Guidelines for the BLOOD TRANSFUSION SERVICE

Second Edition

Amendment 1

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Guidelines for the Blood Transfusion Service

Amendment 1

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GENERAL CONSIDERATIONS

SECTION 1

Chapter 1

Selection of Donors

1.1 General Considerations

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

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

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

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

1.1.3 Hazardous Occupations or Hobbies

1.1.3.1 Service aircrew, whether trained or under training, are not permitted to act as blood donors. Civil aircrew may not donate if on flying duties.

1.1.3.2 Occupations where a delayed faint may present a hazard to the donor and/or others - accept only when the individual is going **off** duty, e.g. train, HGV or bus driver, heavy machine or crane operator, driver, climbing ladders or scaffolding, miner working underground.

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

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

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

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

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

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

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

1.2 Medical Assessment

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

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

1.2.3 Age

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

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

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

1.2.4 Frequency of donations

Usually 2 donations are given in a 12-month period. Many donors are able to give blood more frequently without developing iron deficiency so that an interval of 16 weeks between donations is considered a reasonable minimum. Any further reduction, to an absolute minimum interval of 12 weeks, must be accompanied by appropriate donor monitoring.

1.2.5 Volume of donation

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

1.3 Medical history of donors

1.3.1 General considerations

1.3.1.1 All volunteer donors should clearly understand any information and/or questionnaire presented to them and should sign NBTS 110 (or equivalent document). Any condition declared should be discussed with the medical practitioner or senior nurse in attendance at the blood collection session unless clear, unequivocal instructions regarding the responses are available to the member of staff conducting the questioning.

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

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

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

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

1.3.1.5 Information which is, or may be, of relevance to the health of the recipient and which arises subseqent to the transfusion of the blood, should be reported to the appropriate

party (i.e. National Fractionation Centre or Consultant in charge of the hospital blood transfusion laboratory) so that further action may be taken if deemed necessary.

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

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

1.4 Examples of conditions necessitating permanent exclusion

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

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

1.4.1 Cardiovascular diseases

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

1.4.2 Central nervous system diseases

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

1.4.3 Gastrointestinal diseases

All diseases which may be of immune origin, or which render the individual liable to iron deficiency through impaired iron absorption or blood loss, should be reason for exclusion. Individuals with coeliac disease which is controlled by gluten-free diet alone, may be accepted.

1.4.4 Haematological disease

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

1.4.5 Infectious diseases which are reasons for permanent exclusion:-

AIDS, HIV infection
Brucellosis
Granuloma Inguinale
Kala Azar
Lymphogranuloma venereum
Q fever
Syphilis
Trypanosmiasis cruzi (Chagas' disease)

1.4.6 Metabolic diseases

In general, individuals who are receiving continual therapy which might adversely affect a transfusion recipient should be permanently excluded. Diabetics who are controlled by diet alone are acceptable.

1.4.7 Renal diseases

All chronic renal diseases are a reason for permanent exclusion.

1.4.8 Respiratory diseases

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

1.4.9 Recipient of human growth hormone

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

1.4.10 Auto-immune diseases

All diseases known or suspected to be auto-immune in origin, are a reason for permanent exclusion.

1.4.11 Malignancy

All diseases of malignant origin should be cause for permanent exclusion, although exception may be made for localized conditions such as carcinoma in situ of the cervix and rodent ulcer, at the conclusion of successful therapy. Conditions associated with neoblastic change should debar.

1.4.12 Diseases known to relapse

These should be considered as contra-indications to blood donation.

1.5 Conditions necessitating temporary deferral or qualified acceptance

Reference should be made to the current documents on Medical Assessment of Donors produced by the National Blood Authority and the Scottish National Blood Transfusion Service.

1.6 Pregnancy

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

1.7 Donors on treatment with drugs

1.7.1 Donor deferral for most drugs is based on the underlying illness suffered by the donor rather than for the properties of the drug itself e.g. cardiovascular disease, diabetes, anaemia and malignancies.

However, since, in general, traces of drugs in blood and blood components are harmless to patients, most persons taking medications even when prescribed are acceptable as blood donors as long as the reason for which the medication is taken is acceptable.

A pragmatic view should be taken of treatment of infections with antibiotics. Providing the donor is in good health deferral is limited to one week after cessation of antibiotic therapy. This is based on what may be regarded as a reasonable recovery period for the infection and is not related to the antibiotic therapy itself.

1.7.2 Donors taking drugs which are proven or potentially teratogenic (e.g. vitamin A derivatives) or who are taking drugs which accumulate in tissues over long periods, should not be accepted for blood donation. The period of deferment after finishing a course of treatment should be determined individually for each drug in these categories.

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

1.7.4 If the donor has taken drugs affecting platelet function (aspirin, anti-inflammatory drugs) within the last 5 days, the donation should not be used for the preparation of platelets. A list of all such drugs should be made available to staff at blood donor sessions.

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

1.7.6 Donors taking part in the clinical trial of drug therapy should be deferred until the trial is completed. Acceptability would then depend on the type of drug and its dosage, if the donor continues to take it prophylactically.

1.8 Infectious diseases

1.8.1 HIV infections

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

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

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

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

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

1.8.2 Hepatitis

Individuals with a history of jaundice or hepatitis should only be considered as blood donors 12 months after recovery from the illness. At this stage, approved tests for HBsAg and anti-HCV should be negative. The presence of anti-HBs does not debar.

1.8.2.1 Risk groups

All persons who have received a transfusion of blood or blood products, acupuncture (other than by a registered medical practitioner), tattooing, electrolysis and ear-piercing should be deferred for 12 months, as should those who have been in close contact with a case of hepatitis. Hospital staff involved in caring for patients with hepatitis, or working in hospital laboratories, may be accepted as donors provided they have not suffered an inoculation injury with blood from an individual infected with hepatitis, in which case they should be deferred for 12 months.

1.8.2.2 Circumstantial involvement

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

1.8.2.3 Return of Donors with Acute HBV Infection to Active Panel

Blood donors found to have had an acute hepatitis B infection with or without symptoms of disease can be considered eligible for re-admittance to active donor panel provided one

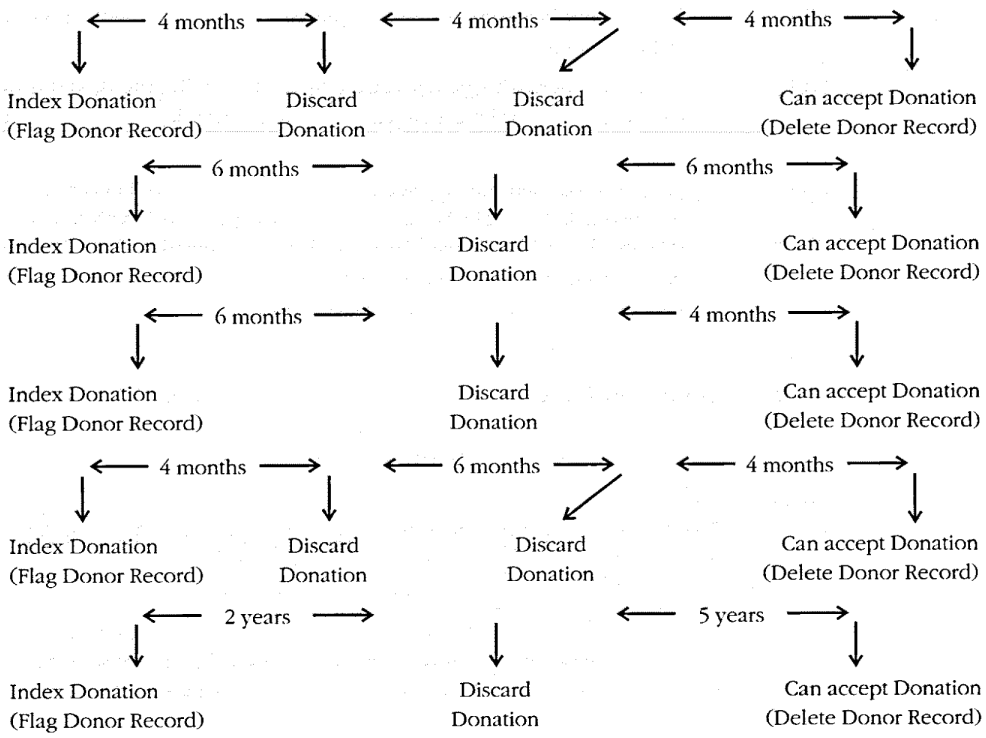
year has elapsed since the acute episode, there was clearance of HBsAg within six months and that a level of >0.1 iu/ml of anti-HBs can be demonstrated in their serum.

This recommendation applies only to donors whose sera have previously been negative for HBsAg and does not apply to known carriers of HBsAg who have lost HBsAg over protracted periods of time. Neither does it apply to donors found to have anti-HBc as a solitary marker in their serum. The reasonable expectation of an absence of integrated hepatic HBV DNA (and hence termination of infection) following recovery is only tenable for those individuals demonstrated to have undergone an acute self-limiting infection.

1.8.3 Reinstatement of seropositive donors

A positive result in microbiological screening tests, unconfirmed by a Reference Laboratory, and particularly those for anti-HIV 1+2 and anti-HCV, may on subsequent testing not be repeatable. Under carefully controlled conditions, such donors may be returned to the active panel. A minimum of 6 months must elapse between a negative/indeterminate result from the Reference Laboratory and a negative result from both RTC and Reference Laboratory before donor can be considered eligible for re-admittance to the active donor panel. At next visit thereafter the donor can be admitted to the active panel and the flag deleted from the donor record ***provided repeat screening tests at RTC are negative.***

Thus the following examples apply:



1.9 Malaria

Donors should be asked if they have visited places abroad (other than in Western Europe, Australia, New Zealand or North America) or have lived in such places at any time during the first 5 years of life.

1.9.1 Acceptability as cell donors

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

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

1.9.2 Acceptability as plasma donors

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

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

1.9.3 Donation after return from malarial areas

See algorithm in Appendix I to this Chapter.

1.10 Other tropical diseases

1.10.1 Trypanosomiasis (Cruzi)

This may lead to an acute or chronic, incurable, and even fatal illness. Blood from donors who have visited or lived in rural South America or Central America including Southern Mexico should ONLY be used for preparing plasma fractions (*not* plasma for clinical use or cryoprecipitate).

Donations from such persons may be used for normal purposes provided they have been shown by suitable test to be free of antibodies to Trypanosma Cruzi.

1.10.2 Filariasis, Kala Azar, Q Fever and Yaws

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

1.10.3 Amoebic dysentery, Schistosomiasis and Arthropod-borne encephalitides

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

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

1.11 Inoculations and immunisations

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

Live vaccines	BCG oral polio yellow fever oral typhoid rubella	3 weeks
Killed vaccines	anthrax cholera influenza diphtheria polio (Salk) rabies tetanus typhoid meningitis	48 hours
Recombinant vaccine	hepatitis B (provided there has been no known exposure)	48 hours

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

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

anti-tetanus Ig	4 weeks
normal human Ig	6 weeks

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

1.12 Physical examination of donors

1.12.1 General considerations

Most donors may be accepted on the basis of medical history, general appearance and haemoglobin estimation, although it is advisable to examine the pulse and check the blood pressure where there are any doubts, particularly in new donors.

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

1.12.2 Inspection of the donor

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

1.12.3 Weight

Healthy individuals can generally donate up to 500 ml of blood (plus small laboratory sample) without any deleterious effect on their health. A standard blood donation is 450±10% with optimum blood/anticoagulant ratio of 7:1 (See Annex I).

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

1.12.4 Haemoglobin estimation

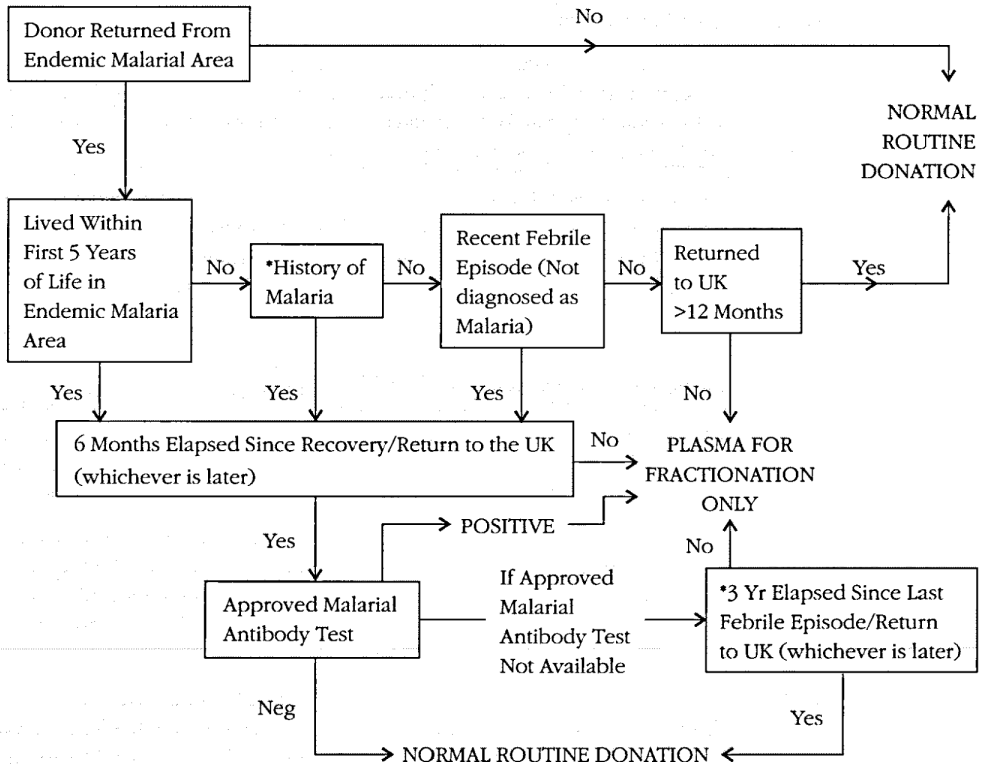
The haemoglobin concentration should be determined each time a potential donor presents. The acceptable lower limits are female donors 12.5 g/dl, or male donors 13.5 g/dl. The type of test used is left to the discretion of the RTD.

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

Where a quantitative method of Hb estimation is employed, an acceptable upper limit for donation should be set at the normal upper limit for the method used. Individuals with significantly high Hb levels should be investigated and referred appropriately.

APPENDIX I

Donation after Return from Malarial Area



**Donor with history of malaria must be sero negative before cellular donation can be used routinely.*

1.4.12 Tests required.

The manufacturer should test, as described in these Guidelines, each batch or sub-batch of a reagent obtained from the immediate container to be supplied for use. The immediate container at the point of test need not be labelled with the final label if it is the manufacturer's practice to apply the final label after testing has assured the satisfactory performance of the reagent. *See Section 3, 2.2.*

1.4.13 Foetal Calf Serum

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

1.5 Guidelines for human source material

1.5.1 Each individual donation or sample of human material in a reagent for blood group serology shall be tested and found negative for HIV antibodies and HBsAg in microbiological tests required by the UK BTS for blood donations (*see Section 1, Annex 3*). A statement is required in the package insert to this effect. Donations collected after 1st September 1991 should be tested and found negative for HCV antibody.

1.5.2 Where a sample from a patient is being used repeatedly for the evaluation of reagents for blood group serology, it should be used, whenever practical, with the patient's consent and tested and found negative for HIV antibodies and HBsAg in microbiological tests required by the UK BTS for blood donations. Samples collected after 1st September 1991 should be tested and found negative for HCV antibody.

1.5.3 To ensure retrospective serological testing, a sample of plasma, or preferably serum, of at least 1ml, collected at the same time as the donation used in the formulation of a particular reagent, should be stored at -20°C or lower until at least six months after the expiry date of the last batch of the reagent made from that material. This recommendation applies also to human donations used in the preparation of monoclonal antibody reagents.

1.6 Immediate container label

1.6.1 The label fixed to the immediate container of a reagent should leave uncovered sufficient area of the full length or circumference of the container to allow ready visual inspection of the contents.

1.6.2 The information printed on the label should be in black ink. The specificity of the reagent for blood group serology should be of a print size which is clearly legible; if possible not less than 12 point for containers of less than 5 ml volume and not less than 18 point for containers of greater than or equal to 5 ml volume. The print size of other information given on the label should not exceed that used for the specificity of the reagent.

1.6.3 The following information should be stated on the label.

1 The name or specificity of the batch or sub-batch of reagent in the same manner as described on the outer container and package insert.

2 If not of human origin, the source of the material, for example, 'mouse monoclonal', or 'Dolichos biflorus'.

3 The name, or unequivocal logo, of the manufacturer or supplier.

4 A reference number or code by which the complete manufacturing history of the batch or sub-batch can be identified.

5 The expiry date after which the reagent is not to be used when stored, within the final container, as recommended by the manufacturer. The expiry date may be stated as day, month and year, or month and year. In the latter case, the expiry date is the last day of the stated month.

6 A space should be indicated for the user to write the expiry date of a freeze-dried product after it has been reconstituted and stored as recommended.

7 The minimum net weight, or net volume, of the reagent within the final container of each batch or sub-batch, or the average net weight, (or net volume) of an immediate container together with the 'e' as defined by the Weights and Measures Act 1979.

8 A statement that the reagent contains or does not contain a preservative. If the preservative is an azide its identity and concentration should be stated.

9 Where reagent red cells are to be washed before use, a statement to that effect.

10 The recommended temperature and conditions of storage. If the reagent is to be stored only in the liquid state, a statement that the reagent is not to be frozen.

11 Any colour appearing on the main panel of the label should comply with the permitted colour coding of reagents, see 1.4.8.1, except :-

(a) The main panel of labels of enzyme-treated reagent red cells should be coloured pink in order to be distinguishable from non enzyme-treated reagent red cells. Pantone colour reference Pink 432 is recommended.

(b) The company logo or name, which if coloured, should be located away from the main panel of the label where details of the specificity are given, and should not cause confusion with the permitted colour coding of reagents. Pantone colour reference 223 is recommended.

12 If reagent red cells are suspended in a low ionic strength medium, this should be stated on the label.

13 A statement that the reagent is for *in vitro* use only.

14 A statement that the user should refer to the package insert for details on the use of the reagent, e.g. 'Read package insert before use.'

15 Further labelling guidelines specific to a particular reagent may be described under the appropriate paragraphs.

16 Where for reasons of rarity of the reagent, the immediate container is of a size insufficient to support a label with the information detailed above, the information provided on the label should conform to the minimum requirements of EN 375: 1990, that is:

the product name,
the supplier or logo,
the reference number or code to identify the batch or sub-batch,
the expiry date, and
the appropriate cautionary statements or symbols.

1.7 Package insert

1.7.1 Each reagent for blood group serology should be supplied with an accompanying document (package insert). If two or more immediate containers requiring identical package inserts are placed in a single package, only one package insert is necessary.

1.7.2 Information in the package insert should include that required for the label of the immediate container together with the following.

1 The batch reference on the label of the immediate container to which the package insert refers.

2 The detailed methods of use recommended by the manufacturer for the stated batch or sub-batch of reagent, including the serological controls to assure the specificity of tests, any limitations or precautions, together with other information relevant to the safe use, storage and disposal of the reagent and its immediate container. This requirement includes information on the type of specimen to be used, any pretreatment, and the conditions of storage prior to use by any method recommended for use by the manufacturer.

The duration and temperature of incubation or of other procedures should be explicit; terms such as 'room temperature' or 'immediately centrifuge' are to be avoided.

3 Contaminating antibodies to antigens having a prevalence of greater than 99 per cent in the general population of the UK should be excluded by negative results in tests using samples of red cells from four different individuals who lack the antigen corresponding to the antibody specificity under test.

If tests using all methods recommended for use by the manufacturer do not exclude the presence of antibodies to the following antigens, these antibody specificities should be stated in the package insert as not having been excluded in specificity testing.
Xg^a, Do^a, Yt^a, Co^b, Wr^a, Bg^a and V^w.

4 If the reagent contains material of human origin, a statement that the human material has been tested at source and found negative for HIV and HCV antibodies and HBsAg in microbiological tests required by the UK BTS for blood donations. See 1.5.

5 If the reagent contains material of animal or human origin, a statement that the reagent cannot be assumed to be free from infectious agents and care is to be taken in the use and disposal of the container and its contents.

6 A statement that the reagent is for *in vitro* use only.

7 If the reagent is supplied at the optimal dilution for use, a statement that the reagent is to be used as supplied without dilution or addition.

8 If the reagent is supplied to the user in a form requiring dilution for use, the extent of which is determined by the user, full details of the diluent and dilution procedure, together with a statement that the user is to perform the tests to assure the correct performance of the diluted reagent. That is, at the dilution and for the techniques selected for use, tests for potency, specificity, stability and, for a reagent to be used by a slide technique, for avidity.

9 If the reagent is supplied in a freeze-dried form, full details of the reconstitution and reconstitution medium, together with the period during which it may be used following reconstitution, when stored as recommended by the manufacturer. The manufacturer should include a statement to the effect that after reconstituting the dried reagent the user should record the recommended expiry date on the space provided on the label.

10 A statement that the reagent has been characterised by the procedures recommended in the package insert and that its suitability for use in other techniques must be determined by the user.

11 For reagents other than reagent red cells, a statement that the reagent is not to be used if a precipitate, fibrin gel, or particles are present.

12 A statement that storage of the reagent at temperatures outside the recommended range may result in an acceleration in the rate of loss of reactivity.

13 The nature of any colourant added to the reagent.

14 If a blood grouping reagent is supplied for use with a reagent control, a statement that each test red cell sample is to be tested in parallel with the blood grouping reagent and control, and that no determination of the blood group is possible with that reagent if the reagent control effects agglutination of the test cell sample.

In addition, a statement that caution should be exercised in the interpretation of results of tests performed with such reagents at temperatures other than the temperature recommended by the manufacturer.

15 For blood grouping reagents containing monoclonal antibodies, the identity of the cell line(s) from which the monoclonal antibodies have been derived.

16 For blended reagents other than anti-human globulin, polyclonal preparations obtained from the same individual and different culture supernatants of the same monoclonal preparation, details of the blend (see 1.3.12).

17 For reagent red cells a statement that the reagent is not to be used if it is obviously discoloured or if the suspension medium indicates obvious haemolysis.

18 For reagent red cells, one or more of the following statements, as appropriate:

'for ABO grouping'
'for Rh D grouping'
'for antibody identification'
'for antibody screening'
'for the control of the anti-human globulin technique'

19 For reagent red cells that are to be washed prior to use, instructions on the washing and resuspension of the red cells.

20 A statement that loss of reactivity may occur during the stated shelf life of the red cells and that since this loss is partly determined by characteristics of individual blood donations or donors, which cannot be predicted or controlled, the conditions of storage and use recommended by the manufacturer should be rigidly applied.

21 When red cells are preserved in LISS, especially in the presence of aminoglycoside antibiotics, e.g. neomycin sulphate, they should be discarded within 24 hours of resuspension. (*Note: there is accelerated deterioration in the reactivity of* Fy^a, Fy^b, $\overline{s}$ *and, to a lesser extent, S antigens under these conditions*).

22 Reagent red cells which have been washed and resuspended in saline or LISS solution are to be discarded not more than 24 hours after their preparation.

23 For enzyme-treated reagent red cells, information should be given concerning those antigens which are rendered inactive or less active by the enzyme treatment used. In addition, a statement that the efficacy of enzyme-treated reagent red cells should be confirmed daily or each time the reagent is used, whichever is shorter.

24 If the user is required to add a potentiator, such as enzyme or albumin, a statement that the potentiator should comply with the requirements of these Guidelines.

25 A list of any additional reagents that are required by the user to undertake any method recommended by the manufacturer but which is not provided.

26 A statement that the reagent does or does not comply with the recommendations contained in the latest issue of Section 3 of the Guidelines for the Blood Transfusion Services in the United Kingdom.

27 Further package insert guidelines specific to a particular reagent may be described under the appropriate paragraph of these Guidelines.

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Guidelines for the Blood Transfusion Service

Amendment 1

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GENERAL CONSIDERATIONS

SECTION 1

Chapter 1

Selection of Donors

1.1 General Considerations

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

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

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

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

1.1.3 Hazardous Occupations or Hobbies

1.1.3.1 Service aircrew, whether trained or under training, are not permitted to act as blood donors. Civil aircrew may not donate if on flying duties.

1.1.3.2 Occupations where a delayed faint may present a hazard to the donor and/or others - accept only when the individual is going **off** duty, e.g. train, HGV or bus driver, heavy machine or crane operator, driver, climbing ladders or scaffolding, miner working underground.

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

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

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

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

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

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

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

1.2 Medical Assessment

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

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

1.2.3 Age

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

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

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

1.2.4 Frequency of donations

Usually 2 donations are given in a 12-month period. Many donors are able to give blood more frequently without developing iron deficiency so that an interval of 16 weeks between donations is considered a reasonable minimum. Any further reduction, to an absolute minimum interval of 12 weeks, must be accompanied by appropriate donor monitoring.

1.2.5 Volume of donation

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

1.3 Medical history of donors

1.3.1 General considerations

1.3.1.1 All volunteer donors should clearly understand any information and/or questionnaire presented to them and should sign NBTS 110 (or equivalent document). Any condition declared should be discussed with the medical practitioner or senior nurse in attendance at the blood collection session unless clear, unequivocal instructions regarding the responses are available to the member of staff conducting the questioning.

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

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

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

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

1.3.1.5 Information which is, or may be, of relevance to the health of the recipient and which arises subseqent to the transfusion of the blood, should be reported to the appropriate

party (i.e. National Fractionation Centre or Consultant in charge of the hospital blood transfusion laboratory) so that further action may be taken if deemed necessary.

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

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

1.4 Examples of conditions necessitating permanent exclusion

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

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

1.4.1 Cardiovascular diseases

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

1.4.2 Central nervous system diseases

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

1.4.3 Gastrointestinal diseases

All diseases which may be of immune origin, or which render the individual liable to iron deficiency through impaired iron absorption or blood loss, should be reason for exclusion. Individuals with coeliac disease which is controlled by gluten-free diet alone, may be accepted.

1.4.4 Haematological disease

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

1.4.5 Infectious diseases which are reasons for permanent exclusion:-

AIDS, HIV infection
Brucellosis
Granuloma Inguinale
Kala Azar
Lymphogranuloma venereum
Q fever
Syphilis
Trypanosmiasis cruzi (Chagas' disease)

1.4.6 Metabolic diseases

In general, individuals who are receiving continual therapy which might adversely affect a transfusion recipient should be permanently excluded. Diabetics who are controlled by diet alone are acceptable.

1.4.7 Renal diseases

All chronic renal diseases are a reason for permanent exclusion.

1.4.8 Respiratory diseases

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

1.4.9 Recipient of human growth hormone

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

1.4.10 Auto-immune diseases

All diseases known or suspected to be auto-immune in origin, are a reason for permanent exclusion.

1.4.11 Malignancy

All diseases of malignant origin should be cause for permanent exclusion, although exception may be made for localized conditions such as carcinoma in situ of the cervix and rodent ulcer, at the conclusion of successful therapy. Conditions associated with neoblastic change should debar.

1.4.12 Diseases known to relapse

These should be considered as contra-indications to blood donation.

1.5 Conditions necessitating temporary deferral or qualified acceptance

Reference should be made to the current documents on Medical Assessment of Donors produced by the National Blood Authority and the Scottish National Blood Transfusion Service.

1.6 Pregnancy

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

1.7 Donors on treatment with drugs

1.7.1 Donor deferral for most drugs is based on the underlying illness suffered by the donor rather than for the properties of the drug itself e.g. cardiovascular disease, diabetes, anaemia and malignancies.

However, since, in general, traces of drugs in blood and blood components are harmless to patients, most persons taking medications even when prescribed are acceptable as blood donors as long as the reason for which the medication is taken is acceptable.

A pragmatic view should be taken of treatment of infections with antibiotics. Providing the donor is in good health deferral is limited to one week after cessation of antibiotic therapy. This is based on what may be regarded as a reasonable recovery period for the infection and is not related to the antibiotic therapy itself.

1.7.2 Donors taking drugs which are proven or potentially teratogenic (e.g. vitamin A derivatives) or who are taking drugs which accumulate in tissues over long periods, should not be accepted for blood donation. The period of deferment after finishing a course of treatment should be determined individually for each drug in these categories.

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

1.7.4 If the donor has taken drugs affecting platelet function (aspirin, anti-inflammatory drugs) within the last 5 days, the donation should not be used for the preparation of platelets. A list of all such drugs should be made available to staff at blood donor sessions.

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

1.7.6 Donors taking part in the clinical trial of drug therapy should be deferred until the trial is completed. Acceptability would then depend on the type of drug and its dosage, if the donor continues to take it prophylactically.

1.8 Infectious diseases

1.8.1 HIV infections

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

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

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

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

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

1.8.2 Hepatitis

Individuals with a history of jaundice or hepatitis should only be considered as blood donors 12 months after recovery from the illness. At this stage, approved tests for HBsAg and anti-HCV should be negative. The presence of anti-HBs does not debar.

1.8.2.1 Risk groups

All persons who have received a transfusion of blood or blood products, acupuncture (other than by a registered medical practitioner), tattooing, electrolysis and ear-piercing should be deferred for 12 months, as should those who have been in close contact with a case of hepatitis. Hospital staff involved in caring for patients with hepatitis, or working in hospital laboratories, may be accepted as donors provided they have not suffered an inoculation injury with blood from an individual infected with hepatitis, in which case they should be deferred for 12 months.

1.8.2.2 Circumstantial involvement

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

1.8.2.3 Return of Donors with Acute HBV Infection to Active Panel

Blood donors found to have had an acute hepatitis B infection with or without symptoms of disease can be considered eligible for re-admittance to active donor panel provided one

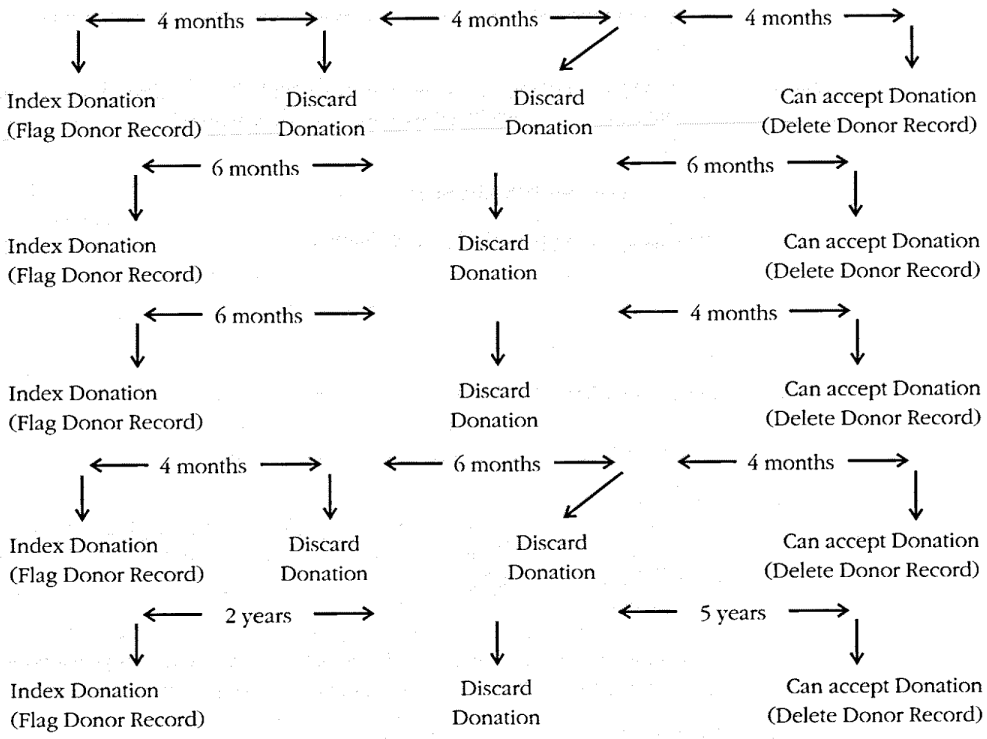
year has elapsed since the acute episode, there was clearance of HBsAg within six months and that a level of >0.1 iu/ml of anti-HBs can be demonstrated in their serum.

This recommendation applies only to donors whose sera have previously been negative for HBsAg and does not apply to known carriers of HBsAg who have lost HBsAg over protracted periods of time. Neither does it apply to donors found to have anti-HBc as a solitary marker in their serum. The reasonable expectation of an absence of integrated hepatic HBV DNA (and hence termination of infection) following recovery is only tenable for those individuals demonstrated to have undergone an acute self-limiting infection.

1.8.3 Reinstatement of seropositive donors

A positive result in microbiological screening tests, unconfirmed by a Reference Laboratory, and particularly those for anti-HIV 1+2 and anti-HCV, may on subsequent testing not be repeatable. Under carefully controlled conditions, such donors may be returned to the active panel. A minimum of 6 months must elapse between a negative/indeterminate result from the Reference Laboratory and a negative result from both RTC and Reference Laboratory before donor can be considered eligible for re-admittance to the active donor panel. At next visit thereafter the donor can be admitted to the active panel and the flag deleted from the donor record ***provided repeat screening tests at RTC are negative.***

Thus the following examples apply:



1.9 Malaria

Donors should be asked if they have visited places abroad (other than in Western Europe, Australia, New Zealand or North America) or have lived in such places at any time during the first 5 years of life.

