BLOOD TRANSFUSION

A Guide to the Formation and Operation of a Transfusion Service

Edited by

C. C. BOWLEY, K. L. G. GOLDSMITH & W. D'A. MAYCOCK

on behalf of

the World Health Organization, the International Society of Blood Transfusion, and the League of Red Cross Societies

World Health Organization

Geneva

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In many developing countries, blood transfusion services are still insufficiently organized or wholly lacking. This situation is mainly due to a great shortage of the qualified staff, both professional and technical, needed to create and develop such services where resources and equipment may be unavoidably restricted.

The present book is intended to help physicians and pathologists who, after receiving a basic training in blood transfusion, are entrusted with the responsibility of establishing and developing transfusion services in their own countries, either under the ministry of health or through the agency of a voluntary organization, such as a national Red Cross Society. It provides practical information on the four main aspects of blood transfusion : organization of a service ; recruitment of donors ; collection, preservation, and distribution of blood ; and laboratory techniques.

The World Health Organization, the International Society of Blood Transfusion, and the League of Red Cross Societies have given constant support to the preparation of this guide and have reviewed the final manuscript. This was prepared by Dr C. C. Bowley in collaboration with Dr K. L. G. Goldsmith, under the general guidance of Dr W. d'A. Maycock, on the basis of contributions received from the directors of Red Cross and other national transfusion services. A full list of contributors is given on page 8.

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Organization of a National Blood Transfusion Service

The primary task of a national blood transfusion service is to meet the country's needs for blood and blood derivatives. This entails co-operation with the public health authorities, hospitals, and medical associations and the promotion of good relationships with the general public.

A national blood transfusion service cannot be built up quickly. Its development is a laborious and lengthy task which w l be successful only if there is, from the beginning, a clearly defined organizational structure and complete agreement about the responsibilities allotted to each organization or group participating in the enterprise. Depending upon national policy, the country's ministry of health may assume responsibility for organizing and administering the transfusion service at all levels or may delegate some or all of the responsibility to a suitable organization such as the national Red Cross Society.

The introduction of a national blood transfusion service where none exists should preferably be accompanied or preceded by the establishment of a national transfusion committee, whose task will be to co-ordinate the programme at a national level. Membership of this body should include representatives of the public health authorities, the medical services of the armed forces, national Red Cross, Red Crescent, or Red Lion and Sun Societies, and the leading medical faculties, as well as individual experts interested in blood transfusion.

The first task of the transfusion committee will be to appoint, as director of the national blood transfusion service, a suitably qualified medical practitioner with the necessary experience to implement the committee's policy.

The first task of the director is to assess the transfusion needs of the country and to advise the committee on how best they can be met.

To implement its agreed policy, the committee must first establish a centre where both organizational and technical matters relating to blood transfusion are co-ordinated for the whole country. Initially this may be established within an existing unit of the health laboratory service. This centre will be placed under the director of the national blood transfusion service and will function, in the early stages, as a regional transfusion centre.

The responsibilities of a regional transfusion centre are :

(*a*) the recruitment of blood donors and the maintenance of donor records ;

(*b*) the medical examination of donors and the collection of blood ;

(*c*) the preparation and distribution of transfusion equipment ;

(*d*) the testing, storage, and distribution of donated blood ;

(*e*) the examination of special blood samples—for example, from suspected transfusion reactions or possible cases of haemolytic disease of the newborn.

In view of the increasing complexity of antibody identification, the examination of prenatal blood samples and the determination of blood genotypes, when indicated, are also particularly appropriate work for a regional transfusion centre and materially assist in obtaining antisera, but this development may have to come after the more immediate objective of providing blood for transfusion has been achieved.

These tasks are extensive and may be undertaken step by step.

Similar regional transfusion centres can gradually be established as necessary for other areas, the staff receiving their preliminary training at the original centre which, in the natural course of development, will eventually become recognized as the national reference centre for transfusion problems. At some stage it may be necessary for the director and co-ordinator of the national transfusion service, while retaining these functions, to hand over responsibility for the centre to another director.

A national reference centre for transfusion problems would have the following additional duties :

(*a*) to issue directions or recommendations on matters of organization, equipment, or technique pertaining to blood transfusion ;

(*b*) to co-ordinate and supervise the activity of regional blood transfusion centres in order to carry out the policy decisions of the national transfusion committee ;

(*c*) to undertake blood group serological examinations of specimens referred from regional transfusion centres because of their particular difficulty or significance ;

(*d*) to manufacture and distribute stable blood derivatives (dried plasma, plasma fractions) and diagnostic reagents (blood grouping test sera) ;

(*e*) to collect rare sera from other laboratories and act as a central distribution point for them ;

(*f*) to train doctors and medical auxiliary personnel (e.g., technicians) from the regional transfusion centres and other postgraduate students in the

more advanced aspects of blood group serology and blood transfusion organization ;

(*g*) to institute and maintain a national panel of blood donors of rare groups ; and

(*h*) to maintain contact with national reference laboratories in other countries in order to keep abreast of recent developments and facilitate the exchange of sera.

In some circumstances the reference centre might also undertake the preparation and distribution of equipment (containers, taking and giving sets) to regional transfusion centres, but this would depend on local circumstances.

The director and co-ordinator of the national transfusion service might wish to retain control of the reference work, in which circumstances he and the director of the regional transfusion centre would work in close co-operation, so that the reference centre would remain in close contact with daily problems in the field and thus be in less danger of issuing instructions or recommendations relating only partially, or not at all, to practical conditions.

Finally, one of the service's laboratories would be designated by the commission as the national blood group reference laboratory, which, after recognition by WHO, would collaborate with other national blood group reference laboratories and with the WHO International Blood Group Reference Laboratory, Medical Research Council's Blood Group Reference Laboratory, London, England.

During the development of the service and its specialized units, it will become necessary to provide separate accommodation with appropriate services and equipment for the work involved. The following chapters describe the basic work of a transfusion service and contain further suggestions for organizing a national blood transfusion service and regional transfusion centres.

PART I

RECRUITMENT AND SELECTION OF BLOOD DONORS

MAINTENANCE OF DONOR RECORDS

Recruitment of Blood Donors

Basic systems

There are three basic systems of obtaining blood donors : (*a*) paid donation ; (*b*) the bank system ; and (*c*) voluntary unpaid donation.

Paid donation

Under the paid donation system the donor receives financial recompense. The transfusion service is thereby relieved of some responsibility towards the donor, and may sell the blood and blood derivatives to a hospital or to the recipient at a profit. Apart from the disadvantage of commercializing so precious a product as human blood, the system may lead to too frequent donation. In the absence of careful medical supervision this will result in damage to health from anaemia and hypoproteinaemia, especially as paid donors are often already in poor health and may show nutritional and other deficiencies. Moreover, there is a danger that donors in need of money will conceal previous illnesses, such as jaundice. Added to this, "professional" donors may form syndicates and from time to time demand an increase in financial recompense. This, together with the indisputable fact that such donors mostly come from the lowest social strata where alcoholics and drug addicts are often found, has brought paid blood donation into disrepute in many places.

Bank system

Under the so-called bank system, donors are recruited by the recipient himself, who has to pay if he is unable to replace the blood he needs by donations from his family, relatives, or friends. In this system, high transfusion fees provide the incentive for donor recruitment ; it works, therefore, only where the patient has to bear the costs of treatment himself. If this is not the case, if for example the expenses are born by a sickness insurance company or a state health service, then the stimulus to recruit donors will be lacking. The credit principle entails an exceptionally heavy administrative burden, since complicated calculations involving different blood banks have to be made. The bank system has also been organized on a community basis. For example, workers at a certain factory and their

immediate dependents are entitled to free blood transfusion only as long as donor sessions at the factory maintain a credit balance. This system originated, and is still widely prevalent, in the USA, where regional and national clearing houses have been set up to settle the accounts involved.

Voluntary unpaid donation

The voluntary unpaid blood donation is a humanitarian act towards the sick by the healthy. A blood transfusion service based upon unpaid donations is obliged to work on a non-profit basis, since blood given free of charge should not be used for profit. If the service is not sub-sidized by the state, it may charge the patients, the hospitals, or their sickness insurance companies, but only for the costs of storing, distributing, or processing blood and blood derivatives. Any surplus funds accruing from such charges should be used only to improve or extend the service, not for other purposes. Under this system it is easier to verify the donor's state of health, since—unlike some paid donors—he has no reason to try to conceal illness.

At the XVIIth International Conference of the Red Cross in Stockholm in 1948 a resolution was adopted recommending the voluntary free gift of blood as the ideal system. Since then its obvious advantages have everywhere brought good results. In a number of countries the blood transfusion services have successfully changed over from the paid system to that of free donation.

It is most strongly recommended that new transfusion services should make every effort to adopt the voluntary unpaid system from the beginning.

Size of donor panel

In countries with modern health services and extensive blood transfusion services, it is possible to arrive at an approximation of the amount of blood used each year in terms of the number of hospital beds. The figure should be based on the number of emergency beds in general hospitals, i.e., it should exclude beds for infectious diseases, chronic sickness, mental deficiency, and mental illness, and pre-convalescence and convalescence. The following is a representative example:

England and Wales (1969)	*Units issued per emergency bed*
Whole human blood:	6.76
Plasma:	0.42

Estimates linked to the number of hospital beds may be misleading as they cannot take the number of patients treated into account. More or fewer patients may occupy the same number of beds a year, depending upon the effectiveness of treatment, the efficiency of the hospital, the

diseases involved, and other factors. It is therefore more realistic to base estimates of need upon the number of patients treated in a year.

A convenient figure to use is the number of patients discharged or dying in a year. Thus :

England and Wales (1969)   *Units issued per 100 patients*
                                discharged or dying

                                Blood :   24.66
                                Plasma :   1.55

A record of the number of patients discharged or dying however is less likely to be available than the number of beds.

In the United Kingdom the unpaid donor system is used. It is national policy to ask for donations twice a year, at approximately six-monthly intervals. The national donor response rate varies from time to time and place to place; the average is between 50% and 55%. About 7% of donors reporting at blood collecting sessions are deferred on medical grounds, usually in their own interests rather than those of the potential recipients.

Assuming similar conditions elsewhere, it may be said that to meet the blood requirements of 1000 emergency hospital beds a panel of about 7000 donors is required. Experience has shown, however, that these figures are applicable only after a service has been operating for five to ten years, and the initial activities need not be on such a large scale. A frequently quoted estimate is that, in countries with fully operational public health and blood transfusion services, the need for blood can be met if about 2% of the population are regular donors. With the development of open heart surgery, renal dialysis, and other procedures using transfusion, it is probable that this figure is now too low.

Recruiting methods

Philanthropic organizations, such as national Red Cross Societies, can be very helpful in recruiting donors. It is unwise, however, to separate the recruitment and care of donors from the medical and technical services in the regional transfusion centre. All belong under the same roof and should come under the responsibility of the medical director of the centre. The donor organizer and his staff, responsible to the medical director for donor recruitment and care, will be able to carry out their work usefully and correctly only if they are constantly aware of the centre's needs. Similarly, the medical director must be constantly aware of the problems of donor recruitment and care. The motto of the medical director should be : " Without the donor panel there would be no blood transfusion service ;

therefore the convenience, comfort, and wishes of the donors should be given every consideration." For the donor organizer the motto should be : " The blood must be available in the quantity needed, at the place and time required. All other considerations are subservient to this."

Initial steps to form a panel of donors are best taken within such groups and communities as the armed forces, the police, large industrial or commercial undertakings, universities, prisons, and social or religious foundations. The advantages are that information about the need for donors and the speed and ease of donation can easily be given directly to the members of the community or group in question and that blood collection can be arranged and carried out without delay. It is also possible to introduce a competitive atmosphere in which public comparisons of the results of blood collections will encourage more donors to come forward. Success is more likely in a community if the leaders set a good example.

It is of primary importance to dispel donors' fears at the outset. If a new donor is bled skilfully, treated well, and convinced by personal experience that blood donation is harmless, he will usually return to make further donations. This is the best advertisement for blood donation. If donor recruitment in groups or communities meets with difficulty in the initial stages of the service, it may be stimulated by arranging some small advantage for the donors, such as additional free time for soldiers, policemen, or factory workers, but this is not generally necessary. The fact that the management allows the donor session, usually in its premises during working hours, is normally sufficient encouragement. Financial remuneration should be avoided in all circumstances.

Recruitment among the general public may be started once experience has been gained with special groups or organizations. Apart from local publicity to pave the way for the opening of large blood donor clinics, general information must be given regularly to the public through modern information media, i.e., the press, radio, films, and television. General articles on the meaning of blood donation and reports on donor clinics should be placed in newspapers. Material on dramatic blood transfusions must be handled very carefully by experienced publicity officers as many newspaper editors are unwilling to publish an account of something that has already happened and is therefore no longer news ; on the other hand journalists with advance information have been known to violate the privacy of both donor and recipient in a totally unacceptable manner. While it is essential to maintain good relations with the press it is usually inadvisable to release personal details about either donors or patients. Radio and television are suitable for urgent appeals and information on blood donation, which should always be based on actual fact if the public is to feel personally involved. Advertising to impart general information about the existence and work of the service may, however, be ineffective, if it is not followed up by an intensive local campaign.

In addition to such general publicity, specific local recruiting propaganda is needed. Local publicity is particularly effective if carried out in close co-operation with associations willing to adopt blood donation as one of their interests. The Red Cross and other philanthropic and civic organizations can render especially valuable service at the local level. At meetings of such organizations, lectures on transfusion, illustrated by films, can be very effective, particularly if they are given by doctors well-known to the audience. Propaganda pamphlets containing enrolment forms should be distributed on these occasions. Great assistance in recruiting donors can be given by public figures—magistrates, religious and political leaders, film stars, and sportsmen—if they are prepared to volunteer as donors and allow their example to be made public.

The decisive factor in the success of a transfusion service is that as many people as possible should become regular donors after their first donation. They must therefore be treated with every consideration during donation ; waiting periods must be kept short ; venepuncture must be perfect. If venepuncture on a new donor is unsatisfactory it should not be repeated in the other arm. Very occasionally a regular donor may be upset if less than a full donation is obtained for technical reasons ; only in such a case and at the donor's request, should a second venepuncture be considered. Attractive light refreshments should await the donor in a bright, clean, and pleasant room, and above all the staff must at all times appear courteous, interested, and cheerful. The donor must be thanked for his donation. A thank-you card or badge given after the first donation is usually appreciated and is effective in encouraging donors to return. The possibility of earning a badge after a given number of donations is also effective; for example, a bronze badge could be awarded for 5–10 donations, a silver one for 15–25 donations, and a gold or silver gilt one for 30–50 donations.

The anonymity of donor and recipient should always be preserved. Although telling a donor how his blood has been used might help to retain his interest, it is most unwise to institute this practice because it will be increasingly difficult and finally impossible to continue it as the transfusion service grows. There might also be occasions on which it would be embarrassing to the service to inform the donor of the use made of his blood. It is preferable to tell donors about the use of blood and other aspects of the transfusion service in a more general way—for example, by providing informative leaflets. Some services give donors regular bulletins and in some places donors form their own associations to maintain their interest in transfusion and stimulate that of others.

Other ways of stimulating donor recruitment include :

1. The collection of blood from athletes the day before they take part in a sports meeting or match, to demonstrate that blood donation in no way affects physical effort.

2. The use of specially designed stamps and postal cancellation marks.

3. The distribution of enrolment cards to hospital visitors whose relatives or friends have received transfusions.

4. The presentation of silver or gold donors' badges (see above) at a meeting of an organization to which the donor belongs or by a local personality at a special ceremony.

Samples of propaganda brochures, posters, badges, etc., for the recruitment of unpaid donors may be obtained from the League of Red Cross Societies, 1211 Petit-Saconnex, Geneva, Switzerland.

Possible Complications of Blood Donation and Diseases Transmissible by Transfusion

Neither blood donation nor blood transfusion is completely free from risk. The risks of donation are generally very slight, but none the less real, when the donors are fit and well; they are somewhat greater if volunteers unfit to give blood are accepted. Some of the risks to which the recipient is exposed are inherent in every transfusion, but others may be due to uncritical acceptance of donors. It is necessary to consider some of the possible complications before proceeding to the medical examination of donors.

Protection of the donor's health

When considering how to protect the donor's health, it is convenient to consider first the possible risks each time blood is given.

Risks associated with the act of donation

1. *Local damage*

If the technique of venepuncture is imperfect, a haematoma may result. This rarely leads to thrombosis, but may cause temporary discomfort and some limitation in the use of the arm and may render the veins less suitable for further donations or injections.

If the skin is thoroughly cleansed and covered with a dressing after donation, there will be little chance of infection at the site of venepuncture.

Occasionally the taking needle may in error be inserted into an aberrant artery. The resulting donation is very rapid with a pulsating flow of bright red blood. Provided the incident is recognized, prompt elevation of the limb, application of a dressing and a suitable pressure bandage to the puncture site, followed by a period of observation, are generally the only treatment required.

Local dermatitis due to hypersensitivity to a skin disinfectant, a local anaesthetic, or plaster is sometimes observed.

2. *Systemic reactions*

Circulatory. The most frequent reaction to blood donation is fainting. The symptoms in their usual order of appearance are : abdominal discomfort, increasing pallor, sweating, yawning, giddiness, blackness before the eyes, hypotonia of the muscles, and finally loss of consciousness. Contrary to what might be expected, although the blood pressure is low the pulse rate is retarded, which is typical of a vasovagal attack rather than a condition of shock due to loss of circulating blood volume. The treatment consists of discontinuing the donation, reassuring the donor, loosening any tight clothing, and maintaining the donor in a supine position, possibly with the legs raised. Recovery is usually rapid. This reaction appears to be more common among new donors and particularly among more intelligent and imaginative persons. Emotions play a considerable role in causing fainting, and once one donor is seen to faint an " epidemic " of fainting due to mass suggestion may occur.

In occasional instances the fainting syndrome is due to inadequate reaction of the circulation to the decrease in blood volume. Temporary vasodilatation in the muscles and splanchnic vessels results in momentary cerebral hypoxia.

Fainting occurs in 1%–6% of donors ; the higher incidence, however, includes mild cases with only early signs and symptoms. If more than 400–420 ml of blood are taken, the frequency of fainting will increase, especially in young people and asthenic donors.

Donors with circulatory disorders are especially subject to cardiovascular disturbances due to the rapid haemodynamic alterations. Coronary and cerebral thrombosis have occasionally been observed several hours after blood has been taken from persons suffering from arteriosclerosis and high blood pressure. Hypertensives with average systolic blood pressure of over 200 mm Hg or diastolic pressure of over 110 mm Hg must be excluded from donation. Many experienced medical officers exclude donation at a lower systolic level than this. It is also advisable to reject hypotensives with an average systolic blood pressure below 100 mm Hg or a diastolic pressure below 60 mm Hg.

Air embolism. Air embolism can be avoided by carefully preparing the equipment and by following the correct technique of blood collection (see Chapter 8, p. 66).

Evidence of air embolism may vary from cessation of blood flow and the sight of air bubbles passing up the taking set into the donor's vein to anxiety in the donor, accompanied by cough and chest pain, with cyanosis, cardiac irregularity, and abnormal heart sounds in extreme cases.

If an air embolus is suspected, pressure in the sphygmomanometer cuff must be immediately increased and the taking needle rapidly removed. These actions must be undertaken promptly without worrying about leakage

from the puncture site. The donor should be turned on his left side to keep any air bubbles in the right atrium, and his head should be lowered over the side of the couch.

Tetany. Hyperventilation tetany is a very rare complication which may be observed as part of the fainting syndrome in anxious donors.

Hepatitis. To avoid the possible transmission of serum hepatitis from donor to donor all needles (i.e., intravenous, hypodermic, and skin lancets) must be adequately sterilized and fresh ones used for each donor.

The possibility of transmitting serum hepatitis in the course of blood transfusion is discussed on p. 24.

Reactivation of previous illnesses. There is no reason to exclude potential donors who have recovered from a minor tuberculous infection without complications more than five years previously. However, volunteers who have suffered from advanced tuberculosis, e.g., of the lungs, bones and joints, or urogenital system, and in whom the disease has left permanent lesions, should be rejected. Those suffering from chronic diseases, such as diabetes, kidney disease, epilepsy, asthma, or psychosis, or known to have had cancer should be rejected. There is always danger of exacerbation which can raise the legal question of responsibility. It must be clear to the general public that only persons in good health are accepted as donors.

Risks associated with repeated donation

With repeated donation, disturbances in the haemopoietic system may occur.

The human body varies in its reaction to the rapid removal of nearly half a litre of whole blood. In some donors it is difficult to detect any change in haemoglobin level, red cell count, or plasma protein fractionation. In healthy donors the plasma volume is restored in a few hours and the plasma protein concentration, if lowered immediately after giving blood, returns to normal in 1–2 days. Replacement of the lost red cells is a slower process. If 400–420 ml of blood are taken the haemoglobin level will fall by an average of 1 g/100 ml, while approximately 200 mg of iron will be lost. If the donor has no iron deficiency, the erythrocytes and the haemoglobin level will generally return to normal within 3–4 weeks.

With frequent donations at short intervals, there is a serious danger of sideropenia, particularly in women, who lose about 600 mg of iron yearly through menstruation. During pregnancy approximately 400 mg of additional iron is supplied by the mother to the foetus; during breast-feeding 1–1.5 mg of iron is lost daily. Sideropenia may be suspected from symptoms of fatigue, weakness, headaches, and palpitations. The longer it lasts the more likely it is that a secondary iron deficiency anaemia will develop, with

the usual signs of deterioration of the nails, fissures at the corners of the mouth, and dysphagia.

