood transfusion may be divided into two main groups: immunological and non-immunological.

Immunological complications

Immunological complications of blood transfusion may be caused by red-cell, leukocyte, or platelet incompatibility, or by allergic reactions to plasma components.

The manifestations of red-cell incompatibility include acute intravascular haemolysis, which is usually associated with incompatibility in the ABO system, and extravascular haemolysis, which is usually due to Rh-incompatibility. In some cases, extravascular haemolysis develops after a delay of up to 2 weeks after transfusion.

Haemolytic transfusion reactions are frequently the result of clerical errors in the ward or in the laboratory. Methods for reducing the risk of clerical errors in the ward are outlined above (pages 142–143). Clerical errors in the laboratory can be reduced by meticulous attention to labelling of samples, and to accurate recording of data as a part of overall quality assurance in the blood bank.

Haemolytic transfusion reactions may also result from careless technical errors, or from the use of inappropriate techniques in the blood bank. An example of the latter is cross-matching by simply mixing donor’s cells and recipient’s serum at room temperature on microscope slides. Recommended methods for blood-grouping and cross-matching are outlined in Chapter 6.

A third cause of haemolytic transfusion reactions is the transfusion of group O whole blood, or plasma, to group A, group B, or group AB recipients. This usually results from the
indiscriminate use of group O whole blood that has not been screened to ascertain anti-A or anti-B titres, and may be avoided by using as “universal donor blood” only group O units that have been screened by the blood bank and certified as suitable for the purpose. This will be unnecessary in those services in which the red cells are routinely suspended in additive solutions; otherwise, the problem can be avoided by using crystalloids and/or colloids to stabilize the patient while blood-grouping and cross-matching are carried out.

Leukocyte and platelet incompatibility may cause fever, and leukocyte incompatibility may cause pulmonary infiltrates and symptoms such as dyspnoea, non-productive cough and chest pain. The risk of these episodes can be reduced by using leukocyte-poor red cells.

Allergic reactions may be caused by interaction of a patient’s preformed reagins with transfused allergens, or by the passive transfer of reagins in donor blood. Ascertain whether a donor has a history of allergic reactions may reduce this risk. When allergic reactions occur, it may be necessary to administer antihistamines during the transfusion.

Fever is sometimes a manifestation of one of the complications of transfusion, e.g. allergy, red-cell, leukocyte or platelet incompatibility, or infection. Leukoagglutinins are probably the commonest cause of fever, but very often no cause is found. In these cases, the fever may occur at the time of the transfusion or may be delayed for up to 24 hours.

**Non-immunological complications**

Non-immunological complications of blood transfusion include transmission of infectious agents and circulatory overload, and those resulting from massive transfusions.

Measures for reducing the risk of transmitting blood-borne infectious agents are indicated in Chapter 4. Blood products that appear to carry a low risk, or none at all, for transmitting HIV, viral hepatitis B, and non-A non-B hepatitis are albumin and immunoglobulins. However, these infections are transmissible by whole blood, red cells, platelets, and cryoprecipitate. It is therefore worth re-emphasizing that blood and blood products should be transfused or administered only when they are clearly indicated, and that safer alternatives should be used whenever possible.
Bacterial contamination during the collection or processing of blood can cause sepsicaemia on transfusion. This risk is very small in those transfusion services where plastic packs, rather than glass bottles, are used, particularly when a closed multi-pack system is used for preparation of blood components. Even in these circumstances, however, there are still occasional cases of contamination, many of which are due to a tiny breach in the plastic bag, which is more likely when the pack has been frozen. Thus the bag (and its contents) must be examined before transfusion: the pack must be examined for leaks and the contents for evidence of infection (although this is not always noticeable).

Contamination can occur at the time of venepuncture, and it is therefore important to clean the skin very carefully. Care should also be taken during centrifugation: the packs should be put into centrifuge bags, and the buckets should be cleaned periodically with 2% gluteraldehyde. Because they are stored for several days at room temperature, contaminated platelet concentrates carry the risk of bacterial growth.

The risk of circulatory overload is increased when large volumes of fluid are given too rapidly. Patients who are chronically anaemic, especially the elderly, are particularly at risk. It is always a sound principle to use for replacement therapy only the blood component(s) that the patient needs, transfused in the smallest volume possible.

Massive transfusion, i.e. transfusion of a volume equivalent to the patient’s total blood volume in less than 24 hours, can cause metabolic and haemostatic derangements. Fresh whole blood, fresh frozen plasma, or platelet concentrates may be required for treatment of diffuse intravascular coagulation or thrombocytopenia, and appropriate management of hyperkalaemia, hypokalaemia, or acute respiratory distress syndrome may be necessary.

Because of the hazards associated with homologous blood transfusion, use of autologous blood transfusion has been advocated. Most patients who are fit for elective surgery will be fit for autologous blood transfusion, making this an attractive alternative for such individuals. Autologous blood transfusion presents logistical problems, and should be undertaken only when the infrastructure of the transfusion service has been developed satisfactorily. Decisions about the disposal of units that have not been used by the donors (e.g. for donation to another patient) should be included in the country’s national
blood policy. The criteria for selecting donors should also be clearly defined.

Another alternative to homologous blood transfusion is the salvaging of blood during operations for transfusion to the patient. For example, blood in the abdominal or thoracic cavity has been salvaged and transfused. Contraindications are the presence of infection, gastrointestinal contents, or malignant cells.

ROLE OF THE HOSPITAL TRANSFUSION COMMITTEE

An efficiently functioning hospital transfusion committee will contribute to increased safety of blood and blood products. This committee should comprise representatives of all the departments in the hospital that use blood, the director of the transfusion services, and representatives of the nursing staff, the hospital administration, and the medical records department. The functions of this committee include setting guidelines for the transfusion of blood and blood components, carrying out audits on transfusion practice, investigating undesirable effects of transfusion (and, where necessary, instituting corrective measures), and promoting continuing education in transfusion medicine for hospital staff.

CONCLUSIONS

Blood and blood products should be transfused only when they are clearly indicated, and the possibility of using alternative therapy should be examined. In particular, plasma substitutes should be considered for the management of patients with acute haemorrhage.

Replacement therapy should consist of only the blood component(s) that the patient needs, administered in the smallest volume possible.

Ward and laboratory staff must be meticulous in collecting and processing samples, and in setting up transfusions, in order to reduce the risk of transfusion reactions. Ward staff must be meticulous in documenting the details of all transfusions given to the patient.

The physician must know the potential complications of transfusion of blood and its components, and how to prevent and treat them.

An efficient hospital transfusion committee will increase the safety of transfusion of blood and blood products.
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