1.9.1 Acceptability as cell donors

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

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

1.9.2 Acceptability as plasma donors

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

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

1.9.3 Donation after return from malarial areas

See algorithm in Appendix I to this Chapter.

1.10 Other tropical diseases

1.10.1 Trypanosomiasis (Cruzi)

This may lead to an acute or chronic, incurable, and even fatal illness. Blood from donors who have visited or lived in rural South America or Central America including Southern Mexico should ONLY be used for preparing plasma fractions (*not* plasma for clinical use or cryoprecipitate).

Donations from such persons may be used for normal purposes provided they have been shown by suitable test to be free of antibodies to Trypanosma Cruzi.

1.10.2 Filariasis, Kala Azar, Q Fever and Yaws

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

1.10.3 Amoebic dysentery, Schistosomiasis and Arthropod-borne encephalitides

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

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

1.11 Inoculations and immunisations

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

Live vaccines	BCG oral polio yellow fever oral typhoid rubella	3 weeks
Killed vaccines	anthrax cholera influenza diphtheria polio (Salk) rabies tetanus typhoid meningitis	48 hours
Recombinant vaccine	hepatitis B (provided there has been no known exposure)	48 hours

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

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

anti-tetanus Ig	4 weeks
normal human Ig	6 weeks

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

1.12 Physical examination of donors

1.12.1 General considerations

Most donors may be accepted on the basis of medical history, general appearance and haemoglobin estimation, although it is advisable to examine the pulse and check the blood pressure where there are any doubts, particularly in new donors.

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

1.12.2 Inspection of the donor

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

1.12.3 Weight

Healthy individuals can generally donate up to 500 ml of blood (plus small laboratory sample) without any deleterious effect on their health. A standard blood donation is 450±10% with optimum blood/anticoagulant ratio of 7:1 (See Annex I).

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

1.12.4 Haemoglobin estimation

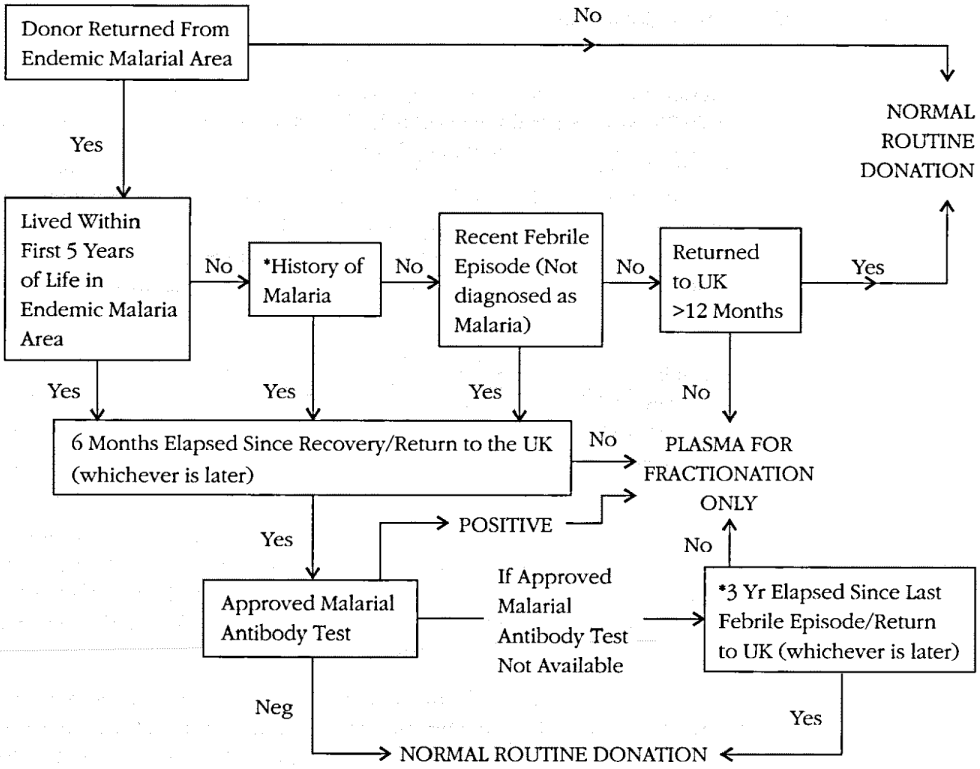
The haemoglobin concentration should be determined each time a potential donor presents. The acceptable lower limits are female donors 12.5 g/dl, or male donors 13.5 g/dl. The type of test used is left to the discretion of the RTD.

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

Where a quantitative method of Hb estimation is employed, an acceptable upper limit for donation should be set at the normal upper limit for the method used. Individuals with significantly high Hb levels should be investigated and referred appropriately.

APPENDIX I

Donation after Return from Malarial Area



**Donor with history of malaria must be sero negative before cellular donation can be used routinely.*

1.4.12 Tests required.

The manufacturer should test, as described in these Guidelines, each batch or sub-batch of a reagent obtained from the immediate container to be supplied for use. The immediate container at the point of test need not be labelled with the final label if it is the manufacturer's practice to apply the final label after testing has assured the satisfactory performance of the reagent. *See Section 3, 2.2.*

1.4.13 Foetal Calf Serum

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

1.5 Guidelines for human source material

1.5.1 Each individual donation or sample of human material in a reagent for blood group serology shall be tested and found negative for HIV antibodies and HBsAg in microbiological tests required by the UK BTS for blood donations (*see Section 1, Annex 3*). A statement is required in the package insert to this effect. Donations collected after 1st September 1991 should be tested and found negative for HCV antibody.

1.5.2 Where a sample from a patient is being used repeatedly for the evaluation of reagents for blood group serology, it should be used, whenever practical, with the patient's consent and tested and found negative for HIV antibodies and HBsAg in microbiological tests required by the UK BTS for blood donations. Samples collected after 1st September 1991 should be tested and found negative for HCV antibody.

1.5.3 To ensure retrospective serological testing, a sample of plasma, or preferably serum, of at least 1ml, collected at the same time as the donation used in the formulation of a particular reagent, should be stored at -20°C or lower until at least six months after the expiry date of the last batch of the reagent made from that material. This recommendation applies also to human donations used in the preparation of monoclonal antibody reagents.

1.6 Immediate container label

1.6.1 The label fixed to the immediate container of a reagent should leave uncovered sufficient area of the full length or circumference of the container to allow ready visual inspection of the contents.

1.6.2 The information printed on the label should be in black ink. The specificity of the reagent for blood group serology should be of a print size which is clearly legible; if possible not less than 12 point for containers of less than 5 ml volume and not less than 18 point for containers of greater than or equal to 5 ml volume. The print size of other information given on the label should not exceed that used for the specificity of the reagent.

1.6.3 The following information should be stated on the label.

1 The name or specificity of the batch or sub-batch of reagent in the same manner as described on the outer container and package insert.

2 If not of human origin, the source of the material, for example, 'mouse monoclonal', or 'Dolichos biflorus'.

3 The name, or unequivocal logo, of the manufacturer or supplier.

4 A reference number or code by which the complete manufacturing history of the batch or sub-batch can be identified.

5 The expiry date after which the reagent is not to be used when stored, within the final container, as recommended by the manufacturer. The expiry date may be stated as day, month and year, or month and year. In the latter case, the expiry date is the last day of the stated month.

6 A space should be indicated for the user to write the expiry date of a freeze-dried product after it has been reconstituted and stored as recommended.

7 The minimum net weight, or net volume, of the reagent within the final container of each batch or sub-batch, or the average net weight, (or net volume) of an immediate container together with the 'e' as defined by the Weights and Measures Act 1979.

8 A statement that the reagent contains or does not contain a preservative. If the preservative is an azide its identity and concentration should be stated.

9 Where reagent red cells are to be washed before use, a statement to that effect.

10 The recommended temperature and conditions of storage. If the reagent is to be stored only in the liquid state, a statement that the reagent is not to be frozen.

11 Any colour appearing on the main panel of the label should comply with the permitted colour coding of reagents, see 1.4.8.1, except :-

(a) The main panel of labels of enzyme-treated reagent red cells should be coloured pink in order to be distinguishable from non enzyme-treated reagent red cells. Pantone colour reference Pink 432 is recommended.

(b) The company logo or name, which if coloured, should be located away from the main panel of the label where details of the specificity are given, and should not cause confusion with the permitted colour coding of reagents. Pantone colour reference 223 is recommended.

12 If reagent red cells are suspended in a low ionic strength medium, this should be stated on the label.

13 A statement that the reagent is for *in vitro* use only.

14 A statement that the user should refer to the package insert for details on the use of the reagent, e.g. 'Read package insert before use.'

15 Further labelling guidelines specific to a particular reagent may be described under the appropriate paragraphs.

16 Where for reasons of rarity of the reagent, the immediate container is of a size insufficient to support a label with the information detailed above, the information provided on the label should conform to the minimum requirements of EN 375: 1990, that is:

the product name,
the supplier or logo,
the reference number or code to identify the batch or sub-batch,
the expiry date, and
the appropriate cautionary statements or symbols.

1.7 Package insert

1.7.1 Each reagent for blood group serology should be supplied with an accompanying document (package insert). If two or more immediate containers requiring identical package inserts are placed in a single package, only one package insert is necessary.

1.7.2 Information in the package insert should include that required for the label of the immediate container together with the following.

1 The batch reference on the label of the immediate container to which the package insert refers.

2 The detailed methods of use recommended by the manufacturer for the stated batch or sub-batch of reagent, including the serological controls to assure the specificity of tests, any limitations or precautions, together with other information relevant to the safe use, storage and disposal of the reagent and its immediate container. This requirement includes information on the type of specimen to be used, any pretreatment, and the conditions of storage prior to use by any method recommended for use by the manufacturer.

The duration and temperature of incubation or of other procedures should be explicit; terms such as 'room temperature' or 'immediately centrifuge' are to be avoided.

3 Contaminating antibodies to antigens having a prevalence of greater than 99 per cent in the general population of the UK should be excluded by negative results in tests using samples of red cells from four different individuals who lack the antigen corresponding to the antibody specificity under test.

If tests using all methods recommended for use by the manufacturer do not exclude the presence of antibodies to the following antigens, these antibody specificities should be stated in the package insert as not having been excluded in specificity testing.
Xg^a, Do^a, Yt^a, Co^b, Wr^a, Bg^a and V^w.

4 If the reagent contains material of human origin, a statement that the human material has been tested at source and found negative for HIV and HCV antibodies and HBsAg in microbiological tests required by the UK BTS for blood donations. See 1.5.

5 If the reagent contains material of animal or human origin, a statement that the reagent cannot be assumed to be free from infectious agents and care is to be taken in the use and disposal of the container and its contents.

6 A statement that the reagent is for *in vitro* use only.

7 If the reagent is supplied at the optimal dilution for use, a statement that the reagent is to be used as supplied without dilution or addition.

8 If the reagent is supplied to the user in a form requiring dilution for use, the extent of which is determined by the user, full details of the diluent and dilution procedure, together with a statement that the user is to perform the tests to assure the correct performance of the diluted reagent. That is, at the dilution and for the techniques selected for use, tests for potency, specificity, stability and, for a reagent to be used by a slide technique, for avidity.

9 If the reagent is supplied in a freeze-dried form, full details of the reconstitution and reconstitution medium, together with the period during which it may be used following reconstitution, when stored as recommended by the manufacturer. The manufacturer should include a statement to the effect that after reconstituting the dried reagent the user should record the recommended expiry date on the space provided on the label.

10 A statement that the reagent has been characterised by the procedures recommended in the package insert and that its suitability for use in other techniques must be determined by the user.

11 For reagents other than reagent red cells, a statement that the reagent is not to be used if a precipitate, fibrin gel, or particles are present.

12 A statement that storage of the reagent at temperatures outside the recommended range may result in an acceleration in the rate of loss of reactivity.

13 The nature of any colourant added to the reagent.

14 If a blood grouping reagent is supplied for use with a reagent control, a statement that each test red cell sample is to be tested in parallel with the blood grouping reagent and control, and that no determination of the blood group is possible with that reagent if the reagent control effects agglutination of the test cell sample.

In addition, a statement that caution should be exercised in the interpretation of results of tests performed with such reagents at temperatures other than the temperature recommended by the manufacturer.

15 For blood grouping reagents containing monoclonal antibodies, the identity of the cell line(s) from which the monoclonal antibodies have been derived.

16 For blended reagents other than anti-human globulin, polyclonal preparations obtained from the same individual and different culture supernatants of the same monoclonal preparation, details of the blend (see 1.3.12).

17 For reagent red cells a statement that the reagent is not to be used if it is obviously discoloured or if the suspension medium indicates obvious haemolysis.

18 For reagent red cells, one or more of the following statements, as appropriate:

'for ABO grouping'
'for Rh D grouping'
'for antibody identification'
'for antibody screening'
'for the control of the anti-human globulin technique'

19 For reagent red cells that are to be washed prior to use, instructions on the washing and resuspension of the red cells.

20 A statement that loss of reactivity may occur during the stated shelf life of the red cells and that since this loss is partly determined by characteristics of individual blood donations or donors, which cannot be predicted or controlled, the conditions of storage and use recommended by the manufacturer should be rigidly applied.

21 When red cells are preserved in LISS, especially in the presence of aminoglycoside antibiotics, e.g. neomycin sulphate, they should be discarded within 24 hours of resuspension. (*Note: there is accelerated deterioration in the reactivity of* Fy^a, Fy^b, $\overline{s}$ *and, to a lesser extent, S antigens under these conditions*).

22 Reagent red cells which have been washed and resuspended in saline or LISS solution are to be discarded not more than 24 hours after their preparation.

23 For enzyme-treated reagent red cells, information should be given concerning those antigens which are rendered inactive or less active by the enzyme treatment used. In addition, a statement that the efficacy of enzyme-treated reagent red cells should be confirmed daily or each time the reagent is used, whichever is shorter.

24 If the user is required to add a potentiator, such as enzyme or albumin, a statement that the potentiator should comply with the requirements of these Guidelines.

25 A list of any additional reagents that are required by the user to undertake any method recommended by the manufacturer but which is not provided.

26 A statement that the reagent does or does not comply with the recommendations contained in the latest issue of Section 3 of the Guidelines for the Blood Transfusion Services in the United Kingdom.

27 Further package insert guidelines specific to a particular reagent may be described under the appropriate paragraph of these Guidelines.

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PREFACE TO SECOND EDITION

Since the publication of the first edition of these Guidelines, in 1990, they seem to have been adopted as the benchmark for the practice of Transfusion Medicine and Science in the U.K. The apparent enthusiasm of those who received the document engendered a great deal of satisfaction in the hearts of those involved in its formulation, tinged with a certain amount of apprehension as they waited to see the results of putting into universal practice the principles embodied in the Guidelines.

Feedback from users has been positive, with wide encouragement that the path being followed was the right one. The many suggestions received from those who adopted the first edition have been taken into account, together with the inevitable expansion of knowledge and experience since its publication, in the production of this second edition. Its users will also see some alterations in the style of presentation, designed to make the format of the document compare with that of similar guides being produced in other communities. Hopefully, the style will continue to be regarded as "user friendly".

At the time of publication, there is a declared intention that updating will be carried out at shorter intervals, by the production of individual pages to replace those which contain superseded material. This, of course, should not preclude the assembly of a completely new edition, when full revision is obviously required.

In the mean time, comments and criticism (hopefully constructive) will continue to be welcomed.

The Guidelines are intended for the use of Blood Transfusion Centres and, where appropriate, hospital Departments of Transfusion Medicine. The advice offered is believed to represent the knowledge and opinions current at the time of printing, but neither the UKBTS/NIBSC Liaison Group nor the various groups contributing to the Guidelines can be held responsible for any damage resulting from their application.

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OCTOBER 1992

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GENERAL CONSIDERATIONS

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Chapter 1

Introduction

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

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

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

1.4 Working Groups were formed to consider:-
(i) RTC derived blood components
(ii) Fractionated plasma products
(iii) Reagents for blood group serology and HLA typing
(iv) Microbiological aspects, providing advice to the other Working Groups

Following publication of the first edition of the Guidelines for the Blood Transfusion Service in the United Kingdom (1989), these Groups were reorganized into:

(i) Standing Committee on Donor Selection, with (a) a Working Party on Donor Sessions and (b) a Working Party on Apheresis Sessions,

(ii) Standing Committee on Components,

(iii) Standing Committee on Reagents, with a Working Party on Histocompatibility Testing,

(iv) Standing Committee on Plasma for Fractionation,

This organization undertook revision of the original document, in the light of experience in its application and of current developments.

1.5 The resulting Guidelines give advice, guidance and, where appropriate, general specifications. Details of methods have been included when relevant. Reference preparations have been identified within the Guidelines. At the present time, preparations for anti-A, anti-B have been developed, as have those for IgM anti-D, rabbit complement and anti-HLA-A2. Other British and International Standards are available at NIBSC (*see Annex 2*)

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

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

1.8 The Guidelines, in general, should be used in conjunction with the current Guide to Good Pharmaceutical Manufacturing Practice (HMSO) and the Requirements for the Collection,

Processing and Quality Control of Blood, Blood Components and Plasma Derivatives; (WHO: Requirements for Biological substances, No. 27, revised 1992). European texts which are particularly relevant include:

(i) Council Directive 89/381/EEC, which covers medicinal products derived from blood and plasma donations.

(ii) Council Directive 91/356/EEC, which sets out the principles of Good Manufacturing Practice.

(iii) Preparation, use and quality assurance of blood components (Council of Europe 1991).

1.9 There are many reasons why UKBTS should achieve and maintain the highest standard of operations. That some uniformity should be engendered in the determination of those procedures which will ensure maximum safety of blood and its products has been highlighted by two events, viz;

(i) Crown Privilege, under which the UKBTS and its associated Fractionation Centres have operated, was withdrawn on 31st March 1991. Thus, fractionated plasma products require to be licensed and whilst it is unlikely that licensing procedures will be required for cellular products, licences could not be approved for fractionated products if the RTC producing the source plasma was not operating within agreed guidelines.

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

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

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

1.10 Section 1 contains guidelines for blood components prepared at RTCs.

1.10.1 Chapters 1, 3 and 4 contain recommendations for the selection of donors, in general and specifically for apheresis and immune plasma.

1.10.2 Chapters 2 and 3 give guidance on the requirements for the conduct of blood and plasma donor sessions.

1.10.3 Chapters 5 and 6 give general and specific guidelines for the production of blood components.

1.10.4 Annexes 1 to 4 contain in general those specifications and requirements which are common to the procedures previously described.

1.11 Section 2 contains Guidelines for the preparation of plasma fractions from human source plasma although the word "human" will not be included in the text.

1.11.1 Chapter 1 contains general specifications for plasma intended for fractionation and this has been divided for convenience into those features common to all types of plasma and those specific for defined plasma types. Detailed plasma specifications have been agreed between the NBTS and the SNBTS and their respective Fractionation Centres.

1.11.2 Product characteristics, the assignment of potency to batches of Factor VIII and IX concentrates and steps to achieve viral inactivation can be found in Chapters 2, 3 and 4.

1.12 Section 3 contains recommendations for the production and use of reagents used in blood group serology and HLA typing.

1.12.1 In Chapters 1 and 2 there are general guidelines for the preparation and use, respectively, of reagents relating to red cell serology.

1.12.2 Subsequent chapters contain guidelines for specific reagents, reagent red cells, low ionic strength solutions, enzymes, anti-human globulin reagents, bovine serum albumin, the use of reagents in manual, microplate and automated systems and HLA typing reagents.

Chapter 2

Guidelines on Quality System Elements for the Collection and Processing of Blood and Blood Products

Introduction

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

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

Many manufacturing and service industries have adopted the British Standard 5750 Part 1 (or its International and European equivalents IS0 9001 and EN29001) as the foundation of their quality assurance systems. The principles in these standards can apply to the UKBTS.

BS 5750 Part 1 sets out requirements for organisations in which the design and development of new processes and products are carried out. Within RTCs, the collection and processing of blood and blood products are under continuous development to improve product quality and it is therefore appropriate that their quality systems should cover design and development activity.

The intention of this chapter is to provide guidance and assistance in the use of BS 5750 Part 1 for quality assurance systems in RTCs. The chapter cannot for copyright reasons reproduce BS 5750 Part 1 and should therefore be read in conjunction with it.

Although the chapter is written with examples drawn from Transfusion Centres, the same principles apply to Fractionation Centres.

For ease of reference, the paragraph headings below correspond with those in BS 5750 Part 1.

2.1 Management Responsibility

2.1.1 Quality Policy

Each RTC should develop a policy for achieving and maintaining quality. The policy should define what quality means to the RTC; should describe the commitment of management; should explain how quality will be achieved and maintained; should establish the involvement of staff and the steps to be taken, such as training, to ensure they can understand and contribute to the furtherance of the policy.

The NBTS Quality Policy developed in England and Wales which may be used as a model is given in Appendix 1.

2.1.2 Organisation

2.1.2.1 Responsibility And Authority

Each RTC should have an organisational structure that enables its quality policy to be achieved. Staff should have their responsibilities and objectives affecting quality clearly defined and documented.

Particular duties which should be covered and which may feature in the job descriptions of several senior managers include:

(a) The control of the quality system to ensure all blood products meet their specifications.

(b) The identification of quality problems and the initiation and control of corrective actions.

(c) The assessment of the effectiveness of corrective action.

(d) The control of production and release of products until any quality problems have been resolved.

2.1.2.2 Verification, Resources And Personnel

Each RTC should have an ongoing programme to ensure it is producing blood products in accordance with its quality objectives. The programme should include quality monitoring, quality audit, NEQAS participation and feedback from donors and product users. The programme should identify outside laboratory services which may be used for confirmatory testing, environmental monitoring etc.

2.1.2.3. Management Representative

Each RTC should appoint a person responsible for quality, independent of production and preferably of other functions, who should have the necessary authority and the responsibility for ensuring that the requirements of these Guidelines are implemented and maintained.

The person responsible for quality should report directly to the Chief Executive/RTD or to another designated person who is entirely independent of production.

In the event of conflict arising between the person responsible for quality and the Chief Executive/RTD (or designated person), the circumstances and the decision taken should be fully documented and discussed at the time of the next quality audit.

2.1.3 Management Review

Each RTC should have a documented procedure for the review of its quality system. The object of the review is to ensure that the quality system and programme of the RTC is suitable and effective. The review should examine the RTC's quality policies and review the effectiveness of those policies in terms of hospital user satisfaction, donor response and satisfaction, conformance of product to specification, outcome of internal and external quality audits, MCA assessments and other appropriate measures.

2.2 The Quality System

The quality system should be defined and recorded in a Quality Manual. The Manual should include or refer to the location of all the key elements of the system. It should show the inter-relationship of management and computer systems with the quality system and be sufficiently detailed to show that systems a) exist, b) are operating and c) are effective. The manual should cover the elements of the quality system identified in BS 5750 Part 1 and these Guidelines.

2.3 Contract Review

Most RTCs now have a formal contract between themselves and hospital users in which product quality, quantity and service details are specified. These contracts should be reviewed during negotiation to ensure that commitments are clear to both contracting parties and can be met without compromising quality. This review should be a formal, documented step in the contracting process. Subsequent reviews should be carried out periodically during the duration of the contract

2.4 Design Control

2.4.1 The Design Process

The design of a blood product includes the process of defining a need for the product; drawing up a specification; deciding the best way to obtain it; deciding which pack to use; deciding how to label it; how to prepare it for use; devising quality criteria; and monitoring how effective the product is clinically.

2.4.2 Product Master File

For each product the design process should be documented in a product master file which should include or refer to the location of the following information.

(a) Specifications.

(b) Standard operating procedures.

(c) Quality assurance procedures and specifications including quality control checks and the apparatus used.

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

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

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

(g) The record in the product master file should be maintained for at least fifteen years.

(h) The master file should be prepared, dated and signed by a designated person(s). Any changes should be authorised in writing by the signature of a designated person(s).

2.4.3 Design Changes

All design changes should be covered by appropriate documented procedures which ensure their identification, validation, documentation and review. Common examples of changes covered by this requirement include changes in donor selection criteria, centrifuge parameters, virology testing protocols and the introduction or trial of new pieces of equipment.

2.4.4 Computer Systems

Computer systems are frequently critical to the quality of blood products. They are used in donor management, laboratory and issue systems for the recording, manipulation and analysis of data. In addition, automated laboratory equipment contains software governing its operation. In all cases the responsibility for final validation of the software is that of the RTC. Particular attention should be given to the validation of integrated systems involving disparate software from different sources. A designated person in each RTC should ensure that the design, validation, documentation and changes to software are controlled in a systematic way. Written procedures should exist to ensure that the quality system embraces all aspects of the computer system which may affect quality. A designated person should be responsible for auditing the quality aspects of the computer system.

2.5 Document Control

2.5.1 Document Approval And Issue

This section covers all documentation which may affect the quality of the product. Particularly, forms, work-sheets, laboratory work-books, computer generated lists, machine

outputs, standard operating procedures, software documentation specifications and contract documentation may all fall into this category.

A system should exist to review and approve documents before issue and to ensure that current revisions of appropriate documents are available at all locations, including mobile blood collection sites.

2.5.2 Document Changes/Modifications

Only authorised, up-to-date documents should be used and any changes should themselves be authorised. Changes to computer screens should be treated in the same way as changes to a form or similar document.

Master copies of each issue of any quality system document should be clearly marked and retained in a manner which cannot lead to confusion with current documents. Superseded master copies should be marked with their date of issue and the date on which they were superseded and retained for the Product Master File.

All superseded documents should be removed from use.

2.6 Purchasing

2.6.1 General

Every RTC purchases items which are critical to the product quality, examples are printed bar code labels, blood packs, reagents and computer software. It is the responsibility of the purchaser to ensure that the requirements which suppliers are expected to meet have been specified. It is also the responsibility of the purchaser to ensure conformance to the specification.

2.6.2 Assessment of suppliers to the RTC

The extent of control exercised by the purchasing RTC in assessing its suppliers will depend on the type of product being purchased and the supplier's demonstrated capability.

Assessment of a supplier should ensure that the supplier's capability and quality system, and the RTC's capability of monitoring product received, together provide a system that ensures that the specified requirements are met. The limits of the responsibilities accepted by each of the parties of the contract should be clearly defined.

The supplier should not pass a contract or any part of it to another manufacturer to fulfil on their behalf without the prior consent of the RTC.

The purchaser RTC should ensure that the supplier has adequate quality systems, premises, equipment and staff with sufficient knowledge and experience to carry out the work satisfactorily. This may require the RTC to carry-out a quality audit on its supplier and adequate access to the supplier's premises should be negotiated.

2.6.3 Purchasing Data

The purchasing documents including the specification determine the quality of the product received. They should be drawn up using a formal procedure to ensure that the supplier of the product has all the necessary information.

2.6.4 Verification Of Purchased Product

In most cases it will not be possible for the RTC to verify conformance to all the aspects of a specification on receipt of the product. However, where visual checks are carried out they should be specified and documented.

Verification of software by designated RTC staff in a working environment should be specified in the contract.

2.7 Purchaser Supplied Product

An example of this category of product is an autologous donation. Blood may be accepted from the donor/patient using special criteria. However, the RTC should have systems in place to ensure that the quality and identification are maintained through appropriate processing, labelling and storage.

2.8 Product Identification and Traceability

There should be an audit trail allowing traceability of a blood product from the patient to the donor. The key to traceability is the donation number. Using the donation number it should be possible to audit every step in the manufacturing history of that blood product.

2.9 Process Control

2.9.1 General

The person responsible for quality in each RTC should ensure that blood and plasma collection and processing are carried out under appropriately controlled conditions. Guidance on process control in the pharmaceutical industry may be found in the "Rules Governing Medicinal Products in the European Community Volume IV (Guide to Good Manufacturing Practice For Medicinal Products)."

Those items which contribute to a controlled process are detailed below.

2.9.1.1 Standard Operating Procedures

Each procedure which affects the quality of the product should have a standard operating procedure (SOP). The SOP should be agreed between those responsible for Quality Assurance and Collection or Processing. It should provide step-by-step instructions for the procedures; details of any quality control checks to be performed; instructions on the procedure to be followed in the event of unforeseen problems; instructions on the presentation of the product to the next processing stage or disposal if appropriate.

2.9.1.2 Monitoring Of Procedures And Equipment

The effectiveness of procedures and equipment should be monitored with sufficient frequency to provide assurance that the process is in control. An example is the scrutiny of changes in the number of deferred donors which may reflect changes in the effectiveness of the donor screening programme.

2.9.1.3 Equipment Maintenance

All equipment should be cleaned and maintained in accordance with documented procedures. Records of cleaning and maintenance should be kept.

2.9.1.4 Cleaning And Environmental Monitoring

There should be a written cleaning schedule for all areas. Records should show that the schedule has been followed. Suitable techniques should be used to show that the appropriate level of cleanliness is maintained, these techniques may include bacteriological monitoring.

Appropriate standards should be set and monitored for blood collection sites which are hired by RTCs.

2.9.2 Special Processes

There are many processes in the production of blood products where the results cannot readily be verified by inspection and testing. Examples are the effect of agitation during collection and the effect of freezing schedules on plasma quality. All such processes should be identified and special care taken to document adherence to the SOP or other measures which may be taken to ensure the specification is achieved.

2.10 Inspection And Testing

2.10.1 Receiving Inspection And Testing

The quality of blood products relies in the first instance on the procedures used to screen blood donors. These procedures should be documented and appropriate records kept to demonstrate that they were effectively carried out.

Other raw materials which require documented acceptance procedures will include blood packs, reagents and labels.

2.10.2 In-Process Inspection And Testing

During blood processing, the blood products and the blood samples taken at session are handled separately. Each RTC should have appropriate systems in place to uniquely identify the sample with the product.

The test results from the samples should enable products to be appropriately handled, quarantined or disposed of.

The frequency of quality control tests on the products should conform to those set-out in these Guidelines.

2.10.3 Final Inspection And Testing

Products should only be released for issue when all tests have been satisfactorily completed. Release should be a defined step where all records on the product are reviewed for conformance to specified requirements. Customarily these records include donor records, blood grouping and microbiology results and processing records. Computers may be used to facilitate accurate review of records, and may permit or withhold the release of an individual unit. However, the correct release of products is the responsibility of the designated person carrying out the procedure. The computer system should be shown to be fully secure against the possibility of uninspected or defective product being released.

Procedures should be established for a back-up manual or computer system to assist the release of products when the main computer system is not operational.

2.10.4 Inspection And Test Records

In addition to records of the tests and inspections which have been carried out, each RTC should have defined procedures for the retention and retrieval of test samples for each blood donation tested.

2.11 Inspection, Measuring And Test Equipment

Each RTC should have a system to ensure that the criteria and tools which it uses for measurement are able to produce consistent results. This applies to the reagents and equipment used in the laboratory which should be stored, used, cleaned, calibrated and maintained in accordance with written procedures and the manufacturer's instructions. Any deviations from manufacturer's instructions should be validated and documented.

Consistent measurement techniques should be used for measuring non-laboratory activities such as the satisfaction of donors, the effectiveness of publicity techniques and the service provided to blood users.

2.12 Inspection And Test Status

Each RTC should have a system to ensure that at all times, the status of all products can be identified. This identification is usually by labelling on the pack or by a particular physical location which is itself labelled, segregated and appropriately secured. The identification should be confirmed by the computer record. Particular care should be given to identification and security of packs at blood collection sessions. Care should be taken to preserve the status of products, for example by adopting a left to right discipline when making up orders for issue etc.

The practice of labelling blood packs with blood groups at donor sessions should be viewed with caution. The groups are unvalidated and the labels do not reflect the test status of the pack.

Where packs are to be transferred from one RTC to another, the labelling should use a common format to ensure the product and its test status are clearly identified to hospital users.

2.13 Control Of Non-Conforming Product

Blood products may be found to be unacceptable for further processing or issue at any stage in the production process from collection to release. Each RTC should have systems in place at every stage to record, identify and segregate the non-conforming products. Records of these products should be reviewed in a timely way so that trends may be observed and appropriate corrective action may be taken.

Disposal of a non-conforming product should be documented so that there is a positive record of disposal which completes the audit trail for that product. Disposal should comply with the requirements of the Environmental Protection Act 1990 and/or local water authority regulations.

2.14 Corrective Action

Each RTC should have a system to ensure that the records of non-conforming products which originate from collection, production and quality control departments are reviewed. The review, the corrective action and the person responsible for carrying it out should be recorded. The effectiveness of any corrective action should be assessed.

2.14.1 Complaints And Complaints Files

Each RTC should have procedures for recording product quality complaints, and complaints from blood donors. These should be reported to the person responsible for quality and where appropriate an investigation should be made by a designated person. Where no investigation is made the RTC should maintain a record that includes the reason and the name of the person responsible for the decision not to investigate.

The procedure should ensure that any complaint relating to injury, death or any hazard to safety, is immediately reviewed, evaluated and investigated by a designated person, and the record of the complaint and the investigation is maintained in a separate portion of the complaints file.