To prevent iron deficiency in donors, an interval of at least 3 months between donations must be maintained. Women should never give more than three nor men more than four donations of 400–420 ml per year, and ideally the aim should be to recruit enough donors to meet the need for blood with only two donations a year from each donor. Donors with a haemoglobin level of 12.4 g/100 ml or less should be rejected and referred to their doctor for advice and treatment. In some countries it is customary to reject male donors with a haemoglobin level of 13.1 g/100 ml, or less, as this degree of anaemia in males is frequently of greater clinical significance than one of 12.4 g/100 ml in women.

If, in view of local conditions, it is considered that donors would benefit from supplementary iron given orally, then a sufficient quantity of an easily absorbed preparation of iron, for example, 100 mg of ferrous sulfate or an equivalent preparation, should be taken 3 times a day for 7 days.

On any container used for dispensing iron tablets, it must be clearly marked that the contents are poisonous to children.

Protection of the recipient's health

Risks to the recipient of a transfusion may arise from:

(*a*) transmission of disease or allergy from donor to recipient;

(*b*) accidents through careless preparation of blood and blood derivatives;

(*c*) immunological incompatibility between donor and recipient.

Transmission of disease by transfusion

1. Viral hepatitis

The transfusion of blood, plasma, and certain plasma fractions may transmit viral hepatitis. Two forms of viral hepatitis transmitted by transfusion are recognized: infectious hepatitis, and serum hepatitis. Both have the same clinical picture, and human beings are the only known source of infection. Patients with clinical jaundice are not the main source of the disease; far more significant sources are the mild anicteric case, the convalescent carrier, those incubating the disease, and the healthy contact carrier, all of whom at one time or another may be viraemic.

Infectious hepatitis is an " open " infection. Towards the end of the incubation period and during illness, the virus is present in the faeces. In the majority of cases infection is peroral, transmitted either by direct close contact or through the consumption of contaminated food (water, milk, etc.). Parenteral infection by transfusion or the use of contaminated

instruments for injection or scarification is possible during the viraemic stage, i.e., towards the end of the incubation period and in the 3–4 weeks following the appearance of the first signs. The incubation period is short: 15–30, rarely up to 50, days. Infectious hepatitis chiefly attacks younger people. Incidence reaches a peak in late summer and early autumn, and the infection is endemic in most countries. The case fatality is about 0.2%.

Serum hepatitis on the other hand is almost always a " closed " infection. As far as is known the causative agent is not excreted. It is, however, present in the blood during the incubation period, several weeks before the appearance of jaundice, and it may persist for a long time ; cases have been recorded in which the blood was still icterogenic 5½ years after recovery from the disease. The causative agent is apparently almost always transmitted by procedures in which the skin or mucous membranes are pierced (transfusion of contamined blood or blood products ; the use of contaminated syringes, scarifiers, vaccination lancets, tattooing needles, dental instruments, etc.) or by contamination of a wound with infected material.

The disease is characterized by an extraordinarily long incubation period (50–160 days) and there is no seasonal variation in incidence. Most cases occur in adults. The case-fatality rate is higher than that for infectious hepatitis.

Sporadic cases or outbreaks of the disease have been reported among the staff of transfusion laboratories and haemodialysis units. Staff handling blood, blood derivatives, transfusion equipment, or any other equipment soiled with blood or blood derivatives should take care to minimize contact with blood.

Recently an antigen, known as Australia (hepatitis-associated) antigen has been shown to be closely associated with serum hepatitis. The presence of the antigen and its antibody can be detected in human beings by appropriate laboratory tests (Informal consultation on viral hepatitis, 1970). This is of great potential significance to transfusion services, because it seems probable that the detection and exclusion of donors shown to carry the antigen or its antibody will reduce the risk of transmitting serum hepatitis by the transfusion of blood or blood products.

2. Syphilis

The transfusion of blood infected with *Treponema pallidum* is usually followed within 9–10 weeks by the appearance of a typical secondary eruption. The transmission of syphilis has become less frequent with the increasing use of stored blood. It has been shown experimentally that refrigeration for 72–96 hours at a temperature of 2°–6°C will kill the *T. pallidum* in whole blood and liquid plasma. The organism is also killed by freezing and freeze-drying. No guarantee of safety is given by a negative

syphilis test of the donor's blood since serum reactions are negative during the 3–4 weeks' incubation period following infection, and in about one-third of patients with a clinical primary infection.

Experience has shown it is usually sufficient to test donations by a reliable flocculation reaction with a cardiolipin antigen; tests with both a flocculation and a complement-fixation reaction may be preferred. Unless the result is negative, the tests should be repeated and additional tests, such as the treponemal immobilization test, used.

3. Yaws (framboesia)

As for syphilis.

4. Post-transfusion brucellosis, salmonellosis, and leptospirosis

Potential donors who have a recent history of brucellosis or salmonellosis should be excluded. Donors with a recent history of leptospirosis should be excluded for one year.

5. Tropical diseases

The risk of transmitting malaria is only slightly reduced by the use of stored blood since all three malaria parasites (*Plasmodium vivax*, *P. malariae*, and *P. falciparum*) survive refrigeration for several days and even weeks with little change in their powers of infection. Donors with a history of malaria and those returning from malarious areas should therefore be excluded from donation for whole blood. Their plasma, if freeze-dried, may be used. In endemic areas the use of malarious blood may be unavoidable, in which case the recipients should be given 200 mg of chloroquine daily for 4 days. In areas where trypanosomic diseases (African sleeping sickness, Chagas' disease), leishmaniasis (kala-azar, oriental sore), and relapsing fever are endemic, a negative history is of little significance because of the high incidence of the disease. Potential donors should therefore undergo an erythrocyte sedimentation test and blood film examination. These tests are discussed in the next chapter.

Helminthiasis, amoebic dysentery, yellow fever, dengue, Rift Valley fever, sandfly fever, and arthropod-borne encephalitides are not absolute contraindications to blood donation. Volunteers who have suffered from any of these conditions may donate once the disease has been cured.

6. Post-vaccinal states

Primary smallpox vaccination causes, after 6–7 days, a generally febrile reaction of several days' duration, during which a viraemia is present. After revaccination, the reaction is quicker and less severe. To prevent transmission of virus by blood transfusion, potential donors should not

be bled if they have been vaccinated against smallpox in the previous 3 weeks.

The same rule should apply for 3 weeks after yellow fever vaccination.

Potential donors vaccinated against rabies should be suspended from donation for at least 3 weeks.

Immunization against poliomyelitis may be carried out by the oral administration of an attenuated live vaccine. This vaccine causes little or no generalized reaction, does not increase in virulence as a result of passage through the body, and should not in any way endanger the health of a patient to whom it is accidentally transmitted by transfusion. While it is considered advisable that donors should not give blood for 2 weeks after receiving oral poliomyelitis vaccine, this is not obligatory.

7. *Allergies*

Transfusion of whole blood or plasma from a donor suffering from an acute allergic attack may temporarily sensitize the recipient by introducing allergens or reagins. Allergic donors should therefore be excluded from donation during attacks. Potential donors who have been immunized with animal sera within the preceding 3 weeks should be excluded, in order to prevent primary sensitization to animal protein or allergic complications in recipients already sensitive to animal protein.

Accidents due to errors in collection or storage of blood

Risks to recipients arising from errors during the collection or storage of blood are generally attributable to the use of incorrectly prepared anticoagulant, bacterial contamination, storage at the wrong temperature, or any cause which increases the number of non-viable or, in extreme cases, haemolysed red cells (e.g., heating, freezing, or excessive age of red cells).

Methods of routine organization designed to eliminate these risks are discussed in later chapters.

Immunological incompatibilities

Frank incompatibility between antibodies already present in recipients and donor red cell antigens can be avoided by the use of adequate cross-matching techniques, as described in Chapter 13.

In rare instances, such tests may be inadequate since a patient may have no detectable antibody, but nevertheless already be sensitized to an antigen in the donor's cells. In these circumstances the donated cells are rapidly eliminated. It is still not possible to examine routinely all samples for leucocyte antibodies. Those prescribing blood transfusions

should always be aware of these admittedly rare, but nevertheless real, dangers.

Any reaction occurring in the patient within 48 hours of transfusion, and possibly due to the transfusion, should be reported immediately by the clinician in charge of the patient to the transfusion laboratory so that appropriate investigation and treatment can be undertaken without delay.

Selection and Medical Examination of Donors

The problems that arise in the selection and medical examination of donors vary considerably from one country to another. The use of unpaid volunteer donors is an important safeguard in itself, as it ensures that there is less temptation for the donor to attend if he does not feel fit. Fortunately, in most countries the development of the general medical services is at least parallel to, if not in advance of, the development of the transfusion services. Thus the busiest donor sessions will usually be held where the selection and medical examination of donors have become less onerous, because of the general high standard of health education and public health supervision. In countries where the health services are less highly developed, and in particular where there are endemic tropical diseases that may be transmitted by blood transfusion, a more searching medical examination of potential donors is required. In some centres this may be extended to include a chest X-ray and a complete urinalysis. It is possible that in some countries the opportunity of such a comprehensive medical examination free of charge might even assist donor recruitment. Examinations of this type should hardly be necessary where there is a free national health service.

Although it is possible to formulate general rules for the selection and examination of donors, these will need to be modified or extended in the light of local circumstances and experience.

Selection

1. The lower and upper age limits for blood donation are generally 18 and 65 years. In some countries donors under 21 years of age must have the consent of a parent or guardian.

2. The normal donation of blood (usually 420 ml) should not be taken more than 3 times annually from women and 4 times annually from men. There should be a minimum interval of 3 months between donations. The aim should be to recruit enough donors to ensure that none of them is asked to give blood more than twice a year.

3. Donors with hazardous occupations, for example aircrew, train, bus, and crane drivers, steeplejacks, etc., should give blood only after finishing work for the day.

Medical examination

Before each donation the donor should be examined to determine his suitability. The findings of this examination should be strictly confidential. It should include : medical history ; physical examination ; and laboratory tests.

Medical history

The medical history may be taken by a doctor, a nurse, or a specially trained clerk who interviews the donors, or by means of a questionnaire completed by the donor. The points that should be covered include the health of the donor in recent months or since the last donation, and previous medical treatment or hospitalization. There should also be a brief interrogation dealing with the central nervous system, heart, blood circulation, lungs, and the gastro-intestinal and urogenital systems, as well as questions on allergies, diabetes, epilepsy, and hepatitis.

Pregnant women should not be bled. Nursing mothers should not be bled for at least 6 months after childbirth or for as long as they are breast-feeding. An exception may be made where blood is taken, under strict medical supervision, to obtain blood-grouping sera. Menstruation, apart from acute menorrhagia, is not a contraindication to blood donation.

Potential donors should not :

(*a*) be bled within 3 days following tooth extraction ;

(*b*) have had any serious febrile illness or major surgical operation during the preceding year or have received a transfusion in the previous 6 months ;

(*c*) have had any minor febrile illness during the previous 3 weeks ;

(*d*) be bled during any acute attack due to allergy.

Persons with chronic illness, cardiac insufficiency, renal damage, nervous complaints, psychosis, diabetes, etc., should not be accepted as donors.

Blood taken from patients with polycythaemia vera or hypertension should not be used, and in general it is not desirable to undertake therapeutic venesections at public donor sessions. If blood is taken from patients with haemochromatosis, it should be used only for plasma or fractionation.

The previous chapter gives guidance on the management of potential donors who have recently been vaccinated or who have a history of any of the following infectious diseases : amoebic dysentery, arthropod-borne encephalitis, brucellosis, Chagas' disease, dengue, helminthiasis, hepatitis, leishmaniasis, leptospirosis, malaria, Rift Valley fever, salmonellosis, sandfly fever, syphilis, tuberculosis, undulant fever, yaws, yellow fever.

If the medical history raises any doubt as to the wisdom of blood dona-
tion, the doctor should ask further questions and, if necessary, arrange for
laboratory investigations.

Physical examination

Depending on local conditions the physical examination will vary
from a comprehensive clinical examination, including special tests, to
the very simplest assessment of weight, pulse rate, and blood pressure.
During this examination the medical officer should be able to pick out
those prospective donors who may be, for example, undernourished,
crippled, mentally unstable, alcoholics, or drug addicts.

It is customary to take less blood from donors weighing under 60 kg;
300–400 ml can be taken according to weight. Smaller amounts should not be
collected into the usual volume of anticoagulant.

Potential donors should be rejected if:

(*a*) they are found to be hypertensive;

(*b*) they are found to be hypotensive with blood pressures below
100/60 mm Hg;

(*c*) they show clinical signs of disorders of the circulatory system
(decompensation, angina, irregular pulse, etc. — those with pulse rates
over 120, or below 50, beats per minute should also be excluded);

(*d*) they are found to have a raised body temperature.

Laboratory tests

Haemoglobin. No donor with a haemoglobin level of less than 12.4 g/
100 ml (85%) should be accepted, and an increasing number of transfusion
services are using a minimum acceptable haemoglobin concentration of
13.1 g/100 ml (90%) for male donors. The Phillips-Van Slyke copper
sulfate technique (Phillips et al., 1950; Van Slyke et al., 1950a, 1950b)
has proved a reliable method for estimating haemoglobin at donor sessions
and is now in general use. The equipment needed is portable and thus
suitable for mobile sessions. From those failing this test a small venous
sample should be collected into anticoagulant for examination in the labor-
atory by alternative methods of haemoglobinometry and for stained film
examination, if indicated.

Syphilis. A screening test for syphilis should always be made. One
sensitive flocculation reaction is usually sufficient, e.g., the VDRL test,
the Kline test, or other cardiolipin reaction. Unless the result is negative
a full investigation should be made (see Chapter 2, p. 25).

Tropical diseases. In the case of donors resident in tropical areas, or
recently returned from such areas, blood film examination for parasites

and a Westergren sedimentation test should be carried out. Persons with blood parasites or a high sedimentation rate (over 20 mm in the first hour for men, and over 30 mm in the first hour for women) should be excluded.

Other examinations. On occasion the medical history or examination may indicate that additional examinations should be made before accepting a person as a donor, e.g., urinalysis, biochemical examination of the blood, X-ray.

In all cases it is the responsibility of the medical officer in charge of the donor session to decide whether or not an individual is fit to give blood. Those who are rejected should be tactfully informed of the reason and if necessary referred to their own doctor. In this way the blood transfusion service can play a helpful part in the supervision of public health.

Donor Records

All transfusion centres usually have at least one thing in common : the volume and scope of their work tend to expand rapidly beyond the limits originally foreseen. It is important, therefore, that donor record and office filing systems should be so designed that they can be expanded without being basically changed. From only a few hundred records, the number may grow in a few years to tens of thousands.

Anyone responsible for the introduction of a new transfusion service is advised first to study the systems used at several established centres. It is suggested that at least the following records on donors should be maintained.

The master index

Small cards (e.g., International Paper Size A6) may be used to maintain an alphabetical index of every donor. A card should be issued only after the first donation has been given and need contain only the following data :

Mr, Mrs, Miss
Family name
First names

ABO and Rh groups	Cards may be printed in different colours corresponding to blood groups (see p. 36).
Date of invitation to give blood	The use of a date stamp with two different coloured inks, one to indicate an unanswered call and the other an actual donation, is helpful.

A master index on these lines gives sufficient information to permit immediate identification of a donor, to determine his eligibility for further donation, and to permit his personal record card to be found, when required.

It may sometimes be convenient to allot a personal number to each donor, but identification of donors should never rely solely upon a numerical system because such systems are liable to error.

The master index should never be removed from the organizing office.

The donor's record card

This card is the essential record of the donor panel department. It is larger than the master index card (e.g., International Paper Size A5) and shows all the important administrative details. Cards may be filed vertically or horizontally, with indicator clips attached to the edge to show different classifications of donors, e.g., those available for further donation. Alternatively, the cards of all donors bled at the same time, e.g., on the same day or in the same week, may be kept together so that their future availability can be predetermined; this system saves a great deal of extra sorting and filing and is readily combined with the use of the master index card which is brought up to date after each donation.

The obverse of the donor's record card should have space for at least the following:

Mr, Mrs, Miss   Private address
Family name   Telephone number (private and place of work)
First names   Profession or occupation
Date of birth   Doctor's observations
ABO & Rh group (cards   Preferred times for giving blood
    may be printed in dif-
    ferent colours corre-
    sponding to blood
    groups—see p. 36)

The reverse of the card is reserved for recording donations and should have spaces (usually columns) for:

Date of donation   Remarks (e.g., on state of veins, fainting,
Volume collected   haematoma)
Haemoglobin concentration
Result of syphilis test

These data permit a rapid assessment of the availability of each donor and the elimination of those who, for administrative or medical reasons, should no longer be requested to donate blood.

The medical examination sheet

Depending on local circumstances (see Chapter 3 p. 30), it may be possible for the necessary medical details to be entered on the donor's record card, but separate medical examination sheets may be preferred. In either case the details are confidential, and this should be indicated on the record.

The donation register or blood collection record sheet

This is completed by a clerk during the donor session and is retained at the centre as a record. An example is shown in Fig. 1. Such sheets

FIG. 1. BLOOD COLLECTION RECORD SHEET

Date ....   Place of blood collecting session ....

Team No. ....   Clerk ....   Doctor ....

Surname and Initials (Block Capitals) State Mr, Mrs, or Miss	FULL POSTAL ADDRESS	Volume	Comments	Bottle No.	For Laboratory Use Only							
					Cell Group	Serum Group	Rh	O Cells	O Serum	Haem.	Blood Bank	Remarks

should be completed for each blood collecting session, showing the date and place of collection. Against the reference numbers of the bottles of blood are entered the family names and initials (or first names) of the donors concerned. In the columns headed "Comments" and "Remarks" may be noted any abnormal event or special observation, e.g., that a donor fainted or that a donation should not be used for transfusion. It is desirable to complete the record sheet in the laboratory by entering the results of the tests performed on each donation. These results should also be entered on the corresponding donor record cards and compared with previous results (if any); any discrepancy found should be investigated at once. This co-operation between the donor registration department and the laboratory is essential for an efficient service.

Donor's personal card or book

This document is given to the donor after the first donation and confirms his membership of the panel of the blood transfusion centre concerned. It is usually folded double to wallet-size. It records the donor's title (Mr, Mrs, Miss), family name, first names, date of birth, and address, and also clearly indicates the donor's ABO and Rh groups. It may be printed in different colours corresponding to blood groups (see below). The card should bear the date of issue and the stamp of the blood transfusion centre. Each blood donation may be entered directly or recorded on a certificate to be stuck in the book or card, which may then be used as a check before awarding badges. Donors are usually proud of this document. For this reason and also because it is a means of publicity for the service, it should be well produced.

Colour code to denote the ABO blood groups

There is no internationally accepted colour code for blood groups and it seems unlikely that agreement on one will be achieved. The existence of different colour codes is a potential source of danger, for example, when staff move to work in another country or when blood is sent from one country to another. In some instances, blood of the wrong ABO group has been transfused because the person giving the transfusion was accustomed to a different colour code.

Donor records

Within the transfusion centre the handling of donor records is greatly facilitated by adopting a four-colour code to denote the ABO groups, particularly if it also includes a simple means of differentiating Rh-negative blood, such as a vertical red bar. The choice of colours for use in a trans-

fusion centre is essentially an internal matter, but it is highly desirable to adopt a system already used by other transfusion centres or services in the same country. The ABO group letter or letters should be printed in large clear type whether a colour code is adopted or not.

Container labels

On the other hand, if the use of a colour code is extended to the labels of blood containers it is essential, if other transfusion centres or services already exist in the country, that the same code should be used not only within the country but also, if possible, in neighbouring countries with which blood may be exchanged. If such uniformity cannot be achieved, the organizers of a new transfusion service should hesitate before adding to the profusion of colour codes and consider using container labels in black type on white paper, bearing the ABO group letter or letters printed the full width of the label. These letters may be superimposed in outline across the other printed matter. (The ABO group letter or letters should also be printed in large clear type on coloured container labels.)

The problem may be simpler if no other transfusion services already exist in a country. It may then be decided to adopt the colours already in use in neighbouring countries, if these are uniform, but before doing so the advantages of adopting labels printed in black type on white paper should always be carefully considered.

PART II

CENTRAL ORGANIZATION — THE PREPARATION
OF EQUIPMENT

Staffing and Accommodation

Since both the number of staff and the space needed will vary with the commitments of each transfusion centre, precise recommendations cannot be made. However there are certain basic requirements common to every centre. Whatever the size of the centre, there will be six main categories of staff:

1. *Medical staff* to interpret the requirements of the national service and formulate local policy for the centre, examine donors, collect blood, supervise the laboratory work, and advise hospital staff on blood transfusion matters.

2. *A donor organizer* to arrange publicity, recruit and call donors, and maintain records on them ; for these tasks clerical assistance will be needed.

3. *An administrative officer* to maintain staff records, order equipment, pay accounts and salaries, and be responsible for the cleaning and maintenance of the premises, etc.

4. *Donor attendants*, with a nurse in charge, to assemble the blood-collecting equipment and care for the donors.

5. *Laboratory staff* to prepare anticoagulants and other solutions, supervise sterilizing, perform bacteriological checks, and undertake serological tests and haematological investigations. Duties performed by less skilled staff such as washing of glassware and the storage and issue of blood and blood products are of such importance that they should be supervised by technically qualified staff.

6. *Transport staff* to take other staff and equipment to mobile blood collecting clinics and deliver blood from the centre to hospital blood banks.

A small transfusion unit attached to a hospital or clinic and collecting only a dozen or so donations each week would need a staff of four or five part-time workers (medical, technical, and nursing) ; arrangements would also have to be made for the clerical and administrative work. At the other end of the scale, large centres collecting one to two thousand donations each week require quite large departments staffed by full-time workers and may have 100-200 employees. The staff required for the actual laboratory tests is considered in more detail in Chapter 19.