2.14.2 Product Recall

The RTC should have a documented recall procedure. It should be capable of being put into operation at all times. A person should be formally designated to initiate and co-ordinate the procedure and to monitor its progress.

2.15 Handling, Storage, Packaging And Delivery

Each RTC should have procedures to ensure that all materials used in the collection and production process are appropriately handled and stored to avoid deterioration. This requirement applies to items such as unused packs, reagents and labels in addition to blood products.

Specific guidelines for products are given in Chapter 6 and general guidance in Chapter 5.

2.16 Quality Records

Each RTC should have procedures to maintain a product record in the form of a production history file. The file should record or refer to the location of the following records in whatever format they are kept.

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

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

(c) The processing record incorporating the date performed, the designated person or when appropriate, the names of team members performing each operation and, when applicable, the major equipment use.

(d) The inspection checks and quality control tests performed, the methods and equipment used, results, the date and record of the person carrying out the inspection or tests.

(e) A record of the label or, if appropriate, the package insert for each product produced.

(f) A record of the hospital to which the product was issued.

(g) The records for each product should be such that the origin of the product can be traced to the donor of the whole blood or plasma.

2.17 Internal Quality Audits

The person responsible for quality in each RTC should have a documented quality audit programme to ensure that the quality system is operating effectively. Two approaches to audit are needed to ensure a thorough review of the system. First, the systems audit should examine the documentation and procedures that define the system and the way in which its component parts such as the computer system and training programmes support its objectives. Second, the compliance audit should examine the way in which the system is implemented and its effectiveness in ensuring products and services meet their requirements.

The internal quality audits should be carried out by trained RTC personnel who do not have responsibilities in the procedures being audited.

The internal audit results should be formally recorded with a record of the deficiencies found; the corrective action required; the timetable for corrective action and the person responsible for carrying out the corrective action.

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

2.18 Training

Each RTC should have a system for identifying the training needs of all staff. Designated person(s) should be responsible for ensuring that the training needs are met through a written training programme.

Training records of staff should be maintained so that their competence to carry out a particular procedure can be demonstrated.

2.19 Servicing

This paragraph of BS 5750 is not likely to apply to products or services provided by RTCs.

2.20 Statistical Techniques

Appropriate statistical techniques should be used to measure and control the range of activities covered by the RTC's quality system.

The principles of statistical process control may be used to ensure that quality control parameters remain within defined limits and reduce losses.

Statistical techniques may also be used to justify and modify the frequency of inspection and testing; for the analysis of donor recruitment campaigns; and for the analysis of test and clinical data etc.

APPENDIX 1

NBTS Quality Policy Statement

The National Blood Transfusion Service is dedicated to a system of quality management which will ensure that its blood products and services meet the requirements of clinicians and their patients. Because our products are administered to patients our quality system will be comparable in excellence to those used in the pharmaceutical industry by licensed manufacturers.

The quality policy rests on four principles:-

Our definition of quality is **conformance to requirements**. We will carefully specify the requirements for our suppliers (donors and manufacturers), our processes (collection, laboratory, distribution) and our product users.

We will improve and maintain quality through a planned system of quality assurance management which will cover **every** part of our activity. Audit and review will be an essential part of this system.

We will ensure that under the guidance of trained QA management each member of staff recognizes their responsibility for quality improvement.

We will ensure that education and training of staff are sufficient to maintain and improve quality.

Annex 1

Current Membership of UKBTS/NIBSC Liaison Group and Groups contributing to the Guidelines

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(For General Considerations, Chapter 2 - Dr R J Moore, Deputy National Director)

Annex 2

British (BS) and International (IS) Standards available at NIBSC

4th IS	Factor VIII, Concentrate (88/804)
10th BS	Factor VIII, Concentrate (90/552)
2nd IS	Factor VIII-related activities in plasma (87/718)
18th BS	Factor VIII:C, Plasma (90/550)
5th BS	Blood Coagulation Factors, Plasma (91/516)
1st IS	Factors II, IX, X, Concentrate (84/681)
1st IS	Factors II, VII, IX, X, Plasma (84/665)
2nd BS	Factor IX, Concentrate (87/532)
1st IRP	Antithrombin III, Plasma (72/1)
1st IS	Antithrombin III, Concentrate (88/548)
1st IS	Protein C, Plasma (86/622)
1st IS	Anti-D Immunoglobulin, Human (68/419)
1st BS	Anti-D Antibodies, Human (72/229)
1st IS	α-Thrombin, Human (89/588)
1st IS	Plasma Fibrinogen (89/644)

British Working Standards

Anti-CMV
Anti-endotoxin
Anti-HBsAg (plasma)
Anti-measles
Anti-pseudomonas
Anti-zoster (varicella)

SECTION 1

Guidelines for blood components prepared at Regional Transfusion Centres

Chapter 1

Selection of Donors

1.1 General Considerations

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

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

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

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

1.1.3 Hazardous Occupations or Hobbies

1.1.3.1 Service aircrew, whether trained or under training, are not permitted to act as blood donors. Civil aircrew may not donate if on flying duties.

1.1.3.2 Occupations where a delayed faint may present a hazard to the donor and/or others - accept only when the individual is going **off** duty, e.g. train, HGV or bus driver, heavy machine or crane operator, driver, climbing ladders or scaffolding, miner working underground.

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

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

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

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

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

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

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

1.2 Medical Assessment

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

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

1.2.3 Age

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

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

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

1.2.4 Frequency of donations

Usually 2 donations are given in a 12-month period. Many donors are able to give blood more frequently without developing iron deficiency so that an interval of 16 weeks between donations is considered a reasonable minimum. Any further reduction, to an absolute minimum interval of 12 weeks, must be accompanied by appropriate donor monitoring.

1.2.5 Volume of donation

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

1.3 Medical history of donors

1.3.1 General considerations

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

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

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

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

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

1.3.1.5 Information which is, or may be, of relevance to the health of the recipient and which arises subseqent to the transfusion of the blood, should be reported to the appropriate

party (i.e. National Fractionation Centre or Consultant in charge of the hospital blood transfusion laboratory) so that further action may be taken if deemed necessary.

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

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

1.4 Examples of conditions necessitating permanent exclusion

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

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

1.4.1 Cardiovascular diseases

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

1.4.2 Central nervous system diseases

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

1.4.3 Gastrointestinal diseases

All diseases which may be of immune origin, or which render the individual liable to iron deficiency through impaired iron absorption or blood loss, should be reason for exclusion. Individuals with coeliac disease which is controlled by gluten-free diet alone, may be accepted.

1.4.4 Haematological disease

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

1.4.5 Infectious diseases which are reasons for permanent exclusion:-

AIDS, HIV infection
Brucellosis
Granuloma Inguinale
Kala Azar
Lymphogranuloma venereum
Q fever
Syphilis
Trypanosmiasis cruzi (Chagas' disease)

1.4.6 Metabolic diseases

In general, individuals who are receiving continual therapy which might adversely affect a transfusion recipient should be permanently excluded. Diabetics who are controlled by diet alone are acceptable.

1.4.7 Renal diseases

All chronic renal diseases are a reason for permanent exclusion.

1.4.8 Respiratory diseases

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

1.4.9 Recipient of human growth hormone

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

1.4.10 Auto-immune diseases

All diseases known or suspected to be auto-immune in origin, are a reason for permanent exclusion.

1.4.11 Malignancy

All diseases of malignant origin should be cause for permanent exclusion, although exception may be made for localized conditions such as carcinoma in situ of the cervix and rodent ulcer, at the conclusion of successful therapy. Conditions associated with neoblastic change, such as neurofibromatosis, should debar.

1.4.12 Diseases known to relapse

These should be considered as contra-indications to blood donation.

1.5 Conditions necessitating temporary deferral or qualified acceptance

Condition	*Comment*
Abortion, (see pregnancy)	gestation >6/12 - wait 1 year gestation <6/12 - wait 6 months
Accident, minor	wait 3 months
Accident, major	wait 6 months
Acupuncture - performed by a registered medical practitioner	accept
- performed by others	wait 12 months
Allergy including - hayfever	wait until asymptomatic
- desensitising injections	wait 72 hours after last injection
- drug allergy	wait 12 months after last exposure to offending drug
Anaemia	wait until cause is determined; assess each potential donor
Asthma	defer if symptomatic or receiving systemic therapy. Prophylactic treatment by inhalation only need not debar
Blood donation within 3 months	wait until 16 weeks since last donation
Blood transfusion (including cellular and plasma products)	wait 12 months
Contact with infectious fevers	wait for duration of incubation period - 4 weeks if this is unknown

Dental treatment - complicated surgery	wait 3 months
- uncomplicated extraction	wait 24 hours
- others	accept
Drugs - prescribed by physician or self-medication (e.g. aspirin)	individual assessment - see 1.7
Ear-piercing	wait 12 months
Electrolysis	wait 12 months
Epilepsy	individual assessment - some individuals with epilepsy react to minor stress by having fits and it is important that additional risks should be avoided. Anyone on regular medication for epilepsy should not be bled. A known epileptic who has not required anticonvulsant therapy and has not been subject to fits for 3 years may with discretion be considered as a possible donor.
Fractures	wait until recovered
Gilbert's disease	accept unless visibly icteric
Glandular fever	wait 2 years from recovery
Heart operations	may be accepted after appropriate consultation when corrective surgery was carried out for congenital defects
Hepatitis (confirmed)	review 12 months from recovery
Herpes simplex (cold sore)	wait until lesions are beginning to heal
Hereditary Hb disorders	may be accepted. Providing the donor is well and has acceptable Hb concentration there is little risk to donor or recipient, but it may be preferred to use the cells for laboratory purposes only (with the donor's consent)
Infections - boils, sore throat etc	wait until recovered
Infectious diseases - recent measles, mumps etc	wait until 4 weeks after recovery
Infectious mononucleosis	wait 2 years from recovery
Inoculations and immunisations	see 1.11
Inoculation injury	wait 12 months
Jaundice	wait 12 months
Legionnaire's Disease	wait until fully recovered
Leptospirosis	wait until fully recovered
Malaria	see 1.9
Marfan Syndrome	accept only if documented confirmation of no cardiac complication
Meningitis	wait until fully recovered
Peptic ulcer - on active therapy	wait 6 months after completion of treatment
Petit mal	wait until off all therapy and asymptomatic for 3 years (see Epilepsy)

Pregnancy	wait until 1 year after delivery (see 1.6)
Psoriasis	defer if widespread and/or requiring current systemic therapy
Renal disease (acute)	normally wait 5 years after recovery but seek advice from donor's medical adviser
Renal disease (chronic)	see 1.4.7
Respiratory tract	wait until acute symptoms resolve
Retinitis pigmentosa	accept only on written validation by appropriate medical officer
Sarcoidosis	accept only if the attack was short, mild and did not require treatment
Sexually Transmitted Diseases	
- gonorrhoea	wait until 12 months after the end of treatment
- non-specific urethritis (NSU)	wait until symptoms resolve
- genital herpes	wait until symptoms resolve
Spina bifida	accept only if mobility allows unaided progress through donor session, is not suffering from an infection and is not on medication
Surgery, minor e.g.	
- tonsillectomy	
- herniorraphy	
- appendicectomy	wait until fully recovered
Surgery, major e.g.	
- hysterectomy	
- cholecystectomy	wait 6 months
Tattoing	wait 12 months
Thrombosis deep venous (of calf)	wait 6 months
Thrombophlebitis - unrelated to venepuncture	wait 6 months
Thyroid disease	
- hypothyroidism	accept if on stable replacement therapy
- hyperthyroidism	accept when off treatment for 2 years
Toxoplasmosis	wait 2 years from recovery, absence of IgM antibodies
Tropical diseases	see 1.10
Tuberculosis	wait until off all therapy for 2 years

1.6 Pregnancy

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

1.7 Donors on treatment with drugs

1.7.1 Donors receiving a course of prescribed medication should be deferred until treatment is complete. This is to ensure that the blood collected is as near normal as possible, and to minimize risks to donors themselves. It is recommended that each RTC should prepare a list of the commonly used drugs with rules for acceptability of donors, and that in any doubtful situation it is wiser to defer than to take a donation. Drug half-life and possible interactions should be taken into account.

1.7.2 Donors taking drugs which are proven or potentially teratogenic (e.g. vitamin A derivatives) or who are taking drugs which accumulate in tissues over long periods, should not be accepted for blood donation. The period of deferment after finishing a course of treatment should be determined individually for each drug in these categories.

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

1.7.4 If the donor has taken drugs affecting platelet function (aspirin, anti-inflammatory drugs) within the last 4 days, the donation should not be used for the preparation of platelets. A list of all such drugs should be made available to staff at blood donor sessions.

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

1.7.6 Donors taking part in the clinical trial of drug therapy should be deferred until the trial is completed. Acceptability would then depend on the type of drug and its dosage, if the donor continues to take it prophylactically.

1.8 Infectious diseases

1.8.1 HIV infections

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

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

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

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

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

1.8.2 Hepatitis

Individuals with a history of jaundice or hepatitis should only be considered as blood donors 12 months after recovery from the illness. At this stage, approved tests for HBsAg and anti-HCV should be negative. The presence of anti-HBs does not debar.

1.8.2.1 Risk groups

All persons who have received a transfusion of blood or blood products, acupuncture (other than by a registered medical practitioner), tattooing, electrolysis and ear-piercing should be deferred for 12 months, as should those who have been in close contact with a case of hepatitis. Hospital staff involved in caring for patients with

hepatitis, or working in hospital laboratories, may be accepted as donors provided they have not suffered an inoculation injury with blood from an individual infected with hepatitis, in which case they should be deferred for 12 months.

1.8.2.2 Circumstantial involvement

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

1.8.2.3 Return of Donors with Acute HBV Infection to Active Panel

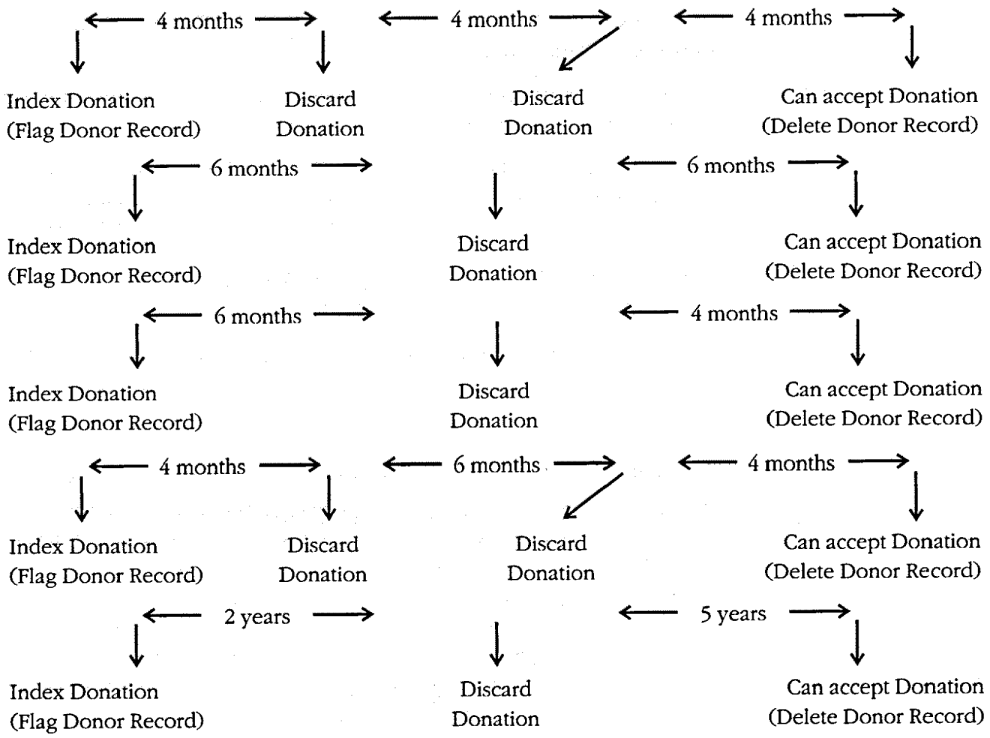
Blood donors found to have had an acute hepatitis B infection with or without symptoms of disease can be considered eligible for re-admittance to active donor panel provided one year has elapsed since the acute episode, there was clearance of HBsAg within six months and that a level of >0.1 iu/ml of anti-HBs can be demonstrated in their serum.

This recommendation applies only to donors whose sera have previously been negative for HBsAg and does not apply to known carriers of HBsAg who have lost HBsAg over protracted periods of time. Neither does it apply to donors found to have anti-HBc as a solitary marker in their serum. The reasonable expectation of an absence of integrated hepatic HBV DNA (and hence termination of infection) following recovery is only tenable for those individuals demonstrated to have undergone an acute self-limiting infection.

1.8.3 Reinstatement of seropositive donors

A positive result in microbiological screening tests, unconfirmed by a Reference Laboratory, and particularly those for anti-HIV 1+2 and anti-HCV, may on subsequent testing not be repeatable. Under carefully controlled conditions, such donors may be returned to the active panel. A minimum of 6 months must elapse between a negative/indeterminate result from the Reference Laboratory and a negative result from both RTC and Reference Laboratory before donor can be considered eligible for re-admittance to the active donor panel. At next visit thereafter the donor can be admitted to the active panel and the flag deleted from the donor record ***provided repeat screening tests at RTC are negative.***

Thus the following examples apply:



1.9 Malaria

Donors should be asked if they have visited places abroad (other than in Western Europe, Australia, New Zealand or North America) or have lived in such places at any time during the first 5 years of life.

1.9.1 Acceptability as cell donors

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

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

1.9.2 Acceptability as plasma donors

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

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

1.9.3 Donation after return from malarial areas

See algorithm in Appendix I to this Chapter.

1.10 Other tropical diseases

1.10.1 Trypanosomiasis (Cruzi)

This may lead to an acute or chronic, incurable, and even fatal illness. Blood from donors who have visited or lived in rural South America or Central America including Southern Mexico should ONLY be used for preparing plasma fractions (*not* plasma for clinical use or cryoprecipitate).

Donations from such persons may be used for normal purposes provided they have been shown by suitable test to be free of antibodies to Trypanosma Cruzi.

1.10.2 Filariasis, Kala Azar, Q Fever and Yaws

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

1.10.3 Amoebic dysentery, Schistosomiasis and Arthropod-borne encephalitides

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

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

1.11 Inoculations and immunisations

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

Live vaccines	BCG oral polio yellow fever oral typhoid rubella	3 weeks
Killed vaccines	anthrax cholera influenza diphtheria polio (Salk) rabies tetanus typhoid meningitis	48 hours
Recombinant vaccine	hepatitis B (provided there has been no known exposure)	48 hours

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

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

anti-tetanus Ig	4 weeks
normal human Ig	6 weeks

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

1.12 Physical examination of donors

1.12.1 General considerations

Most donors may be accepted on the basis of medical history, general appearance and haemoglobin estimation, although it is advisable to examine the pulse and check the blood pressure where there are any doubts, particularly in new donors.

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

1.12.2 Inspection of the donor

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

1.12.3 Weight

Healthy individuals can generally donate up to 500 ml of blood (plus small laboratory sample) without any deleterious effect on their health. A standard blood donation is 450±10% with optimum blood/anticoagulant ratio of 7:1 (See Annex I).

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

1.12.4 Haemoglobin estimation

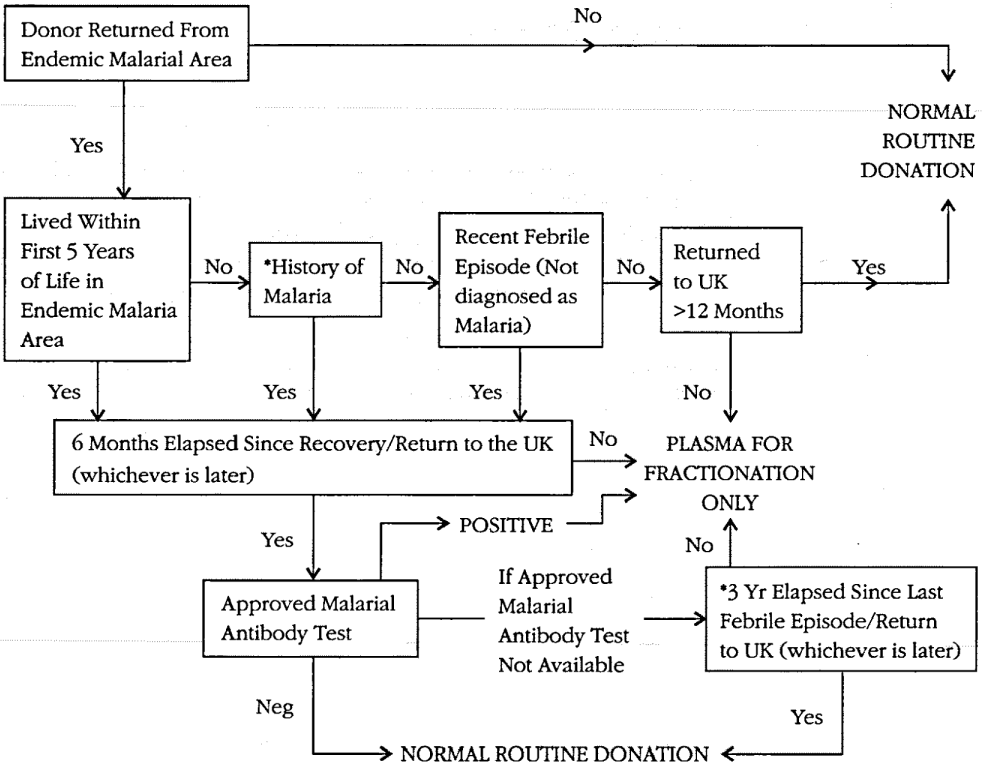
The haemoglobin concentration should be determined each time a potential donor presents. The acceptable lower limits are female donors 12.5 g/dl, or male donors 13.5 g/dl. The type of test used is left to the discretion of the RTD.

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

Where a quantitative method of Hb estimation is employed, an acceptable upper limit for donation should be set at the normal upper limit for the method used. Individuals with significantly high Hb levels should be investigated and referred appropriately.

APPENDIX I

Donation after Return from Malarial Area



**Donor with history of malaria must be sero negative before cellular donation can be used routinely.*

Chapter 2

Quality Assurance at Blood Donor Sessions

2.1 General Specifications

2.1.1 General Comments

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

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

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

2.1.2 Guidance for the following procedure is given in Annex 1:

- DONOR IDENTIFICATION
- HAEMOGLOBIN OR HAEMATOCRIT SCREENING
  Copper sulphate haemoglobin screen
  Copper sulphate storage
  Copper sulphate for routine use
  Copper sulphate procedure: fingerprick blood sample
  Spectrophotometric method for haemoglobin screening
  The microhaematocrit method for haemoglobin screening
- PREPARATION OF THE VENEPUNCTURE SITE
- PREPARATION OF THE BLOOD PACK
- PERFORMANCE OF THE VENEPUNCTURE
- BLOOD DONATION

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

2.1.3 Guidance for laboratory testing procedures is given in Annex 3.

2.2 Records

2.2.1 Donor identification

2.2.1.1 The identity of the donor must be recorded and linked to the donation records.

2.2.2 Donation identification

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

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

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

2.2.3 Labelling

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

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

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

2.2.4 Donor Session Records

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

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

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

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

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

2.3 Documentation

2.3.1 Selection of donors - see Chapter 1.

2.3.2 Specification of blood and blood products - see Chapter 5 and 6.

2.4 Control of Inspection of Test Materiel

Guidance on the calibration and control of laboratory tests is given in Annex 3.

2.5 Control of Purchased Materiel and Services

2.5.1 Specification and inspection of blood bags

2.5.1.1 Blood collection shall be by aseptic techniques using a sterile closed system and a single venepuncture. The integrity of the system must be checked prior to use and measures must be taken to prevent unsterile air entering the system.

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

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

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

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

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

2.5.1.7 Prior to release from the blood collection session the pack and its associated tubing should be reinspected for defects and its integrity should be checked by applying pressure to the pack to detect any leaks. Any defective pack should be marked for *disposal* and held separately from intact packs. Details of the defect (s) should be recorded for future analysis and action (see Annex 1, 6.8 and 6.9)

2.5.2 Inspection of labels for printing errors

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

2.6 Collection Control

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

2.7 Protection and Preservation of Product Quality

Guidance on requirements for labelling, storage and transportation is given in Chapter 5.

Chapter 3

Guidelines for Apheresis of Volunteer Donors within the UK Transfusion Service.

3.1 Introduction

3.1.1 These Guidelines relate to the collection of blood components by apheresis (including manual techniques) from UK BTS donors. Their purpose is to ensure the safety of volunteer donors undergoing apheresis procedures, particularly in sites removed from hospital facilities and to ensure the quality of collected apheresis components. They relate only to the apheresis of healthy volunteer donors and not to the clinical use of cell separators for plasma exchange and other therapeutic procedures, which are covered by Guidelines for the use of therapeutic cell separators, prepared by the Clinical Haematologists Task Force of the British Committee for Standards in Haematology. (Clin.Lab.Haemat.1990 12, 141-158)

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

3.1.3 Extreme care should be taken to ensure that undue pressure is not put on persons to donate.

3.2 Criteria for Acceptance of Donors

3.2.1 Other than in exceptional circumstances (to be decided by a designated medical practitioner), donors for apheresis procedures shall meet the usual criteria for ordinary whole blood donations as described in Chapter 1. They should preferably have given at least 2 routine blood donations without untoward effect.

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

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

3.2.2.2 Donors should not normally be less than 50 Kg in weight.

3.2.2.3 In accordance with specifications set by the fractionation facility volunteers who are donating plasma for fractionation may be acceptable if receiving medical treatment or taking self medication provided that the donor is fit enough to undergo plasmapheresis. Information about fitness to donate should be given to volunteers prior to donation and before being asked to sign the NBTS110 or equivalent. In particular they must be given information about AIDS, such that those at risk of HIV infection will refrain from donating.

3.2.2.4 Persons with sickle cell trait should not be accepted as apheresis donors.

3.2.2.5 For platelet collections the donor should not have taken any aspirin containing compounds for a period of 4 days prior to donation.

3.2.3 Informed Consent

3.2.3.1 Informed consent must be obtained by a doctor or registered nurse, fully conversant with the procedure. The consultant in charge of the apheresis programme must be personally responsible for delegating this task.

3.2.3.2 Leaflets about donor apheresis should be available at the session to assist in the process of obtaining fully informed consent. A consent form, examples of which can be found in Appendix I Form A & Form B, must be signed by each donor before the first donation. At each attendance he/she should sign the fitness to donate form (NBTS110 or equivalent).

3.2.3.3 Separate informed signed consent for leucapheresis should be obtained by a medical officer on each occasion. (see Appendix I Form C)

3.2.3.4 In obtaining donor consent, the doctor or registered nurse must give the donor the following information:

- The purpose of the donation
- A description of the proposed apheresis procedure and the likely duration of the donation
- Explain that a voluntary donor can withdraw consent at any stage of the procedure or of the apheresis programme
- Describe the common risks and discomfort involved
- Recognised risks and discomforts associated with donor plasma and/or plateletpheresis include:
  - Dizziness & fainting (anxiety and/or fluid loss)
  - Haematoma formation (e.g. during pumped red cell return)
  - Citrate toxicity (speed of red cell return and the volume of citrated plasma also returned)
  - Red cell loss if the procedure has to be aborted and it is considered unsafe to return the red cells
  - Chilling on reinfusion

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

3.2.4. Medical Examination of Donors

3.2.4.1 On entry to an apheresis programme the donor's health and general suitability should be assessed. (see chapter 1, section 1.2 & 1.3). In addition as a minimum requirement for donors under 45 years of age the blood pressure, pulse and weight should be examined. For donors over 45 years of age it may be necessary to extend the examination, e.g. ECG prior to recruitment.

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

3.2.4.3 Special care should be taken to assess the health of donors entering a programme which requires deliberate immunisation or boosting, e.g. for anti-D immunoglobulin collection. (see chapter 4 and Annex 4).

3.2.4.4 If necessary, with the donor's signed consent, his/her G.P. may be contacted for further information.

3.2.5 Blood Tests

3.2.5.1. At the initial visit, the following blood tests should be performed: full blood count, serum albumin and total serum protein levels.

3.2.5.2 It is recommended that volunteers with a platelet count of less than 150 x 10^9/L should not undergo plateletpheresis.

3.2.5.3 In general the lower limit of acceptibility for haemoglobin level should be 13.5 g/dl for men and 12.5 g/dl for women as for normal whole blood donation. However donors may be accepted for an individual donation at the discretion of the medical officer in charge, with a minimum level of haemoglobin of 12.5 g/dl for men and 11.5 g/dl for women, since few red cells are lost during plasma or plateletpheresis procedures.

3.2.5.4 In exceptional circumstances, the consultant in charge of the apheresis programme may decide that an individual donor with a haemoglobin level lower than those recommended above, may undergo plasma, platelet or leucocyte apheresis.

3.2.5.5 For all types of donor apheresis procedures, the haemoglobin and microbiological screening tests must be performed at each donor attendance. In addition the platelet count should be performed at each visit for plateletpheresis donors.

3.2.5.6 The full blood count, serum albumin and total serum proteins, should normally be measured at least every 6th visit or annually, whichever is the shorter interval. Differences in the values for pre and post donation samples should be taken into account when assessing the results. The donor's fitness to continue on an apheresis programme should be assessed by a designated medical officer, in the light of these results.

3.3 General Specifications for Apheresis Sessions

3.3.1 Simple Apheresis Procedures

Defined as: - Single Arm
- Intermittent flow
- Procedure normally takes less than 60 minutes.
- Minimal return of citrated plasma i.e. not more than 0.015 mmol/Kg/min (see Appendix III)
- The total extracorporeal blood volume at any one time should not exceed 20% of the estimated total blood volume (or 1 litre). (see section 3.4.3)

3.3.2 Simple apheresis procedures may be carried out at fixed or mobile blood collection sites.

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

3.3.4 The consultant in charge of apheresis must ensure that all doctors and registered nurses who undertake the supervision of apheresis procedures must be trained in cardiopulmonary resuscitation techniques and must receive updating at least annually.

3.3.5 Resuscitation equipment must be available at each apheresis session and must include the following:

- An airway
- An Ambu bag (or equivalent)
- A suction apparatus

3.3.6. **More complex donor apheresis procedures must be undertaken at a fixed collection site with more extensive resuscitation facilities immediately available.**

3.4 Frequency of Apheresis and Volume Collected

3.4.1 Donors should not normally undergo plasmapheresis more often than once a fortnight. A donor should not generally undergo a total of more than 24 plateletpheresis procedures per annum and not more than 12 leucapheresis procedures per annum. There should normally be a minimum of 48 hours between procedures and a donor should not normally undergo more than 2 procedures within a 7 day period.

3.4.2 In apheresis procedures the peak extracorporeal volume during any one cycle (excluding the volume already collected but including the plasma collected during that cycle) should not exceed 15% of the estimated total blood volume. The final collection volume (exclusive of anticoagulant) should not exceed 15% of the total donor blood volume and should not exceed 600ml (excluding anticoagulant) unless intravenous fluid replacement is given.

3.4.3 Some complex procedures may result in a total extracorporeal blood volume of as much as 1L when the plasma collected during any one cycle, plus the already collected plasma volume and the blood in the remaining extracorporeal circuit are accounted for. In donors under 70 Kg in weight this may represent greater than 20% of their total blood volume. The procedures may need to be adjusted accordingly to suit each individual donor's safety tolerance limits. (see total blood volume table, appendix II).

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

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

3.4.6 Not more than 1 litre should be donated by one donor in any one week period.

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

3.4.8 After a whole blood donation, or the loss of an equivalent number of red cells during a failed apheresis procedure, a donor should not normally donate plasma, platelets or leucocytes for a period of 8 weeks.

3.5 Staffing & Training Principles for Apheresis Sessions

3.5.1. The consultant in charge of the apheresis programme must be responsible for establishing adequate staffing levels and for ensuring that staff are properly trained. This consultant may delegate responsibility for the day to day running of apheresis to appropriately trained staff. A suitably trained doctor must be immediately available on the premises at all times when donors are undergoing apheresis.

3.5.2 One or more suitably trained doctors or registered nurses must be responsible for performing venepunctures and for the supervision of machine procedures. During donation, donors should never be left in a room without the presence of an appropriately trained doctor or registered nurse.

3.5.3 Health Authorities should be aware that the training and certification of registered nurses undertaking apheresis procedures including training and monitoring of staff, performing venepunctures and obtaining informed consent, must be in accordance with the relevant Department of Health Circulars relating to the extending role of the nurse (PL/CMO (89) 7, PL/CNO (89) 10 England and Wales and (79(Gen)46) Scotland).

3.5.4 For simple procedures (for definition see 3.3.1) planned staffing levels should ensure that normally there is at least one member of staff present for every 2 machines in use. If a staff member is responsible for 2 machine procedures taking place simultaneously, then these should preferably both be of the same type of procedure using the same type of machine.

3.5.5 Care of volunteers before, during and after the donation procedure, may be undertaken by donor attendants/assistants who have received appropriate training.