The classification of staff into the six categories listed above is helpful whatever the size of the centre. Recommendations on the space required are less easy as so much depends on the equipment to be installed and the scope and organization of the work, e.g., whether all donations are collected at the centre. The following basic estimates for small centres (Examples 1 and 2) may be useful and can be modified according to circumstances. The space given in both estimates allows for a subsequent increase in the number of donations collected.

Example 1

A blood centre producing approximately 3000–4000 units of stored blood annually (about 10–15 daily) and located in a hospital, from which it receives certain services and facilities, will need the following space and staff:

Space	m^2
Waiting room	20
Doctor's room, medical examination room	20
Blood collection and rest room	30
Laboratory	30
Office records, etc.	30
Washing and sterilizing room	20
Miscellaneous (stores, toilets, circulation area, etc.)	50 (minimum)
Total accommodation	200 (minimum)

Staff

1 doctor (part-time)
1 laboratory technician
1 nurse (donor attendant)
1 clerk
1 cleaner (for glassware and domestic cleaning)
1 other employee (as local conditions require)

Example 2

An independent blood centre, producing approximately 6000–8000 units of stored blood annually (about 20–30 daily), will need the following space and staff:

Space	m^2
Waiting room	20
Examination room	15
Blood collecting room	30
Rest room	15
Donor canteen	20
Office, records, etc.	35
Laboratories	60
Doctor's room	15
Staff room	20
Storeroom	30

Space	m^2
Washing and sterilizing room	30
Miscellaneous (toilets, circulation area, etc.)	50
Total accommodation . . . approximately	340

Staff

1 doctor (full-time)
1 assistant doctor (part-time)
3 laboratory technicians
1 nurse and 1 donor attendant
1 donor organizer and office administrator
1 secretary
2 cleaners
1 other employee (as local conditions require)

In both examples it has been assumed that all donors would attend at the centre. If this should not be possible arrangements will have to be made to collect blood from time to time at some suitable place outside the centre and for this purpose suitably equipped vehicles and some additional staff will be needed.

Example 3

The following example lists the equipment, staff, and transport needed to collect 48 units of blood in about 2 hours at a blood-collecting session organized away from the centre.

Equipment

4 couches [1] or beds for collecting blood
6 couches [1] or beds for resting
10 blankets, 10 pillows, 4 sphygmomanometers or tourniquets. Collecting sets and bottles with citrate; local anaesthetic, syringes, and needles; swabs, skin disinfectant; refreshments, cups, etc. (It is advisable to have enough of these items for 55 to 60 donors.)

Staff

1 doctor
1 nurse
1 laboratory technician or donor attendant (for measurement of haemoglobin)
2 donor attendants
1 secretary or clerk
1 driver

Transport

Vehicle or vehicles to carry staff and equipment, which must include a 4°–6°C refrigerator to contain 60 bottles of blood.

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[1] The number of couches has been calculated on the assumption that, on the average, each donor will occupy a collecting couch for 10 minutes and a resting couch for 15 minutes.

At the other end of the scale, some of the new Regional Transfusion Centres in the United Kingdom, which are designed to collect, test, and distribute 120 000–150 000 donations per annum, have a total area of 4000–5000 m². This includes laboratories for testing prenatal blood specimens and for preparing blood-grouping sera and cryoprecipitate, animal houses, etc.

Preparation of Containers, Closures, and Anticoagulants

It is not always possible to give precise data or specifications concerning the preparation of containers, closures, and anticoagulants. For example, washing agents differ in composition and name from country to country so that there is no point in mentioning a particular product. The same applies to test regulations. Precise details regarding the testing of glass, quality of chemicals, the preparation of anticoagulant solution, pyrogen and toxicity testing, and methods of sterilization may be found in the *International Pharmacopoeia* (World Health Organization, 1967) and various national pharmacopoeias, whose specifications are binding in their own countries and have been adopted and recognized, in part, by other nations without pharmacopoeias of their own. Reference will be made to these pharmacopoeias, but no one pharmacopoeia should be regarded as the sole criterion.

The International Standard entitled *Transfusion Equipment for Medical Use* (International Organization for Standardization, 1966) should be used as a reference. This Standard has been formulated by a technical committee including representatives of Australia, Canada, Denmark, Finland, France, Germany, Israel, Italy, the Netherlands, Norway, Portugal, South Africa, Sweden, Switzerland, the United Kingdom, and the USA. In addition, Argentina, Austria, Belgium, Chile, Czechoslovakia, Greece, India, Iran, Ireland, Japan, Mexico, New Zealand, Pakistan, Poland, Romania, Spain, Turkey, and Yugoslavia are " observer " members. This committee has the support of WHO, the International Society of Blood Transfusion, the International Committee of the Red Cross, and the League of Red Cross Societies.

The object of the Standard is to define certain key dimensions and minimal standards of performance for transfusion equipment, in order to make such equipment interchangeable between different countries and satisfactory in use. The Standard, which covers glass containers, bottle closures, and screw caps, and both disposable and reusable taking and giving sets, is obtainable from International Organization for Standardization, 1 Rue de Varembé, 1211 Geneva 20, Switzerland.

with distilled or demineralized water (pyrogen-free). Dry at 60°C and store in closed dust-free containers.

Alternatively, soak for 24 hours in 2% hydrochloric acid, rinse overnight in running water, boil in clean tap water for 20 minutes, rinse with pyrogen-free distilled water or demineralized water, and dry and store as above.

The use of a washing-machine and a suitable detergent may render these time-consuming treatments unnecessary.

When preparing bottles containing anticoagulant, it is convenient to keep the stock of rubber diaphragms or stoppers in pyrogen-free distilled or demineralized water in order to minimize contamination with dust. Any unused closures must be removed from the water, drained, dried, and returned to closed storage.

Screw caps

Screw caps are used to secure the diaphragms and stoppers. If these are supplied dirty or greasy they must be cleaned with a suitable detergent, thoroughly rinsed in water, and dried.

Pilot tubes

Shape

So that a sample need not be removed from the actual stored blood for carrying out compatibility tests, a pilot tube is supplied with each anticoagulant bottle. This is usually a thick-walled test tube or small bottle of 5-ml or 10-ml capacity which contains 1 ml or 2 ml respectively of anticoagulant solution and is closed with a rubber stopper that can be pierced. The tubes are evacuated so that they can be filled with blood (from the taking set) without removing the stopper.

These tubes must be firmly attached to the bottles. There are also specially constructed types combined with the bottle closure.

Quality

Although the contents of the pilot tube are not used therapeutically, good quality glass and rubber are important, as test results may be upset by a high alkali content of the glass and by poor quality rubber.

Cleaning

As for bottles and rubber closures.

Evacuation

Pilot tubes should be evacuated before sterilization, through a fine-bore needle attached to a mechanical vacuum pump.

The anticoagulant solution

Only acid citrate dextrose solution (ACD solution) is described, since it is used in almost all transfusion services for preparing stored human blood for general use and gives the best *in vitro* and *in vivo* survival of red cells. Citrate prevents coagulation by removing ionized calcium, and glucose provides the erythrocytes with a suitable medium for their metabolism (anaerobic glycolysis).

ACD-preserved blood is suitable for use for at least twenty-one days after donation, provided it is stored continuously at 4°C ($\pm$ 2°).

Chemicals

Since glucose has a tendency to caramelize during sterilization, particularly in alkaline media, the anticoagulant solution should have a slightly acid reaction. This is produced by using disodium hydrogen citrate, or trisodium citrate with citric acid, as a source of citrate.

Trisodium citrate ($C_6H_5Na_3O_7 + 5\frac{1}{2} H_2O$) and citric acid ($C_6H_8O_7 + H_2O$) are standard substances appearing in practically all pharmacopoeias. Disodium hydrogen citrate ($C_6H_6Na_2O_7 + 1\frac{1}{2} H_2O$) is much used in English-speaking countries and is described in the *British Pharmaceutical Codex* (Pharmaceutical Society of Great Britain, 1968).

Glucose (dextrose) $C_6H_{12}O_6$ is described in all pharmacopoeias, which also give full qualitative and quantitative tests.

Distilled water

Unless the water is particularly soft, it is advisable to soften it before distillation if possible, since this greatly reduces calcareous deposits in the distillation apparatus. For producing small amounts of distilled water, glass equipment is usually employed, whereas for large quantities, stills made from tinned copper, for example, can be used, or any of the other types of still available commercially. All stills must be adequately baffled to prevent the carry-over into the distillate of any unvaporized droplets which may contain pyrogens. The distillate must meet the physical and chemical requirements and pass the test for freedom from pyrogens described in one of the pharmacopoeias already referred to.

Distilled water receivers and any tubing leading from the stills to the receivers must be scrupulously clean. The safest course is to clean and sterilize this equipment daily.

In order to prepare pyrogen-free solutions, the proliferation of pyrogen-producing bacteria in the distillate must be prevented. This can be achieved:

(*a*) by using the water, immediately after distillation, to prepare ACD and other parenterally used solutions, which are then immediately sterilized in their final containers;

(b) by sterilizing the water, immediately after distillation, in suitable containers and keeping these under sterile conditions until the water is needed for preparing solutions (which, when made, must be sterilized)— this method is less satisfactory than (a) and is not recommended ;

(c) by cooling the distillate to 80°C and storing it at this temperature— this method is suitable only when large amounts of water are being used.

The chemicals (citrate, glucose, sodium chloride) used for preparing solutions are usually pyrogen-free ; pyrogenic batches are occasionally encountered and it may be necessary to test the raw materials when investigating the cause of pyrogenicity in solutions.

Demineralized water

Opinion is not unanimous on the use of demineralized water prepared by ion exchange for infusion solutions. Certain pharmacopoeias do not permit its use for this purpose.

Composition of solution

The composition of ACD solution varies, often within the same country. Some of the more widely used formulae are shown in Table 2.

All the pharmacopoeias referred to in the table include instructions for preparing and testing ACD solutions.

TABLE 2

COMPOSITION OF ACD SOLUTION

	US Pharma- copoeia a	Scandinavian Pharma- copoeia b	Swiss Pharma- copoeia c	British Pharma- copoeia d
Glucose	2.45 g	1.47 g	2.50 g	3.00 g
Disodium hydrogen citrate	—	—	—	2.0—2.5 g
Trisodium citrate	2.20 g	1.32 g	1.60 g	—
Citric acid	0.89 g	0.48 g	0.47 g	—
Distilled water	100 ml	100 ml	100 ml	120 ml
Ratio of ACD vol. to whole blood	75 ml/500 ml	125 ml/500 ml	100 ml/400 ml	120 ml/420 ml

a United States, Pharmacopeial Convention, Inc. (1965). The composition given is that of Solution A.

b Scandinavia, Nordic Pharmacopoeia Council (1963). The composition given is the same as that of Solution B in the US Pharmacopoeia.

c Switzerland, Commission fédérale de la Pharmacopée (1934).

d Great Britain, General Medical Council (1963).

Preparation

The method employed to prepare the solution depends on the quantities involved. Small amounts (up to about 10 litres) can be prepared by weighing out the ingredients directly and dissolving them in hot distilled water.

When larger volumes are required, it is advisable to make up concentrated stock solutions, the concentration of which may advantageously be 10 or 20 times that of the dilute ACD solution. For example, glucose and citric acid can be combined in one stock solution and trisodium citrate in another. A concentrated solution of glucose alone cannot be sterilized without causing excessive caramelization. Since the concentrated stock solutions are viscous it is an advantage if the mixing vessel is heated and provided with a stirrer to accelerate solution. The stock solutions must be filtered through sintered glass filters (G3 or G4 grade), after activated charcoal has been added to absorb any pyrogenic impurities present. Other filters (e.g., Seitz filters, sintered metal filters) can be used if they yield a filtrate free from suspended matter. The filtered stock solutions are bottled and sterilized.

Final ACD solution is made by mixing and diluting appropriate volumes of the stock solutions.

The dispensing of ACD solutions into bottles can be carried out by means of gravity or compressed air. An automatic dispensing machine is an advantage, particularly in preparing large batches. Provided the stock solutions and the distilled water used for dilution are free from suspended matter, and air-borne contamination can be controlled, the final ACD solution need not be filtered.

Sterilization

The stock solutions and the filled ACD bottles should be autoclaved immediately after preparation. Maintaining the fluid temperature at 120°–125°C for 20 minutes is a satisfactory sterilizing procedure.

After the bottles have been sterilized they must be cooled to below 80°C before removal from the autoclave.

The use of modern " fluids " autoclaves, in which the sterilized bottles are rapidly cooled with cold water sprays whilst under compressed air pressure, accelerates the cooling process so much that a batch of 250 bottles can be loaded, pre-heated, sterilized, cooled, and removed from the autoclave in less than an hour and a quarter, compared with 10–12 hours by the older methods. This rapid cycle permits the installation of fewer or smaller autoclaves than might otherwise be required.

It is now generally accepted that if autoclaves are properly maintained and tested at regular intervals and fitted with adequate temperature recorders and pressure gauges which provide a record of each sterilizing run, no routine biological tests are necessary. From time to time tests can be made using chemical/physical indicators and bacterial spore-papers

packed within the load. With less adequate equipment, routine sterility tests may be indicated. Thioglycolate broth incubated at 20°C and 37°C is a suitable culture medium for test organisms.

Final control tests

Pyrogens. If initial tests have shown the chemicals to be pyrogen-free and routine control tests on the distilled water are satisfactory, pyrogen tests on the sterilized ACD may be omitted. If pyrogen tests are to be carried out, it should be borne in mind that injection of untreated ACD solution often produces a violent reaction in rabbits. Before injection into rabbits, it should therefore be mixed with 20% calcium gluconate (one part of calcium gluconate to four parts of ACD solution) and brought to pH 7 by adding sterile pyrogen-free sodium hydroxide solution, drop by drop, using phenolphthalein indicator.

Suspended matter. ACD bottles in which there are suspended particles visible to the unaided eye should be eliminated. The test is best carried out against a black background with light falling from above at an angle. It is difficult to give precise directions here, since the detection of suspended matter is to some extent subjective and calls for a certain amount of experience. Rejection of an unduly high percentage of bottles because of the presence of particulate matter suggests that certain measures should be taken, for example, the adoption of fibre-free protective working clothes, alternative methods of cleaning the rooms concerned, or air-conditioning. Another cause of deposit within the ACD bottle after sterilizing or after storage is breakdown of the internal surface of the bottle due to hydrolytic attack on the glass by the contents. In a dry bottle, this shows up as white etched marks on the inside surface which cannot be removed. Unfortunately these signs are not easily seen in a wet bottle but a deposit results in the solution after sterilizing. The bottles affected must be discarded. This complication can be greatly reduced by the use of a good quality boro-silicate type of relatively soda-free glass.

Protective caps and labelling

The sterilized bottles may be fitted with metal or plastic screw caps to protect the rubber closure. Tear-off caps or dust-covers are also fitted and the bottles labelled with the date of sterilization and the production batch number.

The bottle label should also indicate the number of ml of ACD solution in the bottle, its composition, and how many ml of blood should be added.

Storage

No particular precautions are necessary for the storage of ACD bottles although extreme fluctuations of temperature and prolonged exposure to

bright light are best avoided. Owing to the acid reaction of the solution (pH 5.5), any failure of sterility is likely to become apparent as a growth of mould. Some centres deliberately store their prepared bottles in a warm room (about 28°C) for a week or two before use, during which time any such failure becomes obvious on inspection.

Keeping properties. The properties of ACD solution are little affected by storage. After unduly prolonged storage, rubber closures will become hard and brittle and may no longer ensure a tight seal. Any vacuum in the bottle may then draw in contaminated air. The fact that the upper surface of a rubber diaphragm is concave does not necessarily indicate the persistence of a satisfactory seal—a partial leak may have occurred or the rubber may have taken a " permanent set ". Normally, the interval between production and use should not exceed a few weeks.

Use of plastic containers

Disposable plastic bags are now available as an alternative to the customary glass bottles.

Bags undoubtedly have some advantages. They weigh much less than bottles, take up less space, and need not be returned after use, so that washing is eliminated. No air-venting system is necessary during blood taking or giving and, because of the direct connexion between the bag and the blood-taking set, the risk of bacterial contamination is theoretically less. On the other hand, bags are expensive for routine use and there is as yet no evidence that blood keeps any better in plastic bags than in glass bottles. Some recent experience has suggested the possibility of contamination due to defects in plastic bags.

If it is decided to use plastic containers, it should be ascertained that the plastic fulfils the requirements for freedom from toxicity and pyrogenicity contained in Appendix 7 of *European Agreement on the Exchange of Therapeutic Substances of Human Origin* (Council of Europe, 1967) or documents of similar authority.

In most cases, the " home production " of plastic bags is out of the question, so that in practice it will have to be decided whether to rely upon a commercial source of sterile bags already filled with ACD solution, whether to procure empty bags and undertake the difficult task of filling, sealing, and sterilizing them, or whether to use glass containers.

Preparation of Taking and Giving Sets

Blood transfusion equipment may be made from components that may be used repeatedly, or it may be prepared for use once only. Alternatively some components may be disposable (e.g., tubing) and some re-usable (e.g., needles). Any component intended for re-use must be designed so that it can be thoroughly cleaned.

In some countries national standards exist only for sets of the single-use (disposable) type. This type is widely used but the decision whether to use disposable or re-usable equipment will depend on local circumstances. Although disposable equipment has certain advantages and is, perhaps, to be preferred, properly designed and maintained re-usable equipment is entirely satisfactory. The availability and cost of both labour and materials may well dictate which type will be adopted.

The design of the two types of equipment is basically the same, so that the diagrams given—Fig. 2, Fig. 3, and Fig. 4—are applicable to both. Full details on materials, dimensions, and the assembly and testing of disposable and re-usable taking and giving sets are to be found in the various national standards for transfusion equipment for medical use or in the International Standard obtainable from the International Organization for Standardization (for address, see p. 45).

Taking sets

Re-usable

Re-usable taking sets are almost always made with rubber tubing. Many manufacturers produce special tubing satisfying the requirements of the standards just mentioned. Plastic tubing is generally unsuitable for re-use since it will not withstand repeated sterilization. Silicone tubing is suitable, but comparatively expensive. If the tubing is not sufficiently transparent for the flow of blood or air bubbles to be seen, a glass tube "window" must be inserted about 10 cm from the taking needle. The overall length of the taking set should be not less than 61 cm.

Needles should be made of high quality stainless steel and have an olive-shaped hub or one of similar design to ensure strong airtight connexion with the rubber tube. A new taking (arm) needle should be ap-

FIG. 2. GIVING SET, ONE CHAMBER SYSTEM (UPRIGHT FILTER)

Needle cover ——————————— Cotton wool plug

Bottle needle —————

Needle hub ————————

Clamp

Filter chamber ————————

Filter ————

Latex tubing

Transfusion tubing ——————

Needle adaptor

Cap

Protective cap ——————

Protective cap

Air-inlet needle

Protective case ————————

Needle hub

Giving needle ————————

Air-inlet tube

Cotton wool plug ——————

Cotton wool plug

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FIG. 3. OTHER TYPES OF FILTER CHAMBER

Infusion   2 - chamber system   1 - chamber system
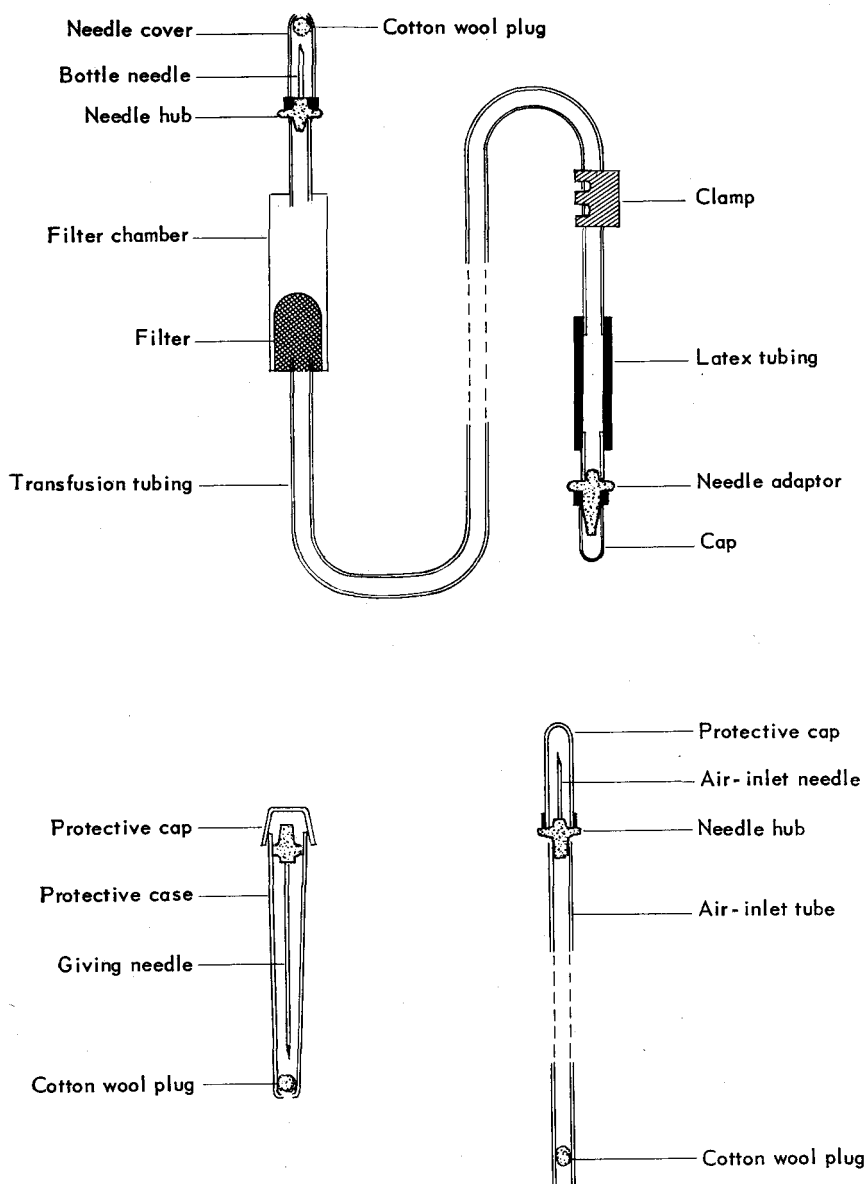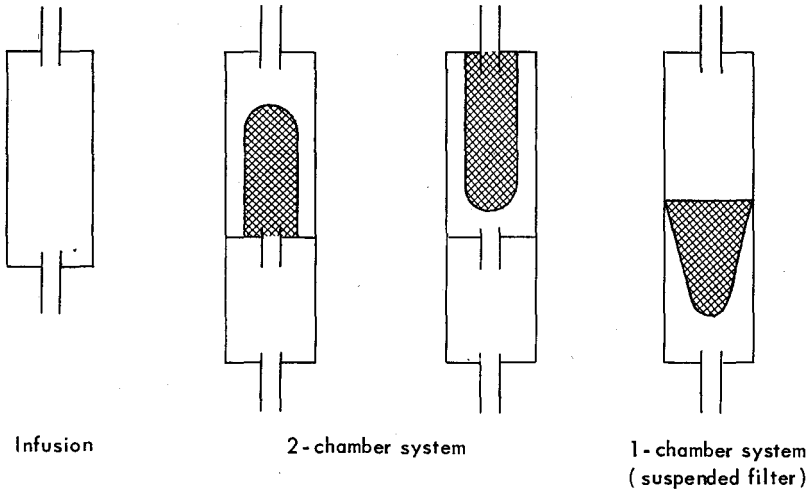(suspended filter)

proximately 2 mm in external diameter and, to allow for re-sharpening, not less than 40 mm in length. The bottle needle should be not less than 35 mm in length and should have a bore slightly greater than that of the taking needle, but its external diameter must not exceed 2.4 mm since it will be used with a self-sealing bottle closure. The air outlet needle must be significantly shorter than the bottle needle to avoid possible entry of blood into the air outlet during collection.

Needle caps may be made from glass or rubber, but the assembled sets must not be hermetically closed at both ends before autoclaving. At least one of the needle caps must be fitted with a cotton-wool plug so that air can leave and steam enter.

Disposable

The design and dimensions of the disposable taking set are the same as for the re-usable set. A glass window is not required with transparent tubing. Because plastic tubing lacks elasticity, needle mounts designed for use with rubber tubing are generally unsuitable. Needles with conical mounts designed specifically for use with plastic tubing are recommended.

Because disposable needles are expensive and rubber tubing is difficult to clean properly, a possible variation that might find favour in certain circumstances would be the use of carefully sharpened re-usable needles with disposable tubing—a " semi-disposable " taking set with the advantage of good, relatively inexpensive needles and cheap tubing which is discarded after use.

FIG. 4. TAKING SET

Needle cap

Taking needle

Needle hub with holder

Tubing

Needle hub

Bottle-needle

Needle cap

Cotton wool plug

Needle cap

Air-outlet needle

Needle hub

Air-outlet tube

Cotton wool plug
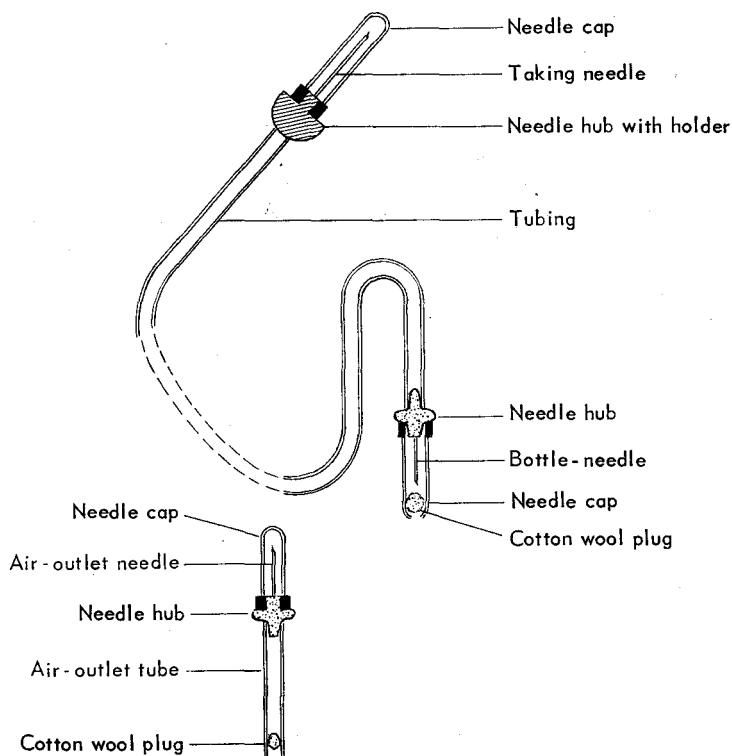
Giving sets

Re-usable

Each set should consist of two parts : (*a*) a closure piercing needle, a filter, a drip counter, and a length of rubber tube bearing a flow regulator, joined by a cone and socket joint to the arm assembly—a shorter section of tubing carrying a giving needle ; (*b*) an air-inlet assembly consisting of a separate air-inlet needle connected to a length of tubing fitted with an air filter.

The comments already made concerning materials and components for re-usable taking sets apply also to all parts of a re-usable giving set. The filter chamber is therefore generally made of glass, but provided with a removable rubber stopper at one or both ends to facilitate cleaning. The material of the actual filter may be re-usable wire gauze, but this is difficult to clean and keep free of pyrogens, and a disposable cotton or nylon filter on a rigid re-usable support, e.g., in nylon, is preferable. The filter should precede the drip counter.

Adjustable metal screw clamps are the best form of flow regulator.

The giving needle recommended for general use is one 35–40 mm long with an external diameter of 1.50 mm and an internal diameter (bore) not less than 70% of the external diameter, but other types of needle or a cannula may be used.

All cone and socket joints should have the Luer 6% conical taper fitting and the overall length of the giving set should not be less than 170 cm.

As transfusions may be given under pressure, the completed assembly should be airtight ; samples should be tested under water with compressed air, any leaks being revealed by the escape of bubbles.

The needle of the air-inlet assembly must extend at least 25 mm beyond the end of the closure-piercing needle fitted in position, and the tubing of the air-inlet assembly must be of such a length that the end carrying the filter can be fixed above the level of the blood to be transfused.

Disposable

The component parts are the same as for the re-usable giving set with the addition of a device for the injection of drugs or other solutions as the plastic tubing is not self-sealing. The air inlet and blood outlet may be combined into a single closure-piercing device. In other respects the dimensions and performance of disposable sets are the same as those of re-usable sets.

Bottle and air-inlet needles on the combined closure-piercing device may be made of plastic or metal. In some cases the shaft of the needle is of metal while the hub is made of plastic. Screw clamps may be made of plastic or metal.

Preparation of re-usable equipment

Cleaning

All items of re-usable equipment must be thoroughly cleaned. There are many satisfactory ways of doing this. One routine procedure is to boil all components for 20–30 minutes in 2%–5% sodium carbonate solution, rinse them, transfer them to 1% acetic acid, rinse them again, and then give them a final rinse in pyrogen-free distilled or demineralized water and dry them in a hot air oven. Another procedure is to wash the components thoroughly in soap and water or detergent solution, rinse them in distilled water, and dry them in an oven. The insides of needles and tubing should be cleaned with a " pull-through " and then thoroughly rinsed under pressure.

All new needles should have the lumen rinsed through before being put to use.

Plastic tubing may be used in association with re-usable components.

Plastic disposable tubing that has the ends capped at the time of extrusion should be absolutely clean (and virtually sterile internally); it will require no special treatment before assembly.

New disposable cotton or nylon filters may be heavily contaminated with dust and fibre, which can be removed by washing or shaking.

Assembly

Ideally, assembly should take place in air-conditioned premises. During the whole process of assembly the greatest cleanliness is essential, and staff should wear working clothing free from fibres (e.g., nylon). Equipment must be sterilized on the day of assembly in order to prevent the development of pyrogens.

Packing

Giving and taking sets must be packed and sterilized in such a way that the interior of each set remains sterile during storage and no part is deformed or kinked when the equipment is required for use. Cotton-wool filters used to maintain sterility must be non-absorbent and protected from becoming moist during storage.

Sterilization

The method of sterilization may depend on the material employed.

For re-usable equipment, steam sterilization in an autoclave is to be preferred. Steam under pressure at a temperature of 121°–125°C for 20 minutes should be used. Because transfusion sets basically consist of long thin tubes of material of poor heat-retaining property, with needles and/or filters at each end, they are amongst the most difficult pieces of medical equipment to sterilize, and even more difficult to dry afterwards in order to maintain sterility. Some centres therefore remove their sterilized sets from the autoclave while they are still moist and dry them in a hot-air oven with fan-assisted circulation. This unsatisfactory arrangement can be avoided by using autoclaves fitted with a special vacuum-drying cycle, during which the sets are flushed with hot sterilized air until dry.

Plastic tubing that is sufficiently heat-resistant can be sterilized by autoclaving. Not all types of plastic behave in the same way during autoclaving, but experience has shown that all polyvinyl chloride (PVC) tubing will withstand steam under pressure at 110°–115°C for 45 minutes, and that some PVC mixtures can be autoclaved at a higher temperature for a shorter time. Careful packing, in which the sets are interleaved with absorbent paper sheets to prevent coils of tube sticking to each other, is required.

Sets including parts made of material that is not heat-resistant cannot be steam-sterilized. In such cases gas sterilization, e.g., with ethylene oxide, or sterilization by radiation may be employed.

Testing

Pyrogen tests

After sterilizing, sample sets from occasional batches should be subjected to tests for pyrogens. Not less than ten sets should be washed through separately, at room temperature, with 40 ml of sterile pyrogen-free isotonic saline solution at a flow rate of approximately 10 ml per minute, and the effluent pooled. The solution thus obtained should then be used for a pyrogen test selected from one of the national pharmacopoeias.

Sterility tests

For equipment which has been steam sterilized in autoclaves, see the comments already made concerning sterility tests (Chapter 6, p. 52). After any other method of sterilization, samples from each batch should be subjected to bacteriological sterility tests. In addition to the use of spore papers, sterilized sets should be rinsed through under aseptic conditions with sterile water, samples of which are then cultured in thioglycolate at 20°C and 37°C.

Storage

After sterilization, sets must be labelled with the date of sterilization and a sterilizing batch number. The dry sterilized sets may then be stored, for many months, provided they are protected from dust, strong sunlight, and extremes of temperature.

In no circumstances should attempts be made to clean, re-assemble, resterilize, and re-use equipment designed specifically for use on one occasion only.

PART III

BLOOD COLLECTION AND STORAGE

Conduct of a Donor Session

In permanent donor clinics, the following procedure may be adopted.

The donor is first registered. At his first attendance, a clerk records the details needed to complete his personal record card after the donation has been tested in the laboratory and the results entered on the blood collection record sheet and returned to the donor records department (see Chapter 4, p. 33). Later, in the same office, a master index card will be prepared from the donor's personal record card.

In the case of a regular donor already on the register, his personal record card and any other documents (e.g., his medical examination sheet) are brought up to date at each attendance.

As a haemoglobin test is obligatory and the medical officer needs to know the result, it can conveniently be performed next and the result entered on the donor's documents before he has his medical examination. At the same time it may be desirable, depending upon the method of labelling the blood donations, to determine the donor's ABO group, if it is not already known, by a rapid (tile) method.

If the donor is passed by the medical officer, he is shown to the collection room and installed on a couch or reclining chair. Venesection is then performed by a doctor or by a qualified nurse, trained in the technique, working under the doctor's supervision. After the blood has been taken, the donor should remain recumbent for fifteen minutes; towards the end of this rest period the site of venepuncture is inspected and a dressing applied. He is then given light refreshment and a drink, which he must take sitting down in the rest room. This intake of fluid and the time spent sitting down—which should be at least ten minutes— play an important part in adjusting the circulation after donation. Should any donor refuse to stay for a reasonable time, a note to that effect should be made on his records and witnessed by the medical officer in charge, in case the donor experiences any untoward effects after leaving the clinic.

In mobile clinics, i.e., when the blood collection takes place in premises not permanently equipped for the purpose, the procedure is the same, but has certain special features.

In some cases, the success of the operation may depend on thorough preparations by a local voluntary organization, such as a Red Cross section, and its close co-operation with the mobile team (doctors, nurses, drivers,

and aides) from the blood transfusion centre. The local organization may be asked not only to recruit donors but to suggest suitable premises; it is important to have sufficient seats and waiting-room space, cloakroom and toilet facilities, and adequate heating and lighting when required. Large operations will run more smoothly if arrangements can be made for the donors to come at specific times.

The donors pass from registration to medical examination, to the blood collection room, and then to rest and refreshment.

With a steady flow of donors there is greater danger of confusion and steps must be taken to see that no donation can be attributed to the wrong donor. One well-tried system is for the clerk performing the registration to allot each donor a bottle number for the donation he is about to give. This number is printed in triplicate (or perhaps quadruplicate or quintuplicate according to local arrangements) on perforated gummed labels, one of which can be affixed during registration to the donor's personal record card and another to the blood collection record. (With well-trained and reliable clerks, of course, the number may be added to the records in writing.) During donation, the remaining copies of the label may be affixed to the bottle, pilot tube, and laboratory sample as required. If the donor fails the haemoglobin test, one label should be used to identify the venous blood specimen taken for further investigation. The donor may be given bottle number labels to take to the collection room and, if desired, his name may be written on the one to be attached to the laboratory sample (but *not* on the label that is to be attached to the actual blood donation). An alternative procedure is to write the number on the donor's hand or arm with a wax pencil. To avoid any possibility of mixing up bottles, the bottle number label and laboratory sample label should be attached at the couch-side immediately after insertion of the needle. In the unlikely event of needing to change the taking set, or if only a few ml of blood are obtained, all containers must be identified. The pilot tube should be fixed to the bottle so that it cannot be easily removed and, depending upon the efficiency of this arrangement, may or may not need to be numbered. In any event the donation, pilot tube sample, and laboratory samples must all be adequately labelled at the couch-side before the bottle and samples are placed with other newly collected donations.

Technique of blood collection

During blood collection attention should be paid to the following points:

(1) prevention of bacterial contamination of the donation (careful disinfection of the skin, " no touch " blood-taking technique, disinfection and sealing of the bottle top before refrigeration);

(2) preservation of the quality of the blood (adequate mixing to avoid any coagulation, optimal proportion of blood and anticoagulant) ;

(3) avoidance of discomfort to the donor (good blood-taking technique, sharp needles, and use of local anaesthetic if possible).

To take blood the following equipment is needed :

(1) sterile containers with pyrogen-free anticoagulant solution ;

(2) sterile pyrogen-free taking sets ;

(3) an efficient and non-irritant antiseptic (tincture of iodine, organic mercury disinfectant, quaternary ammonium compound, 70 % alcohol) ;

(4) sterile swabs, dressings, bandages, and adhesive plaster ;

(5) rubber tourniquet or sphygmomanometer, elbow pad, and hand grip ;

(6) local anaesthetic and syringes, capsules with needles, or multi-dose compressed air injectors.

Blood is withdrawn as follows :
The bottle is prepared for receiving blood. The cover over the screw cap is removed and the rubber diaphragm swabbed with antiseptic. The needle of the air-outlet assembly is first pushed into position through the rubber closure so that atmospheric pressure is equilibrated in the event of the bottle having a negative pressure after sterilization, and the bottle needle of the taking set is then inserted. It should be recalled at all times that the parts of the apparatus that come into contact with the blood (taking needle, interior of taking set, and bottle) must remain sterile. After preparation of the bottle, the donor's arm is prepared. A hand grip and a pad behind the elbow to assist full extension are valuable aids. Venous stasis is produced in the donor's arm in such a way that the veins of the antecubital fossa are distended ; arterial circulation should not be interrupted. A type of padded tourniquet may be used, but ideally an aneroid sphygmomanometer cuff should be positioned round the upper arm and inflated to an indicated level of 80–100 mm Hg. The antecubital fossa is then swabbed with a liberal quantity of antiseptic. After this has had time to take effect, any excess is removed with a sterile swab and a local anaesthetic may be given. The cleansed skin must not be touched after this stage.
The needle is then inserted into the vein.
When this has been done the donor should loosen his grip and the pressure in the cuff should be reduced to, and maintained at, about 40 mm Hg until just before the needle is removed.
After filling the bottle, the pilot tube and laboratory samples must be obtained. The blood remaining in the taking set should be used for

this purpose. After the needle is removed the point of venepuncture is compressed with a sterile swab, and pressure is maintained by the donor during his rest period, towards the end of which, when bleeding has ceased, a sterile dressing and bandage are applied.

Technical difficulties during blood collection

The air filter may be blocked if it is moist or too tightly packed. In such cases the increasing compression of the air in the bottle slows, and may eventually stop, the flow of blood. If the tourniquet is released in such circumstances, there is a possibility of air from the bottle entering the vein and producing an air embolism which may endanger the donor's life (see Chapter 2, p. 22).

Other causes of interruption in the flow of blood may be :

1. Too much compression of the upper arm obstructing arterial circulation.

2. Formation of a haematoma if the needle has pierced the opposite wall of the vein.

3. The bevel of the needle lying against the vein wall ; this is easily remedied by slightly moving the needle.

4. Spasm of the vein in cold weather, which can be remedied by resting the arm on a hot water bottle.

5. Clotting in the needle or tube.

6. Primary fainting symptoms.

For details on donor reactions during donation and on treatment of the immediate complications of blood collection, see Chapter 2.

Labelling of donations

The use of printed gummed labels giving a " bottle number " for each donation has been described above (p. 66). In many cases these are likely to be plain labels (e.g., white with black print) so that another label indicating the ABO and Rh group and repeating the bottle number will need to be affixed to the bottle before issue. It is possible to arrange for the numbered label used at the blood collecting session to be the final bottle label, if the donors have been bled before and their groups are known, or if new donors are " tile grouped " before donation. This procedure reduces the possibility of error when re-labelling all the bottles after collection and testing, but it does not absolve the centre from the responsibility of grouping every donation, no matter how many times the donor may previously have been tested. The use of colour coded bottle labels is discussed in Chapter 4.

The final bottle label must also give the address of the transfusion centre, the date of collection, the expiry date, instructions to store at $4 \pm 2°C$, and details of the volume and nature of the anticoagulant used. If so desired, it may also include instructions to check the ABO and Rh group on the label with the ABO group and Rh group of the recipient, to cross-match before use, and to transfuse through a filter.

Storage and Transport of Blood

All donations and samples of whole blood should be stored at a temperature of $4° \pm 2°C$. Lowering the temperature reduces the metabolism of the red cells, thereby improving their viability in the recipient. Freezing causes lysis of the red cells. As long as this is avoided, the lower the storage temperature the better the cell preservation. A temperature of $4° \pm 2°C$ is considered optimal and inhibits the growth of bacteria normally pathogenic in humans, should any minor degree of contamination have occurred in spite of the usual precautions. However, refrigeration is not in itself a complete safeguard against bacterial growth in blood, as some cryophilic organisms that are widely dispersed in dust, air, and water may multiply at this temperature. Although not normally pathogenic to humans they produce toxic products which can be lethal if transfused ; hence the importance of the meticulous sterilization and sealing of anticoagulant containers and taking sets, and the use of air filters and antiseptic precautions during blood collection.

Blood may be stored in walk-in cold rooms, in free-standing, reach-in refrigerated cabinets, or in refrigerators on vehicles. For transporting small volumes over short distances, use may be made of suitably constructed insulated containers, preferably with some form of cold source (eutectic solution or ice) which will maintain a temperature of $4° \pm 2°C$ for several hours.

The installation requirements of walk-in cold rooms and reach-in refrigerators are identical. A large dial-type thermometer indicating the air temperature within the refrigerator should be placed in a prominent position. Because the air temperature will fluctuate more rapidly and over a wider range than the temperature of fluid stored in containers, there should be a battery-operated audible and visual alarm designed to give the earliest possible indication of any undesirable change in the air temperature, whether this be due to electrical or mechanical failure, to loss of refrigerant, or to nothing more than a door left open. The use of batteries presents no problem as units are now available requiring re-charging no more than twice a year ; alternatively arrangements can be made for continuous automatic charging from the mains supply during normal operation, thus ensuring the availability of a fully charged battery in the event of

mains failure. It is desirable to extend the alarm indicator from the laboratory to some place likely to be staffed at all times, e.g., a telephone switchboard. In practice a lower air temperature of just below 2°C and an upper air temperature of 8°C have been found to be satisfactory levels for activating the alarm.

For the recipients' safety and for medico-legal reasons, it is also necessary to have a permanent record of the actual storage temperature of the blood. This can be obtained by immersing a sensor (thermocouple or thermometer bulb) in 500 ml of water in an appropriately placed standard bottle linked to a recording thermometer. The recorder should be driven by an eight day clockwork mechanism (i.e., entirely independent of the power supply to the refrigerator) and a seven-day circular chart is to be preferred to a strip chart so that any variations over several days may be seen at a glance. In some cases such variations may be retrospectively significant. The recorder should be mounted on an adjoining wall not subject to any vibrations from closing doors, etc.

To avoid accidental switching off or the disconnexion of a refrigerator's power supply, all free-standing blood storage refrigerators should be wired back to a fuse box or distribution board in a permanent way. If an ordinary socket outlet has to be used the plug head should be firmly taped in position. Switched outlets should not be used.