3.5.6. More complex apheresis procedures must be more closely supervised by appropriately trained individuals. The consultant in charge of apheresis in consultation with the nurse manager must ensure that there is an appropriate staffing level and skill mix to ensure donor safety and adequate monitoring of the equipment in use.

The nurse manager should refer to the guidance document issued by the Royal College of Nursing on blood transfusion nursing standards of care, to assist in this process.

3.5.7 The consultant in charge of apheresis in consultation with the nurse manager should determine the duties and responsibilities of all apheresis staff. A programme should be established for initial and continued training to ensure an appropriate level of proficiency.

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

of the relevant manufacturer's manual for each type of machine in use must be available on site.

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

3.6 Collection, Storage and Testing of Apheresis Components

3.6.1 Specification and inspection of apheresis sets

3.6.1.1 Blood components must be collected by apheresis using sterile, single use, disposable items from manufacturers who have been registered under the Department of Health Manufacturers' Registration scheme. The apheresis set should preferably be preconnected to ensure a sterile pathway once the venepuncture needle is in place and designed in such a way that whole blood can be collected, separated and cellular and humoral elements safely returned to the donor.

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

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

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

3.6.1.5 Any faults detected before or during a procedure must be recorded in accordance with local quality systems. Any significant defect of a potentially hazardous nature must be reported. (see 3.7)

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

3.6.2 Specifications for automated donor apheresis machines

3.6.2.1 Machines must be correctly installed and commissioned according to manufacturers instructions.

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

3.6.2.3 Machines used must comply with the relevant aspects of the Health and Safety at Work Act. Additionally such machines must comply with the requirements of British Standard BS 5724 : Part I : Safety of Medical Electrical Equipment.

3.6.2.4 On sites where manual plasmapheresis is still performed, a blood bag centrifuge is required for plasma separation. The operating procedure for using this centrifuge must be available at the session. The centrifuge must be calibrated at installation and subsequently at each service visit and after repair. Records of calibration maintenance & repair details must be maintained according to local quality systems.

3.6.2.5 Automated apheresis machines should have the following features:-

- A manual override system so that the operator can stop the automatic cycle at anytime during the procedure.
- A blood flow monitor, to monitor blood flow during blood withdrawal and return. The purpose is to ensure that the selected donor flow rate does not collapse the donor's vein and to monitor the venous pressure during the donor blood return cycle such that if any obstruction to flow occurs, the blood pump will automatically reduce speed and/or stop. In either event a visual and audible alarm system should operate.
- An in line air detector to protect the donor from air embolism. In the event of air entering the extracorporeal circuit a visible and audible alarm must be activated, the return blood pump must automatically stop and the venous return line must automatically be occluded.
- A blood filter integral with the harness to prevent any aggregates formed during the procedure from being returned to the donor.
- An anticoagulant flow indicator, providing a visible means of monitoring the rate of delivery throughout the procedure.
- A device for presetting the collection volume, monitoring the collection volume during the procedure and automatically ending the procedure. A manual system with a visual and audible alarm to notify the operator of the completion of the procedure may be provided.
- Automated plasma filtration machines must be provided with a monitoring device to record the transmembrane pressure, and a protective system to ensure that the machine will only operate between preset ranges. If the transmembrane pressure falls outside the preset ranges an audible and visual alarm must be activated.
- Automated plasma filtration machines must have a means of detecting haemolysis or red cell leakage. Once haemoglobin or red cells have been detected an audible and visual alarm must be activated, the blood return pump must stop and the venous return line must automatically be occluded.
- In the event of a power failure the machine must automatically enter a standby mode once power returns. Also a manual system for returning any remaining donor blood is desirable if the extracorporeal red cell volume is in excess of 200 ml and the power failure continues.

3.6.2.6. Apheresis machines must be serviced in accordance with the manufacturer's instructions. A planned maintenance scheme should be followed. Machine maintenance and servicing must be in accordance with the procedures outlined in Health Equipment Information HEI 198 : Management of Equipment.

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

3.6.3 Anticoagulant

3.6.3.1 A licensed citrate anticoagulant must be used at a ratio which achieves a final concentration of 15 - 25 mmol/l citrate (*see Appendix III*).

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

3.6.4 Guidance for Collection Procedures are identical to that for normal whole blood donations except where indicated.

3.6.4.1 Donor Identification

3.6.4.2 Labelling

Apheresis packs and donor sample tubes must be labelled in accordance with local SOPs.

3.6.4.3 Preparation of the Venepuncture Site

3.6.4.4 Performance of the venepuncture

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

3.6.4.5 Anticoagulation

Occurs automatically in machine apheresis, but requires a standard mixing procedure for manual apheresis

3.6.4.6 Blood flow

Occurs automatically in machine apheresis, unless a satisfactory flow rate cannot be maintained.

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

3.6.4.7 Blood flow monitoring

Occurs automatically in machine apheresis, but requires monitoring in manual apheresis

3.6.4.8 Blood Volume Monitoring

Occurs automatically in machine apheresis, but requires monitoring in manual apheresis

3.6.4.9 Sample Collection

In machine apheresis sampling may take place at the beginning of a donation. The methods employed shall ensure an aseptic technique with no risk of contamination and be clearly defined in the sessional procedures SOP manual.

Extra samples are required at specified intervals from regular apheresis donors. (*see 3.2.5.6*). A system should be in operation for regular review of these results, together with a documented protocol of the action to be taken in the light of any abnormal findings.

3.6.4.10 Completion of the donation and quality control samples

A length of tubing should only be left attached to the plasma collection pack if samples are required for testing purposes, as any side tubing left attached to the plasma bag is more likely to fracture when the plasma bag is frozen. Any tubing containing samples required for testing should be separated from the collection packs prior to freezing without compromising the sterility of the containers. Regular quality control of the plasma and platelets collected should be undertaken on these samples. All used disposable equipment must be discarded in such a way as to prevent any risk to personnel, according to Health & Safety regulations.

3.6.4.11 Final Donation Inspection

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

3.6.5 Apheresis component storage & testing

3.6.5.1 Plasma donations may be frozen and stored on fixed collection sites removed from the Regional Transfusion Centre. If a freezer is installed on site, there must be a system to monitor temperature as well as an alarm system to warn personnel before storage temperatures reach unacceptable limits, as described for blood bank refrigerators in compliance with BS 4376:1982.

3.6.5.2 All apheresis components must be transported, tested and stored in accordance with the specifications for blood components in chapter 5.

3.7 Notification of Hazards

3.7.1 Any safety related defects in the equipment, including the single use items, must be reported promptly to the Department of Health in accordance with the requirements of Health Notice HN(88)51. For Scotland 1991(GEN)24. For Wales WHC(89)29. For Northern Ireland. DHSS NI Circular HSS(ESD)3/90.

3.7.2 Any potentially serious procedural problem should be reported **as soon as possible** to the Central Collator who will ensure that all other practising centres are informed of the potential hazard without delay.

3.7.3 A national register for hazards of donor apheresis is being maintained for statistical purposes by the Central Collator. Centres should ordinarily complete an annual return of such data, on request.

Central Collator's Office
Leeds Regional Transfusion Centre
Tel: (0532) 645091
Ext. GRO-C (Cell Separator Unit)
Fax: (0532) 603925

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

3.8 Donor Compensation

Health Authorities should consider sympathetically and decide promptly any claim by a donor for compensation for any injury or loss allegedly attributable to having donated by apheresis. Regional Health Authorities (RHAs) have delegated powers to make ex-gratia payments (Health Circular HC(78)42 & HC(80) 3 refers). The Department of Health has arranged that claims for a volunteer or his/her family which cannot be resolved at RHA level, will be considered by a small tribunal established for this purpose (Appendix A to AC(84) 11 refers). A similar system of ex gratia payments and compensation operates in Scotland, through the Scottish Home & Health Department.

APPENDIX I

Form A

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

DONOR CONSENT FORM (APHERESIS)

I ..... (full name)

of ..... (full address)

.....

.....

confirm that I have read and understood the explanatory literature relating to **plasmapheresis/ plateletpheresis.**

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

Medical Officer ..... (full name)

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

Signature of volunteer donor .....

Date .....

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

Signature of Medical Officer .....

Date .....

APPENDIX I

Form B

A recommended format for a donor consent form where a Registered Nurse is obtaining informed consent, this duty having been delegated by the Consultant in charge.

DONOR CONSENT FORM (APHERESIS)

I ..... (full name)

of ..... (full address)

.....

.....

confirm that I have read and understood the explanatory literature relating to **plasmapheresis/ plateletpheresis.**

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

Registered Nurse ..... (full name)

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

Signature of voluntary donor .....

Date .....

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

Date .....

I confirm that the above Registered Nurse has received training in obtaining informed consent from donors volunteering to undergo apheresis and has reached a satisfactory standard. I have delegated the task of obtaining informed consent to the Registered Nurse.

Signature of Consultant
in charge of apheresis .....

Date .....

APPENDIX I

Form C

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

DONOR CONSENT FORM (LEUCAPHERESIS)

I ..... (full name)

of ..... (full address)

.....

.....

confirm that I have read and understood the explanatory literature relating to **leucapheresis.**

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

Medical Officer ..... (full name)

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

Signature of volunteer donor .....

Date .....

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

Signature of Medical Officer .....

Date .....

APPENDIX II

Total Blood Volume (TBV)* & Extracorporeal Blood Volume (ECV)

Weight: Kg.	40	45	50	55	60	65	70	75	80	85	90	95	100
Weight: St.	6.3	7.1	7.9	8.6	9.4	10.2	11.0	11.8	12.6	13.4	14.1	14.9	15.7
TBV - ml.	2840	3195	3550	3905	4260	4615	4970	5325	5680	6035	6390	6745	7100
10% TBV	284	320	355	391	426	462	497	533	568	604	639	675	710
15% TBV	426	479	533	586	639	692	746	799	852	905	959	1012	1065
20% TBV	568	639	710	781	852	923	994	1065	1136	1207	1278	1349	1420

* *based on the assumption that in the normal healthy adult TBV = 71ml/Kg.*
i.e. TBV of a 70Kg. adult 5.0L

N.B. (1) Total ECV at any point in a donor apheresis procedure must not exceed 20% TBV (excluding anticoagulant)

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

APPENDIX III

Citrate Anticoagulants and the Avoidance of Citrate Toxicity

Based on recent studies (1989/90) the following recommendations can be made to avoid citrate toxicity during apheresis procedures.

1. Intermittent flow cell separator machines

The re-infusion rate of citrated blood or plasma should not exceed 0.015 mmol/Kg/min.

2. Continuous flow cell separator machines

The continuous reinfusion rate of citrated plasma should not exceed 0.01 mmol/Kg/min.

For the four citrate anticoagulants commonly used in the UK, the above recommendations are represented in the table below:

Maximum Acceptable Reinfusion Rates (Ml/Min for a 70 Kg Donor)

AC	AC:Blood ratio	Average plasma citrate mmol/L	PLASMA Int.	PLASMA Cont.	WHOLE BLOOD Int.	WHOLE BLOOD Cont.	PACKED CELLS
ACD-B	1+7(1:8)	13	80	43	135	75	*
CPD-50	1+15(1:16)	17	60	33	100	60	*
Acid CPD	1+11(1:12)	19	55	29	90	50	*
ACD-A	1+11(1:12)	16	60	33	100	55	*
ACD-A	1+7(1:8)	23	45	24	75	40	*

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

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

N.B. **For donors less than 70 Kg,** these reinfusion rates need to be suitably adjusted downwards to avoid citrate toxicity occurring. They may also be adjusted upwards for donors above 70Kg.

If different anticoagulant formulations or ratios are used other than these represented in this table, the procedure should be validated (i) to ensure plasma citrate levels are within the required range for fractionation purposes, i.e. 15-25 mmol/L, (ii) to ensure the citrate molar reinfusion rate does not exceed these recommended maximum acceptable limits.

Chapter 4

Donors for immune plasma and/or immunisation

4.1 General considerations

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

Subjects for primary immunisation should normally be regular donors under the age of 50 with a good attendance record. Older subjects may be considered for boosting.

4.2 Immune plasma donors

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

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

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

4.3 Immunisation of donors

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

4.3.2 When immunisation is intended, the donor must be:

- informed of the procedures by a qualified physician and encouraged to take part in a discussion. This may be achieved by discussing the procedures with small groups of potential donors.
- encouraged to seek advice from his/her GP before agreeing to immunisation.
- informed that any physician of his/her choice will be sent all information about the proposed immunisation procedure.
- required to indicate his/her agreement by signing an informed consent form.
- informed, and must understand, that he/she is free to withdraw consent at any time.

- encouraged to follow the advice of the Association of British Insurance Companies, by informing any insurer providing life cover of intention to participate in the programme.

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

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

4.3.5 Specific recommendations on the selection of RhD negative donors for immunisation and/or boosting, for the production of anti-D immunoglobulin have been formulated by the Immunoglobulin Working Party (*see Annex 4a*).

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

Where production of immune anti-D is the reason for immunisation, the red cell donor and the subject should be matched as far as possible for major blood group antigens other than D. Mismatching in the Ss, Kell, Fy, Jk systems is generally unacceptable. Mismatching within the Rh system for C and/or E is acceptable.

4.3.7 Criteria for the selection of donors of erythrocytes for immunisations are established by the Immunoglobulin Working Party of the UK Transfusion Directors, who also hold a register of suitable erythrocyte donors.

Only erythrocytes obtained from donors who fulfil the criteria of the Immunoglobulin Working Party should be used for immunisation, and the recommendations of the Working Party should be followed (*see Annex 4*).

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

4.3.9 Donors entering immunisation programmes and producing anti-D or other significant antibodies should carry an identity/hazard information card.

4.3.10 The maintenance of comprehensive records on subjects entering an immunising programme is essential and these records should be retained indefinitely.

4.3.11 Subjects who are being actively immunised or boosted require an annual medical assessment.

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

4.3.13 After five years' participation in an immunising/boosting programme, all subjects should be thoroughly assessed for general fitness to continue, and at the same time a review of the pattern of immunising/boosting doses should be undertaken.

Chapter 5

General Guidelines for Blood Component Manufacture

5.1 Scope of the Guidelines

5.1.1 These guidelines provide a framework on which RTCs should assemble standard operating procedures for the manufacture of blood components.

5.1.2 These guidelines apply to single donor and small pool components prepared from units of whole blood or by apheresis.

5.2 Setting and Maintenance of Specifications

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

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

5.2.3 If the results of analysis show a consistent trend away from the minimum requirements the cause should be investigated. The criteria to be investigated will be detailed in the relevant Standard Operating Procedure (SOP) together with the corrective action to be taken, although the steps to be considered should include the following:

1 An investigation of the testing and production procedures.

2 Checking that SOPs are up to date and followed.

3 Checking the operation of equipment and storage conditions.

4 The quality assurance manager may initiate investigations beyond the scope of written procedures. If no causative factors are revealed, then management procedures and training should be reviewed.

5.3 Component and Process Monitoring Tests

5.3.1 Certain tests are performed on every component unit. Red cell serology and certain microbiological screening tests are mandatory quality control procedures since the test results have a direct bearing on the release of the final component.

5.3.2 These guidelines also indicate the minimum level of additional testing necessary to ensure components are prepared to specification.

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

5.3.4 Sampling Procedures

1 Sampling procedures should be designed and validated to ensure the sample truly reflects the contents of the component pack.

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

5.3.5 Frequency of Tests

The regularity with which components are made influences the frequency with which component and process monitoring tests are required.

1 The target minimum testing frequency is 1% of the annual production or 4 component units per month, whichever is greater.

2 If a component is made, on average, less than 4 times per month the additional tests described in the specifications should be performed on every component unit.

3 The testing protocol should take into account all variables and ensure samples are taken across these variables.

5.3.6 Component Weight : Volume

1 To provide information which is useful for clinicians, the component specifications given in Chapter 6 generally require the component label to indicate a nominal volume. Depending on the component this volume may be a national specification or locally established nominal volume.

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

i. Whole blood volume is most appropriately calculated by deducting the weight of the pack assembly and anticoagulant and dividing the resulting weight by the nominal specific gravity of 1.06.

ii. For red cell components, volume is calculated by weighing the pack, deducting the weight of the pack assembly only and dividing the resultant weight by the nominal specific gravity of 1.09. The weight of anticoagulant and, if relevant, additive solution are not deducted when calculating the volume of red cell components.

iii. For platelets and plasma components, volume is calculated by weighing the pack, deducting the weight of the pack assembly and dividing the resulting weight by the nominal specific gravity of 1.03.

5.3.7 Requirement to Meet Specifications

For sterility tests and mandatory microbiology screening tests, all components must meet their specification.

With the exception of these tests, because of biological variability, it is acceptable if a minimum of 75% of the results of component and process monitoring tests achieve the specification.

5.4 Processing

5.4.1 The Starting Material

The starting material for component preparation is whole blood or the products of apheresis collected from donors who satisfy the selection criteria given in Chapter 1. Starting material for component preparation should be transported as described in **5.11.2**.

5.4.2 Separation of Components for Direct Clinical Use

The timing and method of separation depends on the components to be prepared from a given donation. Platelets and plasma components should be separated and placed under validated, controlled storage conditions, normally within 8 hours of venepuncture.

5.4.3 Prevention of Microbial Contamination

1 The use of presterilised multiple blood pack assemblies has greatly reduced the incidence of infection associated with microbial contamination of blood and blood components. Occasional reports of such events still occur. Although there is no evidence to suggest that routine sterility testing of blood components diminishes or eliminates such incidents of infection, the following measures will contribute to a higher degree of safety:

i. Creating and maintaining the highest level of awareness among all personnel of the constant care and attention to detail needed to minimise microbial contamination. Well planned training and refresher programmes are essential.

ii. Establishment of well-conceived and validated procedures designed to minimise microbial contamination of the environment and prevent microbial contamination of components.

iii. Monitoring of the microbial load in equipment and in the environment of component preparation areas.

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

3 Closed Systems

i. A closed system is one in which the blood pack assembly is manufactured under clean conditions, sealed to the external environment and sterilised by an approved method. Apart from the act of blood collection, when a needle is exposed and enters the donor's arm, the integrity of this assembly must not be breached in any way.

ii. When a Sterile Connecting Device is used the system can be regarded as closed providing that it has been validated that the process of joining and sealing does not lead to the possibility of microbial contamination of the component.

iii. A closed system is also provided by the use of an approved microbial filter where the filter is an integral part of the pack assembly.

4 Open System

i. An open system is one in which a breach has occurred but where every effort is made to prevent microbial contamination by operating in a clean environment, using sterilised materials and aseptic handling techniques.

In such circumstances, positive pressure should be exerted on the original container and maintained until the container is sealed. Open system processing should be undertaken in a designated clean environment as defined in the current Rules Governing Medicinal Products in the European Community. Vol IV. Good Manufacturing Practice for Medicinal Products.

The sterility of components prepared in an open system should be monitored following methods outlined in the current European Pharmacopoeia.

ii. Blood components prepared by an open system should be used as soon as possible. If storage is unavoidable, components with a recommended storage temperature of 22°C ± 2°C should be used within 6 hours. Components with a recommended storage temperature of 4°C ± 2°C should be used within 24 hours.

5 Components are rendered unsuitable for clinical use when accidentally or intentionally breached and the requirements necessary to achieve an open system have not been observed.

6 Any new development in component preparation by an open procedure must be validated to ensure the maintenance of sterility.

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

5.5 Component Shelf Life

To provide an unequivocal definition of component dating periods the following conventions should apply:

5.5.1 Where components are pooled, the maximum shelf life of the pool must not exceed the expiry date of the oldest constituent component.

5.5.2 Blood components prepared by an open system should be used as soon as possible. If storage is unavoidable, components with a recommended storage temperature of 22°C ± 2°C should be used within 6 hours. Components with a recommended storage temperature of 4°C ± 2°C should be used within 24 hours.

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

5.6 Component Labelling

5.6.1 Barcoded labels and associated technology must be used whenever possible.

5.6.2 The design and use of labels should conform with specifications set out in appropriate sections of the current British and European Pharmacopieas.

Specific guidelines on labels for blood components are given in Chapter 6 of these guidelines and in the current edition of 'Specifications for the Uniform Labelling of Blood and Blood Products' compiled for the UKBTS.

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

5.6.4 Care should be taken in the storage and use of labels to be attached to blood components.

5.6.5 Donation/Donor Identification

1 The use of a unique bar coded/eyereadable donation number links the donation to its donor. Donation numbers must be attached to all integral packs at the time of donation.

2 When component production requires the use of subsidiary packs which are not an integral part of the pack assembly, a secure system must be in place to ensure that the correct eyereadable and barcoded donation number is placed on each additional pack used.

3 To ensure that all constituents of a component pool can be traced, a unique batch number must be assigned to the pool and placed on the pack containing the pool. Alternatively, the unique donation number of each constituent component should appear on the pack containing the component pool.

4 When a component is divided for neonatal use a secure system must be in place to ensure that all sub batches can be traced. This also applies to components prepared by apheresis.

5.7 Component Storage

5.7.1 Specifications for Component Storage Areas

1 Storage areas for blood components should provide adequate space, suitable lighting and be arranged and equipped to allow dry, clean and orderly storage.

2 Good Manufacturing Practice requires that components of different status are appropriately identified and effectively separated. Recognised status categories include:

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

Secure and exclusive quarantine storage should be available for known biohazard material awaiting disposal (see **5.8.2**).

ii. Non-conforming
Components which do not comply with the specification for mandatory tests or are otherwise unsuitable for transfusion should be categorised as non-conforming.

iii. Returned
Components which are returned to the RTC but which remain within specification should be held securely pending possible re-instatement to stock by a designated person.

Components which are returned from blood transfusion laboratories outside the direct control of the RTC normally should not be returned to stock.

iv. Stock
Components which have been deemed satisfactory for issue by a designated person should be held in stock.

3 Appropriate security and status labelling of component storage areas is essential.

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

5 Areas/equipment in which components are to be stored should be validated before their introduction into routine use and periodically thereafter.

A permanent continuous record of storage temperatures should be made and stored.

5.7.2 Procedures for Component Storage

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

1 A procedure to ensure components are not released to stock unless authorised by a designated person.

2 Definitions of the designated storage areas including the storage specification, the status of components to be stored in each area and the persons who are authorised to access each area.

3 Procedures for validating and monitoring the conditions of storage.

4 Procedures for ensuring the good order and cleanliness of storage areas.

5 Procedures to ensure the storage of blood components does not jeopardise their identity, integrity or quality.

6 A procedure which ensures appropriate stock rotation.

5.8 Discard of Non-Conforming Components

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

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

5.8.2 Biohazards

1 Components from donations that are repeatably positive in mandatory microbiological screening tests or from donors whose records indicate their components should be destroyed because of previous mandatory test results are classified as biohazards.

2 Secure and effective procedures are required to ensure that all components and samples from biohazard donations are retrieved and inactivated before their disposal. In addition to **5.8.1**, this includes:

- a system which ensures all components prepared from any donation can be traced.
- maintaining a record of the person who retrieves each biohazard component, including laboratory samples.
- a procedure to ensure biohazard material has been inactivated before disposal.

3 When biohazard material, eg plasma, is retained for laboratory use, it must be stored in a secure and appropriately labelled freezer, separate from any components or products which might be used for therapeutic purposes and in a manner which will prevent it ever being used mistakenly for transfusion.

5.9 Component Release

5.9.1 All components must be appropriately labelled in accordance with these guideline specifications including those general guidelines outlined in **5.6**.

5.9.2 Standard procedures must ensure that blood and blood components cannot be released to stock until all the required laboratory tests, mandatory and additional, have been completed, documented and approved within a validated system of work. Compliance with this requirements may be achieved by:

1 The use of a computer program, or suite of programs, which requires the input of valid and acceptable test results for all the mandatory and required laboratory tests before permitting, or withholding, the release of each individual unit.

2 Where a computer-based system is not used, documented approval for the release of each individual unit by a designated person.

3 Where the computer-based system is temporarily unavailable, it is necessary to revert to the procedure in **5.9.2.2**.

5.10 Release of Components which do not Conform to Mandatory Requirements

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

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

5.10.1 That components which have not been subjected to all the mandatory test requirements are only issued by a senior member of the medical staff of the RTC to a registered medical practitioner.

5.10.2 That the reason for the issue is fully documented.

5.10.3 That a warning indicating an increased level of risk is given by a senior member of the medical staff of the RTC to the receiving registered medical practitioner who should sign a statement indicating that he/she is willing to accept these risks.

5.10.4 That the name of the recipient is entered on the issue documentation and on the component.

5.10.5 That the component is clearly identified with a label indicating that it has not been subjected to all mandatory tests.

5.11 Transportation of Blood Components

See also Annex 5

5.11.1 General Considerations

1 Blood components should be transported in containers which have been validated for the purpose.

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

3 Documentation should accompany components in transit to permit their identification.

4 Transport containers should not be exposed to extremes of temperature during transportation.

5 Where a coolant is used to achieve an appropriate storage temperature, if melting ice is used direct contact between coolant and components should be avoided.

6 Dead air space in packaging containers should be minimised.

7 Written procedures for the transportation of components should be established and should ensure that the guidance given in **5.11.1.1** to **5.11.1.6** is complied with. In addition, written procedures should include the following:

i. Definition of approved systems of packaging, transportation and transport conditions required for each component (see Chapter 6).

ii. A procedure for monitoring approved systems of packaging and transportation.

5.11.2 Transportation from Collection Site to Processing Centre

1 Blood from donor sessions must be transported under conditions validated to be suitable for the various components to be prepared from the donations.

2 Blood being transported from donor sessions must be accompanied by documentation which ensures that all donations can be accounted for.

5.11.3 Transportation of Components from RTCs to Hospitals/Users

1 Blood components should be transported under conditions which are as close as possible to their specific storage requirements. (See Chapter 6). Transport time should be kept to a minimum.

2 Components despatched from an RTC should be accompanied by a despatch note detailing as a minimum the donation number of each component and, if relevant, their blood group. The despatch note should also contain the signature(s) and designation of the person(s) responsible for the issue and of the person receiving the consignment. A copy of the signed and annotated despatch note should be returned to the RTC for storage.

5.12 Component Recall

5.12.1 There must be a documented system available in each RTC whereby adverse effects caused by the administration of any component can enable the recall, if appropriate, of all unused components derived from that donation or all donations which are a constituent of a component pool.

5.12.2 Similarly, there must be a documented system in each RTC for the recall of any component or constituent of a component pool where reasonable grounds exist for believing it could cause adverse effects.

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

Chapter 6

Guidelines Specifications for Blood Components

6.1 Whole Blood

6.1.1 General Description

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

Whole blood from which certain cellular or plasma elements have been removed as an operational expediant rather than for a specific clinical indication can be labelled as whole blood provided it meets the specification given at **6.1.5**.

6.1.2 Technical Information

A unit of whole blood consists of 450mL ± 45mL of blood from a suitable donor (*see Chapter 1*) in an approved container containing anticoagulant (normally 63mL).

6.1.3 Labelling (for general guidelines see **5.6**)

The following shall be included on the label

- whole blood and total nominal volume (ie including anticoagulant)
- the producer's name
- the donation number
- the ABO group
- the RhD group stated as positive or negative (reference may also be made to tests for the C or E antigens of RhD negative units)
- the composition and volume of the anticoagulant solution
- the date of collection and expiry date
- the temperature of storage
- that the component must not be used if there are visible signs of deterioration
- that the component must be administered through a suitable transfusion set incorporating a 170µm filter
- that the component may transmit infection

6.1.4 Storage (*for general guidelines see* **5.7**)

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

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

3 Exceptionally, eg due to equipment failure, whole blood which has been exposed to a core temperature not exceeding 10°C and not less than 1°C may be released for transfusion provided:

- that the component has been exposed to such a temperature increase on one occasion only
- that the duration of the temperature increase has not exceeded 5 hours
- that a documented system is available in each RTC to cover such eventualities
- that adequate records of the incident are compiled and retained

6.1.5 Testing

1 In addition to the mandatory and other tests required for blood donations and which are described in annex 3, a minimum of 75% of those units tested for the parameter shown at **6.1.5.2** below shall meet the specified value.

2 Additional Tests

PARAMETER	FREQUENCY OF TEST	SPECIFICATION
Volume	1%	450mL ± 45mL

6.1.6 Transportation (for general guidelines see **5.11**)

The air temperature of transport containers for units of whole blood should be maintained between 2°C and 10°C during transportation from the RTC to the place that they are intended for use. Transport time under these conditions normally should not exceed 12 hours.

6.2 Red Cells

6.2.1 General Description

A component prepared by removing most of the plasma from whole blood after centrifugation.

6.2.2 Technical Information

The volume of remaining plasma will influence the haematocrit of this component.

6.2.3 Labelling (*for general guidelines see* **5.6**)

The following shall be included on the label

- **red cells** and nominal volume
- the producer's name
- the donation number
- the ABO group
- the RhD group stated as positive or negative (reference may also be made to tests for the C or E antigens of RhD negative units)
- the composition and volume of the anticoagulant solution
- the date of collection and expiry date
- the temperature of storage
- that the component must not be used if there are visible signs of deterioration
- that the component must be administered through a suitable transfusion set incorporating a 170µm filter
- that the component may transmit infection

6.2.4 Storage (*for general guidelines see* **5.7**)

1 Red cells may be stored for a maximum of 35 days at a core temperature of 4°C ± 2°C if an adenine supplemented anticoagulant is used, otherwise the maximum period of storage is 28 days at a core temperature of 4°C ± 2°C.

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

3 Exceptionally, eg due to equipment failure, red cells which have been exposed to a core temperature not exceeding 10°C and not less than 1°C may be released for transfusion provided:

- that the component has been exposed to such a temperature increase on one occasion only
- that the duration of the temperature increase has not exceeded 5 hours
- that a documented system is available in each RTC to cover such eventualities
- that adequate records of the incident are compiled and retained

6.2.5 Testing

1 In addition to the mandatory and other tests required for blood donations and which are described in annex 3, a minimum of 75% of those units tested for the parameters shown at **6.2.5.2** below shall meet all the specified values.

2 Additional Tests

PARAMETER	FREQUENCY OF TEST	SPECIFICATION
Volume	1%	280mL ± 60mL
Haematocrit	1%	0.55 to 0.75

6.2.6 Transportation (*for general guidelines see* **5.11**)

The air temperature of transport containers for units of red cells should be maintained between 2°C and 10°C during transportation from the RTC to the place that they are intended for use. Transport time under these conditions normally should not exceed 12 hours.

6.3 Red Cells in Additive Solution

6.3.1 General Description

A component prepared by removing most of the plasma from whole blood after centrifugation. The red cells are suspended in a licensed additive solution.

6.3.2 Technical Information

The volume of remaining plasma will influence the haematocrit of this component.

6.3.3 Labelling (*for general guidelines see* **5.6**)

The following shall be included on the label

- **red cells in additive solution** and nominal volume
- the producer's name
- the donation number
- the ABO group
- the RhD group stated as positive or negative (reference may also be made to tests for the C or E antigens of RhD negative units)
- the composition and volume of the additive solution
- the date of collection and expiry date
- the temperature of storage
- that the component must not be used if there are visible signs of deterioration
- that the component must be administered through a suitable transfusion set incorporating a 170µm filter
- that the component may transmit infection

6.3.4 Storage (for general guidelines see 5.7)

1 Red cells in additive solution may be stored for a maximum of 35 days at a core temperature of 4°C ± 2°C.

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

3 Exceptionally, eg due to equipment failure, red cells in additive solution which have been exposed to a core temperature not exceeding 10°C and not less than 1°C may be released for transfusion provided:

- that the component has been exposed to such a temperature increase on one occasion only
- that the duration of the temperature increase has not exceeded 5 hours
- that a documented system is available in each RTC to cover such eventualities
- that adequate records of the incident are compiled and retained

6.3.5 Testing

1 In addition to the mandatory and other tests required for blood donations and which are described in annex 3, a minimum of 75% of those units tested for the parameters shown at **6.3.5.2** below shall meet all the specified values.

2 Additional Tests

PARAMETER	FREQUENCY OF TEST	SPECIFICATION
Volume	1%	350mL ± 70mL
Haematocrit	1%	0.50 - 0.70

6.3.6 Transportation (*for general guidelines see* **5.11**)

The air temperature of transport containers for units of red cells in additive solution should be maintained between 2°C and 10°C during transportation from the RTC to the place that they are intended for use. Transport time under these conditions normally should not exceed 12 hours.

6.4 Red Cells in Additive Solution, Buffy Coat Removed

6.4.1 General Description

A component prepared by removing most of the plasma, leucocytes and platelets from whole blood after centrifugation. The red cells are suspended in a licensed additive solution.

6.4.2 Technical Information

The volume of remaining plasma will influence the haematocrit of this component.

6.4.3 Labelling (*for general guidelines see* **5.6**)

The following shall be included on the label

- **red cells in additive solution, buffy coat removed** and nominal volume
- the producer's name
- the donation number
- the ABO group
- the RhD group stated as positive or negative (reference may also be made to tests for the C or E antigens of RhD negative units)
- the composition and volume of the additive solution
- the date of collection and expiry date
- the temperature of storage
- that the component must not be used if there are visible signs of deterioration
- that the component must be administered through a suitable transfusion set incorporating a 170μm filter
- that the component may transmit infection

6.4.4 Storage (*for general guidelines see* **5.7**)

1 Red cells in additive solution, buffy coat removed may be stored for a maximum of 35 days at a core temperature of 4°C ± 2°C.