Refrigerators in vehicles should be designed so that they can be plugged into an electric socket when the vehicle is stationary. In addition, if the use of the vehicle justifies the extra cost, a small diesel generator can be installed inside or bolted to the chassis beneath the floor, to provide current when the mains supply is not available. Another arrangement, less expensive and suitable for smaller vehicles, is to install a compressor for the refrigerant driven by a belt from an electric motor plugged into the mains or alternatively by a single-cylinder petrol engine installed beside it. Any installation can be arranged so that the motor shuts off automatically when the required temperature is achieved. The use of a refrigerated water jacket on these cabinets allows a considerable " hold-over " period of stable low temperature while making accidental freezing of the blood almost impossible. For these mobile cabinets, rather less stringent alarms and controls are acceptable but at least a dial-type indicating thermometer should be provided.

Insulated boxes for the carriage of smaller volumes of blood need to be made of light-weight but durable material to facilitate movement and to minimize transport costs and damage in transit. In this respect the older type of plywood case with cork insulation is being replaced by cases of fibre, fibre glass, or plastic with an insulation of expanded plastic or plastic foam, e.g., polystyrene. Such cases, if equipped with an ice insert and particularly if kept in the cold room when not in use, will give satisfactory

" hold-over " (i.e., below 10°C) for at least 12 hours and possibly for over 24 hours depending on the ambient temperature. No container should have a capacity of more than 10 units ; anything larger becomes too heavy and bulky for convenient handling.

PART IV

LABORATORY ORGANIZATION AND TECHNIQUES

Organization and Management of a Blood Bank

Anyone who is to be responsible for the management of a blood bank should first be trained in a recognized institution where he can obtain first-hand knowledge of the problems he will meet. He will require, for reference, various books and journals (see list of references, p. 131); for the investigation of more complex serological problems, he will need the help of a blood group reference laboratory.

A series of serological techniques, which form the basis for all the bench work, are set out at the end of Part IV. They are described in deliberately simplified form, and it is expected that, as a transfusion service develops and its experience increases, use will be made of the refined techniques described in the more advanced books and papers in the list of references.

No other department in a pathological laboratory carries the same degree of responsibility as the blood bank, for in no other department is an error so likely to result in the death of a patient. It must be organized so that errors—clerical, serological, or other—will be detected before any harm has been done. Moreover, although the blood bank may not be directly responsible for the collection of blood specimens from patients or for giving transfusions, its staff should nevertheless be prepared to instruct clinicians and nursing staff on the part that they will have to play in the routine functioning of the bank, familiarizing them with the types of specimens required and with transfusion hazards.

The need for double checking

In the blood bank itself, if only one person had to bear all the responsibility both for setting up tests and reading the results, he would need to be superhuman to avoid errors, particularly as his services would be required at all times of the day and often at night as well. Every effort should be made, therefore, to divide this responsibility between two people so that each can check the other's work. In the ward, any blood specimen taken from a patient should be put into a container that has been previously labelled with the patient's full name, hospital number, and ward number, the name of the hospital, and the date. The procedure of taking the blood should be carried out by a medical officer, assisted by a member of the

nursing staff who should check carefully to see that the blood is put into the correct container. Similarly, when a bottle of blood is received from the blood bank for transfusion, two people should check that the blood is given to the person for whom it is intended.

Specimens—usually 5 ml of clotted blood—labelled as described above, should be sent from the ward to the blood bank, together with forms requesting that they be grouped and, if necessary, that the serum samples be used to perform compatibility tests on one or more bottles of blood. Details on a suitable form for this purpose are found in the chapter dealing with documentation (p. 110).

Use of the Pasteur pipette

When dispensing cells, sera, and antisera, a fine-bore pipette should be used for distributing equal drop volumes of the reagents. The volume of a single drop delivered from this pipette should be of the order of 0.03 ml. An even finer pipette with a drop volume delivery of the order of 0.01 ml should be used for dispensing rare antisera. For reading results, a pipette with a wider bore should be used to help preserve fragile agglutinates. A third pipette and a teat should be reserved by each worker for use solely with anti-human-globulin serum.

Care in grouping

The grouping tests can be conveniently divided between two workers. For example, in the case of ABO grouping, one can test for the presence of the agglutinogens and the other for the presence of agglutinins. In Rh grouping, two anti-D sera will be used, one by the first staff-member and the other by the second. Further details on techniques are given in Chapter 11 ; at this stage it is sufficient to say that the two workers should read their results independently and compare them afterwards. Should there be any discrepancies, then both sets of tests must be repeated. The responsibility of matching may likewise be divided, one worker reading saline and albumin results while the other takes charge of antiglobulin tests. Results of all tests, whether of grouping or of compatibility, must be recorded as described in the chapter on documentation (p. 106).

All blood grouping tests must be adequately controlled; in general, there should be at least one positive control to demonstrate that the serum is potent.

During the grouping of blood samples by a two-hour tube technique in a particularly dry tropical climate, it was noticed that water was evaporating from the red-cell serum mixtures so that the tests could not be read. All the capped tubes which contained tests were accordingly covered with a moist cloth to increase humidity, and no further trouble was encountered.

Storage of blood for transfusion

The collection and storage of blood from donors is dealt with in Chapters 8 and 9. The grouping will be the responsibility of the laboratory. The blood received from the blood-collecting team must be labelled, after testing, with the correct group, and the bottles stored in a refrigerator reserved for the purpose.

Blood containers in the bank should be clearly separated according to groups. Blood on which compatibility tests have been performed should be labelled with the prospective patient's name, and kept separate from other containers; outdated containers should likewise be segregated from all others.

Unless for some reason the blood is required for use absolutely fresh, all containers should be allowed to stand until the red cells have sedimented. Containers in which the plasma shows evidence of haemolysis or in which there is a change in colour of the red cells should be rejected. Plasma from time-expired blood may be used for the preparation of dried plasma, if local facilities exist for this.

Storage and use of antisera

Grouping sera should always be kept at −25°C when not in use, unless the instructions issued with a particular antiserum state that it should not be stored frozen. Like the bottles of blood, the antisera should be carefully labelled and always kept in the same place, known to all who need to use them, so that in an emergency they will be immediately obtainable. Every care must be taken to avoid contamination of antisera with other reagents or with micro-organisms. Before use, each reagent must be completely thawed and shaken. Instructions accompanying the antisera should always be followed. The principles of grouping described in the following chapters may be used, in the absence of specific instructions, as laboratory methods.

Grouping Techniques (ABO and Rh)

ABO grouping

Clearly labelled samples of clotted blood (5 ml) are needed for grouping and, in the case of patients, for compatibility tests. On receipt in the laboratory, each specimen is centrifuged at 1000–2000 rev/min for a few minutes, after which the supernatant serum is pipetted off into another suitably labelled tube. In the case of donors, 2-ml amounts of serum are set aside at this stage so that the appropriate tests for syphilis may be performed (See Chapter 2). The red cells will be used for agglutinogen tests, the serum for agglutinin and compatibility tests. Any serum not used for the tests should be stored at $-25°C$, and the red cell samples should be retained at $4°C$ for a few days for reference purposes.

A suitable quantity of red cells from each sample should be washed three times in an excess of saline and made up to a 5% cell suspension in saline. The instructions which normally accompany the antisera should always be followed. In the absence of instructions, follow the technique described below.

Technique

Basic Technique No. 1 (Chapter 23, p. 122) should be used. Precipitin tubes are arranged in two wooden blocks, one for the agglutinogen tests, and the other for the agglutinin tests, as shown in Fig. 5 and Fig. 6. Ideally one of the blocks should be set up and read by one worker, and the other by another, so that each may check the other's results. Apart from cells and serum samples from the specimens under test, known 0, A_1, A_2, and B red cells are required, as well as anti-A, anti-B, and anti-A plus anti-B (group 0) sera. It may be that the A subgroup of the cells available will not be known, in which case one batch of A red cells should be used regardless of whether A_1 or A_2 cells are specified. The A controls are arranged so that weaker A_2 cells check the anti-A and the anti-A plus anti-B (group 0) sera, while strongly reacting A_1 cells are tested against the anti-B serum to ensure that the latter does not, as a result of some error, react with A cells.

Equal volumes (approximately 0.03 ml) of the appropriate grouping serum and cell suspensions are put into the tubes as indicated. The optimum

temperature of incubation is about 20°C and results are read after 2 hours, the degree of agglutination being recorded in a suitable permanent register.

FIG. 5. ABO AGGLUTINOGEN BLOCK SET UP TO TEST RED CELLS OF FOUR SPECIMENS AND CONTROLS, WITH RESULTS a

	Red cells of specimen 1	Red cells of specimen 2	Red cells of specimen 3	Red cells of specimen 4	Known O cells	Known A_1 cells	Known A_2 cells	Known B cells
Anti-(A+B) serum in this row	0	c	c	c	0		c	c
Anti-B grouping serum in this row	0	0	c	c	0	0		c
Anti-A grouping serum in this row	0	c	0	c	0		c	0

Insert label here to identify block and state time of setting up tests and time at which they are read.

a c = complete agglutination v = visual agglutination 0 = no agglutination

The spaces on the block in which no symbol (c, v, or 0) appears were not used for the tests.

FIG. 6. ABO AGGLUTININ BLOCK SET UP TO TEST SERA OF 4 SPECIMENS, WITH RESULTS a

	Serum of specimen 1	Serum of specimen 2	Serum of specimen 3	Serum of specimen 4
Known O cells in this row	0	0	0	0
Known B cells in this row	c	c	0	0
Known A_1 cells in this row	c	0	c	0

Insert label here to identify block and state time of setting up tests and time at which they are read.

a See footnote to Fig. 5.

Recording results

Fig. 5 and Fig. 6 show the position of the precipitin tubes in the wooden blocks and also the results that might be obtained. The same results, as they might be recorded in the register, are given in Table 3.

TABLE 3

ABO GROUPING *a*

(Extract from register showing results of tests illustrated in Fig. 5 and Fig. 6)

	Anti-A	Anti-B	Anti-(A+B)	Serum check against A_1 cells	Serum check against B cells	Serum check against O cells
Specimen 1	0	0	0	c	c	0
Specimen 2	c	0	c	0	c	0
Specimen 3	0	c	c	c	0	0
Specimen 4	c	c	c	0	0	0
Red cells, group O (control)	0	0	0			
Red cells, group A_1 (control)	0					
Red cells, group A_2 (control)	c		c			
Red cells, group B (control)	0	c	c			

a c = complete agglutination   v = visual agglutination   0 = no agglutination.

Where no symbol (c, v, or 0) is given, the test was not performed.

As will be seen, specimen 1 is group O, specimen 2 is A, specimen 3 is B, and specimen 4 is AB.

Causes of anomalous results

False positive reactions. The sera may be infected, may be contaminated with another sample, or may contain an unexpected antibody or a rouleaux-maker (pseudo-agglutinin). The rouleaux formation of red cells is a phenomenon in which red cells collect in lines, like stacks of coins. Infected cell samples will sometimes agglutinate spontaneously.

False negative reactions. The serum may be inactive, may have been omitted from the tube, or may have a haemolysin that has destroyed red cells ; the red cells may not be in good condition ; or the technique may have been wrongly performed.

Emergency technique

The test should be set up as already described, and after a minimum of 15 minutes' incubation each tube should be centrifuged at 1000 rev/min for 2 minutes before reading microscopically as described in Basic Technique No. 1 (Chapter 23, p. 122). All such emergency grouping tests should be repeated, with the normal incubation period, to ensure that no error has occurred through using the shorter procedure.

Rh grouping

The number of investigations that may be performed in determining the Rh group vary greatly. In this manual only the Rh grouping problems of clinical significance will be discussed, i.e., those involved in the Rh grouping of the patient, of the donor, and of the husband of an Rh-negative woman whose child may suffer from haemolytic disease of the new-born.

Nomenclature

At the present time there are two main systems of Rh nomenclature : that based on the work of A.S. Wiener, and that based on the work of Sir Ronald Fisher. Here, the Fisher nomenclature will be used, according to which there are six common Rh antigens, called C, c, D, d, E, and e. It will be noted that the antigens fall into pairs, e.g., C, c. The antigens result from genes carried on the chromosomes of the nucleated cells, and these genes, which may for our purpose be given the same letters as the antigens they govern, are in a relationship of genetic allelomorphism. Thus a particular chromosome responsible for the development of Rh antigens will carry a C or c, a D or d, and an E or e gene. A sex cell possesses one such chromosome while body cells carry two, one inherited from each parent. Thus, for example, a child may inherit the combination CDe from its mother, and cde from its father, giving it the complete genotype of CDe/cde.

The hypothesis is that each of the antigens can, under suitable circumstances, stimulate the production of the corresponding antibody, though anti-d has never been detected. On the other hand anti-D is by far the commonest of the Rh antibodies. D-negative people are very likely to produce anti-D if they are transfused with D-positive blood. A D-

negative woman, immunized by previous D-positive transfusion or preg-
nancies, is almost certain to have anti-D in her serum, and this antibody
will react with the cells of any D-positive child she may bear, causing it
to suffer from haemolytic disease of the newborn. Details of tests for
haemolytic disease of the newborn are given in the chapter on prenatal
and postnatal serology (Chapter 21, p. 118).

" Rh-positive " patients are those who have been found to be D-positive,
and " Rh-negative " patients are D-negative. In a routine hospital labor-
atory, anti-D is usually the only Rh serum available, or indeed necessary,
for tests. Anyone tested with anti-D serum only and found negative will
usually be cde/cde, but may possibly be Cde/cde or cdE/cde ; in either case,
if he or she is to receive a transfusion, D-negative blood will be indicated.
On the other hand, the transfusion of blood from a Cde/cde donor to a
cde/cde recipient could give rise to the development of anti-C in the reci-
pient. Similarly cdE/cde blood could give rise to anti-E. Therefore, before
a donor is accepted as Rh-negative, he or she must be tested with anti-C,
anti-D, and anti-E sera and shown to react negatively with all three. Any
potential donor or recipient whose red cells fail to agglutinate in the pres-
ence of anti-D serum must also be tested for a D variant, known as D^u.
D^u is a weak D variant which may give rise to minimal, or no, agglutination
of red cells when suspended in anti-D serum. Nevertheless, such red cells are
antigenic, giving rise to anti-D when injected into Rh-negative recipients.
There is no specific anti-D^u antibody. Details of tests for D^u are given on
page 84.

Even if a donor's red cells do not react with anti-D, he or she will be
classified as an Rh-positive donor, if positive results are obtained with
anti-C or anti-E. This gives rise to an apparent anomaly, for such a person
is an Rh-negative (D-negative) *recipient*, but an Rh-positive (C-positive
or E-positive) *donor*. It might be argued that D-positive people should
receive only D-positive blood lest they develop anti-d, or that CDe/CDe
people should not receive cDE/cDE blood lest they develop anti-E. How-
ever, as antibodies other than anti-D develop only rarely and anti-d proba-
bly never develops, any further subdivision of groups as a routine measure
is neither practical nor necessary. The compatibility tests described later
(Chapter 13) will detect any untoward reaction should the patient already
be sensitized to one of these other factors.

For reasons more fully considered in the section on prenatal serology
(Chapter 21), it is sometimes necessary to know not just whether the hus-
band of an Rh-negative woman is Rh-negative or Rh-positive but whether
he is homozygous DD or heterozygous Dd Rh-positive. To determine this
may necessitate the use of some or all of the sera anti-C, anti-D, anti-E,
anti-c, and anti-e. Such work should if possible be left to a reference
laboratory. If further information is required, then a suitable work of
reference (e.g. Race & Sanger, 1968) should be consulted.

Grouping of patients

The technique will vary according to whether the anti-D available reacts in saline or in albumin. This information will normally be given in the instructions accompanying the antisera which should always be followed. In the absence of instructions, the following techniques should be used. In the case of a saline or an albumin anti-D serum, equal volumes (approximately 0.01 ml) of serum and red cell suspension should be used, and Basic Technique No. 1 or No. 2 (Chapter 23, pp. 122 and 123) should be followed. The incubation period is two hours in either case, and the temperature 37°C. The reagents required include a batch—or preferably two different batches—of anti-D serum, as well as red cells known to be D-positive (preferably Dd) and D-negative, to act as controls. If possible, the D grouping should be set up in parallel by two different workers, so that each may confirm the other's findings. The arrangement of the block is illustrated in Fig. 7. The results given should be compared with the extract from the register (Table 4); here two anti-D sera have been used, and the results from the two blocks are recorded. The tests indicate that specimens 1, 2, and 4 are D-positive, while 3 is D-negative.

FIG. 7. BLOCK SET UP FOR Rh (D) GROUPING OF PATIENTS, WITH RESULTS *a*

Insert label here to identify block and state time of setting up tests and time at which they are read.

Anti-D serum: v v 0 v v 0

Specimen 1, Specimen 2, Specimen 3, Specimen 4, D-positive cells, D-negative cells

a See footnote to Fig. 5, p. 79.

Emergency D grouping

Many anti-D sera will react after a much shorter incubation period than two hours, particularly if the tubes containing the tests are gently centrifuged. Instructions giving the necessary details for emergency use are frequently issued with the antiserum, but, if not, then incubation for 15 minutes, followed by centrifugation of the tubes at 1000 rev/min often gives clear-cut results. Great care must be taken to include controls, which

TABLE 4

Rh GROUPING OF PATIENTS *a*

(Extract from register showing results of tests illustrated in Fig. 7)

	Anti-D batch 1	Anti-D batch 2
Specimen 1	v	v
Specimen 2	v	v
Specimen 3	0	0
Specimen 4	v	v
D positive cells	v	v
D negative cells	0	0

a For explanation of symbols, see Table 3, p. 80.

must be treated in exactly the same way as the unknown sample, since the recording of false positive results is a recognized hazard with centrifuge methods. A confirmatory routine two-hour test must also be done. Where any doubt exists, the patient should be regarded as D-negative until proved otherwise.

D^u-testing

Unless the anti-D sera give clear-cut positive results with a particular sample of red cells, they should be tested by an indirect antiglobulin technique (Basic Technique No. 3 (*a*), Chapter 23, p. 124).

Controls should consist of known D-positive (heterozygous Dd-positive or D^u-positive if available) and D-negative cells. Albumin anti-D—preferably a serum specifically shown to react with the majority of D^u-positive cells by the indirect antiglobulin technique—is used as the grouping serum, with incubation at 37°C for two hours, before washing and testing with antiglobulin serum.

Rh grouping of donors

The potential donor's red cells are tested with anti-C, and anti-E as well as anti-D. Anti-C+D, anti-D+E, or a mixture containing all three antibodies are all suitable, as long as care is taken to ensure that

FIG. 8. BLOCK SET UP FOR THE Rh (C, D, AND E) GROUPING
OF DONORS, WITH RESULTS [a]

								:—	Insert label here to identify block and state time of setting up tests and time at which they are read.
Anti-E	0	v	0	v			v	0	
Anti-D	v	v	0	v		v		0	
Anti-C	v	0	0	v		v		0	

Specimen 1	Specimen 2	Specimen 3	Specimen 4	CDe/cde cells	cDE/cde cells	cde/cde cells

[a] See footnote to Fig. 5, p. 79.

antibodies for the three antigens, C, D, and E, are included in the combination of sera used. Tests should be performed in saline or albumin as recommended for each batch of serum. Any donor whose red cells give a positive reaction to any one of the sera must be classified as an Rh-positive donor. Red cells giving negative reactions with anti-C, anti-D, and anti-E must be tested for the presence of D^u, as just described, to make sure that they are not cD^ue/cde. Rare in Europe, this group is fairly common in Africa and in certain other parts of the world. Donors possessing the antigen D^u must be classified as Rh-positive.

TABLE 5

Rh GROUPING OF DONORS [a]

(Extract from register showing results of tests illustrated in Fig. 8)

	Anti-C	Anti-D	Anti-E	D^u Test
Specimen 1	v	v	0	
Specimen 2	0	v	v	
Specimen 3	0	0	0	0
Specimen 4	v	v	v	
Positive controls	v	v	v	v
Negative controls	0	0	0	0

[a] For explanation of symbols, see Table 3, p. 80.

Fig. 8 shows a block set up with tests and controls for grouping donors, using anti-C, anti-D, and anti-E sera, as well as the results that might be obtained.

The relevant part of the grouping register is shown in Table 5.

The tests indicate that specimen 1 bears the antigens CDe, specimen 2 cDE, specimen 3 cde, and specimen 4 CDE.

Causes of false positive and false negative reactions

These causes are the same as those listed in the section on ABO grouping. Haemolysis will occur only if the Rh antisera are infected with haemolytic bacteria, as Rh antibodies do not themselves haemolyse red cells. Errors in the indirect antiglobulin test are most frequently due to dirty apparatus, inefficiently washed red cells, or the use of antiglobulin serum that has lost its potency.

Screening of Donor Blood for Unwanted Antibodies

Detection of the dangerous group O donor

At one time it was thought that group O blood could be safely given to any patient, provided that the Rh group was compatible. It is now known that, even though the recipient's serum may not react with the donor's red cells, group O donor's plasma may contain potent anti-A or anti-B, capable of reacting with the red cells of a group A, B, or AB recipient and sometimes causing a severe transfusion reaction. The antibodies may take the form of high-titre agglutinins or of haemolysins.

Technique

Agglutinins. As a routine, the sera of all group O donors should be tested in the following way. Each serum is diluted 1 in 200 in saline and AB serum. To each of two precipitin tubes is added approximately 0.03 ml of serum diluted in saline ; an equal volume of a 5% suspension of A_1 red cells in saline is added to one tube and of a similar suspension of B cells to the other. Similarly, to one of a pair of tubes containing serum diluted in AB serum is added an albumin suspension of A_1 cells and to the other an albumin suspension of B cells. Before reading, the saline tests should be incubated for 2 hours at 20°C and the albumin tests for 2 hours at 37°C. If any agglutination of A or B cells occurs, the donor should be regarded as a dangerous group O donor, and his or her blood should be given only to group O patients.