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

3 Exceptionally, eg due to equipment failure, red cells in additive solution, buffy coat removed which have been exposed to a core temperature not exceeding 10°C and not less than 1°C may be released for transfusion provided:

- that the component has been exposed to such a temperature increase on one occasion only
- that the duration of the temperature increase has not exceeded 5 hours
- that a documented system is available in each RTC to cover such eventualities
- that adequate records of the incident are compiled and retained

6.4.5 Testing

1 In addition to the mandatory and other tests required for blood donations and which are described in annex 3, a minimum of 75% of those units tested for the parameters shown at **6.4.5.2** below shall meet all the specified values.

2 Additional Tests

PARAMETER	FREQUENCY OF TEST	SPECIFICATION
Volume	1%	280mL ± 60mL
Haematocrit	1%	0.50 to 0.70
Leucocyte count	1%	$<1.2 \times 10^9$/unit

6.4.6 Transportation (for general guidelines see **5.11**)

The air temperature of storage containers for units of red cells in additive solution, buffy coat removed should be maintained between 2°C and 10°C during transportation from the RTC to the place that they are intended for use. Transport time under these conditions normally should not exceed 12 hours.

6.5 Red Cells, Leucocyte Depleted

6.5.1 General Description

A red cell component from which most of the leucocytes have been removed by filtration.

6.5.2 Technical Information

The sequential use of buffy coat removal and subsequent depletion of leucocytes by filtration provides a method to achieve the specification. To ensure optimal leucocyte removal the recommended capacity of the filter should not be exceeded.

6.5.3 Labelling (*for general guidelines see* **5.6**)

The following shall be included on the label

- **red cells, leucocyte depleted** and nominal volume
- the producer's name
- the donation number
- the ABO group
- the RhD group stated as positive or negative (reference may also be made to tests for the C or E antigens of RhD negative units)
- the composition and volume of the anticoagulant solution
- the date of collection and expiry date and time
- the temperature of storage
- that the component must not be used if there are visible signs of deterioration
- that the component must be administered through a suitable transfusion set incorporating a 170µm filter
- that the component may transmit infection

6.5.4 Storage (*for general guidelines see* **5.7**)

1 If prepared in a closed system, red cells, leucocyte depleted may be stored for a maximum of 35 days at a core temperature of 4°C ± 2°C if an adenine supplemented anticoagulant is used, otherwise the maximum period of storage is 28 days at a core temperature of 4°C ± 2°C.

2 Red cells, leucocyte depleted prepared in an open system should be used as soon as possible. If storage is unavoidable, the component should be stored at a core temperature of 4°C ± 2°C and used within 24 hours.

3 Variation from the core temperature of 4°C ± 2°C must be kept to a minimum during storage and restricted to any short period necessary for examining, labelling or issuing the component.

4 Exceptionally, eg due to equipment failure, red cells, leucocyte depleted which have been exposed to a core temperature not exceeding 10°C and not less than 1°C may be released for transfusion provided:

- that the component has been exposed to such a temperature increase on one occasion only
- that the duration of the temperature increase has not exceeded 5 hours
- that a documented system is available in each RTC to cover such eventualities
- that adequate records of the incident are compiled and retained

6.5.5 Testing

1 In addition to the mandatory and other tests required for blood donations and which are described in annex 3, a minimum of 75% of those units tested for the parameter shown at **6.5.5.2** below shall meet the specified value.

2 Additional Tests

PARAMETER	FREQUENCY OF TEST	SPECIFICATION
Leucocyte count	1%	$<5 \times 10^6$/unit*

* *electronic particle counters should not be used*

6.5.6 Transportation (*for general guidelines see* **5.11**)

The air temperature of transport containers for units of red cells, leucocyte depleted should be maintained between 2°C and 10°C during transportation from the RTC to the place that they are intended for use. Transport time under these conditions normally should not exceed 12 hours and if open processing has been used should be as short as possible.

6.6 Red Cells, Washed

6.6.1 General description

A red cell component from which most of the plasma, leucocytes and platelets have been removed by washing with an isotonic saline solution.

6.6.2 Technical Information

1 The amount of residual protein will depend on the washing protocol. Washing can be performed by interrupted or continuous flow centrifugation.

2 **A secure system must be in place to ensure the correct donation identification number is put on the component pack of red cells, washed.**

6.6.3 Labelling (*for general guidelines see* **5.6**)

The following shall be included on the label

- **red cells, washed** and nominal volume
- the producer's name
- the donation number
- the ABO group
- the RhD group stated as positive or negative (reference may also be made to tests for the C or E antigens of RhD negative units)
- the composition and volume of the suspending solution
- the date and time of preparation and expiry date and time
- the temperature of storage
- that the component must not be used if there are visible signs of deterioration
- that the component must be administered through a suitable transfusion set incorporating a 170µm filter
- that the component may transmit infection

6.6.4 Storage (for general guidelines see **5.7**)

Red cells, washed should be used as soon as possible. If storage is unavoidable, or if an open system of preparation has been used, the component should be stored at a core temperature of 4°C ± 2°C and used within 24 hours.

6.6.5 Testing

1 In addition to the mandatory and other tests required for blood donations and which are described in annex 3, a minimum of 75% of those units tested for the parameters shown at **6.6.5.2** below shall meet all the specified values.

2 Additional Tests

PARAMETER	FREQUENCY OF TEST	SPECIFICATION
Volume	4 per month or, if infrequent, every component	280mL ± 60mL
Haematocrit		0.65 to 0.75
Residual protein		<0.5g/unit

6.6.6 Transportation (*for general guidelines see* **5.11**)

The air temperature of transport containers for units of red cells, washed should be maintained between 2°C and 10°C during transportation from the RTC to the place that they are intended for use. Transport times under these conditions normally should not exceed 12 hours and if open processing has been used should be as short as possible.

6.7 Red Cells, Thawed and Washed

6.7.1 General Description

A red cell component which has been frozen preferably within 5 days of collection and is washed free of cryoprotectant on thawing for use.

6.7.2 Technical Information

1 The concentration and nature of the cryoprotectant must provide appropriate protection of the red cells at the intended storage temperature. The entire process of freezing, thawing and washing must be validated and documented.

2 **A secure system must be in place to ensure the correct donation identification number is put on the pack in which the component is frozen and the pack in which the final component is presented.**

6.7.3 Labelling (*for general guidelines see* **5.6**)

The following shall be included on the label

- **red cells, thawed and washed** and nominal volume
- the producer's name
- the donation number
- the ABO group
- the RhD group stated as positive or negative (reference may also be made to tests for the C or E antigens of RhD negative units)
- the composition and volume of the suspending solution
- the date and time of preparation and expiry date and time
- the temperature of storage
- that the component must not be used if there are visible signs of deterioration
- that the component must be administered through a suitable transfusion set incorporating a 170µm filter
- that the component may transmit infection

6.7.4 Storage (*for general guidelines see* **5.7**)

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

2 After preparation, red cells, thawed and washed should be used as soon as possible. If storage is unavoidable, the component should be stored at a core temperature of 4°C ± 2°C and used within 24 hours.

6.7.5 Testing

1 In addition to the mandatory and other tests required for blood donations and which are described in annex 3, a minimum of 75% of those units tested for the parameters shown at **6.7.5.2** below shall meet all the specified values.

2 Additional Tests

PARAMETER	FREQUENCY OF TEST	SPECIFICATION
Volume	All	Within locally defined nominal volume range
Haematocrit	4 per month or, if infrequent, every component	0.60 - 0.75
Supernatant Hb		⩽ 1g/L

6.7.6 Transportation (*for general guidelines see* **5.11**)

1 The transport requirements for red cells in the frozen state will be influenced by the nature and concentration of cryoprotectant used: eg a component containing <20% glycerol should not be transported using dry ice as a refrigerant.

2 The air temperature of transport containers for units of red cells, thawed and washed should be maintained between 2°C and 10°C during transportation from the RTC to the place that they are intended for use. Transport times under these conditions should be as short as possible and should not exceed 12 hours.

6.8 Platelets

6.8.1 General Description

A component prepared from whole blood normally within 8 hours of venepuncture, which contains platelets as the major cellular product suspended in 50mL ± 10mL of anticoagulated plasma or in an appropriate volume of a licensed isotonic suspension medium.

6.8.2 Technical Information

50mL ± 10mL plasma (or an appropriate volume of a licensed isotonic suspension medium) containing most of the platelets in a unit of whole blood. The component must be prepared at ambient temperature before the red cell component is cooled to its storage temperature.

6.8.3 Labelling (*for general guidelines see* **5.6**)

The following shall be included on the label

- **platelets** and nominal volume
- the producer's name
- the donation number
- the ABO group
- the RhD group stated as positive or negative
- the composition and volume of the additive solution, if used
- the date of collection and expiry date
- the temperature of storage and a comment that continuous gentle agitation throughout storage is recommended
- that the component must not be used if there are visible signs of deterioration
- that the component must be administered through a suitable transfusion set incorporating a 170µm filter
- that the component may transmit infection

NOTE: If pooled, the donation number of all contributing platelet components, or a unique batch or pool number, must appear on the component label.

6.8.4 Storage (*for general guidelines see* **5.7**)

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

2 Plastics currently in use for this purpose allow for storage at a core temperature of 22°C ± 2°C for up to 5 days in a closed system. Gentle agitation must be maintained throughout the storage period.

3 After any open system manipulation, platelets should be used as soon as possible. If storage is unavoidable the component should be stored at a core temperature of 22°C ± 2°C with continuous gentle agitation and used within 6 hours.

6.8.5 Testing

1 In addition to the mandatory and other tests required for blood donations and which are described in annex 3, a minimum of 75% of those units tested for the parameters shown at **6.8.5.2** below shall meet all the specified values.

2 Additional Tests

PARAMETER	FREQUENCY OF TEST	SPECIFICATION
Volume	1%	50mL ± 10mL
Platelet Count	1%	$\geq 55 \times 10^9$/unit
Leucocyte Count	1%	$<0.2 \times 10^9$/unit
Erythrocyte Count	1%	$<1.0 \times 10^9$/unit
pH at end of shelf life	1%	6.4 - 7.4

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

6.8.6 Transportation (*for general guidelines see* **5.11**)

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

6.9 Platelets, Pooled, Leucocyte Depleted

6.9.1 General Description

A pool of platelets which has been filtered to remove leucocytes.

6.9.2 Technical Information

To ensure optimal leucocyte removal the recommended capacity of the filter should not be exceeded.

The minimum platelet count should be $\geq 47 \times 10^9$ per contributing platelet unit and the maximum leucocyte count should be $<1.0 \times 10^6$ per contributing platelet unit.

6.9.3 Labelling (*for general guidelines see* **5.6**)

The following shall be included on the label

- **platelets, pooled, leucocyte depleted** and nominal volume
- the producer's name
- the donation number of all contributing platelet units or a unique batch or pool number
- the ABO group
- the RhD group stated as positive or negative
- the composition and volume of the additive solution, if used
- the date and time of preparation and expiry date and time
- the temperature of storage and a comment that continuous gentle agitation throughout storage is recommended.
- that the component must not be used if there are visible signs of deterioration
- that the component must be administered through a suitable transfusion set incorporating a 170µm filter.
- that the component may transmit infection

6.9.4 Storage (*for general guidelines see* **5.7**)

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

2 Plastics currently in use for this purpose allow storage with continuous gentle agitation at a core temperature of 22°C ± 2°C for up to 5 days in a closed system.

3 If pooling and/or filtration is by an open system, after preparation the component should be used as soon as possible. If storage is unavoidable, the component should be stored at a core temperature of 22°C ± 2°C with continuous gentle agitation and used within 6 hours.

6.9.5 Testing

1 In addition to the mandatory and other tests required for blood donations and which are described in annex 3, a minimum of 75% of those units tested for the parameters shown at **6.9.5.2** below shall meet all the specified values.

2 Additional Tests

PARAMETER	FREQUENCY OF TEST	SPECIFICATION
Volume	1% or 4 per month whichever is greater. If less than 4 per month, every component	Within locally defined nominal volume range
Platelet Count		$\geq 47 \times 10^9$/ contributing platelet unit
Leucocyte Count		$<1.0 \times 10^6$/ contributing platelet unit
pH at end of shelf life		6.4 - 7.4

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

6.9.6 Transportation (*for general guidelines see* **5.11**)

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

6.10 Platelets, Apheresis

6.10.1 General Description

A component prepared from anticoagulated blood which is separated into components by a suitable apheresis machine with retention of the platelets and a portion of the plasma. The remaining elements may be returned to the donor.

6.10.2 Technical Information

Platelets may be collected by a variety of apheresis systems using different protocols. Since platelet yields may vary, each procedural protocol must be fully validated, documented and specifications set accordingly. The volume of residual plasma must be sufficient to maintain the pH within the range 6.4-7.4 throughout the shelf life of the component.

6.10.3 Labelling (*for general guidelines see* **5.6**)

The following shall be included on the label

- **platelets, apheresis** and nominal volume
- the producer's name
- the donation number
- the ABO group
- the RhD group stated as positive or negative
- the date of collection and expiry date and time
- the temperature of storage and a comment that continuous gentle agitation throughout storage is recommended
- that the component must not be used if there are visible signs of deterioration
- that the component must be administered through a suitable transfusion set incorporating a 170µm filter
- that the component may transmit infection

6.10.4 Storage (*for general guidelines see* **5.7**)

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

2 Plastics currently in use for this purpose allow storage with continuous, gentle agitation at a core temperature of 22°C ± 2°C for up to 5 days in a closed system.

3 If the apheresis harness is not preconnected prior to use or where an approved microbiological filter is used but is not part of the pack assembly, the system must be regarded as open and the platelets used as soon as possible after collection. If storage is unavoidable the component should be stored at a core temperature of 22°C ± 2°C with continuous gentle agitation and used within 6 hours.

6.10.5 Testing

1 In addition to the mandatory and other tests required for blood donations and which are described in annex 3, a minimum of 75% of those units tested for the parameters shown at **6.10.5.2** below shall meet all the specified values.

2 Additional Tests

PARAMETER	FREQUENCY OF TEST	SPECIFICATION
Volume	1% or 4 per month whichever is greater If less than 4 per month, every component	> 40mL per 55×10^9 platelets
Platelet Count		$\geqslant 165 \times 10^9$ per unit
Leucocyte Count		$< 0.2 \times 10^9$ per 55×10^9 platelets
Erythrocyte Count		6.4 - 7.4
pH at end of shelf life		

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

6.10.6 Transportation (*for general guidelines see* **5.11**)

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

6.11 Platelets, Apheresis, Leucocyte Depleted

6.11.1 General Description

A component prepared by the filtration of apheresis platelets to remove leucocytes.

6.11.2 Technical Information

To ensure optimal leucocyte removal the recommended capacity of the filter should not be exceeded.

The minimum platelet count should be $\geqslant 140 \times 10^9$ per unit and the maximum leucocyte count should be $< 1.0 \times 10^6$ per 55×10^9 platelets. The volume of residual plasma must be sufficient to maintain the pH within the range 6.4 - 7.4 throughout the shelf life of the component.

6.11.3 Labelling (*for general guidelines see* **5.6**)

The following shall be included on the label

- **platelets, apheresis, leucocyte depleted** and nominal volume
- the producer's name
- the donation number
- the ABO group
- the RhD group stated as positive or negative
- the date and time of preparation and expiry date and time
- the temperature of storage and a comment that continuous gentle agitation throughout storage is recommended
- that the component must not be used if there are visible signs of deterioration
- that the component must be administered through a suitable transfusion set incorporating a 170µm filter
- that the component may transmit infection

6.11.4 Storage (*for general guidelines see* **5.7**)

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

2 Plastics currently in use for this purpose allow storage with continuous, gentle agitation at a core temperature of 22°C ± 2°C for up to 5 days in a closed system.

3 Where filtration is by an open system the platelets should be used as soon as possible after collection. If storage is unavoidable the component should be stored at a core temperature of 22°C ± 2°C with continuous gentle agitation and used within 6 hours.

6.11.5 Testing

1 In addition to the mandatory and other tests required for blood donations and which are described in annex 3, a minimum of 75% of those units tested for the parameters shown at **6.11.5.2** below shall meet all the specified values.

2 Additional Tests

PARAMETER	FREQUENCY OF TEST	SPECIFICATION
Volume	1% or 4 per month whichever is greater If less than 4 per month, every component	> 40mL per 55×10^9 platelets
Platelet Count		$\geqslant 140 \times 10^9$ per unit
Leucocyte Count		$< 1.0 \times 10^6$ per 55×10^9 platelets
pH at end of shelf life		6.4 - 7.4

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

6.11.6 Transportation (*for general guidelines see* **5.11**)

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

6.12 Leucocytes, Buffy Coat

6.12.1 General Description

A component prepared from whole blood by separation of the buffy coat within 8 hours of venepuncture and which contains granulocytes as a major cellular product suspended in anticoagulated plasma or an appropriate volume of a licensed isotonic suspension medium.

6.12.2 Technical Information

The component must be prepared at ambient temperature before the red cell component is cooled to its storage temperature. The component must not be agitated during storage.

6.12.3 Labelling (*for general guidelines see* **5.6**)

The following shall be included on the label

- **leucocytes, buffy coat** and nominal volume
- the producer's name
- the donation number
- the ABO group
- the RhD group stated as positive or negative
- the composition and volume of the additive solution, if used
- the date of collection and expiry date and time
- the temperature of storage
- that the component must not be used if there are visible signs of deterioration
- that the component must be administered through a suitable transfusion set incorporating a 170µm filter.
- that the component may transmit infection.

NOTE: If pooled, the donation number of all contributing buffy coats or a unique batch or pool number must appear on the component label.

6.12.4 Storage (*for general guidelines see* **5.7**)

Leucocytes, buffy coat should be used as soon as possible after their preparation. Whether prepared in an open or closed system, if storage is unavoidable, the component should be stored at a core temperature of 22°C ± 2°C and used within 6 hours of preparation.

6.12.5 Testing

1 In addition to the mandatory and other tests required for blood donations and which are described in annex 3, a minimum of 75% of those units tested for the parameters shown at **6.12.5.2** below shall meet all the specified values.

2 Additional Tests

PARAMETER	FREQUENCY OF TEST	SPECIFICATION
Volume	1% or 4 per month whichever is greater. If less than 4 per month, every component	50mL ± 10mL per contributing unit
Total Leucocyte Count		> 1.0×10^9 per contributing unit

6.12.6 Transportation (*for general guidelines see* **5.11**)

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

6.13 Leucocytes, Apheresis

6.13.1 General Description

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

6.13.2 Technical Information

Leucocytes may be collected by a variety of apheresis systems using different protocols. Since yields may vary, each procedural protocol must be fully validated, documented and specifications set accordingly. The component must not be agitated during storage.

6.13.3 Labelling (*for general guidelines see* **5.6**)

The following shall be included on the label

- **leucocytes, apheresis** and nominal volume
- the producer's name
- the donation number
- the ABO group
- the RhD group stated as positive or negative
- the date of collection and expiry date and time
- the temperature of storage
- that the component must not be used if there are visible signs of deterioration
- that the component must be administered through a suitable transfusion set incorporating a 170µm filter
- that the component may transmit infection

6.13.4 Storage (*for general guidelines see* **5.7**)

Leucocytes, apheresis should be used as soon as possible after their preparation. Whether prepared in an open or closed system, if storage is unavoidable the component should be stored at a core temperature of 22°C ± 2°C and used within 6 hours of preparation.

6.13.5 Testing

1 In addition to the mandatory and other tests required for blood donations and which are described in annex 3, a minimum of 75% of those units tested for the parameters shown at **6.13.5.2** below shall meet all the specified values.

2 Additional Tests

PARAMETER	FREQUENCY OF TEST	SPECIFICATION
Volume	1% or 4 per month whichever is greater. If less than 4 per month, every component	> 40mL per 1.0×10^9 leucocytes
Total leucocyte Count		> 10×10^9 per unit

6.13.6 Transportation (*for general guidelines see* **5.11**)

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

6.14 **Fresh Frozen Plasma** (for direct clinical use)

6.14.1 General Description

Fresh frozen plasma is plasma that has been obtained from whole blood or by apheresis and rapidly frozen to a temperature that will maintain the activity of labile coagulation factors.

6.14.2 Technical Information

1 Ideally the plasma should be separated at 22°C ± 2°C before the red cell component is cooled to its storage temperature.

2 The method of preparation should ensure the component has the maximum level of labile coagulation factors with minimum cellular contamination.

3 A rapid freezing process should be used to ensure that a core temperature of -30°C or below is achieved within 2 hours of commencing the freezing process.

4 The maximum time period from venepuncture to obtaining a core temperature of -30°C or below is normally 8 hours.

6.14.3 Labelling (*for general guidelines see* **5.6**)

The following shall be included on the component label

- fresh frozen plasma and nominal volume
- the producer's name
- the donation number
- the ABO group
- the RhD group stated as positive or negative
- the date of preparation and expiry date of the frozen component
- the temperature of storage
- a warning that the component must be used within 2 hours of thawing
- that the component must not be used if there are visible signs of deterioration
- that the component may transmit infection

6.14.4 Storage (*for general guidelines see* **5.7**)

1 Fresh frozen plasma should be stored at a core temperature of -30°C or below for a maximum of 12 months.

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

3 Once thawed, fresh frozen plasma must not be refrozen and should be used immediately. If delay is unavoidable, the component should be stored at ambient temperature and used within 2 hours.

6.14.5 Testing

1 In addition to the mandatory and other tests required for blood donations and which are described in annex 3, a minimum of 75% of those units tested for the parameters shown at **6.14.5.2** below shall meet all the specified values.

2 Additional Tests

PARAMETER	FREQUENCY OF TEST	SPECIFICATION
Volume	1%	⩾ 150 mL and within locally defined nominal volume range
Platelets	1%	$< 30 \times 10^9$/L*
Factor VIIIc	1%	> 0.7 iu/mL

* prefreeze

6.14.6 Transportation (*for general guidelines see* **5.11**)

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

6.15 Cryoprecipitate

6.15.1 General Description

The component contains the major portion of Factor VIII, von Willebrand factor, fibrinogen, Factor XIII and fibronectin from a unit of fresh frozen plasma.

6.15.2 Technical Information

1 Cryoprecipitate is the cryoglobulin fraction of plasma obtained by thawing a single donation of fresh frozen plasma at 4°C ± 2°C and concentrating to a final volume of 20mL ± 5mL.

2 After preparation cryoprecipitate should be used immediately or rapidly frozen to a core temperature of -30°C or below within 2 hours of preparation.

6.15.3 Labelling (*for general guidelines see* **5.6**)

The following shall be included on the component label

- **cryoprecipitate** and nominal volume
- the producer's name
- the donation number
- the ABO group
- the RhD group stated as positive or negative
- the date of preparation and expiry date of the frozen component
- the temperature of storage
- a warning that the component must be used within 2 hours of thawing
- that the component must not be used if there are visible signs of deterioration
- that the component may transmit infection

6.15.4 Storage (*for general guidelines see* **5.7**)

1 Cryoprecipitate should be stored at a core temperature of -30°C or below for a maximum of 12 months.

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

3 Once thawed, cryoprecipitate must not be refrozen and should be used immediately. If delay is unavoidable, the component should be stored at ambient temperature and used within 2 hours.

6.15.5 Testing

1 In addition to the mandatory and other tests required for blood donations and which are described in annex 3, a minimum of 75% of those units tested for the parameters shown at **6.15.5.2** below shall meet all the specified values.

2 Additional Tests

PARAMETER	FREQUENCY OF TEST	SPECIFICATION
Volume	1%	20mL ± 5mL
Fibrinogen	1%	> 140 mg/unit
Factor VIIIc	1%	> 70 iu/unit

6.15.6 Transportation (*for general guidelines see* **5.11**)

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

6.16 Plasma, Cryoprecipitate Depleted

6.16.1 General Description

The supernatant plasma removed during the preparation of cryoprecipitate. The content of albumin and immunoglobulins is comparable with fresh frozen plasma; factor VIII, von Willebrand factor, fibrinogen, factor XIII and fibronectin are reduced.

6.16.2 Technical Information

Plasma, cryoprecipitate depleted should be frozen to a core temperature of -30°C or below within 2 hours of separation from its cryoprecipitate.

6.16.3 Labelling (*for general guidelines see* **5.6**)

The following shall be included on the component label

- **plasma, cryoprecipitate depleted** and nominal volume
- the producer's name
- the donation number
- the ABO group
- the RhD group stated as positive or negative
- the date of preparation and expiry date of the frozen component
- the temperature of storage
- a warning that the component must be used within 2 hours of thawing
- that the component must not be used if there are visible signs of deterioration
- that the component may transmit infection

6.16.4 Storage (*for general guidelines see* **5.7**)

1 Plasma, cryoprecipitate depleted should be stored at a core temperature of -30°C or below for a maximum of 12 months.

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

3 Once thawed, plasma, cryoprecipitate depleted must not be refrozen and should be used immediately. If delay is unavoidable, the component should be stored at ambient temperature and used within 2 hours.

6.16.5 Testing

1 In addition to the mandatory and other tests required for blood donations and which are described in annex 3, a minimum of 75% of those units tested for the parameter shown at **6.16.5.2** below shall meet the specified value.

2 Additional Tests

PARAMETER	FREQUENCY OF TEST	SPECIFICATION
Volume	1%	Within locally defined nominal volume range

6.16.6 Transportation (*for general guidelines see* **5.11**)

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

6.17 Components for Neonatal Use

6.17.1 General Guidelines

1 It is good practice to provide neonates with components of lower volume by dividing standard components into sub batches. This minimises wastage of valuable components and provides the potential to reduce donor exposures. However, provided they meet the specifications outlined below, blood components already specified in this Chapter are suitable for neonatal use.

2 When a component is divided for neonatal use, each sub batch must be identified by a unique number to ensure all sub batches of a component can be accounted for.

3 When a component is to be divided for neonatal use the index pack must first be mixed thoroughly by a validated procedure to ensure that the contents are homogeneous.

4 When components for neonatal use are prepared by dividing standard components the specifications shown below shall apply.

5 RTCs should ensure hospital laboratories in their Region are informed of UKBTS/NIBSC guidelines concerning components for neonatal use.

6.17.2 **Whole Blood for Neonatal Use**

1 GENERAL DESCRIPTION

A unit of blood collected into a licensed anticoagulant and, in a closed system, divided into approximately equal volumes. The component is otherwise not further processed.

Whole blood for neonatal use from which certain cellular or plasma elements have been removed as an operational expendiant rather than for a specific clinical indication can be labelled as whole blood for neonatal use provided it meets the specification given at **6.17.2.5.**

2 TECHNICAL INFORMATION

For exchange and massive transfusion of neonates, whole blood should be less than 5 days old. For 'top up' transfusion of neonates, whole blood may be used at any time within the shelf life of the original component.

3 LABELLING (*for general guidelines see* **5.6**)

The following shall be included on the label

- **whole blood for neonatal use** and nominal volume
- the producer's name
- the donation/unique batch number and sub batch identity
- the ABO group
- the RhD group stated as positive or negative (reference may also be made to tests for the C or E antigens of RhD negative units)
- the identity of anticoagulant solution
- the date of collection and expiry date
- the temperature of storage
- that the component must not be used if there are visible signs of deterioration
- that the component must be administered through a suitable transfusion set incorporating a 170µm filter
- that the component may transmit infection

4 STORAGE (*for general guidelines see* **5.7**)

i. Whole blood for 'top up' transfusion of neonates may be stored for a maximum of 35 days at a core temperature of 4°C ± 2°C if an adenine supplemented anticoagulant is used, otherwise the maximum period of storage is 28 days at at a core temperature of 4°C ± 2°C.

ii. Whole blood for exchange or massive transfusion of neonates should be used within 5 days of venepuncture.

iii. Variation from the core temperature of 4°C ± 2°C must be kept to a minimum during storage and restricted to any short period necessary for examining, labelling or issuing the component.

iv. Exceptionally, eg due to equipment failure, whole blood for neonatal use which has been exposed to a core temperature not exceeding 10°C and not less than 1°C may be released for transfusion provided:

- that the component has been exposed to such a temperature increase on one occasion only
- that the duration of the temperature increase has not exceeded 5 hours
- that a documented system is available in each RTC to cover such eventualities
- that adequate records of the incident are compiled and retained

5 TESTING

i. In addition to the mandatory and other tests required for blood donations and which are described in annex 3, a minimum of 75% of those units tested for the parameter shown at **6.17.2.5.ii** below shall meet the specified value.

ii. Additional Tests

PARAMETER	FREQUENCY OF TEST	SPECIFICATION
Volume	1% or 4 per month. If less than 4 per month, every component	Within locally defined nominal volume range
High titre anti-A and/or anti-B	Every component	Within locally defined limits of potency

6 TRANSPORTATION (*for general guidelines see* **5.11**)

The air temperature of transport containers for units of whole blood for neonatal use should be maintained between 2°C and 10°C during transportation from the RTC to the place that they are intended for use. Transport time under these conditions normally should not exceed 12 hours.

6.17.3 **Red Cells, Plasma Reduced for Neonatal Use**

1 GENERAL DESCRIPTION

A component prepared by removing 100-150mL of plasma from a unit of whole blood and, using a closed system, dividing the remaining elements into approximately equal volumes.

2 TECHNICAL INFORMATION

The volume of remaining plasma will influence the haematocrit of the component.

3 LABELLING (*for general guidelines see* **5.6**)

The following shall be included on the label

- **red cells, plasma reduced for neonatal use** and nominal volume
- the producer's name
- the donation/unique batch number and sub batch identity
- the ABO group
- the RhD group stated as positive or negative (reference may also be made to tests for the C or E antigens of RhD negative units)
- the identity of the anticoagulant solution
- the date of collection and expiry date
- the temperature of storage
- that the component must not be used if there are visible signs of deterioration

- that the component must be administered through a suitable transfusion set incorporating a 170µm filter
- that the component may transmit infection

4 STORAGE (*for general guidelines see 5.7*)

i. Red cells, plasma reduced for top up transfusion of neonates may be stored for a maximum of 35 days at a core temperature of 4°C ± 2°C if an adenine supplemented anticoagulant is used, otherwise the maximum period of storage is 28 days at a core temperature of 4°C ± 2°C.

ii. Red cells, plasma reduced for exchange or massive transfusion of neonates should be used within 5 days of venepuncture.

iii. Variation from the core temperature of 4°C ± 2°C must be kept to a minimum during storage and restricted to any short period necessary for examining, labelling or issuing the component.

iv. Exceptionally, eg due to equipment failure, red cells, plasma reduced for neonatal use which have been exposed to a core temperature not exceeding 10°C and not less than 1°C may be released for transfusion provided:

- that the component has been exposed to such a temperature increase on one occasion only
- that the duration of the temperature increase has not exceeded 5 hours
- that a documented system is available in each RTC to cover such eventualities
- that adequate records of the incident are compiled and retained

5 TESTING

i. In addition to the mandatory and other tests required for blood donations and which are described in annex 3, a minimum of 75% of those units tested for the parameters shown at **6.17.3.5.ii** below shall meet all the specified values.

ii. Additional Tests

<table>
<tr><th>PARAMETER</th><th>FREQUENCY OF TEST</th><th>SPECIFICATION</th></tr>
<tr><td>Volume</td><td rowspan="2">1% or 4 per month whichever is greater
If less than 4 per month, every component</td><td>Within locally defined nominal volume range</td></tr>
<tr><td>Haematocrit</td><td>0.50 - 0.65</td></tr>
<tr><td rowspan="2">High titre anti-A and/or anti-B</td><td>Every component</td><td rowspan="2">Within locally defined limits of potency</td></tr>
</table>

6 TRANSPORTATION (*for general guidelines see* **5.11**)

The air temperature of transport containers for units of red cells, plasma reduced for neonatal use should be maintained between 2°C and 10°C during transportation from the RTC to the place that they are intended for use. Transport time under these conditions normally should not exceed 12 hours.

6.17.4 Red Cells in Additive Solution, for Neonatal Use

1 GENERAL DESCRIPTION

A component prepared by removing most of the plasma from whole blood after centrifugation. The red cells are suspended in a licensed additive solution and, using a closed system, divided into approximately equal volumes.

2 TECHNICAL INFORMATION

The volume of remaining plasma will influence the haematocrit of this component.

This component is not suitable for exchange or massive neonatal transfusion.