Haemolysins. Haemolysins can be detected only in the presence of complement, which occurs in fresh serum. Unless the donor sample is less than 6 hours old, one cannot be sure that it still contains complement and it is therefore advisable to add to it an equal volume of fresh AB serum. To approximately 0.09 ml of the donor serum (fresh AB serum having been added or not according to the age of the donor serum) is added approximately 0.01 ml of a 5 % suspension of A_1 red cells in one tube and of a similar suspension of B cells in another. The two tubes are incubated at 37°C for two hours, the contents are tapped to resuspend the red cells in the serum, and the tubes spun at 2000 rev/min for two minutes. The supernatant serum is then examined with the unaided eye

for the presence or absence of haemolysins. Controls should be included, consisting of sera known to contain haemolysins. If any donor sample shows haemolysis, then the donor concerned should be considered a dangerous group O donor and his or her blood transfused only into group O patients.

Investigation of donors' sera for the presence of irregular antibodies

Not only anti-A and anti-B but other antibodies present in donor plasma may react with the recipient's red cells to cause a transfusion reaction. To detect such antibodies it is usual in some laboratories to test the donor's plasma against the recipient's red cells but this adds to the complexity of the matching test. To make this test as simple as possible, it is better to screen the plasma of all donors for the presence of blood group antibodies using the techniques for the detection of irregular antibodies described in Chapter 14.

Compatibility Tests

Compatibility tests are essential to ensure that a particular donation of blood may be safely transfused to a given patient. Normally blood of the same ABO and Rh group as that of the patient will be chosen. Occasionally it may be necessary to give a bottle of group O blood to an A or B patient, or A or B blood to an AB patient. Such a practice is not recommended, however, as it leads to excessive bleeding in donors of certain groups as compared with others and to the administration of agglutinins that are incompatible with the patient's cells. It is essential that Rh-negative females who have not reached the menopause should be given only ABO-compatible Rh-negative blood. Every effort should be made to give only Rh-negative blood to Rh-negative post-menopausal women and to Rh-negative male patients, but sometimes it may be necessary to select Rh-positive blood for elderly Rh-negative male patients and for nulliparous women past the menopause who have not had a transfusion before. The administration of even a small quantity of Rh-positive blood to an Rh-negative female child may sensitize her to the D antigen and may render her incapable of bearing normal healthy Rh-positive children free from haemolytic disease of the newborn.

The principle of the compatibility test

The donor's red cells are tested against the recipient's serum, to discover whether the latter does or does not contain antibodies which will react with the donor cells. If incompatibility is not detected, then it is likely that the donor blood, if transfused into the patient, will survive normally. A finding of incompatibility indicates that the transfusion of such blood is potentially dangerous, and further steps should be taken to identify the antibody.

Limitations of compatibility tests

It is emphasized that the compatibility test demonstrates only those antibodies present in the patient's serum at the time of testing and gives no indication of any sensitization that may follow the particular transfusion for which the tests are being performed. For example, the serum of an

Rh-negative patient who has not been previously sensitized will not contain Rh antibodies, but he or she is very liable to develop them if transfused with Rh-positive blood. The results of compatibility tests between Rh-positive red cells and the serum of such a patient will be entirely satisfactory, giving no indication of the danger that would result from the administration of Rh-positive blood and the consequent sensitizing of the recipient. In other words, the performance of a compatibility test does not in any way eliminate the necessity for accurate grouping of both donor and recipient. Similarly, ABO and Rh grouping of donor and recipient does not remove the need for a compatibility test ; antibodies to antigens other than ABO and D may be present in the serum of the recipient, and would be detected only by such a test.

Methods of compatibility testing

The methods recommended are :

    (a) saline agglutination technique at 20°C ;
    (b) albumin agglutination technique at 37°C ;
    (c) indirect antiglobulin technique at 37°C.

Saline agglutination technique

Basic Technique No. 1. (Chapter 23, p. 122) is followed. The red cells used are those of the donor samples, and it is useful to test the patient's red cells in the same way so that any unexpected clumping of the donor cells may be compared with the effect of the patient's serum on his own red cells. Occasionally the observation that the patient's own red cells are similarly affected is a great help in excluding a more serious cause, such as a specific iso-antibody causing true agglutination. The serum used is that of the patient, and, as any antibody present therein may be extremely weak, it is useful to use three volumes of serum to every one of red cell suspension. Incubation is for two hours at 20°C.

Albumin agglutination technique

Either Basic Technique No. 2 (a) or Basic Technique No. 2 (b) (Chapter 23, p. 123) may be used. As in the case of the saline agglutination technique, the use of three volumes of patient's serum to one of cells is recommended. Likewise, the patient's own red cells should be tested against his serum. The total incubation period is two hours at 37°C.

Indirect antiglobulin technique

The technique used is Basic Technique No. 3 (a) (Chapter 23, p. 124). The various samples of donor red cells (and the red cells of the patient)

are tested against the patient's serum, and once again three volumes of the patient's serum should be used per test. Positive and negative controls are included to ensure that the antiglobulin serum is potent. These consist of known D-positive cells incubated respectively with a weak pure incomplete anti-D and with AB serum.

Layout of blocks

Fig. 9, Fig. 10, and Fig. 11 show the three blocks that will be set up to match three bottles of blood for a patient, together with results that might be obtained.

FIG. 9. COMPATIBILITY TESTING:
SALINE AGGLUTINATION TECHNIQUE
AT 20°C, WITH RESULTS *a*

Insert label here to identify block and state time of setting up tests and time at which they are read.

Serum of patient: 0 0 0   0

Red cells of donor 1
Red cells of donor 2
Red cells of donor 3
Red cells of patient

a See footnote to Fig. 5, p. 79.

Recording results

Results obtained should be recorded in the appropriate compatibility register as shown in Table 6 (see p. 93). Red cells of donors 1 and 2 appear compatible, but the red cells of donor 3 react with the patient's serum by the albumin and indirect antiglobulin technique, and should not be used for this patient. It is nevertheless important that the antibody be identified (see Chapter 14, p. 94), since cells which react only very weakly with the antibody may in some future test be wrongly accepted as compatible, with serious results.

Emergency compatibility tests

Occasionally a patient will be in urgent need of transfusion, and the delay involved in the performance of the recommended grouping and

FIG. 10. COMPATIBILITY TESTING:
ALBUMIN AGGLUTINATION TECHNIQUE
AT 37°C, WITH RESULTS a

Insert label here to identify block and state time of setting up tests and time at which they are read.

Serum of patient

Red cells of donor 1	Red cells of donor 2	Red cells of donor 3		Red cells of patient
0	0	v		0

a See footnote to Fig. 5, p. 79.

FIG. 11. COMPATIBILITY TESTING:
INDIRECT ANTIGLOBULIN TECHNIQUE
AT 37°C, WITH RESULTS a

Insert label here stating time of setting up tests and time at which they are read.

Label all precipitin tubes to identify them after centrifuging.

Weak anti-D

AB serum

Serum of patient

				Red cells of patient	
				v	
				0	

Red cells of donor 1	Red cells of donor 2	Red cells of donor 3		Red cells of patient	Rh-positive cells
0	0	v		0	

a See footnote to Fig. 5, p. 79.

compatibility tests, which require two hours' incubation, might endanger life. Under these circumstances an emergency grouping method, as described on page 81, could be used, and an emergency compatibility test should follow. The tests are those described, except that the incubation period is curtailed (though it should be at least 15 minutes) and both

TABLE 6

EXTRACT FROM REGISTER SHOWING COMPATIBILITY TEST RESULTS [a]

Patient's name		20°C saline with patient's serum	37°C albumin with patient's serum	37°C indirect antiglobulin with patient's serum	Weak anti-D serum	AB serum
Miss A.	Red cells of donor 1	0	0	0		
5181	Red cells of donor 2	0	0	0		
	Red cells of donor 3	0	v	v		
	Red cells of patient	0	0	0		
	Controls for antiglobulin test				v	0

[a] For explanation of symbols, see Table 3, p. 80.

saline and albumin techniques should be used. The cell-serum mixtures, after incubation, are centrifuged at 1000 rev/min for not more than two minutes before the results are read. In the albumin test, the serum-albumin method—Basic Technique No. 2 (a)—should be used, as the albumin replacement technique is a two-stage procedure and cannot be carried out so quickly. Only in an extreme emergency should the use of group O Rh-negative blood be considered until the patient's ABO and Rh groups have been determined, and such emergencies are very rare indeed.

It is important to carry out the normal two-hour compatibility test in parallel with the emergency test, as weak antibodies may not be detected after a short incubation period. Clinicians should always be informed that such emergency procedures are not without risk to the patient and that they must accept responsibility for using blood selected at their request by these procedures.

Detection and Identification of Irregular Antibodies Using a Standard Red Cell Panel

Use of fresh and stored red cells

Any laboratory that is to undertake the identification of irregular antibodies must have a suitable panel of red cells fully grouped for as many antigens as possible. In the first instance, it is essential to have the red cells of members of the permanent staff fully grouped by a reference laboratory. In order to make cells of rare groups available at all times, an additional frozen cell panel is of great value. In a small laboratory, red cells of even common groups may have to be stored frozen. Such red cells may have been obtained locally, or may have been received, already frozen, from a laboratory elsewhere. The technique of preserving red cells in a frozen state will be discussed in the next chapter.

Cell groups to be included in panel

Most of the blood specimens constituting a standard control panel should be of group O, so that they will not react with the anti-A and anti-B that are likely to be present in most of the sera tested. Nevertheless, A_1, A_2, and B cells should also be available. For detecting Rh antibodies, cells of the genotypes CDe/CDe, CDe/cde, Cde/cde, cDE/cDE, cDE/cde, cdE/cde, cDe/cde, and cde/cde should be available, as well as C^w-positive cells, if possible. Other antigens and combinations of antigens which must be included among these cells are MM, MN, NN, S+, S—, P_1 and P_2, Lu (a+) and Lu (a—), Le (a+), Le (b+) and Le (a—b—), K+ and K—, and Fy (a+) and Fy (a—). This list does not cover all possible blood group systems or even all possible combinations within a particular system. It should be realized that it may take some years to accumulate an adequate panel such as that described above.

Techniques

The serum to be investigated is tested against the panel of cells, and also against the cells of the patient. If the patient's cells are agglutinated, a diagnosis of an auto-agglutinin or, alternatively, the presence of a non-

specific pseudo-agglutinating rouleaux-maker must be considered. An auto-immune acquired haemolytic anaemia may also present in this way.

The serum under examination is tested against each sample of red cells using the saline and albumin agglutination techniques at 4°C, 20°C, and 37°C, as well as Löw's papain technique, the two-stage papain technique, and the indirect antiglobulin technique, all at 37°C (see Basic Techniques No. 1—No. 4, Chapter 23, pp. 122–126).

Interpretation of results

An antibody may be identified by noting the conditions under which it is detected, the frequency of positive results in a random population, and the groups of the fully tested blood specimens with which it reacts. The frequency of positive reactions in a random population will vary in

TABLE 7

TYPICAL REACTIONS OF COMMONER BLOOD GROUP ANTIBODIES
OTHER THAN ANTI-A OR ANTI-B a, b

Antibodies	Optimum conditions for, and nature of, reactions
A_1	Reacts best in saline, 4°C to 20°C. Agglutinates A_1 cells only.
H or O	Reacts best in saline, 4°C to 20°C. Agglutinates O and A_2 red cells well, B less easily, and A_1 hardly at all.
Rh all antibodies	Optimum temperature 37°C. May react in saline, but nearly always reacts in albumin. Enzymes and antiglobulin techniques give good results.
M and N	React best in saline, 4°C to 20°C. Enzyme-treated cells fail to react. May sometimes be detected by the antiglobulin technique.
S	Usually reacts best at 37°C in saline or albumin. May be detected by antiglobulin technique.
P_1	Reacts best in saline and albumin, 4°C to 20°C. Detected by enzyme technique but not always by the antiglobulin test. On rare occasions has haemolytic activity.
Lu[a]	Usually reacts best at 4°C to 20°C in saline and albumin. Sometimes shows agglutinates among free cells.
Le[a] and Le[b]	React best in saline and albumin at 20°C and by enzyme techniques. Antiglobulin test positive especially if complement present (see Polley & Mollison, 1961). Haemolysin may be present.
K and k	React best by antiglobulin test. May sometimes be detected at 37°C in albumin or by enzyme technique.
Fy[a]	Antiglobulin technique only.

a Adapted from Stratton & Renton (1958).

b The examples given are for guidance only. Exceptions frequently occur. An antibody of a given specificity may occur in different forms in different patients, but a particular sample does not change its characteristics if stored at —25°C or below.

different parts of the world as the blood group frequencies themselves vary. For further information on blood group frequencies throughout the world, the reader is referred to Mourant (1954) and Mourant et al. (1958). Table 7 shows some of the commoner antibodies and their reactions.

Sera containing multiple antibodies

Not infrequently a mixture of antibodies is present in the serum. Some of the techniques involved in their separation and identification, such as absorptions and elutions, are beyond the scope of this manual, but the fact that a technique may be specific for only one antibody may enable that antibody to be identified among others. Complicated problems should be referred to a reference laboratory.

Preservation of Red Cells for Grouping

Advantage of fresh cells

Whenever they are available, fresh cells less than 24 hours old are to be preferred to stored cells, as their antigens are in better condition, and they are not liable to give false positive reactions. The Lewis antigens behave erratically after storage since they are attached only secondarily to red cells (Sneath & Sneath, 1955) from the plasma or serum, while some other antigens are weakened by storage.

Necessity for stored cells

Despite the disadvantages of stored cells, cells of certain rare groups may not always be available, and it is therefore usually necessary to supplement the available fresh cells by red cells stored at either +4°C or frozen at −25°C. Cells stored in their own plasma or serum are easily washed ready for use and will usually keep at +4°C for a maximum of six weeks. Saline suspensions of cells should not be stored. Frozen cells, though more difficult to handle, will keep for several years at −25°C with little deterioration.

Storage technique

Storage at +4°C

Whole blood may be stored in sterile acid citrate dextrose in closed containers. Four volumes of blood are added to one volume of the anticoagulant solution. If sterile acid citrate dextrose solution is not available, clotted samples may be used.

Storage at −25°C

Freezing of red cells. Four parts of blood are taken into one part of acid citrate dextrose solution, which has the following formula :

Disodium hydrogen citrate, monohydrate	2.50 g
Glucose (anhydrous)	2.00 g
Distilled water	100 ml

The cells are packed by centrifuging for 10 minutes at 3000 rev/min. Almost all the supernatant plasma is removed and is replaced by an equal volume of the following mixture :

Tripotassium citrate	1.95 g
Dipotassium hydrogen phosphate	0.36 g
Potassium dihydrogen phosphate	0.28 g
Distilled water	60 ml
Glycerol	40 ml

The tubes should be continually agitated during the replacement process.

The resulting cell suspensions are stored at −25°C in 1-ml volumes and are kept frozen until the cells are to be used.

Recovery of frozen cells. The recovery method of Weiner (1961) is easier to perform than the methods previously used, while cells recovered by this method behave better than those recovered by other techniques.

One ml of thawed red cell suspension is placed in 10 cm of dialysis tubing (about 6 mm lay-flat diameter), the lower end of which has been tied off with strong thread. After the blood has been introduced, the upper end of the tubing is closed with a small screw clamp, such as that used to adjust the flow in a giving set, or with a paper-clip. The tubing is placed in a beaker containing a large excess of 0.85% aqueous sodium chloride solution and is left on the bench for two hours, after which the cell suspension is removed to a 50 mm × 11 mm tube and the cells are washed three times in more saline.

Cleaning Laboratory Glassware

Clean glassware is essential for reliable blood grouping. The techniques used for cleaning glassware are time-consuming and for this reason some laboratories now make use of disposable plastic test tubes and syringes. The suitability of the disposable tubes for blood grouping work depends upon the formulation of the plastic. The following method of cleaning glassware is recommended :

1. All glassware must be rinsed free from blood immediately after use, using cold tap water; tubes 50 mm × 7 mm or 50 mm × 11 mm may be attached together in bundles by rubber bands, all the tubes facing in the same direction.

2. After rinsing, the glassware is put into bowls of tap water and soaked overnight.

3. It is then treated with a solution of a suitable detergent, care being taken to avoid those of the alkyl-sulfuric or sulfonic ester type, as the slightest trace of these has a deleterious effect on red cells. The technique used will depend on the detergent employed, but with most the glassware is heated in the solution for ten minutes. In some cases, if the detergent is strongly alkaline, the glassware may with advantage afterwards be soaked in 3% hydrochloric acid (commercial grade). The glassware is then shaken vigorously to expel the detergent or acid and washed six times in fresh tap water, the brisk shaking being repeated between each wash.

4. The glassware is rinsed once in distilled water.

5. It is then shaken very thoroughly and tubes are removed from the rubber bands and packed, open ends downwards, in wire baskets.

6. The baskets, with all the other glassware, are dried in an oven at 120°C for two hours.

7. The baskets are removed from the oven when cool enough to handle. The items of glassware are then inspected, collectively and individually, under a good light to ensure that all traces of blood, grease, and detergent have been removed. Any piece of glassware which does not pass this test must be cleaned again.

Preparation of Grouping Sera

Whenever possible, grouping sera should be prepared in a specialized reference laboratory. In any event, even if it is necessary to prepare the sera locally, it is most important that a sample of each batch prepared should be examined by another specialized laboratory. It is essential that every grouping serum be fully tested for potency, specificity, avidity and freedom from rouleaux-forming properties. It must contain a powerful antibody that reacts only with red cells carrying the corresponding antigen, and it must agglutinate such red cells quickly to form large clumps.

Donors of grouping serum are bled with the type of sterile taking set used for the venesection of blood donors, but the container in which blood is collected should not contain anticoagulant. When the blood has clotted, the serum is removed aseptically into another sterile container, using apparatus similar to that designed for the removal of plasma from packed red cells. The serum container is then centrifuged, and, if any red cells are deposited, the serum is transferred as before to another sterile container, care being taken to leave the deposit behind. Exceptionally, grouping serum can be prepared from plasma containing rare or particularly valuable antibodies by recalcification or by adsorption of fibrinogen by kaolin or bentonite. Difficulties may be encountered with these techniques, details of which will be found in standard textbooks on the subject, e.g., Dunsford & Bowley (1967) and Stratton & Renton (1958).

A sample of the serum is taken and tested in the manner described below. The bulk of the serum is then transferred aseptically into smaller labelled containers and stored at −25°C, but only if the sample has reacted satisfactorily to all the tests.

Preparation of anti-A and anti-B

Requirements of serum

Donors should have an anti-A or anti-B titre of at least 64, the antibody should not haemolyse red cells, and it should not show a prozone on titration. By prozone is meant the failure of an antibody to give an unequivocal reaction with a red cell suspension unless the serum is further diluted.

Exclusion of other antibodies

The serum should be tested for other unwanted agglutinins using the various techniques described in the chapter on the detection and identification of irregular antibodies (page 94). Particular care must be taken to look for and exclude sera containing anti-H (or anti-O), anti-P_1, anti-Rh, anti-Le^a, anti-Le^b, anti-Kell, or anti-Fy^a, as well as anti-Wr^a, anti-Vw, or anti-Mi^a. (The last three antibodies have not previously been mentioned, the antigens concerned being of low frequency. Wr^a is not known to be related to any other systems, but Vw and Mi^a are linked with MN. Despite the rarity of the antigens, the antibodies are not uncommon. The corresponding antibodies may occur without previous stimulus and their presence in potential grouping sera should be excluded by the use of appropriate Wr(a+) and Vw+Mi(a+) red cells. Cells for this work will have to be supplied by a reference laboratory.)

Titration of antibodies

If the sera have given satisfactory results with all the tests mentioned so far, they are then titrated at 20°C in saline as described in Basic Technique No. 5 (*a*) (Chapter 23, p. 127), using A_1, A_2, and A_2B cells for anti-A (as well as B and O cells as negative controls). B and AB cells (as well as A_1 and O cells as negative controls) are used for anti-B. Care must be taken to ensure that anti-B, which may come from an A_2 donor, does not contain anti-A_1 as well and that the anti-A and anti-B do not haemolyse red cells carrying the corresponding antigens. If visual agglutination is present at a titre of at least 64 with a given serum, the bulk supply is passed through a clarifying filter and then through a sterilizing filter. The serum is put in 4-ml quantities in suitable containers, such as 5-ml screw-capped bottles, and stored at −25°C until required.

Tests for avidity

The avidity of each antibody is determined by adding one drop of 10% saline suspension of washed cells to an equal volume of serum on a microscope slide or opal glass tile and by rocking the mixture gently. Agglutination of A_1 and B cells by anti-A and anti-B respectively should begin to be visible in 10 seconds. A_2B cells should begin to agglutinate with anti-A in 30 seconds.

Preparation of AB serum

AB serum for serological use must be free from all antibodies. Its method of preparation is based on the techniques just described, except that there is no need to perform titrations or tests for avidity, as the serum can be used only if completely inactive.

Anti-A plus anti-B (group O) serum

Anti-A plus anti-B (group O) serum is tested in the same way, special attention being given to titrations against A_2 as well as A_1 and B cells, as this serum is of particular help in detecting the weaker sub-groups of A.

Preparation of anti-Rh sera

Of the Rh antisera, the preparation of anti-D alone is considered here.

Source of the serum

Most, but not all, of the suitable donors will be recently delivered Rh-negative mothers known to have had infants suffering from haemolytic disease of the newborn. The patient's serum may already have been tested using the techniques and cell panel described on page 94 for the identification of irregular antibodies, including also anti-Vw, anti-Mi[a], and anti-Wr[a]. If the serum is shown to contain any antibodies other than anti-A, anti-B, and anti-D, then it will be of no use for preparing anti-D but may be of use as, for example, anti-C + D.