3 LABELLING (*for general guidelines see* **5.6**)

The following shall be included on the label

- **red cells in additive solution, for neonatal use** and nominal volume
- the producer's name
- the donation/unique batch number and sub batch identity
- the ABO group
- the RhD group stated as positive or negative (reference may also be made to tests for the C or E antigens of RhD negative units)
- the identity of the additive solution
- the date of collection and expiry date
- the temperature of storage
- that the component must not be used if there are visible signs of deterioration
- that the component must be administered through a suitable transfusion set incorporating a 170µm filter
- that the component may transmit infection

4 STORAGE (*for general guidelines see 5.7*)

i. Red cells in additive solution for top up transfusion of neonates may be stored for a maximum of 35 days at a core temperature of 4°C ± 2°C.

ii. Variation from the core temperature of 4°C ± 2°C must be kept to a minimum during storage and restricted to any short period necessary for examining, labelling or issuing the component.

iii. Exceptionally, eg due to equipment failure, red cells in additive solution, for neonatal use which have been exposed to a core temperature not exceeding 10°C and not less than 1°C may be released for transfusion provided:

- that the component has been exposed to such a temperature increase on one occasion only
- that the duration of the temperature increase has not exceeded 5 hours
- that a documented system is available in each RTC to cover such eventualities
- that adequate records of the incident are compiled and retained

5 TESTING

i. In addition to the mandatory and other tests required for blood donations and which are described in annex 3, a minimum of 75% of those units tested for the parameters shown at **6.17.4.5.ii** below shall meet all the specified values.

ii. Additional Tests

<table>
<tr><th>PARAMETER</th><th>FREQUENCY OF TEST</th><th>SPECIFICATION</th></tr>
<tr><td>Volume</td><td rowspan="2">1% or 4 per month whichever is greater
If less than 4 per month, every component</td><td>Within locally defined nominal volume range</td></tr>
<tr><td>Haematocrit</td><td rowspan="2">0.50 - 0.70</td></tr>
<tr><td>High titre anti-A and/or anti-B</td><td>Every component</td><td>Within locally defined limits of potency</td></tr>
</table>

6 TRANSPORTATION (*for general guidelines see* **5.11**)

The air temperature of transport containers for units of red cells in additive solution, for neonatal use should be maintained between 2°C and 10°C during transportation from the RTC to the place that they are intended for use. Transport time under these conditions normally should not exceed 12 hours.

6.17.5 Fresh Frozen Plasma for Neonatal Use

1 GENERAL DESCRIPTION

Fresh frozen plasma for neonatal use is plasma that has been obtained from whole blood or by apheresis, sub divided into approximately equal volumes in a closed system and rapidly frozen to a temperature that will maintain the activity of labile coagulation factors.

2 TECHNICAL INFORMATION

i. Ideally the plasma should be separated at 22°C ± 2°C before the red cell component is cooled to its storage temperature.

ii. The method of preparation should ensure the component has the maximum level of labile coagulation factors with minimum cellular contamination.

iii. A rapid freezing process should be used to ensure that a core temperature of -30°C or below is achieved within 2 hours of commencing the freezing process.

iv. The maximum time period from venepuncture to obtaining a core temperature of -30°C or below is normally 8 hours.

3 LABELLING (*for general guidelines see* **5.6**)

The following shall be included on the component label

- **fresh frozen plasma for neonatal use** and nominal volume
- the producer's name
- the donation/unique batch number and sub batch identity
- the ABO group
- the RhD group stated as positive or negative
- the date of preparation and expiry date of the frozen component
- the temperature of storage
- a warning that the component should be used within 2 hours of thawing
- that the component must not be used if there are visible signs of deterioration
- that the component may transmit infection

4 STORAGE (*for general guidelines see* **5.7**)

i. Fresh frozen plasma for neonatal use should be stored at a core temperature of -30°C or below for a maximum of 12 months.

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

iii. Once thawed, fresh frozen plasma for neonatal use must not be refrozen and should be used immediately. If delay is unavoidable, the component should be stored at ambient temperature and used within 2 hours.

5 TESTING

i. In addition to the mandatory and other tests required for blood donations and which are described in annex 3, a minimum of 75% of those units tested for the parameters shown at **6.17.5.5.ii** below shall meet all the specified values.

ii. Additional Tests

PARAMETER	FREQUENCY OF TEST	SPECIFICATION
Volume	1% or 4 per month, whichever is greater. If less than 4 per month, every component	Within locally defined nominal volume range
Platelets		< 30 × 10⁹/L*
Factor VIIIc		> 0.7 iu/mL
High titre anti-A and/or anti-B	Every component	Within locally defined limits of potency

* prefreeze

6 TRANSPORTATION (*for general guidelines see* **5.11**)

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

6.17.6 **Platelets for Neonatal Use**

In circumstances where platelets are required for neonatal use and there are overriding volume considerations, the volume of plasma or licenced additive solution in which platelets are suspended can be reduced to 25mL ± 5mL. Such platelet components should be used as soon as possible after their preparation and disaggregation. The appropriate labelling, testing (except volume) and transportation specifications given in **6.8** through **6.11** should be applied.

6.18 Irradiated Components

6.18.1 This section provides guidance on the irradiation of components in the knowledge that this is an evolving scientific area where further developments are envisaged.

Irradiated components should conform to their appropriate specification previously given in this Chapter. In addition, the guidelines shown below should be observed.

6.18.2 General Description

Irradiated components are components that have been gamma irradiated by a validated procedure.

6.18.3 Technical Information

1 Red cell components should be irradiated within 4 days of collection.

2 For red cells, platelets and granulocytes the recommended minimum dose is 25Gy.

6.18.4 Labelling

1 Irradiated components must be identified by an approved overstick label.

2 Labels which are sensitive to gamma rays and change from 'NOT IRRADIATED' to 'IRRADIATED' are available and are considered a useful indicator of exposure to gamma rays.

3 Irradiated red cell components have a reduced shelf life. This change must be made to the component label.

6.18.5 Storage

1 The maximum shelf life of irradiated red cell components for use in intrauterine, exchange or massive transfusion of neonates is 1 day from the time of irradiation.

The maximum shelf life of irradiated red cell components for use in other clinical applications is 4 days from the time of irradiation.

2 Platelet and granulocyte components should be irradiated and used as soon as possible after their preparation but within the shelf life specified earlier in this Chapter.

6.18.6 Testing

1 Irradiation of cellular components produces increased extracellular levels of potassium, consequently extracellular levels of potassium should be estimated periodically for irradiated components.

2 The mixed lymphocyte culture represents a procedure which can be used to validate the efficiency of the irradiation process.

6.19 Other Components

The specifications given in Chapter 6 cover the majority of components made in most Regional Transfusion Centres. However, the list is not exhaustive. RTCs who wish to prepare components not specified should consider the following aspects:

- The need to establish a local specification for the component.
- The need for consultation with other RTCs to ensure consistency in specifications and commonality of component names and labelling.
- Labelling requirements.
- Storage requirements.
- Testing requirements.
- Transport requirements.

Annex 1

General Specifications for Blood Donor Sessions

1. Donor Identification

1.1 Before the venepuncture the donor must be positively identified and RTC procedures followed to check this action. The identically numbered labels must be checked to ensure that those on the blood packs, sample tubes and donation records are identical.

2. Haemoglobin or Haematocrit Screening

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

2.2 Copper sulphate storage

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

2.3 Copper sulphate for routine use

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

2.4 Copper sulphate procedure: fingerprick blood sample

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

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

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

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

2.4.5 One drop of blood is allowed to fall by unassisted gravity from the tube from a height of 1cm above the surface of the copper sulphate solution. The drop is observed for 15

seconds. If the drop of blood has a higher specific gravity than the solution, it will sink within 15 seconds. If not, the sinking drop will hesitate, remain suspended, or rise to the top of the solution.

2.4.6 Results are recorded as pass or fail.

2.5 Spectrophotometric method for haemoglobin screening

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

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

2.6 The microhaematocrit method for haemoglobin screening

2.6.1 The microhaematocrit centrifuge should be calibrated when first placed in service, after repairs, and annually thereafter.

2.6.2 The time and speed should be checked at a minimum of 6 months and preferably every 3 months by an appropriate qualified person using a precision RPM meter and a stop-watch to check speed, acceleration and retardation.

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

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

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

3. Preparation of the Venepuncture Site

3.1 Blood should be drawn from a suitable vein in the antecubital fossa in an area that is free of skin lesions. The veins can be made more prominent by using a blood pressure cuff inflated to 40-60mm Hg and by asking the donor to open and close his/her hand a few times.

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

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

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

4. Preparation of the Blood Pack

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

4.2 Moisture on the surface of a plastic pack after unpacking should arouse suspicion of a leak and if one or more packs in any packet is found to be abnormally damp, none of the packs in that container should be used.

4.3 The anticoagulated solution should be checked for clarity and must be clear before accepting the packs for use.

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

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

5. Performance of the Venepuncture

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

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

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

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

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

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

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

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

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

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

6. Blood Donation

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

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

6.3 Blood anticoagulation

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

6.4 Blood Flow

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

6.5 Blood Volume Monitoring

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

6.5.2 The most efficient way of measuring the blood volume in plastic bags is by weight. The mean weight of 1ml of blood is 1.06g; a unit containing 405-495ml should therefore weigh 430-525g plus the weight of the container and its anticoagulant.

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

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

6.6 Sample Collection

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

6.7 Completion of the Donation

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

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

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

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

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

6.8 Final Donation Inspection

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

6.9 Safety Related Defects

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

7. Declaration of Health

7.1 Active steps must be taken regarding the medical history of donors to ensure compliance with the requirements listed in Chapter 1, paragraph 1.3 of this section.

Annex 2

Premises

1. General Considerations

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

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

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

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

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

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

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

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

2.5 Social and medical care of donors, including those who suffer reactions. Sufficient seating should be provided for donors and staff, with allowance made for possible queues during busy periods.

2.6 Storage of equipment, reagents and disposables.

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

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

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

3. Health and safety factors to be considered

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

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

3.2.1 The venue should be as close as possible to the centre of population being served. It should be possible for the sessional vehicle(s) to park in close proximity to the access doors, to facilitate off-loading. The ground to be covered by staff carrying equipment should be even and well-lit. Preferably, the space to be used should not entail carriage of equipment on stairs. A similar safe approach should be ensured for donors, with as much provision as possible for the parking of their cars. Notices should be displayed, directing donors to the appropriate entry point of the building, and to the room being used.

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

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

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

3.2.5 Environmental control may not be within the power of a mobile team, but every effort should be made to ensure that the space does not become too hot, too cold or stuffy. Subsidiary cooling fans and heating should be carried on sessional vehicles, and used as necessary. This equipment should be subjected to a planned maintenance programme at the RTC.

3.2.6 Facilities for the provision of refreshments for donors and staff should be separated from the other activities of a donor session whenever possible. Every effort should be made to ensure that equipment used in this area poses the minimum threat of danger to all persons.

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

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

3.2.9 The premises should be free from vermin.

Annex 3

General Specifications for Laboratory Test Procedures

1 General Considerations

1.1 Scope

These specifications provide guidance on the tests required for blood donations in the UK. Specific procedures should be written by individual RTCs in the form of Standard Operating Procedures.

1.2 General Requirements

Secure and effective procedures must be in place to ensure that:

1.2.1 blood donations, components and their laboratory samples are correctly identified by barcoded and eyereadable numbers.

1.2.2 donations can be linked to their donor.

1.2.3 a donor's record is reviewed every time they donate.

1.2.4 donor samples are suitably stored under appropriate conditions of time and temperature to preserve the properties for which they will be tested.

1.2.5 tests are appropriately performed and controlled using validated procedures and the results recorded.

1.2.6 test results and other relevant test information are archived.

1.3 Test Reagents, Kits and Equipment

1.3.1 Unless validated for alternative techniques, test kits and reagents should be stored and used according to the manufacturer's instructions.

1.3.2 All test procedures should be documented and an inventory maintained of kits and reagents in stock.

1.3.3 Procedures should ensure the traceability of the batch number and manufacturer of kits and reagents and, if relevant, the serial number of equipment used to test every donation.

1.3.4 Test equipment should be validated, calibrated and maintained. Appropriate records for these activities should be made and retained.

1.3.5 Appropriate reactivity with control samples must be demonstrated with every series of tests.

A series of tests is defined as the number of tests set up at the same time, under the same conditions and processed in a similar manner. Where a microplate format is used for microbiological testing, each plate constitutes a series even if only a few wells are used.

1.4 Reporting of Results

1.4.1 The laboratory report should indicate the result of each and every test, preferably by a system that provides positive sample identification. Individual test results should be recorded either manually or, ideally, by a computer interfaced to a test reader.

1.4.2 Reporting a series of tests, particularly those of a microbiological nature, by an 'assumed negative' procedure is potentially dangerous and not acceptable.

1.5 Release of Tested Components

Standard procedures must ensure that blood and blood components cannot be released for issue until all the required laboratory tests (mandatory and additional) have been completed, documented and approved within a validated system of work. Compliance with this requirement may be achieved by:

1.5.1 The use of a computer program, or suite of programs, which requires the input of valid and acceptable test results for all the mandatory and required laboratory tests before permitting, or withholding, the release of each individual unit.

1.5.2 Where a computer-based system is not used, a system which requires documented approval for the release of each individual unit by a designated person.

1.5.3 Where the computer-based system is temporarily unavailable, it is necessary to revert to the procedure in **1.5.2**.

1.6 Laboratory Test Categories

Laboratory tests include the following categories:

1.6.1 **Mandatory tests** which are required as part of the criteria for release of all blood donations and components for clinical use currently are ABO and RhD blood grouping; irregular red cell antibody screening; tests for HBsAg, anti-HIV 1 and 2, anti-HCV and syphilis antibodies. These mandatory microbiological tests are determined by the Department of Health and may, from time to time, be extended.

1.6.2 **Additional tests** which are undertaken in special circumstances,

- to increase the safety of transfusion for susceptible patients or clinical effectiveness of specific transfusions eg by providing anti-CMV negative components or HLA matched platelets.
- to immunise volunteers with red cells for the procurement of anti-D immunoglobulin (see Annex 4)

Whilst not required for all blood donations or components, when such tests are performed to meet a specific need the results are an essential part of the criteria for release of that component.

1.6.3 **Component and process monitoring tests** are undertaken on at least 1% of each component type. These tests are a requirement of GMP. With the exception of tests for component sterility, a minimum of 75% of components tested should meet the specified values (see Chapter 6). Tests of this type need not be part of the criteria for component release and are not considered further in this annex.

2 Mandatory Testing of Blood Donations

2.1 Blood Group Serology Tests

2.1.1 ABO Blood Grouping

1 The ABO blood group shall be determined on each blood donation. The primary blood pack and any derived components for direct clinical use shall be labelled appropriately.

2 The ABO blood group shall be determined by testing the red cells and serum (or plasma) of the donor with blood grouping reagents which comply with Section III of these Guidelines.

3 The sample from a donor whose ABO blood group is unknown, for example a first time donor, shall be tested twice and the ABO blood group only accepted when the results are in agreement.

4 There is no need to test with two different examples of ABO reagents if the reagents comply with Section III of these Guidelines.

For those donors whose ABO group is known, a single test can be used provided this shows agreement with the previously recorded ABO blood group of the donor.

5 Quality Control of ABO Blood Grouping

Quality control of procedures recommended by reagent and equipment manufacturers should be observed. However, the following minimum controls are required for each series of ABO blood grouping tests:

Unequivocal, appropriate reactions must be obtained:

for anti-A	with	A_1, A_2B, B and O red cells
for anti-B	with	B, A_1B, A_1 and O red cells
for anti-A,B	with	A_2, B, A_x* and O red cells
for anti-A+B	with	A_2, B and O red cells

A_1; A_2; A_1B; A_2B; B; O; A_x reagent red cells must give unequivocal appropriate reactions with anti-A; anti-B; anti-A,B and/or anti-A+B.

* if anti-A,B is used to detect A_x donations, appropriate reactivity with A_x cells should be confirmed before the anti-A,B is used routinely. Appropriate reactivity with A_x red cells should be confirmed regularly during use, although not necessarily with each series of tests.

2.1.2 RhD Grouping

1 The RhD blood group shall be determined on each donation of blood. The primary blood pack and any derived components for direct clinical use shall be labelled appropriately.

2 The RhD blood group shall be determined by testing the red cells of the donor with two different examples of anti-D blood grouping reagents which comply with Section III of these guidelines.

Whilst it is neither essential nor possible to detect all D^u or D variant samples, the selection of anti-D blood grouping reagents and test procedures should be made to maximise the detection of D^u and D variant red cells as RhD positive. The combination of reagents and methodology used should detect D^{VI} red cells as RhD positive.

i. Donors whose blood gives a positive reaction with both anti-D reagents should be regarded as RhD positive.

ii. Donors whose blood is clearly negative with both anti-D reagents should be regarded as RhD negative.

iii. If the results with the anti-D reagents are discordant or ambiguous the tests should be repeated. If still discordant or ambiguous, the donor's red cells should be tested with reagents which will detect the Rh antigens C, D and E. Anti-c and anti-e may also be used. Where the RhD group is in doubt it is safer to classify donors as RhD positive.

iv. For some patients, eg those who have made anti-CD, it may be necessary to provide blood which is negative for C, D and E antigens. This is achieved by testing RhD negative blood with reagents that contain anti-C and anti-E and applying an approved overstick label, ie 'CDE negative', to components from donors who clearly lack C, D and E antigens.

3 Quality Control of Rh Grouping

Quality control procedures recommended by reagent and equipment manufacturers should be observed. However, as a minimum, for each series of Rh blood grouping tests, unequivocal, appropriate reactions must be obtained:

for anti-D with R_1r, r^1r and $r''r$ red cells
for anti-C with R_1r or r^1r and R_2r red cells
for anti-E with $r''r$ or R_2r and R_1r red cells
for anti-CD with R_2r or R_or, r^1r, $r''r$ and rr red cells
for anti-CDE with R_or, r^1r, $r''r$ and rr red cells

2.1.3 Irregular Blood Group Antibody Detection

1 The release from quarantine of blood components containing an irregular antibody shall be determined by the documented policy of the RTC. This policy should ensure that components which contain potentially harmful blood group antibodies are not placed in stock.

2 The presence of irregular blood group antibodies in blood and blood components intended for neonatal use should be determined by test procedures which are equivalent in sensitivity to those used to screen patient's samples for irregular antibodies prior to transfusion. Group O donations with a high titre of anti-A and/or anti-B and donations with high titres of other antibodies of probable clinical significance should not be made available for neonatal use. Each RTC should define "high titre" according to the sensitivity of the tests used locally.

3 For blood and blood components not intended for neonatal use the presence of relatively potent clinically important red cell antibodies should be determined by testing the donor's serum or plasma by a validated technique capable of detecting anti-D of 0.5IU/ml or lower and other antibodies of high titre and potential clinical significance.

2.2 Microbiology Tests

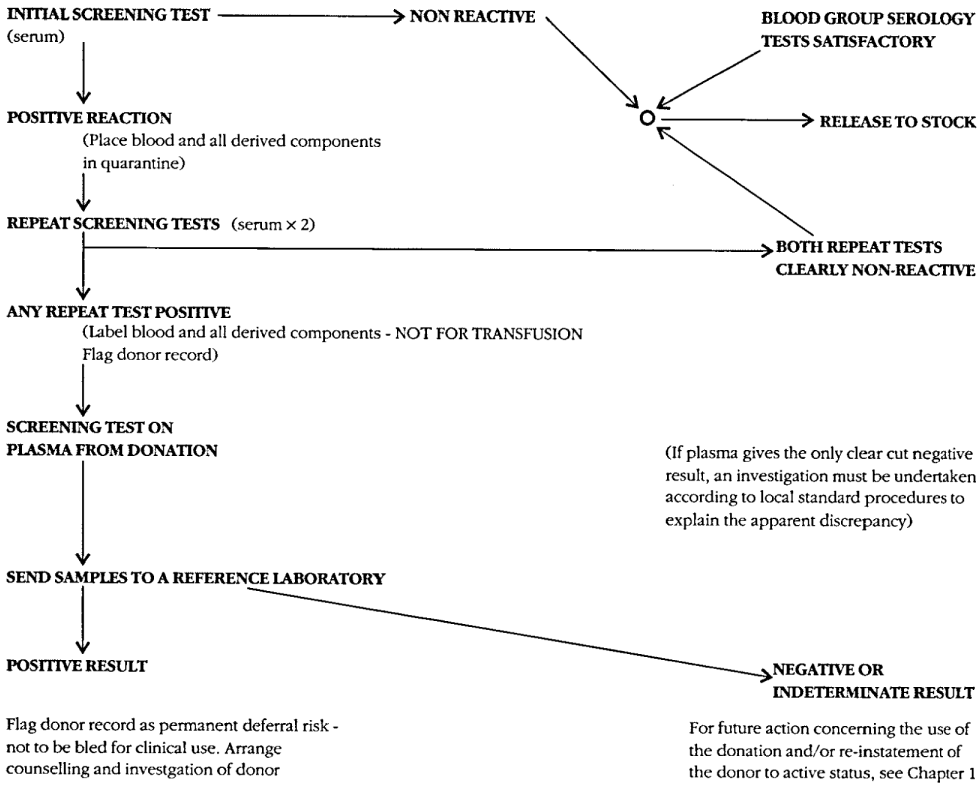
2.2.1 In addition to blood group serology requirements, blood and blood components must not be released to stock unless they have been tested and found negative for HBsAg, anti-HIV 1 and 2, anti-HCV and antibodies to syphilis.

2.2.2 The presence or absence of the microbiology markers described at **2.2.1** above should be determined by testing the serum of the donor. If plasma is used for testing, it should be treated according to the instructions accompanying the test kit. If there is a deviation from the kit manufacturers instructions, the variation should be validated to ensure it meets the required specificity and sensitivity criteria.

2.2.3 Initial Screen Reactive Samples

1. All initially reactive samples must be retested in duplicate. This is an extremely important area of work and requires particular attention to ensure
   i. that the correct sample is retrieved for repeat testing.
   ii. that the actual sampling procedure for repeat testing is undertaken with due care eg some samplers do not accept the same barcode number twice.
   iii. that the results are carefully verified.
   iv. that edits to initial screen reactive results are stringently controlled and recorded.
2. If both the repeat screening tests are clearly non-reactive, the blood and any derived components can be released to stock.
3. If one or both of the repeat screening tests are reactive, the blood and any derived components should be labelled NOT FOR TRANSFUSION and a sample of plasma from the bleedline should be tested. The guidance given at **2.2.3.1** applies.
4. If the plasma sample from the bleedline produces the only clearly negative result an investigation should be initiated according to local standard procedures to explain the apparent discrepancy.
5. If the donor is considered to be reactive for any of the mandatory microbiology tests described at **2.2.1**, samples from the donor/donation should be sent to a reference laboratory for confirmatory testing.
   i. If a positive result is reported by the reference laboratory, the donor record must be flagged as "permanent deferral risk - not to be bled for clinical use" or equivalent. Arrangements should be made to counsel and take repeat samples from the donor to ensure consistency of results and accuracy of donor identity.
   ii. If a negative or indeterminate result is reported by the reference laboratory, the procedure for reinstatement of such donors to active status is covered in Chapter 1.

2.2.4 Algorithm for mandatory microbiological testing



2.2.5 HBsAg

1 Specification

The UK specification for the minimum level of sensitivity for the performance of HBsAg screening is 0.5IU/ml. A UK working standard containing 0.5 IU/ml is available and should give a positive reaction in each series of HBsAg screening tests.

2 Quality Control of HBsAg Screening

i. Each batch of HBsAg test kits should be shown to conform with locally established minimum criteria for specificity and sensitivity.

ii. In addition to the test kit manufacturer's controls, quality control measures should be taken with each series of tests to demonstrate acceptable sensitivity of the test method.

iii. No series of tests should be considered acceptable unless the result of the test kit manufacturer's and the additional quality control samples have satisfied the criteria laid down.

2.2.6 Anti-HIV 1 and 2

1 Specification

The UK specification for the minimum level of sensitivity for the performance of anti-HIV 1 and 2 screening has not yet been defined beyond the requirement that in each series of tests a positive result must be obtained with the national working standard, when it becomes available.

2 Quality Control of Anti-HIV 1 and 2 Screening

i. Each batch of anti-HIV 1 and 2 test kits should be shown to conform with locally established minimum criteria for specificity and sensitivity.

ii. In addition to the test kit manufacturer's controls, quality control measures should be taken to demonstrate acceptable sensitivity of the test method.

iii. No series of tests should be considered acceptable unless the result of the test kit manufacturer's and the additional quality control samples have satisfied the criteria laid down.

2.2.7 Anti-HCV

1 Specification

The UK specification for the minimum level of sensitivity for the performance of anti-HCV screening has not yet been defined beyond the requirement that in each series of tests a positive result must be obtained with the national working standard, when it becomes available.

2 Quality Control of Anti-HCV Screening

i. Each batch of anti-HCV test kits should be shown to conform with locally established minimum criteria for specificity and sensitivity.

ii. In addition to the test kit manufacturer's controls, quality control measures should be taken to demonstrate acceptable sensitivity of the test method.

iii. No series of tests should be considered acceptable unless the result of the test kit manufacturer's and the additional quality control samples have satisfied the criteria laid down.

2.2.8 Syphilis Antibody

1 Specification

The specification for the minimum level of sensitivity for the performance of syphilis antibody screening has not yet been defined beyond the requirement in each series of tests a that positive result must be obtained with the national working standard, when it becomes available.

2 Quality Control of Syphilis Antibody Screening

i. Each batch of syphilis antibody test kits should be shown to conform with locally established minimum criteria for specificity and sensitivity.

ii. In addition to the test kit manufacturer's controls, quality control measures should be taken to demonstrate acceptable sensitivity of the test method.

iii. No series of tests should be considered acceptable unless the result of the test kit manufacturer's and the additional quality control samples have satisfied the criteria laid down.

3 Additional Testing of Selected Donations

3.1 Scope

In certain circumstances additional testing of donations may be necessary to increase the safety of transfusion for specific patients.

3.2 Selected Red Cell Phenotypes

3.2.1 For patients with irregular red cell antibodies, red cell components should be phenotyped to ensure they do not express antigens reactive with these antibodies. Only those blood grouping reagents which meet the requirements of Section III of these Guidelines should be used to phenotype red cell components for this purpose and unless appropriately validated, the technique used should be that for which the reagents are recommended by the manufacturer.

3.2.2 For donors being grouped for the first time or for whom no adequate records exist, two different examples of each blood grouping reagent should be used, or two independent tests should be performed with the same reagent.

3.2.3 If the donor has been grouped previously, and the donor's record clearly states the result of that grouping, one reagent may be used to confirm that the donor lacks the antigen under investigation.

3.2.4 Whole blood or red cell components should not be labelled for this purpose unless the donation has been appropriately tested and the results obtained meet the agreed criteria.

3.3 Selected HLA Phenotypes

3.3.1 HLA matched platelets are obtained from blood donors whose lymphocytes have been typed for HLA A and B antigens. The testing should be performed using HLA typing reagents that comply with Section III of these Guidelines.

3.3.2 Platelets from HLA matched donors may be selected for a specific patient by reference to records of donors' HLA typings. Alternatively, platelets that are crossmatch negative by platelet or lymphocyte tests may be issued for refractory patients.

3.4 Selected Platelet-Specific Phenotypes

3.4.1 Platelets from platelet-typed donors are occasionally required for infants or fetuses with alloimmune thrombocytopenia and, more rarely, for patients whose immunological refractoriness to random platelet transfusions is due to platelet-specific antibodies rather than to HLA antibodies.

3.4.2 HPA-la-negative (Pl-A1-negative) platelets are obtained from donors whose platelets have been typed with anti-HPA-la. Platelets negative for other platelet-specific antigens are required more rarely.

3.5 Antibody to Cytomegalovirus (Anti-CMV)

3.5.1 The presence or absence of anti-CMV should be determined by examination of the serum or plasma of the donor. The UK specification for the minimum level of sensitivity for the performance of anti-CMV screening has not yet been defined beyond the requirement that in each series of tests a positive result must be obtained with the national working standard, when it becomes available.

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

3.5.3 Quality Control of Anti-CMV Tests

1 Each batch of anti-CMV test kits should be shown to conform with locally established minimum criteria for specificity and sensitivity.

2 In addition to the test kit manufacturer's controls, quality control measures should be taken to demonstrate acceptable sensitivity of the test method.

3 No series of tests should be considered acceptable unless the result of the test manufacturer's and the additional quality control samples have saitsfied the critieria laid down.

3.6 Tests for Malarial Antibodies

3.6.1 The exclusion period for donors from malarial areas is given in Chapter 1, Appendix I, which deals with the selection of donors. Certain of these categories may require donations to be tested for malarial antibodies and negative results obtained prior to the release of any blood component for direct clinical use, when an approved test becomes available.

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

3.6.3 No series of tests should be considered acceptable unless both the manufacturers' and the additional quality control tests have satisfied the criteria laid down.

3.6.4 Quality Control of Malarial Antibody Tests

Formulation of appropriate quality control measures for malarial antibody testing awaits the availability of an approved test.

Annex 4

Accredited Donors of Red Cells for RhD Immunisation and Boosting

(Recommendations of the Immunoglobulin Working Party 1992)

Accredited donors are a special panel of donors who because of detailed testing can be relied on to be a very safe source of red cells for immunising and boosting Rh negative volunteers for the production of anti-D Immunoglobulin. The donor is accredited by careful selection, medical assessment and repeated blood tests during the six month period before donation of red cells for immunisation. The donor's red cells become accredited when these tests have given satisfactory results for at least six months after the donation in question.

1 General criteria for donor selection

1.1 A potential cell donor must fulfil all the criteria for blood donation and be an established donor who has given blood or plasma on at least three occasions during the previous two years.

1.2 Particular attention should be given to excluding anyone who may be in an HIV risk group. The donor should complete a questionnaire and have a personal interview to ensure this.

1.3 The donor must never have received a transfusion of blood or blood components.

1.4 The donor should not have submitted to acupuncture, tattooing, ear piercing or hair electrolysis during the accreditation period before donation.

2 Hepatitis history

The donor should not have a history of hepatitis or infectious jaundice and have had no known contact with a case within the past year before donation.

3 Blood grouping of donors

Blood grouping should be carried out beyond routine ABO and RhD typing.

3.1 The donor should preferably be homozygous for the D antigen.

3.2 In addition to full red cell typing by the RTC, red cells from the donor should be typed for all clinically relevant antigens by an external laboratory, e.g. the MRC Blood Group Unit or the IBGRL.

4 Stored serum samples

A generous "library" sample of serum should be stored from the donor at the beginning of his/her accreditation period.

5 Microbiological screening

Extended microbiological screening should be performed.

5.1 Initial screening for donors of frozen cells must include appropriate assays for anti-HIV 1 and 2, HBsAg, anti-HBs, anti-HBc, anti-HCV, anti-HTLV 1, syphilis, and give negative results.

The results of tests for anti-HBc and anti-HTLV 1 should be performed or confirmed by an external reference laboratory employing established testing methods on a regular and routine basis where these tests are not routinely performed by the Centre.

5.2 Tests for additional viral markers should be included as these become available.

5.3 Alanine amino transferase (ALT) levels should be performed monthly during the six month donor accreditation period and give results which lie within the normal range as established locally and should continue monthly thereafter during accreditation of the donation.

5.4 Prior to donation, which must be at least six months after the initial screening (*see 5.1*) the donor should be tested again and found negative for all the assays listed in 5.1.

5.5 The donation should be stored frozen for at least six months before its us for immunisation.

5.6 Before release of the donation for immunisation or boosting, it should be confirmed that the donor has remained free from serious illness since the relevant donation and that ALT tests have remained within the normal range since this donation. The tests listed in 5.1 must be repeated on a sample taken at least 6 months after the donor's cells have been frozen and give negative results. In addition, a test(s) for HIV should be performed, e.g. a PCR assay for HIV1/HIV2-related DNA or RNA.

6 Registration of accredited donors

Accredited donors and their donations should be recorded on a National Register which will be held on behalf of the NBTS by North London Blood Transfusion Centre and on behalf of the SNBTS by Aberdeen and North-East Scotland Blood Transfusion Service.

7 Spouse as donor

Occasionally an Rh negative volunteer may ask to be immunised or boosted using cells from his/her spouse. The donating spouse should complete a questionnaire and take part in a confidential interview to ensure that he/she is not in an HIV risk group. It is important at the outset to carry out red cell typing for the major systems on both volunteer and spouse to ensure that there are no major mis-matches*.

A full range of microbiological screening as listed in 5.1, together with a test for HIV as specified in 5.6, should be undertaken on the spouse prior to each set of immunising or boosting injections.

**(See following section on Selection of RhD Negative Subjects for Immunisation, paragraph 4.2)*

Annex 4a)

Selection of RhD Negative Subjects for Immunisation and/or Boosting for the Production of Anti-D Immunoglobulin

(*Recommendations of the Immunoglobulin Working Party 1991*)

1 General considerations in donor selection

1.1 Deliberate immunisation and/or boosting with red cells will normally only be carried out in RhD negative volunteers for the production of anti-D Immunoglobulin.

1.2 Subjects should be recruited by a system which allows unconditional withdrawal of the donor at any time.

1.3 Subjects for primary immunisation should normally be regular donors under the age of 50 with a good attendance record. Older subjects may be considered for boosting.

1.4 It is preferable to recruit subjects who already have immune anti-D since boosting will produce high levels more quickly than primary immunisation. This approach also keeps to a minimum the number of subjects exposed to the risks, however small, of primary immunisation. Boosting of anti-D levels in women who have been immunised by pregnancy can only be considered if they are post-menopausal or incapable of further pregnancies.

1.5 The subjects should have good venous access.

2 Examination of the Donor

The subjects should undergo an initial full medical examination undertaken by an experienced physician. Tests carried out as part of this examination may include:

Chest X-ray
ECG
Urinalysis
Full blood count
Total serum protein
Serum protein electropheresis
Albumin
Immunoglobulins
Liver function tests
Extended red cell phenotyping
Red cell antibody screening and identification
Microbiological screening to include HBsAg, anti-HBs, anti-HBc, anti-HIV 1 and 2, anti-HCV, anti-HTLV 1 and syphilis

3 The subject should give written informed consent which is witnessed and follow the advice from the Association of British Insurance Companies to inform any insurers providing life assurance cover.

4 Immunising schedules

4.1 Immunising schedules remain a matter for debate and possible future research. However, where a subject has not produced immune anti-D within a year of receiving three adequate primary immunising doses, it is very unlikely that useful anti-D will ever be produced.

4.2 The red cell donor and the subject should be matched as far as possible for major blood group antigens other than D. Mis-matching in the Ss, Kell, Fy, Jk systems is generally unacceptable. Mis-matching within the Rh system for C and/or E is acceptable.

5 Documentation

5.1 Donors entering immunisation programmes and producing anti-D or other significant antibodies should carry an identity/hazard information card.

5.2 The maintenance of comprehensive records on subjects entering an immunising programme is essential and these records should be retained indefinitely.

6 Medical follow-up of donors

6.1 Subjects who are being actively immunised or boosted require an annual medical assessment.

6.2 After five years' participation in an immunising/boosting programme, all subjects should be thoroughly assessed for general fitness to continue and at the same time a review of the pattern of immunising/boosting doses should be undertaken.

Annex 5

Requirements for Safe and Effective Transportation of Blood and Blood Components

1 Transportation between donor sessions and transfusion centre

1.1 Blood and blood components collected at donor sessions must be transported to the receiving transfusion centre in appropriate conditions of temperature, security and hygiene, in accordance with a validated and documented procedure. Where it is intended to prepare platelet concentrates from the donation, the temperature (air) should not be allowed to drop below +18°C; otherwise, the minimum acceptable temperature is +2°C.

2 Whole blood and red cell components

2.1 Whole blood and red cell components issued by a transfusion centre must be transported to the receiving institution at a temperature (air) between +2°C and 10°C, in a containment system that assures adequate security and hygiene, in accordance with a validated and documented procedure.

3 Platelet preparations

3.1 Platelet preparations issued by a transfusion centre must be transported to the receiving institution at a temperature (air) between +20°C and +24°C, in a containment system that assures adequate security and hygiene, in accordance with a validated and documented procedure.

4 Plasma components

4.1. Plasma components issued by a transfusion centre must be transported to the receiving institution in a containment system that assures adequate security and hygiene, in accordance with a validated and documented procedure designed to maintain the core storage temperature of -30°C during transportation.

5 Transfer between centres or hospitals

5.1 Blood and blood components that are to be transferred between transfusion centres or from one hospital blood bank to another should be transported under conditions equivalent to those defined in 2, 3, or 4, whichever is appropriate.

6 Containment systems

6.1 Containment systems used for the transport of blood and blood components must have been validated for performance, be fit for their intended use, and must bear appropriate labelling indicating origin and/or destination, content, and relevant hazard warning notices.

7 Special shipments

7.1 Where a transfusion centre does not have appropriate facilities for special shipments, e.g. overseas deliveries, the task may be subcontracted to another suitably equipped centre, or other contractor, with appropriate validated procedures and facilities.

SECTION 2

Guidelines for Plasma for Fractionation

Chapter 1

Specification for Plasma Intended for Fractionation

1.1 Features common to all plasma types

1.1.1 Donor qualifications

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

1.1.1.2 Each donation must be tested and found negative for HBsAg using an ELISA or RIA test which detects at leat 0.5 iu per ml of HBs antigen.

1.1.1.3 Each donation must be tested and found to be non-reactive for antibody to HIV-1 and HIV-2.

1.1.1.4 Each donation must be tested and found to be non-reactive for antibody to HCV.

1.1.1.5 All tests must be performed according to the manufacturers' instructions.

1.1.2 Donation Handling

1.1.2.1 Handling techniques will comply with Good Manufacturing Practice as set out in Council Directive 91/356/EEC (and amendments) and supporting National Codes of Practice to establish a quality system for the collection and processing of blood and blood products (Chapter 2, General Considerations).

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

1.1.2.3 Plasma is either collected by plasmapheresis or is obtained from anticoagulated whole blood. In either case, the manufacturers will use a licensed anticoagulant solution and the plastic collection systems shall be registered with the UK Medical Devices Director and will indicate that the containers are in compliance with the European Pharmacopoeia sections VI 2.2.2.1 and VI 2.2.2.2 as set out in the Technical Directive 1991/507/EEC.

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

1.1.2.5 Each plasma donation should be frozen as soon as possible after collection. The rate of cooling must be as rapid as possible and ideally should bring the core temperature of the plasma down to -30°C or below within 60 minutes. If this is not possible, the minimum acceptable rate of freezing must bring the core temperature down to -30°C within 4 hours, as demonstrated by regular performance tests.

1.1.2.6 The required storage temperature for plasma is -30°C or below, both within the RTCs and during transit. Temperature fluctuations in the plasma should be kept to a minimum during storage or transportation. A plasma temperature record during storage and transit of frozen plasma shall be available for inspection.

1.1.3 Donation archive samples

1.1.3.1 A uniquely identifiable archive sample must be taken and retained frozen from each plasma donation. Each sample should be retained for as long as is practically possible and in any case for a minimum of 12 months.

1.1.4 Documentation

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

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

1.1.4.2 Frozen plasma packs of an identical plasma type are packaged for transportation in containers approved by the Fractionation Centre. Each container shall clearly carry a unique identification code defining container number, Transfusion Centre of origin and plasma type.

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

1.1.4.4 Each plasma container shall be accompanied by a fully completed despatch document approved by the Fractionation Centre. It should be noted that this is also a release document and must be signed by the appropriate QA manager or approved deputy to state that the plasma despatched conforms to specifications.

1.1.5 Plasma notifications

Once plasma has been sent, the Fractionation Centre must be notified by the Transfusion Centre as rapidly as possible in the following circumstances:

1.1.5.1 If the donor did not meet the current donor health criteria.

1.1.5.2 If it is discovered that HBsAg testing, or HIV or HCV antibody testing has not been carried out according to agreed procedures.

1.1.5.3 If the donor may be implicated in an episode of post-transfusion infection due to hepatitis viruses or HIV.

1.1.5.4 If the plasma is considered to be unsatisfactory in any other manner.

1.2 Features specific to defined plasma types

1.2.1 Fresh frozen plasma (FFP)

Plasma destined for production of coagulation factors may be obtained from the following sources:

1.2.1.1 Recovered plasma from single whole blood donations.

1.2.1.2 Plasma collected by apheresis.

1.2.2 The requirements for FFP are as follows:

1.2.2.1 Recovered plasma, frozen fresh should be prepared only from donations obtained from a clean venepuncture and an uninterrupted procedure (see Section 1, Annex 1).

1.2.2.2 The donation and the anticoagulant should be mixed during collection.

1.2.2.3 Separated plasma should be monitored regularly to demonstrate that the platelet count is consistently low. The target platelet count will depend on the separation technique used as follows:

(a) Manual methods: $<30 \times 10^9$/Litre
(b) Membrane apheresis: $<10 \times 10^9$/Litre
(c) Centrifugal apheresis: $<40 \times 10^9$/Litre

1.2.2.4 Each RTC should establish appropriate quality control procedures to monitor the factor VIII content of the frozen plasma (Section 1, Chapter 6) such that 75% of donations have factor VIII levels greater than 0.7 iu/ml.

1.2.2.5 Recovered fresh plasma should be frozen to a solid state as soon as possible, and in any case, within 24 hours of collection.

1.2.3 Cryosupernatant plasma shall be derived from plasma which meets the requirements for FFP, except that the cryoprecipitate has been removed by an approved technique.

1.2.4 Outdated plasma

1.2.4.1 Plasma in this category may have been held at +4°C for more than 35 days prior to separation.

1.2.4.2 Time-expired plasma recovered from whole blood returned after issue to hospitals is acceptable if the RTC is satisfied that the blood donation has been stored in a manner which has maintained its fitness for clinical use.

1.2.4.3 Separated plasma donations with visible red cell contamination or which are grossly lipaemic should not be sent for fractionation.

1.2.5 Immune plasma

1.2.5.1 Immune plasma is obtained from donors who meet the normal health criteria, but who are found to have circulating plasma antibody concentrations of sufficient potency to warrant inclusion of their plasma in pools destined for the manufacture of hyperimmune immunoglobulin.

1.2.5.2 Suitable donors may be selected in one of three ways (*see also Section 1, Chapter 4*):

1.2.5.2.1 Known natural exposure to an infective agent or to red cell stimulation.

1.2.5.2.2 Known immunisation against infective agent or deliberate red cell stimulation.

1.2.5.2.3 Testing of donations chosen at random.

1.2.5.3 Acceptable minimum antibody potency must be demonstrated using an assay system agreed with the Fractionation Centre. The requirements for each plasma type are specified below:

1.2.5.3.1 Anti-D

Antibody potency should be estimated in international units using an approved AutoAnalyser-based assay system. Minimum potency should be agreed with the Fractionation Centre.

1.2.5.3.2 Anti-Cytomegalovirus

Antibody potency should be assayed using a quantitative assay agreed with the Fractionation Centre (e.g. ELISA, immunofluorescence, complement fixation).

1.2.5.3.3 Anti-endotoxin

Antibody potency should be assayed using a quantitative assay (e.g. ELISA) agreed with the Fractionation Centre. The donation must be shown to be of a potency equal to or greater than that of a control sample provided by the Fractionation Centre.

1.2.5.3.4 Anti-hepatitis A

The potency of antibody to hepatitis A shall be determined using a quantitative assay agreed with the Fractionation Centre. The minimum acceptable potency is 100 iu/ml.

1.2.5.3.5 Anti-hepatitis B

Antibody potency should be calibrated in international units using an approved assay system (e.g. RIA, ELISA) which detects antibody to hepatitis B surface antigen. The minimum acceptable potency is 10 iu/ml.

1.2.5.3.6 Anti-rabies

It is not practicable to assay plasma donations for rabies antibody. However, a donor should be considered to have acceptable antibody titres between 1 and 3 months after a second dose of vaccine, or between 1 and 3 months after a booster dose of vaccine. Plasma should not be collected from persons immunised after exposure to infection by rabies virus.

1.2.5.3.7 Anti-rubella

Antibody potency should be measured in international units using an assay approved by the Fractionation centre (e.g. haemolysis in gel) which detects antibody to rubella haemagglutinin. The minimum acceptable potency is 500 iu/ml.

1.2.5.3.8 Anti-tetanus

Antibody potency should be calculated in international units using an approved assay system which correlates well with the mouse neutralization assay. The minimum acceptable potency is to be agreed with the Fractionation Centre.

1.2.5.3.9 Anti-zoster (varicella)

Antibody potency should be assayed using a quantitative assay (e.g. ELISA, immunofluorescence, complement fixation) agreed with the Fractionation Centre. The donation must be shown to be of a potency equal to or greater than that of a control sample provided by the Fractionation Centre.

Chapter 2

Product Characteristics

2.1 Factor VIII concentrates

In addition to meeting relevant pharmacopoeial requirements, the product has the following characteristics:

2.1.1 Each manufacturer is expected to provide a single type of concentrate suitable, in terms of potency and freedom from adverse effects at high doses, for the treatment of all patients with inherited Factor VIII deficiency and those with acquired inhibitors of Factor VIII. The concentrate should be suitable for self-administration.

2.1.2 The concentrate may not necessarily be designed to be effective in von Willebrand's disease and any claims to be effective should be made only after extensive clinical trial of the product.

2.1.3 The method of preparing the concentrate should ensure maximum Factor VIII:C yield. Factor VIII C yield should, therefore, not be sacrificed in the interest of specific activity except in so far as increased purity has been shown to affect substantially the product's safety or efficacy.

2.1.4 The concentrate should have been treated during processing, in the final container, or both, with the intention of inactivating bloodborne viruses such as human immunodeficiency (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV). Safety from transmission of viruses must not rely solely on donor screening.

2.1.5 The concentrate should dissolve readily within 10 minutes of addition of water, without warming above room temperature, to a potency >10 iu/ml. Vial contents should be in the range 200-1000 iu.

2.1.6 Specific activity should exceed 0.3 iu/mg protein and 0.5 iu/mg fibrinogen. Other major contaminating plasma proteins should be characterized.

2.1.7 The concentrate should not contain more than the equivalent of 0.5 iu endotoxin/iu Factor VIII determined by the European Pharmacopoeial method.

2.1.8 After injection into adult patients with no concurrent bleeding, the recovery of Factor VIII should be approximately 1.8-2.4% rise/iu/kg (75-100% of theoretical values).

2.1.9 After injection into adult patients with no concurrent bleeding, at a dose sufficient to raise the patient's Factor VIII activity to 0.5 iu/ml, the average half-disappearance time as determined in several patients should be comparable to that found after injection of concentrates from other manufacturers.

2.2 Factor IX concentrates

2.2.1 A single type of concentrate should be suitable, in terms of potency and freedom from adverse effects at high doses, for the treatment of all patients with inherited Factor IX deficiency and those with acquired inhibitors of Factor IX. The concentrate should be suitable for self-administration.

2.2.2 The concentrate may not necessarily be designed to be effective in the treatment of liver disease or other acquired deficiencies of the vitamin K-dependent proteins. Claims to be effective should be made only after extensive clinical trials.

2.2.3 The concentrate may contain amounts of Factor II and Factor X which make it suitable for replacement therapy in inherited deficiencies of these factors. The content of these factors should be stated on the vial label.

2.2.4 The concentrate should have been treated during processing, in the final container, or both, with the intention of inactivating bloodborne viruses such as HIV, HBV, and HCV. Safety from transmission of viruses must not rely solely on donor screening.

2.2.5 The concentrate should dissolve readily within 5 minutes of addition of water, without warming above room temperature, to give a potency of >20 iu/ml. Vial contents of Factor IX should be in the range 250-1000 iu.

2.2.6 Specific activity should exceed 1.0 iu Factor IX/mg protein. No specific activity limit is set for the other vitamin K-dependent proteins which may be present. The major contaminating plasma proteins should be characterised.

2.2.7 When first developed, and after any substantial modification to processing methods, typical batches of concentrate should be subjected to tests of thrombogenicity by injecting at least the equivalent of a maximum human dose into experimental animals and monitoring indices of intravascular coagulation.

2.2.8 After injection into adult patients with no concurrent bleeding, the recovery of Factor IX should be approximately 1.0-1.6% rise/iu/kg (40-66% of theoretical values).

2.2.9 After injection into adult patients with no concurrent bleeding, at a dose sufficient to raise the patients' Factor IX activity to 0.5 iu/ml, the average half disappearance time as determined in several patients should be comparable to that found after injection of concentrates from other manufacturers.

2.3 Albumin Products

2.3.1 This section applies to albumin products which are formulated between 43 g/l to 250 g/l.

2.3.2 Albumin may be prepared from any plasma which meets the criteria detailed in the specification for plasma intended for fractionation.

2.3.3 The process yield should be maximized, consistent with the Pharmacopoeial description.

2.3.4 The endotoxin content of the final product should be controlled and would be expected to be less than 0.5 iu/ml for 4-5% albumin preparations.

2.3.5 The metal ion content should be controlled. In particular, the aluminium content should be kept below 200 µg/L.

2.3.6 The level of pre-kallikrein activator should be less than 35 iu/ml.

2.3.7 The product must be pasteurized in the final container at 60°C for 10 hours. Appropriate stabilizers are added to protect the product during pasteurization.

2.4 Immunoglobulin preparations

2.4.1 The products should comply with the relevant Pharmacopoeial monographs and any additional requirements of the Licensing Authority.

2.4.2 Immunoglobulin preparations should be concentrated and purified from human plasma, collected in accordance with the guidelines in 1.1 and 1.2, in a manner which does not affect the structural and functional integrity of the immunoglobulins. The process should maximize yield without unduly reducing product quality.

2.4.3 Normal immunoglobulin products should be prepared from the pooled plasma from at least 1000 normal donors; the range of antibody activities should be representative of the

donor population. The concentration of antibody to at least one viral and one bacterial antigen in a 16% solution of normal immunoglobulin should be greater than 10 times that in the source plasma pool.

2.4.4 Specific immunoglobulin products should be prepared from the pooled plasma from donors who have been selected for elevated antibody levels to the required specific antigen, e.g. Rh(D), tetanus toxin, rabies virus or hepatitis B virus. Specific immunoglobulin products may be prepared from the pooled plasma from fewer donors than used for normal immunoglobulin. Specific immunoglobulin products should meet the potency requirements of the relevant pharmacopoeial monographs or the specifications of the Licensing Authority. When such requirements do not exist, the concentration of the required antibody in the specific immunoglobulin preparations should be at least 5-fold higher than in a normal immunoglobulin preparation with an equivalent protein concentration.

2.4.5 The products should be safe for administration by the chosen route and should not cause undue discomfort or inconvenience to the recipient when administered at the required dose by the chosen route.

2.4.6 The product should not transmit viral infections and evidence should be available for the removal and/or inactivation of viral contaminants by the process used for the preparation of the immunoglobulins. Departures from conventional cold ethanol fractionation processes, which have a long-established safety record on epidemiological grounds, should be fully validated in vitro by methods similar to those described in Chapter 4.

2.4.7 Each batch of product must be tested for the presence of HBsAg, and antibodies to HIV-1 and 2, and shown to be negative.

2.4.8 The concentration of IgG in the plasma of recipients of the products should rise substantially; an increase in antibody activity should be demonstrable in recipients.

2.4.9 The half-life of the preparations should be studied in suitable patients; for example, after injection into hypogammaglobulinaemic patients the plasma half-life of the product should be not less than 15 days.

2.4.10 The products should contain the four IgG subclasses in ratios similar to those in the plasma pools from which they were derived.

2.4.11 Antigen binding and Fc mediated functions of the immunoglobulin should be retained.

2.4.12 The concentration of immunoglobulins, including IgA and contaminating proteins should be specified. The IgG should not be less than 90% of the total protein.

2.4.13 There should be no detectable increase in the concentration of immunoglobulin fragments in preparations after storage at 37°C for four weeks.

2.4.14 Characteristics specific to intramuscular immunoglobulin preparations:

The content of monomeric/dimeric IgG in the product should not be less than 85%, the content of aggregates should not be greater than 10% and the content of fragments should not exceed 5%.

2.4.15 Characteristics specific to intravenous immunoglobulin preparations:

The content of monomeric/dimeric IgG in the product should not be less than 90%, the content of aggregates should not exceed 5% and the content of fragments should not exceed 5%.

The kallikrein activity should not exceed 0.1 units per ml; the pre-kallikrein activator activity should not exceed 35 iu/ml.

The anti-complementary activity should be controlled.

Chapter 3

Assignment of potency to batches of Factor VIII and IX concentrates

3.1 Standards

Factor VIII concentrates should be assayed against the current British Working Standard (BWS) for Factor VIII concentrate. Factor IX concentrates should be assayed for Factor IX (and Factor II and X if appropriate) against the current BWS for factor IX concentrate.

3.2 Assay methods

The method shall be either that described in the European Pharmacopoeia or another method that has been shown to give comparable results on at least 12 batches of product.

3.3 Number of assays

At least two bottles from each batch should be tested, preferably with two independent assays per bottle. Each assay should consist of replicate sets of at least three dilutions of standard and test preparation.

3.4 Analysis of results

3.4.1 Each individual assay should be analysed by the parallel line bioassay method, such as described by Kirkwood and Snape[1]. Results of individual assays should be combined to give a weighted geometric mean if they are homogeneous or an unweighted geometric mean if they are heterogeneous.

3.4.2 The content of each bottle is calculated by multiplying the potency of the solution by the volume of liquid, which should be measure accurately after reconstitution.

3.4.3 The 95% confidence limits on the overall mean potency should not be greater than plus or minus 10% of the mean.

Reference

1. Kirkwood, T.B.L., Snape, T.J. Biometric principles in clotting and clot lysis assays. Clin.Sci.Haematol., 1980,2: 155-167.

Chapter 4

Viral inactivation

4.1 Specification for the validation of virus inactivation procedures used during the manufacture of plasma products

4.1.1 In designing validation studies for virus inactivation procedures, reference should be made to the EEC Note for Guidance: Validation of Virus Removal and Inactivation Procedures. *Biologicals* Vol 19; 247-252 (1991).

4.1.2 The demonstration that HIV can be transmitted by Factor VIII and Factor IX products has led to the requirement that fully validated virus inactivation procedures must be included in the processes used to manufacture each type of plasma product. This specification outlines the minimum requirements for the virus validation of a particular product.

4.1.3 It should be emphasised that the inactivation of viruses in blood products involves a complex interaction of a number of factors, including product type, product concentration, product formulation, presence of stabilisers and type and conditions of inactivation process. For this reason, each manufacturer must validate each product and cannot rely on data from other products or other manufacturers.

4.1.4 For well-established products with a long history of viral safety, eg albumins, intramuscular immunoglobulins, the need for specific validation studies may be obviated provided that the methods of production and viral inactivation can be shown to be identical to those responsible for clinical safety.

4.2 Source plasma

In order to minimise the virus challenge to the manufacturing process, each individual plasma donation used for the manufacture of coagulation factor concentrates must be tested for the presence of hepatitis B surface antigen and for antibodies to the human immunodeficiency viruses (HIV-1 and HIV-2) and hepatitis C virus. In future, it may become necessary to test for other viral contaminants. The assay methods used must comply with the minimum requirements defined in the appropriate specification for each product.

4.3 Process validation

4.3.1 Validation of formal virus inactivation steps

4.3.1.1 Data shall be generated which demonstrate that at least one single stage in the manufacturing process is capable of inactivating at least 10^5 infectious particles of HIV per ml of solution (ie a 5 log reduction in the concentration of viable virus).

4.3.1.2 Despite the identification of HCV as being responsible for many episodes of NANBH it is widely recognised that the transmission of NANBH is a major potential problem. The agent(s) of NANBH, including HCV, have not yet been cultured and it is, therefore, recommended that data are generated on the ability of the process to inactivate a range of other 'model' viruses to include RNA and DNA viruses, both enveloped and non-enveloped.

It is suggested that these might include Vaccinia and model toga viruses as these have both proved fairly resistant to inactivation procedures. Model virus data may not provide information of direct clinical relevance, but are very useful for comparing the overall inactivation potential of different processes.

4.3.1.3 Virus inactivation experiments must simulate the full scale process steps as closely as is possible. For example, if dry-heating of a freeze-dried product is used, every effort must be made to simulate the exact freeze-drying conditions used in large-scale manufacture.

4.3.2 Validation of rest of process

4.3.2.1 In addition to the inactivation data generated from the evaluation of the formal virus inactivation step, further data should be generated to indicate the extent to which other stages in the overall manufacturing process are capable of inactivating/ eliminating infectious HIV particles present in starting plasma.

4.3.2.2 It is, however, recognised that small-scale laboratory simulations of certain processing stages such as cryoprecipitation, centrifugation, precipitation, adsorption, chromatography and diafiltration, will not precisely replicate the full-scale manufacturing process. Therefore, undue weight must not be attached to the data from this part of the validation, which should be considered to be indicative only.

4.4 Virus assay methods

4.4.1 The assessment of the efficiency of virus inactivation can only be made using a validated assay for infectious virus. For example, in work with HIV, it is not acceptable to rely solely on data obtained from measurements of virus-associated reverse transcriptase.

4.4.2 Appropriate infectivity controls must be run in each assay.

4.4.3 The virus culture technique used must be fully validated to demonstrate that the product does not inhibit the detection of virus.

4.5 Validation of process modifications

4.5.1 Once the process is established and virus validation has been completed, then all aspects of the process must be controlled within tightly defined limits representative of the conditions employed during validation.

4.5.2 Process modifications which are expected to influence the extent of virus inactivation must be validated.

SECTION 3

Guidelines for reagents for blood group serology and HLA typing

SECTION 3

Introduction

These Guidelines apply to reagents used or supplied by the UK Transfusion Services for the determination of blood groups or the detection or identification of antibodies in the sera or plasma of donors or patients.

These Guidelines need not apply to reagents used in-house for preliminary screening or for research purposes.

In certain circumstances, it may not be possible to ensure that blood grouping reagents of a rare specificity are tested as required by these Guidelines. In such instances, the package insert should contain a statement to this effect.

These Guidelines give advice, guidance and, where appropriate, general specifications. Details of methods have been included when relevant. UK reference reagents have been identified within the Guidelines. At the present time (January 1993), reference preparations for anti-A, anti-B, and rabbit complement and anti-HLA-A2 for HLA class I serology have been developed and are available from NIBSC, PO Box 1193, Potters Bar, Herts. EN6 3QH; those for IgM anti-D and anti-IgG are in an advanced state of development.

The advice contained in these Guidelines is believed to represent acceptable practice at the time of press. It is policy to revise these Guidelines as new developments occur. However, it may not be possible to do this at the time of such changes and the Guidelines should be used with due regard to current acceptable practice.

Advice on the assessment of new reagents or technologies not represented in these Guidelines may be sought from the UK BTS - NIBSC Standing Committee on Reagents.

Comments are invited to assist the revision of these Guidelines and should be directed to:

Dr Marcela Contreras,
Chairman, UK BTS - NIBSC Standing Committee on Reagents,
North London Transfusion Centre,
Colindale Avenue,
London, NW9 5BG

Chapter 1

General Guidelines and Definitions

1.1 Introduction

General guidelines applicable to all reagents used to determine the group of human red cells, and the detection of red cell antibodies are presented in this section. In other sections additional guidelines are given for particular reagents.

This document uses Fisher's notation to describe the presumed Rh genotype of red cell samples to be used. Where R_zr or r_yr red cells samples are to be used, the probable genotype should be confirmed, for example by appropriate Rh testing.

1.2 Working Standards

It is intended that the following working standards will be available:-

anti-A
anti-B
anti-D (IgM)
anti-D (IgG)
anti-human IgG
anti-human C3c
anti-human C3d (monoclonal IgM)
anti-HLA-A2
rabbit complement for use in HLA serology

1.3 Definitions

1.3.1 'Saline' in this document is an isotonic solution containing 8.5 to 9.0 g/L NaCl (0.145 - 0.154M) containing, unless stated otherwise, sufficient buffer to maintain pH 7.0 ± 0.2 at 22 ± 1 °C.

1.3.2 Low ionic strength solution (LISS) is 0.03M NaCl, 0.003M Na_2HP0_4: NaH_2P0_4 buffer pH6.7 at 22 ± 1°C and 0.24M glycine.

1.3.3 Water. Reagent manufacturers should be aware that ionic and non-ionic contaminants of water may interfere with components of the reagents or may result in a conductivity or osmolality other than that intended. The techniques used to purify water depend on its use and the quality of the water supply. A combination of reverse osmosis and deionisation produces good quality water which has a conductivity of 1.0µS/cm or less or a resistivity of 1.0Mohm/cm or greater. Manufacturers should assure that the quality of water used in the production of a reagent is adequate for that reagent.

1.3.4 Fresh serum for complement activity should be less than 18 hours from donation if used either in the liquid state or after being stored frozen at -70°C or below. Unless validated, the maximum period of storage shall be six months at this temperature.

1.3.5 Inert blood group compatible serum should be from one or more individuals, as appropriate, of an ABO group compatible with the test red cells. The serum should give negative reactions when tested against a panel of red cells for antibody identification (see 4.11) by appropriate methods.

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

A batch is sometimes described as a lot.

1.3.7 In the testing of reagents, the term 'undiluted' in these guidelines means the reagent as intended by the manufacturer for use. This includes diluted reagent if it is supplied to the user in a form requiring dilution prior to use, as specified in the manufacturer's package insert.

1.3.8 A reagent recommended by the manufacturer for the determination of a blood group of an individual should be designated 'blood grouping reagent'.

1.3.9 A reagent recommended by the manufacturer for the detection of A (that is sub groups A_1 and A_2), A_x, and B should be named 'anti-A,B blood grouping reagent'. A reagent recommended by the manufacturer for the detection of A (that is sub groups A_1 and A_2) and B but not of A_x, should be named 'anti-A+B blood grouping reagent.'

1.3.10 Polyspecific anti-human globulin reagent should be the name for a reagent which contains anti-human IgG and anti-human complement activity, and is recommended by the manufacturer for use in both the direct and indirect anti-human globulin techniques, that is for the detection of red cell bound human IgG, and C3 complement in the form EiC3b and EC3d irrespective of the presence of other anti-human immunoglobulin or anti-human complement specificities.

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

1.3.12 The name of reagents produced by mixing preparations of different specificity or different monoclonal preparations from different sources and of the same apparent specificity, should include the word 'blended'. Details of the blend should appear in the package insert.

Reagents formulated by mixing polyclonal preparations obtained from the same individual, or by mixing different culture supernatants of the same monoclonal preparation should not be termed blended.

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

1.3.14 IgM anti-D refers to anti-D blood grouping reagents of IgM class, recommended by the manufacturer for use in direct agglutination tests.

1.3.15 Chemically modified anti-D refers to anti-D blood grouping reagents that have been chemically modified for use in direct agglutination tests.

1.3.16 Other anti-D refers to anti-D blood grouping reagents not described in 1.3.14 and 1.3.15. They are used, for example, with an enzyme, or include in the formulation a potentiating substance or agent such as high molecular weight dextran or albumin for use in direct agglutination tests, or are used by an indirect anti-globulin method.

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

1.3.18 A monospecific reagent is one containing an antibody or blend of antibodies specific for one antigen; e.g. anti-A.

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

1.3.20 Irregular blood group antibodies are those of specificity other than anti-A or anti-B.

1.3.21 Antibody screening is a test or combination of tests designed to detect irregular antibodies.

1.3.22 Antibody identification is a test or combination of tests designed to determine the specificity of irregular antibodies.

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

1.3.24 Shelf-life is the period for which a reagent, if stored under recommended conditions, is certified by the manufacturer to meet the product specification.

1.3.25 Expiry date is the date beyond which it is not recommended by the manufacturer to use a reagent.

1.3.26 HLA serology is the determination of HLA antigens and antibodies by serological methods and immunological reactions. It includes the isolation from blood, solid tissue or cell cultures of suitable cells such as lymphocytes and their typing for HLA antigens.

1.3.27 A reagent for the determination of an HLA antigen is designated 'HLA typing reagent'.

1.3.28 HLA typing reagents produced as a set of reagents in a multi-welled tray or reservoir and used to define particular HLA antigens is known as an 'HLA typing set'.

1.3.29 Rabbit complement recommended by the manufacturer for use in HLA class I serology is designated 'Rabbit complement for HLA class I serology'.

1.3.30 Rabbit complement recommended by the manufacturer for use in HLA class I and class II serology is designated 'Rabbit complement for HLA class I and class II serology'.

1.3.31 A preparation of lymphocytes recommended by the manufacturer for the detection or determination of the specificity of an HLA antibody is designated 'HLA reagent lymphocytes'.

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

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

1.3.34 Quality Audit is a review of the quality system.

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

1.3.36 Sensitivity is a term defining the limit of detectable specific reactions using reagents or test systems. These Guidelines specify levels of sensitivity which must be achieved.

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

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

Validation of a manufacturing method is a part of a Quality Assurance programme and consists of those steps which are taken in advance to ensure that the product will be of the quality required for its intended use and that tests used in monitoring will accurately reflect the quality of the product.

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

1.3.40 An immediate container is a medium adequate to protect the content(s) from contamination and/or physical damage. For example, a sealed vial, ampoule or bottle, a foiled pouch or a sealed plastic bag. (EN 375:1990)

1.4 General manufacturing considerations.

1.4.1 Good manufacturing practice.

Reagents for blood group serology shall be prepared in accordance with Good Manufacturing Practice (see current "Rules Governing Medicinal Products in the European Community, Volume IV, Good Manufacturing Practice for Medicinal Products", HMSO and 'The Guide to Good Manufacturing Practice for *in vitro* Diagnostic Reagents', Association of British Pharmaceutical Industry, London).

1 The method of manufacture should result in a product within an immediate container that is homogeneous and free of properties which adversely affect its intended use throughout its recommended shelf-life. The reagent should have no precipitate, particles or fibrin gel.

2 Each batch or sub-batch should be identified by a distinctive combination of numbers and/or letters (batch reference) which permits its history to be traced.

1.4.2 Representation of certain antigens and antibody specificities on labels of immediate containers and package inserts.

1 For reagent red cells, the antigens represented by the lower case letters c, k and s should be referred to on the labels of immediate containers, package inserts and the antigenic profiles of reagent red cells with an overscored bar, that is as $\overline{c}$, $\overline{k}$ and $\overline{s}$.

2 Similarly, for antisera, the antibody specificities represented by the lower case letters c, k and s should be referred to on the final labels and package inserts, with an overscored bar, that is as anti-$\overline{c}$, anti-$\overline{k}$ and anti-$\overline{s}$.