The patient is bled into a dry container as described above.

Absorption and filtration

In the case of a recently delivered woman, the antibody is usually most potent within one month of delivery and, if the woman's health permits it, she should be bled at this time. The technique of plasmapheresis may prove particularly valuable in these circumstances. The serum is separated under sterile conditions, and should then be absorbed with A_1, B, or A_1 B, cde/cde cells, as appropriate, to remove unwanted anti-A and/or anti-B agglutinins, should they be present.

Red cells used for absorption should be not more than five days old. The blood container should be spun at 2000 rev/min at 4°C for 30 minutes in a large electric centrifuge with a maximum capacity of 2000 ml. As much of the supernatant plasma as possible is removed and replaced with sterile isotonic saline. The cells and saline are well mixed. Centrifugation is repeated as before, and the supernatant is again removed and replaced by sterile saline. The process is continued until the cells have been washed four times.

After the last washing, as much supernatant saline is removed as possible. To one volume of the washed packed red cells is added an equal volume of the serum to be absorbed, the cells and serum being mixed by inversion of the container. After two hours, the container is spun in the refrigerated centrifuge at 2000 rev/min at 4°C for one hour. As much serum as possible is removed and retained without removing the red cells.

The serum is examined for the presence of the unwanted anti-A and anti-B agglutinins. If any are present, the absorption technique must be repeated. The serum is then filtered through both clarifying and sterilizing pads.

Titration

The antibody is next titrated at 37°C in saline and in albumin using Basic Techniques No. 5 (*a*) and No. 5 (*b*) (Chapter 23, pp. 127 and 128). If it reacts in saline to a titre of at least 32, the serum may be used as a saline anti-D ; if it reacts in albumin to a titre of 32 or more, it may be used as an albumin anti-D. Very weak albumin anti-D samples may be used as control sera for antiglobulin tests. For this purpose only, the antiserum chosen should be so weak that red cells sensitized with it will give only weak agglutination after six minutes' exposure to antiglobulin serum.

Storage

Anti-D serum should be dispensed in 0.5 ml quantities in suitable labelled tubes, and stored at −25°C or below until used.

The preparation of anti-human-globulin serum

Anti-human-globulin serum will normally be prepared by a reference laboratory with facilities for the keeping of animals, and possessing the skilled staff necessary to undertake its absorption and standardization.

Only one method of producing anti-human-globulin will be described here—the immunization of rabbits to produce a broad-spectrum reagent. Sheep, goats, and horses may also be immunized for this purpose, and a suitable technique is described in: Great Britain, Medical Research Council (1966).

Choice of technique

The method described below is that of Slavin (1950) and has been chosen because it has been found reliable and may be performed by relatively unskilled personnel. Fairly large, young rabbits are suitable for the purpose and it may be that a cross-breed will give the best results.

Immunization

Preparation of reagents. A 4% solution of sodium alginate (see Chapter 19, p. 114) is prepared in distilled water, which is stirred vigorously while the powder is gradually added. The solution, which is highly viscous, is autoclaved before use. Four ml of the solution are then mixed, using a sterile pestle and mortar, with 1 ml of human group O serum.

Injections. The mixture is drawn up into a 10-ml syringe without a needle. A wide-bore needle is then fitted to the syringe and the mixture is injected intraperitoneally into the lower abdomen of the rabbit, slightly to one side of the midline. Immediately afterwards, using a different syringe and needle, 2.5 ml of a 1% sterile aqueous solution of calcium chloride are injected intraperitoneally into the same site. The area is massaged gently. Three weeks later, the rabbit is given a course of four injections on consecutive days. The first three are intraperitoneal, consisting of 1 ml respectively of 1 in 10, 1 in 2, and undiluted human group O serum, the dilutions being in sterile saline. The fourth injection consists of 1 ml of 1 in 10 human group O serum in saline given intravenously into the rabbit's ear vein. The animal may be bled from the ear 10 days later, 25–35 ml of blood being removed, but a better reagent may be obtained if the animal is left for three or four months, after which it should be given a second course of the four consecutive injections (three intraperitoneal and one intravenous) described above, 25–35 ml of blood being collected from an ear vein ten days later. This procedure may be continued through the life of the animal, unless the potency of the antibody drops, when the animal should be rested for longer periods between courses. Further injections of alginate and calcium chloride are not given. By following this procedure a good anti-human-globulin serum should be obtained. If the animal is bled after the first course of injections, the titre of the first batch of serum may be lower than indicated below.

Absorption of rabbit serum

Once the animal is bled, the blood is allowed to clot and the clot allowed to retract fully; the serum is then removed. After being inactivated at 56°C for half an hour, it is ready for absorption. For this purpose packed red cells of groups O and A_1 B are used, the volume of each being equal to the volume of rabbit serum to be absorbed. The cells are washed at least six times in fresh sterile saline, using a minimum of 20 volumes of saline to one of red cells on each occasion. It is essential that the red cells and all glassware used should be absolutely free from plasma proteins; after the sixth wash, the supernatant is tested by removing a few ml and adding them to an equal volume of 3% sulphosalicylic acid. The appearance of any opalescence shows that protein is still present, and the washing must therefore be continued.

To one volume of the rabbit serum is added an equal volume of the adequately washed, packed group O red cells, the mixture being left at 20°C for one hour, or overnight at 4°C. It is then centrifuged, and the serum removed and absorbed in the same way, this time with A_1 B cells. The need for further absorptions may become apparent only during subsequent testing of the reagent.

Titration of anti-human-globulin serum

In tubes. The absorbed serum is titrated against unsensitized group O and A_1 B red cells and against group O D-positive red cells sensitized with a strong anti-D serum. These preliminary tests are performed in 50 mm × 7 mm tubes, using Basic Technique No. 5 (*a*) (see Chapter 23, p. 127), the tubes being incubated at room temperature for two hours. The tubes containing the unsensitized cells must be examined with the greatest care, for if these cells show any agglutination then the anti-human-globulin serum is not suitable for use and must again be absorbed. The sensitized D-positive cells should be agglutinated to a titre of at least 512 if the serum is to be regarded as satisfactory.

On an opal glass tile. A final titration is carried out on an opal glass tile, each dilution being tested against unsensitized O and A_1 B cells, strongly sensitized group O D-positive cells, and group O D-positive cells sensitized with a weak anti-D serum. A suitable dilution for the serum when issued is three steps lower than the last to give powerful agglutination of weakly sensitized cells. It must again be stressed that any reaction whatever with unsensitized cells indicates the need to re-absorb the rabbit serum. Once a suitable dilution for routine use has been decided upon, Kell-, Duffy-, and Kidd-positive cells, sensitized with their respective antibodies, are tested with the diluted rabbit serum to make sure that it will agglutinate them. If not, then the reagent must be used at a lower dilution capable of detecting these antibodies as well as anti-Rh.

As already indicated, antiglobulin serum may also be used in a complement-binding technique for the detection of Lewis and other antibodies. It is recommended that each batch of antiglobulin should be standardized for this purpose also ; for details the reader is referred to Polley & Mollison (1961).

Laboratory Documentation

Laboratory documentation is often looked upon as a minor aspect of a blood transfusion service, and its organization left to inexperienced staff, yet more fatalities result from errors in record keeping or the absence of correct labels on specimens or bottles of blood than from errors in laboratory technique. It is essential to have a records system that will enable reference to be made to previous investigations and reports. Time and energy will be saved and patients will be exposed to fewer risks by employing enough staff to maintain an adequate recording system, however small the laboratory.

The records may be divided into four main types : registers, card index system, labels, and forms.

Registers

(a) *Blood stock register* giving reference numbers and expiry dates of all containers of blood received. Against the entry for each unit of blood are shown the names and hospital numbers of any patients for

TABLE 8

EXTRACT FROM BLOOD STOCK REGISTER *a*

Group and bottle number	Expiry date	Compat- ible for	Compat- ible for	Compat- tible for	Compat- tible for	Final disposal
O Rh-pos. 8307	22.9.68	Mr A. X/63810 10.9.68	Miss B. M/71770 14.9.68			Used for Miss B.
O Rh-pos. 8308	22.9.68	Miss C. Y/68119 12.9.68	Miss B. M/71770 16.9.68			Used for Miss B.
AB Rh-pos. 8309	22.9.68					Not used. Sent for preparation of dried plasma.

a In practice, the names must be entered in full each time they appear.

whom it was found compatible and the name of the patient for whom it was used. A typical extract is shown in Table 8.

(b) *Grouping register*, details of which have already been discussed in the sections on ABO and Rh grouping (see Tables 3, 4, and 5, pp. 80, 84, and 85). The design of this register will depend upon whether one person

TABLE 9

EXTRACTS FROM BLOOD GROUPING REGISTERS a, b, c

Name, number of specimen	Anti-A	Anti-B	Anti-(A + B)	Anti-C	Anti-D (batch 1)	Anti-E	D^u test	Name, number of specimen	Serum check against A$_1$ cells	Serum check against B cells	Serum check against O cells	Anti-D (batch 2)
Patient X 1924 Mr A.	0	0	0		v			Patient X 1924 Mr A.	v	v	0	v
Donor Mr B. 8400	v	0	v	v	v	0		Donor Mr B. 8400	0	v	0	v
Donor Miss C. 8401	0	0	0	0	0	0	0	Donor Miss C. 8401	v	v	0	0
Red cells group O	0	0	0									
Red cells group A$_1$		0										
Red cells group A$_2$	v		v									
Red cells group B	0	c	c									
Rh+ controls				v	v	v	v	Rh+ controls				v
Rh— controls				0	0	0	0	Rh— controls				0

a Tests performed by two workers using different registers.
b For explanation of symbols, see Table 3, p. 80.
c See footnote to Table 8, p. 106.

reads all the results, or whether the work is divided between two workers as recommended in the section on ABO and Rh grouping. In the latter case, it is useful to have two grouping registers, one ruled up for tests on the sample with anti-A, anti-B, and anti-A plus anti-B (group O) sera, and one anti-D serum, while the other should be used to record serum checks against A$_1$, B, and O cells as well as the results with the other anti-D serum. See Table 9.

In place of two registers, some workers may prefer to use pairs of sheets of specially ruled paper, one sheet recording one set of tests, and the second the other. When the two sets of results have been compared the sheets are clipped together in a file.

If one person alone does the work, then the results may simply be recorded in one book as shown in Table 10.

TABLE 10

EXTRACT FROM BLOOD GROUPING REGISTER *a, b, c*

Name, number of specimen	Anti-A	Anti-B	Anti-(A + B)	Serum check against A₁ cells	Serum check against B cells	Serum check against O cells	Anti-C	Anti-D (Batch 1)	Anti-D (Batch 2)	Anti-E	Dᵘ test
Patient X 1924 Mr A.	0	0	0	v	v	0		v	v		
Donor Mr B. 8400	v	0	v	0	v	0	v	v	v	0	
Donor Miss C. 8401	0	0	0	v	v	0	0	0	0	0	0
Red cells group O	0	0	0								
Red cells group A₁		0									
Red cells group A₂	v		v								
Red cells group B	0	c	c								
Rh+ controls							v	v	v	v	v
Rh— controls							0	0	0	0	0

a All tests performed by one worker.
b For explanation of symbols, see Table 3, p. 80.
c See footnote to Table 8, p. 106.

If the results are read by two persons, they should not use a single grouping book, as each will know what the other has recorded and may thus be biased in his readings.

In either case, space must be left in one register for tests with anti-C and anti-E, as well as Dᵘ testing.

(*c*) *Compatibility register.* Details of this have already been given in Chapter 13, p. 91 (see also Table 6, p. 93).

Apart from the above three important registers, smaller lists giving details on donors whose full blood groups are known and on the panel of frozen cells should also be kept. Finally a general work book is necessary, in which serological tests carried out in investigating problems and performing titrations may be recorded. This may be ruled up as the occasion demands, giving results of all tests done. No laboratory work may be performed without recording the nature of the tests, the reagents used, and all the results.

Card index system

Donors

See Chapter 4, p. 34.

Patients

Appropriately printed cards (e.g. International Paper Size A5) should indicate the name of the patient, the hospital number, date, ward, the physician or surgeon in charge, diagnosis, previous transfusion history, history of pregnancies (if any), date and result of grouping, and dates of all compatibility tests, together with reference numbers for all bottles of blood transfused. Each time a patient is transfused, he or she should be re-grouped and the findings recorded on the card.

Transfusion reactions and, in the case of pregnancies, any serological or clinical evidence of haemolytic disease of the newborn must be recorded.

Labels

All writing on labels must be done with a ball-point pen and not in ink, which may be smeared by condensation in the refrigerator.

Label to be attached to blood container indicating the patient for whom the blood is compatible.

This label should indicate the unit number, its ABO and Rh group, the full name of the patient, the name of the hospital, the patient's hospital number, the ward, and the date of the test. See also Chapter 4, p. 37 for details of container labels and Chapter 10, p. 75 for details of blood sample labels.

Forms

Requests for blood

The design of forms for requesting blood will vary according to local needs, but the following will serve as a guide :

REQUEST FOR BLOOD TRANSFUSION

HOSPITAL _____ WARD _____

FAMILY NAME OF PATIENT _____ SEX _____

(Block letters)

FIRST NAMES _____ DATE OF BIRTH _____

TEST REQUIRED :　　　GROUPING

　　　　　　　　　　　GROUPING AND COMPATIBILITY TESTS

MATERIAL REQUIRED :　UNITS OF WHOLE BLOOD

　　　　　　　　　　　UNITS OF CONCENTRATED RED CELLS

DATE AND TIME WHEN BLOOD IS REQUIRED _____

DIAGNOSIS AND REASON FOR TRANSFUSION _____

PREVIOUS TRANSFUSION HISTORY _____

UNTOWARD REACTIONS TO PREVIOUS TRANSFUSIONS _____

If the patient is a woman, please record history of previous pregnancies, if any, and state whether she has ever had a miscarriage, a stillbirth, or an infant that suffered from haemolytic disease of the newborn.

DETAILS OF LAST Hb AND BLOOD COUNT _____

SIGNATURE OF MEDICAL OFFICER _____ DATE _____

Every request for blood must be accompanied by a specimen of the potential recipient's blood. The specimen should consist of not less than 10 ml of blood collected with a dry syringe and allowed to clot in a dry sterile tube with a label indicating the patient's full name, the hospital, hospital number, ward, and date. If the patient is an infant, so that it is not possible to collect blood by venepuncture, 10–20 drops of blood should be taken by heel stab into a dry sterile tube.

Except in emergencies, requests for grouping should be made at least 24 hours before the transfusion is to be given to allow time for any special investigations that may be necessary.

Laboratory report of patient's group (for attaching to patient's hospital records)

This form must state the patient's full name, the hospital, hospital number, ward, and date of testing. It should also state the patient's group in simple terms. Where the report concerns tests on the husband of an Rh-negative woman, more details should be given, such as " Group O, Rh-positive, probable genotype CDe/cde, HETEROZYGOUS for D Rh-positive ". Here again, the aim should be simplicity, and the operative word should be in capital letters, i.e., HETEROZYGOUS or HOMO-ZYGOUS.

Laboratory Staff and Equipment

Staff in relation to size of laboratory

Medical officers

There must be at least one medical officer in each blood bank; in large establishments, more will be required and one of them may have to spend a considerable time on administrative work. In small units the medical officer may have additional duties in other departments of the laboratory or in the wards, but there must be someone to whom the staff can turn at all times for advice about transfusion, and who is also responsible for the medical examination and bleeding of donors.

Serologists

These may be technicians, scientifically qualified persons, or medical staff. The number of technicians required will depend to some extent on the skill of the staff, but a very rough estimate may be made by dividing the number of samples received each day by fifty.

Clerical staff

At least one clerk will be needed to type letters, file cards and letters, and carry out all the office routine without which no laboratory can operate efficiently. The clerk may be employed either full- or part-time, as circumstances require.

Washing-up staff

All laboratories need efficient washing-up facilities. Only too often washing-up is done under poor conditions by staff ignorant of the need for the absolute cleanliness of all apparatus.

Animal attendant

If antiglobulin serum is to be prepared, rabbits will have to be kept. These will require daily attention, and an animal attendant will be necessary.

Trainees

Larger laboratories will probably train medical officers or technicians before they are sent out to smaller, often more isolated, units. These trainees will be able to play some part in the routine work of the laboratory.

Laboratory equipment

No two laboratories have the same commitments, and their requirements will differ. The list that follows does, however, contain the essential items that any blood grouping laboratory is likely to require.

Laboratory apparatus

Autoclave	
Balance, centrifuge	for 540-ml blood bottles
Balance, chemical, with set of weights	
Baskets, wire mesh, to take glassware in drying oven	
Beakers	100-ml, 250-ml, 500-ml
Blocks, wooden	with 50 holes in rows of 10, to hold 50 mm × 7 mm and 50 mm × 11 mm tubes
Bottles, glass	5-ml, 25-ml, 100-ml, 200-ml, with caps and heat resistant liners (it is preferable to use neutral glass, where possible, and to have caps that can be sterilized on the bottles, by dry heat)
Brushes, test tube	various sizes
Bungs, rubber	various sizes
Burner, Bunsen	to be connected to domestic supply, or to bottled gas apparatus (if no gas supply is available, paraffin burners may be used)
Caps, glass	for 50 mm × 7 mm tubes
Centrifuge, bench model, electric, swing-out head	200 ml maximum capacity (if no electricity supply is available, hand-driven centrifuges should be used)
Centrifuge, large, electric, refrigerated	for spinning blood bottles, 2000 ml maximum capacity
Clamps, screw	small
Cloths and towels	their various purposes to be identified by colour or labels
Clothing, protective	for washing-up staff (against water and acid)
Detergent	suitable for cleaning glassware
Dialysis tubing, 6 mm lay-flat	
Dishes, plastic or stainless steel	various, some with lids

Donor taking sets and cell packing sets	
Dressings, including gauze, lint, non-absorbent cotton-wool, and adhesive plaster	
Dropping bottles	
Elastic bands	of various sizes as found convenient
Flasks, volumetric	25-ml, 50-ml, 100-ml, 250-ml, 500-ml, 1000-ml
Files, glass-cutting	
Filters, bacteriological	with clarifying and sterilizing pads 6 cm diameter, 14 cm diameter
Glass, opal	for anti-human-globulin tests
Glass tubing	5.0–7.5 mm diameter, for Pasteur pipettes, and other uses
Haemocytometer	
Haemoglobinometer	
Hand lens, 8 × magnification	
Incubators, 37°C (electric, gas, or paraffin)	
Labels, adhesive	
Measuring cylinders	50-ml, 100-ml, 250-ml
Microscopes	(a) without moving stage and fitted with low-power objective for reading agglutination tests (b) binocular with mechanical stage and 3 objectives : ×10, ×40, ×100
Microscope lamps	
Microscope slides	
Needles, straight with cutting edges	for taking capillary blood samples
Oven, hot air, sterilizing	
Pencils, grease	
Pestle and mortar	
Pipettes, chemical	1-ml, 2-ml, 10-ml
Pump, suction	water or electric
Refrigerators (+4°C)	(a) for blood storage, electric, gas, or paraffin-operated, fitted with alarm system (b) for storage of blood samples for testing
Refrigerator (−25°C)	for storage of grouping sera or fresh frozen plasma, fitted with alarm system
Rubber tubing	various sizes
Still	for preparation of pyrogen-free distilled water
Syringes, hypodermic	glass or plastic, various sizes
Tape, cellulose, adhesive	

Teats, rubber	of various sizes, for Pasteur pipettes
Test-tubes, neutral glass	various sizes, including 50 mm × 11 mm and 50 mm × 7 mm in large numbers
Thread, linen	for tying off dialysis tubing
Water baths	37°C and 56°C, fitted with racks to take 50 mm × 11 mm and 50 mm × 7 mm tubes

Reagents

Albumin, 20%, bovine

Ammonium oxalate

Calcium chloride

Copper sulfate

Cysteine hydrochloride

Dextrose (anhydrous)

Ethylenediamine tetra-acetic acid (EDTA), dipotassium salt

Dipotassium hydrogen phosphate

Disodium hydrogen citrate, monohydrate

Disodium hydrogen phosphate

Ethanol 70% V/V

Hydrochloric acid (commercial)

Glycerol

Papain

Potassium oxalate

Potassium dihydrogen phosphate

Sodium alginate (Manucol SS/LH Grade is suitable)[1]

Sodium dihydrogen phosphate

Sodium chloride

Sodium citrate

Sodium hydroxide

Stains, haematological

Sulphosalicylic acid

Tripotassium citrate

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[1] A suitable preparation is obtainable from Alginate Industries Ltd, Walter House, Eedford Street, London W.C. 2, England.

Investigation of Transfusion Reactions

The manifestations of transfusion reactions vary, but commonly there is pyrexia, with or without a rigor, and the patient may develop oliguria or anuria after a severe reaction. The transfusion should at once be stopped, and the cause investigated.

The reaction may be due to incompatibility between blood group antigens and antibodies, but before considering this other possible causes may be listed.

Causes of reactions

(a) Transfusion of haemolysed blood. Haemolysis may be due to:

(i) age of blood (blood older than 21 days should not normally be transfused);

(ii) heating of blood above 37°C (blood should not be warmed except for an exchange transfusion of a baby or the rapid massive transfusion of an adult, and it should be warmed under the supervision of a medical officer or trained nurse);

(iii) freezing of blood;

(iv) incorrect anticoagulant.

Under these circumstances, the patient often becomes febrile and jaundice usually develops within 12 or 24 hours.

(b) Transfusion of infected blood, which causes the rapid onset of severe, sometimes fatal, reactions. Gram-negative organisms may multiply at 4°C, without causing haemolysis.

(c) Transfusion of blood containing pyrogens derived from improperly prepared solutions or equipment.

(d) Transfusion of blood containing allergens, to which the patient is sensitive, or antibodies to allergens present in the patient.

(e) Accidental injection of air, usually due to defective apparatus.

(f) Overloading of the circulation by giving too large a volume of blood, or by administering the transfusion too rapidly, particularly in the presence of myocardial defects.

(*g*) Transfusion of blood containing antigens or antibodies incompatible with the antibodies or antigens of the patient.

(*h*) Transfusion of blood containing an agent causing hepatitis. In the event of any unexplained icterus in a patient 30–160 days after a transfusion, the medical history of the donors involved should be checked and special attention should be given to any similar occurrence in any other recipients of their blood. This may be the only way of identifying possible hepatitis carriers among donors. Recent advances suggest that it will become feasible to test donor blood and blood products for the presence of Australia antigen and its antibody, which are associated with the transmission of serum hepatitis (see Chapter 2, p. 24).

Serological investigation of transfusion reactions

Specimens required

(*a*) a fresh clotted post-transfusion sample of the patient's blood, collected with a dry syringe (in order to avoid accidental haemolysis), preferably from a limb other than that in which the transfusion was given ;

(*b*) the pre-transfusion sample of the patient's red cells and serum which will have been retained for reference (see Chapter 11, p. 78) ;

(*c*) any blood remaining in the container or containers ;

(*d*) all urine voided during the first 24 hours after the reaction.

Serological tests

Re-group the donor blood and both the pre- and the post-transfusion samples from the patient. Look especially for clumps of red cells floating among free cells in the post-transfusion sample. Perform a direct antiglobulin test on the patient's pre- and post-transfusion washed red cells. Repeat compatibility tests of donor's red cells against patient's serum, using the pre- and the post-transfusion samples of serum. Screen both donor plasma and the two serum samples from the patient for abnormal antibodies. If the donor was group O, and the patient was of another ABO group, refer to Chapter 12, p. 87, and perform the relevant tests for " dangerous group O donors ".

Biochemical tests

Examine a post-transfusion sample of the patient's serum for free haemoglobin and bilirubin, comparing the results with those on the pre-transfusion sample. Discoloration of the serum may indicate a reaction. A Schumm's test may be performed to detect methaemalbumin.

The urine should be examined for the presence of free haemoglobin and for red cell casts (again indicating a haemolytic reaction).

Bacteriological tests

The donor bottle should be examined bacteriologically, both by staining of direct smears and by culturing the blood remaining in it at 4°C, 20°C, and 32°C. Some of the organisms that cause severe reactions grow best at +4°C.

Further investigations

If the serological tests show that an incompatible transfusion has been given, it will probably be possible, by taking repeated samples from the patient, to check that the transfused red cells are being rapidly removed from the circulation.

It may be possible for a reference laboratory, by using high titre anti-Rh, anti-M, or anti-N sera, or by tagging donor red cells with radioactive substances, to measure the rate of donor cell survival in the recipient's serum. If it can be shown that the donor cells are surviving normally with a loss of approximately 1% per day, then a haemolytic reaction is excluded.

Prenatal and Postnatal Serology

This subject may be conveniently considered under four headings:

1. Tests on the mother
2. Tests on the father
3. Tests on the baby
4. Selection of blood for transfusion of the baby

Tests on the mother

Initial tests in pregnancy

The ABO and Rh groups should be determined as described in Chapter 11, pp. 78 and 81. A sample of the serum should be tested for possible syphilitic infection, and a haemoglobin estimation should also be performed. The serum of all Rh-negative women should be screened for Rh antibodies. The two-stage papain technique (Basic Technique No. 4 (*b*), Chapter 23, p. 126) may be used, suitable cells being CDe/CDe, cDE/cDE, and cde/cde. If possible Kell- and Duffy-positive cells should also be included, in which case tests should also be performed by the indirect antiglobulin technique (Basic Technique No. 3 (*a*), Chapter 23, p. 124). If any abnormal antibodies are found, they should be investigated as described in Chapter 14 on page 94.

Tests later in pregnancy

Irrespective of her Rh group, the serum of any woman with a history of transfusion or of obstetric complications probably due to blood group incompatibility should be examined for the presence of atypical antibodies by testing against a selected panel of red cells by Basic Techniques No. 1, No. 2, No. 3, and No. 4. The sera of Rh-negative women in which no Rh antibody has been found at 12–16 weeks should be screened again, using the same tests, at 34–36 weeks.

Tests on the father

(*a*) If a woman's serum contains no abnormal antibody then her husband need not be tested.

(b) If an antibody is present, then he should be grouped, with special regard to the serological system concerned.

(c) An attempt must be made to determine whether he is homozygous or heterozygous positive, but this will probably have to be done by a reference laboratory having the necessary specialized knowledge and antisera.

(d) Grouping of any other children that the couple have had may help by showing that one or more do not carry the antigen concerned and thus that the father must be a heterozygote. If the ABO group of the husband's red cells is compatible with the mother's serum, they may be tested against her serum to see if it contains any atypical antibody.

Tests on the baby

(a) If the mother is Rh-positive and shows no abnormal serological features, the child need not be tested.

(b) If she is Rh-negative a sample of clotted cord blood should be collected and sent to the laboratory for ABO- and D-typing and for a direct anti-human-globulin test. Should the latter prove positive, the mother's serum must be retained.

(c) If the serum of the mother is known to contain atypical antibodies (Rh or other) at the time of delivery, a sample of cord blood should be collected at birth and divided between two suitable containers, one with dry anticoagulant and one without. Sequestrene is a suitable anticoagulant.

(i) The clotted sample of cord blood should be used for ABO and Rh grouping (see Chapter 11, p. 78) and for a direct antiglobulin test (see Basic Technique No. 3 (b), Chapter 23, p. 125). Serum of the cord blood should be used for a serum bilirubin estimation, which may be performed in a biochemical laboratory, and the remainder kept in case it is needed for compatibility tests.

(ii) If the mother's serum contains immune antibodies other than ABO or Rh, then the infant should be grouped for the appropriate system, but treatment, if required, need not be withheld while this is done.

(iii) The direct antiglobulin test will usually be unequivocably positive if the child has haemolytic disease of the newborn due to anti-Rh. A negative result may, however, be obtained if the baby is affected by ABO haemolytic disease of the newborn.

(iv) The sample of cord blood that contains anticoagulant should be used for haemoglobin estimation. In interpreting the results one must remember that the haemoglobin level of healthy newborn infants is high (e.g., 17.8 g/100 ml), though it may be lower in an otherwise normal child if the mother is very anaemic.

In doubtful cases, an attempt may be made to test the infant's red cells against the mother's serum using all the techniques for the detection of antibodies mentioned in Chapter 14, p. 94. If no incompatibility can be detected, and the direct antiglobulin test on the infant's cells is negative, the child is unlikely to be suffering from haemolytic disease of the newborn.

More detailed information on the subject will be found in Mollison (1967).

Matching blood for transfusion of baby

If the infant requires transfusion, then compatibility tests must be performed, the blood chosen being as nearly as possible homologous with the ABO and the D-type of the child except that it must lack the antigen against which the mother has an antibody. For example, a group O Rh-positive baby suffering from haemolytic disease of the newborn due to anti-D should receive group O Rh-negative blood, but a group A Rh-positive baby suffering from ABO haemolytic disease should receive group O Rh-positive blood from which most of the plasma has been removed (and thus most of the anti-A and anti-B content).

Compatibility tests should preferably be performed by matching the donor's red cells against the mother's serum, as this contains the antibody in higher concentration than does the serum of the cord blood. The tests should be performed as described in Chapter 13, p. 89. If compatibility testing is complicated by ABO incompatibility between the mother's serum and the infant's red cells, blood may be selected as follows :

(a) Ideally, A or B substance should be added to the mother's serum to neutralize her anti-A or anti-B and the absorbed serum used for the compatibility tests.

(b) Less satisfactorily, the baby's cord serum may be used, but in the event of the limited amount of antibody available being totally absorbed on the infant's red cells, a false compatible result may be obtained with incompatible blood.

Auto-Immune Acquired Haemolytic Anaemia

From time to time serological reactions may be observed that are due not to any incompatibility between potential donor and recipient but to one between the red cells and an antibody in the serum of the same individual (donor or recipient). For instance, a positive antiglobulin reaction found in a compatibility test may be due not to an antibody in the recipient's serum reacting with the donor's cells but to an antibody in the donor's plasma reacting with his or her own cells. In this case, if a direct anti-globulin test is performed on the donor's cells, it will be positive.

In unexplained cases of anaemia and jaundice, the diagnosis of acquired haemolytic anaemia may sometimes be established by matching the patient's red cells against his own serum, using all the techniques described for the identification of irregular antibodies in Chapter 14, p. 94. Readers requiring further information on the subject are referred especially to Dacie (1962) and Dacie & Lewis (1963).

Basic Techniques

BASIC TECHNIQUE No. 1

Saline Agglutination

Apparatus, reagents, etc. needed

> 50 mm × 7 mm precipitin tubes
>
> 50 mm × 11 mm tubes
>
> Caps for precipitin tubes
>
> Racks, or wooden blocks, each containing 50 holes in rows of 10, the holes being large enough to take any of the above tubes
>
> Adhesive labels for tubes which will be centrifuged
>
> Small centrifuge
>
> Pasteur pipettes, fitted with rubber teats, one graduated with grease pencil to deliver 0.03 ml and another to deliver 0.01 ml
>
> Microscope with low-power magnification
>
> Microscope slides
>
> Beakers to hold saline
>
> Dish to receive discarded fluids
>
> 0.85% sterile aqueous sodium chloride solution (saline)
>
> The sodium chloride should be of sufficient purity for analytical work
>
> Red cells to be used, washed three times in physiological saline and made up by eye to 5% suspension in saline
>
> Specific antisera or sera to be tested

Routine method

The red cells are spun down in a centrifuge, and the supernatant serum is removed and replaced by an equal volume of fresh sterile saline. This whole process is known as " cell washing ", and should be performed three times, after which the suspension is made up by eye to 5% by adding saline. Each suspension is prepared in a 50 mm × 11 mm tube and placed in a wooden block, while precipitin tubes in the number needed for the tests are placed in one or more similar blocks. To one volume of serum

placed in each precipitin tube is added an equal volume of red cell suspension. The precipitin tubes are tapped to mix cells and serum and to remove bubbles, capped, and then incubated for two hours.

The sedimented red cells are then gently removed with a Pasteur pipette and spread on a microscope slide, which may with advantage be at the same temperature as that at which the mixtures were incubated. The cells are then examined under low-power magnification. The caps are removed from each tube in turn as the contents are transferred to a slide. In the event of any interruption, the caps remaining in place indicate tubes still to be read.

Suitable controls must always be included.

BASIC TECHNIQUE No. 2

Albumin Agglutination

Apparatus, reagents, etc. needed

As listed for Basic Technique No. 1
Also required : 20% bovine albumin

Routine methods

(a) *Serum-albumin method*

The red cells are washed in saline as previously described, and the packed cells are suspended in 20% bovine albumin to give a 5% suspension. To one volume of serum placed in each precipitin tube is added an equal volume of this red cell suspension. Thereafter the method is the same as that already described for saline agglutination. (Owing to the relatively high optical density of albumin, red cells suspended in it may appear to have haemolysed, but microscopic examination will show that this is not so.)

(b) *Albumin replacement method*

The tests are set up exactly as described in Basic Technique No. 1. After incubation for 1½ hours, the supernatant is carefully removed by means of a Pasteur pipette, without disturbing the " button " of red cells on the bottom of each precipitin tube. A volume of 20% bovine albumin equal to the supernatant removed is now added, care being taken not to disturb the red cells, and the tubes are reincubated for a further 30 minutes. The results are then read by microscope in the usual way.

In albumin techniques, as in all others, adequate controls must be included.

BASIC TECHNIQUE No. 3

Antiglobulin Agglutination

The principle involved is that anti-human-globulin serum will agglutinate red cells coated by an antibody which is itself a globulin. If the antibody has become attached *in vivo*, the test performed is said to be " direct ", whereas if normal red cells have first to react with an antibody *in vitro*, the process is known as an " indirect " antiglobulin test. Unless specific instructions accompany the antiserum, use the following techniques :

(a) The indirect antiglobulin test

Apparatus and reagents needed

The apparatus is similar to that listed for Basic Technique No. 1, though the preparation of the red cell suspension is somewhat different (see below). It cannot be too strongly emphasized that all glassware must be perfectly clean, for the slightest trace of human protein left on any glassware that is to be in contact with the antiglobulin serum may completely neutralize the antibody it contains, thus causing a false negative result. Likewise, if the red cells have not themselves been carefully washed, protein in the fluid in which they are suspended may adhere and give false positive results.

Also required :

A sheet of opal glass 15 cm × 30 cm

Red cells to be tested

Serum to be tested

Known Rh (D)-positive red cells

Weak albumin-agglutinating anti-D serum

AB serum

A suitably diluted anti-human-globulin serum

Pasteur pipette and teat reserved for use with anti-human-globulin only

A microscope is not required.

Routine method

The technique has three stages : (i) sensitization, (ii) washing, (iii) addition of antiglobulin serum to the red cells.

(i) Each specimen of red cells to be tested, including the D-positive cells, is washed three times in saline and packed by centrifuging. One volume (0.03 ml) of packed washed cells is added to 3 volumes of the serum

with which it is to be tested, and also to 3 volumes of AB serum. The D-positive control cells are added to the weak albumin-agglutinating anti-D serum and to AB serum, thus giving positive and negative controls. The tubes are incubated at 37°C for 2 hours.

(ii) All cells are now washed 4 times—in at least 20 volumes of fresh sterile saline each time—and centrifuged at 2000 rev/min for 2 minutes. Great care must be taken to mix cells and saline. After the fourth wash the suspensions are adjusted to 30% in saline.

(iii) The opal glass sheet should then be thoroughly scrubbed with soap and water and rinsed well with tap water so that no soap remains. It should be dried on a clean cloth kept specially for this purpose. Many workers like to rule the opal glass, using a grease pencil and a ruler, into squares of approximately 3 cm × 3 cm.

When undiluted, antiglobulin serum is unstable if not kept frozen; once diluted, it is unstable at any temperature. It must therefore be stored frozen undiluted at −25°C or below and diluted with fresh saline immediately before use, employing a clean Pasteur pipette. The dilution used will be that determined during the standardization of the reagent, and the container will normally bear a label giving the necessary instructions. The undiluted antiglobulin serum should be refrozen immediately after use.

One drop of each cell suspension is placed in a separate square in which a drop of diluted antiglobulin serum has already been placed. The cells and antiglobulin serum are then mixed; some workers use the corner of a clean microscope slide for this purpose changing the corner for each sample, while others use a plastic rod which is wiped before mixing each sample.

The tile is left undisturbed for one minute and then rocked very gently, but at no time must it be shaken. After 7 minutes (or the recommended interval for the antiglobulin reagent used), the tile is held horizontally with a light beneath it and examined for agglutination with the naked eye.

If the time of reading is postponed, a weak false-positive (grainy) reaction may appear in the negative control, indicating that the suspensions are beginning to dry up.

(b) The direct antiglobulin test

As the red cells to be tested have been incubated *in vivo*, section (i) of the indirect test is omitted, though control cells are prepared as before. The test cells, together with the control cells, are washed and added to anti-globulin serum on a tile before reading.

The use of the antiglobulin technique for detecting complement-binding antibodies is not described in this manual. Those interested are referred to Polley & Mollison (1961).

BASIC TECHNIQUE No. 4

Enzyme Agglutination

(a) *Löw's papain technique* (Löw, 1955)

Apparatus and reagents needed

As listed for Basic Technique No. 1
Also required :

Pestle and mortar

Papain—a suitable preparation is Papayotin (Merck)

M/15 phosphate buffer pH 5.4, prepared by mixing 3.5 ml of Na_2HPO_4 (9.46 g/litre) and 96.5 ml of KH_2PO_4 (9.07 g/litre)

0.5 m cysteine, prepared by dissolving 1.57 g of L-cysteine hydrochloride in 10 ml distilled water and adding to 5 ml of this solution an equal volume of sodium hydroxide solution (40 g/litre)

Routine method

Two g of papain are ground in a pestle and mortar with 100 ml of the M/15 phosphate buffer pH 5.4. The resulting mixture is filtered, and 10 ml of the 0.5 m cysteine are added to the filtrate. The volume is then made up to 200 ml with the phosphate buffer. The whole is incubated at 37°C for 1 hour and divided into 1-ml amounts, which must be stored at —25°C.

Equal quantities of the preparation and the serum to be tested are mixed. Thereafter, the technique is that described for saline-agglutinating antibodies, incubation being at 37°C for two hours.

(b) *Two-stage papain technique*

Apparatus and reagents needed

As listed for Basic Technique No. 1
Also required :

Papain, 1 g being suspended in 100 ml of 0.85% sodium chloride

M/15 phosphate buffer pH 7.3, prepared by mixing 72 ml of Na_2HPO_4 (9.46 g/litre) with 28 ml of KH_2PO_4 (9.07 g/litre)

Stock buffered saline—1 volume of phosphate buffer pH 7.3 added to 9 volumes of 0.85% sodium chloride

Routine method

Nine volumes of stock buffered saline are added to one volume of stock papain. One volume of the packed washed red cells is added to two volumes of this buffered saline/papain solution. The mixture is incubated

at 37°C in a water bath for 30 minutes. The cells are then washed once and made up to a 5% concentration in 0.85% saline. Before mixing with the serum, this suspension is warmed to 37°C in the water bath.

After 0.1 ml of the serum to be tested has been warmed to 37°C in the water bath, an equal volume of the cell suspension is added to it and the mixture is incubated for one hour.

Examine macroscopically (and check by microscope) for the presence or absence of agglutination.

Some workers prefer to use the enzyme bromelin. A technique for using it is described by Pirofsky & Mangum (1959).

BASIC TECHNIQUE No. 5

Titration

(a) Saline agglutinating antibodies

Apparatus and reagents needed

As listed for Basic Technique No. 1
Also required : Pasteur pipette to deliver 0.2 ml

Routine method

Ten 50 mm × 11 mm tubes are placed in the back row of a wooden block. In front of this is placed one row of ten 50 mm × 7 mm tubes for each batch of cells to be tested against the particular serum under investigation. To all tubes in the back row, except the first, is added 0.2 ml of saline ; to the first two tubes of the row an equal volume of the serum to be tested is also added. The serum and saline in the second tube are mixed, 0.2 ml of the mixture being transferred to the third tube. Here the mixing is repeated, and 0.2 ml is transferred to the fourth tube. This procedure—that of making " doubling dilutions "—is continued to the end of the row, 0.2 ml being discarded from the tenth tube after mixing. Each tube in the row now contains 0.2 ml of diluted serum, in decreasing concentration from left to right.

With another Pasteur pipette, transfer 0.03-ml volumes of the contents of the tenth tube in the back row to each of the precipitin tubes in front of it. Do the same with the contents of the ninth tube, and so on to the first tube. In this way each row of precipitin tubes from left to right should contain equal volumes of doubling dilutions corresponding to the master dilutions made in the larger tubes in the back row. Next, 0.03 ml of 5% washed cells is added to the precipitin tubes, each row of 10 tubes being used for one particular batch of red cells. The tubes are tapped and capped, and thereafter the test follows the saline agglutination technique already described.

All titrations are read from right to left, i.e., from high dilution to low dilution, in order to ensure that no false negative results due to prozone phenomena are recorded; otherwise there is the risk that a test might be incompletely read if the low dilution tubes were read first and found to be negative. Degrees of agglutination at each dilution should be recorded using the scheme described in Basic Technique No. 6.

(b) Albumin agglutinating antibodies

Two titration techniques will be described : the serum/albumin technique, and the albumin replacement method. These two procedures are closely related to Basic Techniques No. 2 (a) and No. 2 (b) on p. 123.

(i) Serum/albumin titration technique

Apparatus and reagents needed

As listed for Basic Technique No. 2 (a)
Also required : Pasteur pipette to deliver approximately 0.2 ml
Group AB serum

Routine method

The block is set up in exactly the same way as for the titration of saline agglutinating antibodies (see above), except that twofold dilutions of the serum to be tested are made in 50 mm × 11 mm tubes using AB serum instead of saline. Thus, 0.2 ml of AB serum will be added in place of saline to each tube in the back row except the first, and 0.2 ml of the serum to be tested will be added to the first and second tubes. The sera in the second tube are then mixed, and tube by tube transference is carried out as for the saline titration. The red cells to be added are made up to a 5% suspension in 20% bovine albumin as in the corresponding albumin agglutination technique (Basic Technique No. 2 (a), p. 123). Continue as indicated in this technique, reading the results in the way described in Basic Technique No. 5 (a).

(ii) Albumin replacement titration technique

Apparatus and reagents needed

As listed for the titration of saline agglutinating antibodies (Basic Technique No. 5 (a))
Also required : 20 % bovine albumin

Routine method

The technique starts in exactly the same way as Basic Technique No. 5 (a). After incubation for 1½ hours, the supernatant is carefully

removed from each 50 mm × 7 mm tube and, as in Basic Technique No. 2 (*b*), replaced by an equal volume of 20% bovine albumin for the detection of albumin antibodies. The results are read by microscope after a further incubation period of 30 minutes, observing the precautions common to all titrations described in Basic Technique No. 5 (*a*).

BASIC TECHNIQUE No. 6

Recording Results

Degrees of agglutination should be recorded. The following symbols are suggested.

C   Complete agglutination

V   Visual agglutination, several agglutinates being clearly visible without using a microscope

++   Very large agglutinates visible under the microscope

+   Large agglutinates visible under the microscope

(+)   Small agglutinates visible under the microscope

W   Small, but definite, agglutinates

0   No agglutination

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