1.4.3 Reagent red cells.

1 The name of reagent red cells intended for use in ABO grouping should include the words 'for ABO grouping'.

2 The name of reagent red cells intended for use in Rh D grouping should include the words 'for Rh D grouping'.

3 The name of reagent red cells intended for use in the control of the anti-human globulin technique should include the words 'for the control of the anti-human globulin technique'.

4 The name of reagent red cells treated with an enzyme, for example papain, should include the words 'x-treated', where x is the name of the enzyme, for example, 'papain-treated'.

5 The name of reagent red cells intended for the detection of red cell antibodies should include the words 'for antibody screening'.

6 The name of reagent red cells intended for the identification of irregular antibodies should include the words 'for antibody identification'.

1.4.4 Immediate container.

The immediate container for a reagent for blood group serology should be transparent to permit visual inspection of the contents and consist of a material which does not cause deterioration of the reagent over the period recommended for use by the manufacturer.

1.4.5 Date of manufacture

1 For reagents other than reagent red cells, the date of manufacture is the date of commencement of the last potency test on the batch or sub-batch that indicates attainment of the required specification.

2 For reagent red cells the date of manufacture is the date of collection from the donor. Where reagent red cells are prepared from more than one donor, the date of collection of the first donation should be recorded as the date of manufacture. (See 1.4.7.1).

Where a freezing process is used to preserve red cells before their preparation for issue as reagent red cells, normally the freezing process should be undertaken not more than 5 days after the donation is collected and should be capable of maintaining a temperature of -65°C or lower. At such temperatures, red cells may be stored for 10 years before their preparation for use as a reagent.

Other systems for the frozen storage of red cells may be appropriate. All systems used to maintain red cells in the frozen state prior to reagent red cell manufacture must be validated to confirm their suitability.

1.4.6 Stability data for reagents other than reagent red cells

1 The manufacturer should have data to validate the expiry date beyond which the reagent for blood group serology is not to be used when stored as recommended by the manufacturer.

2 If the reagent is supplied as a freeze-dried product then in addition to 1.4.6.1, data should be available to validate the expiry date assigned to the reconstituted material as intended for use, stored as recommended by the manufacturer.

3 Before the release of a batch of a new formulation of a reagent, the manufacturer should have data to indicate compliance with the specifications for a non-released batch of that reagent within the immediate container stored as recommended by the manufacturer for a period of at least three months.

4 A provisional expiry date of not more than one year from the date of manufacture may be assigned to the first released batch of reagent made to the same formulation and by the same processes as the non-released batch for which at least three months data are available.

5 Data should be collected and reviewed from both the non-released and released batches of reagent, in order to determine the expiry date ultimately assigned to batches subsequently made to that formulation.

6 If the data indicate an expiry date for that formulation which is earlier than the provisional expiry date assigned to the released batch, the manufacturer should notify immediately all primary consignees of that batch or sub-batch.

1.4.7 Stability data for reagent red cells

1 Unless data are available to support a longer shelf-life, the maximum expiry date of reagent red cells supplied in a preservative medium should be 40 days from the date of manufacture, excluding any period of storage in the frozen state at a temperature of -65°C or lower (see 1.4.5.2).

2 The manufacturer should validate the expiry date assigned to reagent red cells by establishing that, by their processing methods, significant deterioration does not occur in the expression of those antigens stated in the antigenic profile.

3 Unless data are available to support a longer shelf-life, the maximum expiry date of reagent red cells stored as recommended by the manufacturer and supplied in a medium not specifically formulated to preserve the reactivity of antigens, for example CPDA, should be 21 days from the date of manufacture in unopened final containers and not more than 5 days after opening.

1.4.8 Colour coding

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

polyspecific anti-human globulin and anti-IgG reagents may be coloured green, anti-A may be coloured blue, anti-B may be coloured yellow.

2 Tests for specificity, potency, avidity and stability should be performed on the reagent as intended to be supplied for use, that is including the presence of any colourant.

3 The colourant should not interfere with the observation of the test result.

1.4.9 Freedom from microbial contaminants

1 Reagents should be processed in a manner known to produce a final product free from microbial contaminants that adversely affect the product either during storage at the recommended temperature or in use.

2 A preservative may be included in the reagent to minimise the effects of contamination during use if the preservative has been assured not to adversely affect the product during storage or use. The efficacy of the preservative should be assessed during the development of the reagent and should reflect the expected usable period of the reagent. Organisms which have been shown from experience to cause deterioration of the reagent should be included in the test procedure in addition to those listed in the monograph 'Efficacy of Antimicrobial Preservatives in Pharmaceutical Products' (Appendix: XVIC, British Pharmacopoeia 1988, Volume II).

3 Other than reagent red cells, all reagents for blood group serology recommended by the manufacturer for storage in the liquid state, should be filtered through a sterile filter of pore size not exceeding 0.22 µm. All reagents should be dispensed into the immediate container under aseptic conditions.

4 The production process including dispensing into the final containers, should be validated to comply with the requirements of 1.4.9.1.

5 For reagent red cells, the manufacturer should perform viable count estimations on the final product as part of process and product monitoring but these need not form part of product release requirements. In the event of such monitoring disclosing contamination likely to affect adversely the performance of the reagent during its shelf-life, the manufacturer should notify immediately all primary consignees of that batch or sub-batch to withdraw existing stocks from distribution and, if necessary, re-validate the manufacturing process.

6 Tests for sterility alone do not give absolute assurance of freedom from microbial contamination. In addition, the opening of a container may compromise the sterility of the reagent. Bactericidal agents in common use for blood grouping reagents do not guarantee the absence of microbial agents after opening of the container.

1.4.10 Retained samples

1 A minimum of 1% or 3 immediate containers, whichever is less, of each batch of reagents other than reagent red cells should be retained by the manufacturer and stored as recommended by the manufacturer, to enable analysis of reported defects. Such samples should be retained for at least 6 months beyond the expiry date.

2 A minimum of 2 final containers of each batch of reagent red cells should be retained by the manufacturer and stored as recommended by the manufacturer, to enable analysis of reported defects. Such samples should be retained for at least 10 days beyond the expiry date.

1.4.11 Packaging and despatch of reagents

1 The manufacturer or supplier should ensure that the method of despatch does not cause deterioration in the performance of the reagent.

2 If distributed by UK postal services, the packaging shall conform to the UK postal requirements and be sufficient to protect the reagent from foreseeable mechanical damage.

3 Packaging containing solid carbon dioxide should be vented and precautions taken to prevent inactivation of the reagent by entry of carbon dioxide gas.

4 Reagents despatched frozen and intended to remain so during transit should contain an indicator to demonstrate that the recommended temperature for the reagent has not been exceeded.

5 The label on the packaging should be sufficient to ensure the appropriate handling and correct storage on receipt by the addressee.

1.4.12 Tests required.

The manufacturer should test, as described in these Guidelines, each batch or sub-batch of a reagent obtained from the immediate container to be supplied for use. The immediate container at the point of test need not be labelled with the final label if it is the manufacturer's practice to apply the final label after testing has assured the satisfactory performance of the reagent. *See Section 3, 2.2.*

1.5 Guidelines for human source material

1.5.1 Each individual donation or sample of human material in a reagent for blood group serology shall be tested and found negative for HIV antibodies and HBsAg in microbiological tests required by the UK BTS for blood donations (*see Section 1, Annex 3*). A statement is required in the package insert to this effect. Donations collected after 1st September 1991 should be tested and found negative for HCV antibody.

1.5.2 Where a sample from a patient is being used repeatedly for the evaluation of reagents for blood group serology, it should be used, whenever practical, with the patient's consent and tested and found negative for HIV antibodies and HBsAg in microbiological tests required by the UK BTS for blood donations. Samples collected after 1st September 1991 should be tested and found negative for HCV antibody.

1.5.3 To ensure retrospective serological testing, a sample of plasma, or preferably serum, of at least 1ml, collected at the same time as the donation used in the formulation of a particular reagent, should be stored at -20°C or lower until at least six months after the expiry date of the last batch of the reagent made from that material. This recommendation applies also to human donations used in the preparation of monoclonal antibody reagents.

1.6 Immediate container label

1.6.1 The label fixed to the immediate container of a reagent should leave uncovered sufficient area of the full length or circumference of the container to allow ready visual inspection of the contents.

1.6.2 The information printed on the label should be in black ink. The specificity of the reagent for blood group serology should be of a print size which is clearly legible; if possible not less than 12 point for containers of less than 5 ml volume and not less than 18 point for containers of greater than or equal to 5 ml volume. The print size of other information given on the label should not exceed that used for the specificity of the reagent.

1.6.3 The following information should be stated on the label.

1 The name or specificity of the batch or sub-batch of reagent in the same manner as described on the outer container and package insert.

2 If not of human origin, the source of the material, for example, 'mouse monoclonal', or 'Dolichos biflorus'.

3 The name, or unequivocal logo, of the manufacturer or supplier.

4 A reference number or code by which the complete manufacturing history of the batch or sub-batch can be identified.

5 The expiry date after which the reagent is not to be used when stored, within the final container, as recommended by the manufacturer. The expiry date may be stated as day, month and year, or month and year. In the latter case, the expiry date is the last day of the stated month.

6 A space should be indicated for the user to write the expiry date of a freeze-dried product after it has been reconstituted and stored as recommended.

7 The minimum net weight, or net volume, of the reagent within the final container of each batch or sub-batch, or the average net weight, (or net volume) of an immediate container together with the 'e' as defined by the Weights and Measures Act 1979.

8 A statement that the reagent contains or does not contain a preservative. If the preservative is an azide its identity and concentration should be stated.

9 Where reagent red cells are to be washed before use, a statement to that effect.

10 The recommended temperature and conditions of storage. If the reagent is to be stored only in the liquid state, a statement that the reagent is not to be frozen.

11 Any colour appearing on the main panel of the label should comply with the permitted colour coding of reagents, see 1.4.8.1, except :-

(a) The main panel of labels of enzyme-treated reagent red cells should be coloured pink in order to be distinguishable from non enzyme-treated reagent red cells. Pantone colour reference Pink 432 is recommended.

(b) The company logo or name, which if coloured, should be located away from the main panel of the label where details of the specificity are given, and should not cause confusion with the permitted colour coding of reagents.

12 If reagent red cells are suspended in a low ionic strength medium, this should be stated on the label.

13 A statement that the reagent is for *in vitro* use only.

14 A statement that the user should refer to the package insert for details on the use of the reagent, e.g. 'Read package insert before use.'

15 Further labelling guidelines specific to a particular reagent may be described under the appropriate paragraphs.

16 Where for reasons of rarity of the reagent, the immediate container is of a size insufficient to support a label with the information detailed above, the information provided on the label should conform to the minimum requirements of EN 375: 1990, that is:

the product name,
the supplier or logo,
the reference number or code to identify the batch or sub-batch,
the expiry date, and
the appropriate cautionary statements or symbols.

1.7 Package insert

1.7.1 Each reagent for blood group serology should be supplied with an accompanying document (package insert). If two or more immediate containers requiring identical package inserts are placed in a single package, only one package insert is necessary.

1.7.2 Information in the package insert should include that required for the label of the immediate container together with the following.

1 The batch reference on the label of the immediate container to which the package insert refers.

2 The detailed methods of use recommended by the manufacturer for the stated batch or sub-batch of reagent, including the serological controls to assure the specificity of tests, any limitations or precautions, together with other information relevant to the safe use, storage and disposal of the reagent and its immediate container. This requirement includes information on the type of specimen to be used, any pretreatment, and the conditions of storage prior to use by any method recommended for use by the manufacturer.

The duration and temperature of incubation or of other procedures should be explicit; terms such as 'room temperature' or 'immediately centrifuge' are to be avoided.

3 For blood grouping reagents, a list of those antibodies to antigens having a prevalence of greater than 99 per cent in the general population of the U.K., or of the specificity stated below, whose presence has not been excluded.

A; A_1; B; Le^a; Le^b; K; Kp^a; Js^a; P_1; D; C; E; $\overline{c}$; e; C^w; M; N; S; $\overline{s}$; Lu^a; Jk^a; Jk^b; Fy^a; Fy^b; Xg^a, Do^a; Do^b; Yt^b; Co^b; Wr^a; Bg^a and V^w.

4 If the reagent contains material of human origin, a statement that the human material has been tested at source and found negative for HIV and HCV antibodies and HBsAg in microbiological tests required by the UK BTS for blood donations. See 1.5.

5 If the reagent contains material of animal or human origin, a statement that the reagent cannot be assumed to be free from infectious agents and care is to be taken in the use and disposal of the container and its contents.

6 A statement that the reagent is for *in vitro* use only.

7 If the reagent is supplied at the optimal dilution for use, a statement that the reagent is to be used as supplied without dilution or addition.

8 If the reagent is supplied to the user in a form requiring dilution for use, the extent of which is determined by the user, full details of the diluent and dilution procedure, together with a statement that the user is to perform the tests to assure the correct performance of the diluted reagent. That is, at the dilution and for the techniques selected for use, tests for potency, specificity, stability and, for a reagent to be used by a slide technique, for avidity.

9 If the reagent is supplied in a freeze-dried form, full details of the reconstitution and reconstitution medium, together with the period during which it may be used following reconstitution, when stored as recommended by the manufacturer. The manufacturer should include a statement to the effect that after reconstituting the dried reagent the user should record the recommended expiry date on the space provided on the label.

10 A statement that the reagent has been characterised by the procedures recommended in the package insert and that its suitability for use in other techniques must be determined by the user.

11 For reagents other than reagent red cells, a statement that the reagent is not to be used if a precipitate, fibrin gel, or particles are present.

12 A statement that storage of the reagent at temperatures outside the recommended range may result in an acceleration in the rate of loss of reactivity.

13 The nature of any colourant added to the reagent.

14 If a blood grouping reagent is supplied for use with a reagent control, a statement that each test red cell sample is to be tested in parallel with the blood grouping reagent and control, and that no determination of the blood group is possible with that reagent if the reagent control effects agglutination of the test cell sample.

In addition, a statement that caution should be exercised in the interpretation of results of tests performed with such reagents at temperatures other than the temperature recommended by the manufacturer.

15 For blood grouping reagents containing monoclonal antibodies, the identity of the cell line(s) from which the monoclonal antibodies have been derived.

16 For blended reagents other than anti-human globulin, polyclonal preparations obtained from the same individual and different culture supernatants of the same monoclonal preparation, details of the blend (see 1.3.12).

17 For reagent red cells a statement that the reagent is not to be used if it is obviously discoloured or if the suspension medium indicates obvious haemolysis.

18 For reagent red cells, one or more of the following statements, as appropriate:

'for ABO grouping'
'for Rh D grouping'
'for antibody identification'
'for antibody screening'
'for the control of the anti-human globulin technique'

19 For reagent red cells that are to be washed prior to use, instructions on the washing and resuspension of the red cells.

20 A statement that loss of reactivity may occur during the stated shelf life of the red cells and that since this loss is partly determined by characteristics of individual blood donations or donors, which cannot be predicted or controlled, the conditions of storage and use recommended by the manufacturer should be rigidly applied.

21 When red cells are preserved in LISS, especially in the presence of aminoglycoside antibiotics, e.g. neomycin sulphate, they should be discarded within 24 hours of resuspension. (*Note: there is accelerated deterioration in the reactivity of* Fy^a, Fy^b, $\overline{s}$ *and, to a lesser extent, S antigens under these conditions*).

22 Reagent red cells which have been washed and resuspended in saline or LISS solution are to be discarded not more than 24 hours after their preparation.

23 For enzyme-treated reagent red cells, information should be given concerning those antigens which are rendered inactive or less active by the enzyme treatment used. In addition, a statement that the efficacy of enzyme-treated reagent red cells should be confirmed daily or each time the reagent is used, whichever is shorter.

24 If the user is required to add a potentiator, such as enzyme or albumin, a statement that the potentiator should comply with the requirements of these Guidelines.

25 A list of any additional reagents that are required by the user to undertake any method recommended by the manufacturer but which is not provided.

26 A statement that the reagent does or does not comply with the recommendations contained in the latest issue of Section 3 of the Guidelines for the Blood Transfusion Services in the United Kingdom.

27 Further package insert guidelines specific to a particular reagent may be described under the appropriate paragraph of these Guidelines.

Chapter 2

General Guidelines for Serological Tests

2.1 Introduction

General guidelines applicable to all serological tests are presented in this chapter. In other chapters, additional guidelines are given for application in particular test systems.

2.2 Grading system for agglutination tests

The following grading system is used throughout these Guidelines for manual serological testing. If a cumulative (titration) score is required to assess the characteristics of a blood grouping reagent in a titration, then the score as indicated should be used.

Grade 5: Cell button remains in one clump or dislodges into a few large clumps, macroscopically visible. (Titration score of 12).

Grade 4: Cell button dislodges into numerous large clumps, macroscopically visible. (Titration score of 10).

Grade 3: Cell button dislodges into many small clumps, macroscopically visible. (Titration score of 8).

Grade 2: Cell button dislodges into finely granular but definite, small clumps., macroscopically visible. (Titration score of 5).

Grade 1: Cell button dislodges into fine granules, microscopically visible. (Titration score of 3).

Grade 0: Negative result. (Titration score of 0).

2.3 Number of immediate containers to be tested

2.3.1 For reagents other than red cells, a minimum of 1 percent or 3 immediate containers of the batch, whichever is less, should be tested to assure homogeneity. Where possible the test containers should include those from the beginning and end of the filling process. A minimum of one immediate container should be tested to assure compliance with the specifications for serological reagents given in this document.

2.3.2 For reagent red cells, since the number of immediate containers is limited by the volume of the donation of red cells, a minimum of 1 percent or 2 immediate containers, whichever is less, should be tested to assure homogeneity. Where possible the test containers should include those from the beginning and end of the filling process. A minimum of one immediate container should be tested to assure compliance with the specifications for serological reagents.

2.3.3 If the volume of material required for the serological testing exceeds the volume available from a single immediate container, then the contents from two or more containers should be pooled, mixed and used for testing as if it were the contents of one immediate container.

2.4 Test red cells

2.4.1 Records should be kept of all red cells used in the assessment of a reagent during manufacture.

2.4.2 For specificity testing, red cells stored in the liquid state in a medium not specifically formulated to preserve the reactivity of antigens, should be used within 7 days of collection. Red cells stored in a medium proven to preserve the antigens for which the red cells are being used should be used within the shelf-life assigned to red cells stored in that solution.

2.4.3 Red cells of any age may be used to test the potency and avidity of antibodies.

2.4.4 Red cells may be stored frozen and thawed for use in tests for potency, avidity and specificity. For use in tests for specificity the method of freezing, storage and thawing should be known to preserve the antigens for which testing will be performed. Frozen red cells should be used on the day they are thawed and resuspended unless they are suspended in a medium proven to preserve the antigens for which the red cells are being used, when they should be used within the validated shelf-life of red cells stored in that preservative medium.

2.4.5 Red cells for testing reagents for blood group serology that require an anti-human globulin technique, should give a negative test by a direct anti-human globulin test or with a reagent control tested by an indirect anti-human globulin technique.

2.4.6 Unless red cells are recommended for use without washing, red cells should be washed at least twice in saline before use. The supernatant after the last wash should be clear. Red cells to be suspended in LISS should be given at least one additional wash in LISS. Red cells suspended in saline or LISS should be discarded after 24 hours.

2.4.7 Red blood cells from different individuals used for specificity or potency tests should not be pooled except where otherwise stated in the characterisation of antiglobulin reagents. Cord red cells of a given ABO and Rh D group may be pooled.

2.4.8 Unless otherwise stated, the concentration of test red cell suspensions should be 2-3% by volume for normal ionic strength tests and 1.5-2% for low ionic strength tests.

2.4.9 Throughout this Section of the Guidelines, reference is made to the anti-coagulant CPD-A1. However, any anti-coagulant, licensed for the collection of blood for therapeutic use, may be used.

2.5 Test tubes

Unless otherwise stated 10-12mm (diameter) x 75mm glass tubes should be used.

2.6 Centrifugation following the addition of anti-human globulin reagent

In the anti-human globulin technique, after the addition of the anti-human globulin reagent, the reactants should be centrifuged within 15-30 seconds of mixing, unless otherwise stated.

2.7 Centrifugation and reading of serological tests results

2.7.1 Centrifugation.

The centrifugal force should be just sufficient to create a button of cells with clearly defined edges but not such to make the button difficult to dislodge. Many combinations of relative

centrifugal force (g) and time may give similar results e.g. 110 g for 1 minute, 200 g for 30 seconds, 500 g for 15 seconds or 1000 g for 10 seconds.

2.7.2 Reading of tube tests.

1 Haemolysis

Haemolysis should be determined by the visual detection of haemoglobin in the supernatant fluid.

2 Agglutination.

A 'Shake' reading technique should not be used. Its over-vigorous action disrupts agglutination and is responsible for false negative tests in blood group serology.

Any of the following reading techniques may be used.

- Pipette transfer of cell button to microscope slides - the tube is not agitated at any stage. The transfer pipette is clean and has a 1.5-2.0mm internal diameter bore and the tip is free from any irregularities. The cell button is drawn, by the minimum suction possible, into the stem of the clean pipette and then gently ejected onto a slide and simultaneously drawn out over an area of some 2 cm^2. The angle of the pipette above the horizontal controls the width of the spread. The test is observed macroscopically and microscopically if required.

- Tip and roll - the tube is held almost horizontally (70-80° to the vertical) between the thumb and first two fingers and slowly rotated without any shaking or agitation, until the cell button is dislodged from the tube. The free cell button/agglutinates are only allowed to move a maximum of 1cm down the tube. The test is read macroscopically, with the tube held horizontally over an illuminated light source. A x5 or x6 magnifying mirror, or a x6 hand lens can be used.

  Readings can then be obtained by examination of the tube placed horizontally on the stage of an inverted microscope, or by transferring the tube contents to a microscope slide, either by pipette or by touching the lip of the tube on the slide, pouring the contents and moving the tube slowly along it.

- Gentle agitation - the tube is held almost vertically between the thumb and first two fingers and gently agitated using a trembling or vibrating movement. The test is read as described above.

2.7.3 Reading of slide tests

The reagents are mixed thoroughly by rocking the slide for approximately 30 seconds with occasional further mixing during the incubation period. The test is observed macroscopically, and if required microscopically, for agglutination. This may be facilitated by reading over a diffuse light source.

2.7.4 Reading of microplate tests.

1 Resuspension technique in U-well microplates.

After incubation the microplate should be centrifuged at 100g for 40 seconds and the red cells gently dislodged using a microplate shaker. The time required to achieve this will depend on the speed and orbit of the mixer. This has to be defined by observing known controls and turning off the shaker when cells in a negative reaction are fully resuspended and cells in a positive reaction remain clumped but dislodged. Over agitation will reduce the strength of reaction, under mixing will make reading difficult.

2 Streaming technique in V-well microplates.

After the final centrifugation step, the time and speed of which has to be determined for each technique, place the microplate at 70° to the horizontal, this is best done on a purpose built rack. The negative reactions will stream (trail) along the lower edge of the V part of the well, whereas a positive reaction will remain as a button of cells in the apex of the V. The time taken to distinguish between positive and negative reactions depends on several variables, the cell concentration, serum viscosity, speed of centrifugation. The known positive and negative controls act as a reference.

2.8 Non-serological tests.

2.8.1 Manufacturers should ensure the correct concentration of those components of the formulation that may affect the performance of the reagent in blood group serology, for example, EDTA concentration, NaCl concentration, pH and total protein content.

2.9 Specificity tests.

2.9.1 The manufacturer should test the blood grouping reagent as a final product, by all methods recommended by the manufacturer for the specificity and reactivity claimed.

2.9.2 Specificity should be determined by testing the reagent with red cells from a minimum of 4 different donors known to express the antigen corresponding to the specificity of the reagent and 4 different individuals known to lack that antigen. The reagents should be tested for specificity by all methods recommended by the manufacturer for its use.

If a range of incubation times or incubation temperatures is recommended by the manufacturer, the range(s) should be used in these test procedures.

2.9.3 Contaminating antibodies to antigens having a prevalence of greater than 99 per cent in the general population of the UK should be excluded by negative results in tests using samples of red cells from four different individuals who lack the antigen corresponding to the antibody specificity under test.

If tests using all methods recommended for use by the manufacturer do not exclude the presence of antibodies to the following antigens, these antibody specificities should be stated in the package insert as not having been excluded in specificity testing:

Xg^a, Do^a; Yt^a; Co^b; Wr^a; Bg^a and V^w.

Where practicable, red cells used for this testing should have homozygous expression of these antigens.

Subsequent batches, to the same formulation, of stable, well characterised monoclonal reagents need not be tested for contaminating antibodies to low frequency antigens.

2.9.4 Tests for the presence of contaminating ABO antibodies should be performed with red cells from a minimum of 2 individuals of group A_1 and 2 of group B who lack the antigen corresponding to the antibody specificity under test.

2.9.5 A blood grouping reagent recommended for use by a direct agglutination method should be tested against red cells lacking the antigen corresponding to the antibody specificity but coated with IgG blood group antibody to effect a grade 5 reaction in the anti-human globulin technique. IgG blood group antibodies from at least six individuals should be tested separately.

2.9.6 Blood grouping reagents which are chemically modified, and/or contain in their formulation a potentiator of agglutination that require the user to add such a potentiator or proteolytic enzyme reagent shall be tested, by all methods recommended by the manufacturer with red cells lacking the antigen corresponding to the antibody specificity under test but sensitised with an IgG Rh antibody to effect a grade 5 reaction in the anti-human globulin technique.

2.9.7 Requirements.

1 Blood grouping reagents should not produce a positive reaction when tested with red cells lacking the antigen corresponding to the antibody specificity under test, by any method recommended for use by the manufacturer. Should reactivity to a low frequency antigen be observed with subsequent batches of a reagent, this fact should be brought to the attention of all primary users of that reagent.

2 Blood grouping reagents recommended for use by a direct agglutination method should not contain antibodies reactive against red cells coated with IgG when used by direct agglutination methods recommended by the manufacturer, see 2.9.5.

3 A blood grouping reagent producing agglutination by those methods recommended by the manufacturer and outlined in 2.9.6, should be supplied with a reagent control that has been shown to effect a degree of non-specific reaction with IgG coated red cells similar to the corresponding blood grouping reagent.

4 Rouleaux formation, prozone (see 1.3.17) or haemolysis should not occur in any of the methods recommended by the manufacturer.

2.10 Potency tests.

2.10.1 Potency titrations should be performed by the appropriate procedure given in Annex 2, section 2.

2.10.2 Where the appropriate UK reference reagent exists, manufacturers should compare the potency of the reference preparation and test blood grouping reagent in parallel, as detailed in these guidelines. The minimum number and phenotype of red cell samples to be used in potency testing will be given in the appropriate chapter.

2.10.3 Where no appropriate UK reference reagent exists, the manufacturer should assay the potency of each batch or sub-batch of blood grouping reagent recommended for use by tube or microplate method using the appropriate procedure outlined in Annex 2, section 3.

2.10.4 If a blood grouping reagent fails to satisfy the recommended potency requirements with one of the minimum number of red cell samples required for testing, the potency test may be repeated with red cell samples of the required phenotypes but from different individuals from those initially used.

2.10.5 Requirements.

1 A blood grouping reagent is satisfactory if it produces the potency requirements given in the appropriate chapter with all red cell samples tested.

2 A blood grouping reagent is unsatisfactory if, after the repeat testing outlined at 2.10.4, it fails to produce the potency requirements given in the appropriate chapter with more than one of the total number of red cell samples tested.

2.11 Avidity tests

2.11.1 The manufacturer should test the avidity of each batch or sub-batch of blood grouping reagent recommended for use by a slide method using the appropriate procedure given in Annex 2, section 4.

2.11.2 Requirements

The requirements are stated in the chapter appropriate to the reagent.

Chapter 3

Serological Guidelines for ABO and Rh D Blood Grouping Reagents

3.1 Introduction

3.1.1 The determination of the ABO and Rh D group is of prime importance in ensuring the safe transfusion of blood. It is essential that reagents for ABO and Rh D grouping are prepared using reliable manufacturing procedures that are consistently capable of producing safe and efficacious products.

3.1.2 The term D^u is used in these recommendations to indicate a weakened expression of a normal D antigen. There is a gradation of the expression of the D antigen. In general D^u cells are negative or weakly positive with IgM blood grouping reagents but are positive with IgG Rh D blood grouping reagents used in an anti-human globulin technique.

3.1.3 The term D variant is used in these recommendations to indicate the expression of only a part of the normal D antigen. The reactivity of Rh D blood grouping reagents against D variant red cells is determined by the nature of the D variant, the anti-D reagent and the technique used.

3.1.4 RhD grouping of donors: The reactivity of particular D variant cells in blood grouping procedures depends on the methodology and blood grouping reagent used. Although knowledge of D variants is still developing, low-grade D^u red cells, appear to be poorly immunogenic. Some D variant cells may be immunogenic, especially those with a strong expression of D, such as some D^{IV} cells.

RhD grouping procedures should be validated for sensitivity and procedures adopted to maximise the detection of D^u and D variant red cells as RhD positive.

At least two anti-D blood grouping reagents should be used; one detecting D^{VI} red cells, the other not. This would enable female donors of child-bearing age to be identified in order to avoid their exposure to RhD positive blood. D^{VI} individuals may develop 'anti-D' to those epitopes absent from their D antigens, in response to pregnancy or transfusion.

3.1.5 RhD grouping of patients: Two anti-D blood grouping reagents complying with these Guidelines should be used, neither of which should react with D^{VI} red cells using the method(s) recommended for use. 'Follow-on' tests of negative results using an antiglobulin procedure are not required.

3.2 Specificity of ABO blood grouping reagents for use in manual or microplate tests.

3.2.1 Method

With due regard to the requirements of 2.9, as a minimum the following red cells should be tested using all methods recommended for use by the manufacturer.

Blood Grouping Reagent	Number of red cell samples to be tested						
	A_1	A_2	A_1B	A_2B	B	0	A_x
anti-A	2			2	2	2	3*
anti-B	2		2		2	2	
anti-A,B	1	2			2	4	3
anti-A+B	1	2			2	4	

**only if the anti-A is recommended for the detection of A_x cells.*

If further reactivity is claimed by the manufacturer against subgroups of A other than A_1, A_2 and A_x, red cells from at least three individuals of each claimed subgroup should be tested.

3.2.2 Requirements

1 The blood grouping reagent is satisfactory if not less than a grade 5 reaction is effected with all the red cell samples having the antigen corresponding to the blood grouping reagent being assessed, by all the methods recommended for use by the manufacturer.

2 In addition to 3.2.2.1, anti-A blood grouping reagent is satisfactory if not less than a grade 4 reaction is effected with all the A_2B samples tested by all the methods recommended for use by the manufacturer.

3 In addition to 3.2.2.1, anti-A,B blood grouping reagents and anti-A blood grouping reagents recommended for the detection of A_x should effect at least a grade 3 agglutination with each of the A_x red cell samples by those techniques recommended by the manufacturer for the detection of A_x.

4 In addition to 3.2.2.1, blood grouping reagents which are claimed to detect subgroups of A other than A_1, A_2 and A_x should effect at least a grade 3 agglutination with each of the red cell subgroup samples by those techniques recommended by the manufacturer for the detection of those subgroups.

3.3 Potency of ABO blood grouping reagents for use in manual or microplate tests.

3.3.1 Method

The UK minimum potency reference preparations for anti-A and/or anti-B should be tested in parallel with the reagent batch. As a minimum, the following red cell samples should be used by the methods described in 2.10. A 5 minute incubation period at 19°C-25°C should be used for tube potency tests.

Blood Grouping Reagent	Number of red cell samples to be tested				
	A_1	A_2	A_1B	A_2B	B
anti-A	1			3	
anti-B			3		1
anti-A,B	1	2			2
anti-A+B	1	2			2

3.3.2 Requirements

For blood grouping reagents recommended for use by a tube, slide or microplate technique:

1 anti-A blood grouping reagents should effect a potency titre at least equal to the UK reference anti-A preparation with the red cell samples tested.

2 anti-B blood grouping reagents should effect a potency titre at least equal to the UK reference anti-B preparation with the red cell samples tested.

3 anti-A,B blood grouping reagents should effect a potency titre at least equal to the UK reference anti-A preparation with the A_1 and A_2 red cell samples tested and a potency titre at least equal to the UK reference anti-B preparation with the group B red cell samples tested.

4 anti-A+B blood grouping reagents should effect a potency titre at least equal to the UK reference anti-A preparation with the A_1 and A_2 red cell samples tested and a potency titre at least equal to the UK reference anti-B preparation with the group B red cell samples tested.

3.4 Avidity of ABO blood grouping reagents for use in manual slide tests.

3.4.1 Method

As a minimum, the following red cell samples should be used by the methods described in 3.11.

Blood Grouping Reagent	Number of red cell samples to be tested				
	A_1	A_2	A_1B	A_2B	B
anti-A	1			3	
anti-B			3		1
anti-A,B	1	2			2
anti-A+B	1	2			2

3.4.2 The grade of agglutination with the blood grouping reagent should be at least grade 3 within one minute of mixing.

3.5 Specificity of anti-D blood grouping reagents for use in manual or microplate tests.

3.5.1 Method

With due regard to the requirements of 2.9, as a minimum the following red cells should be tested using all methods recommended for use by the manufacturer:

1 R_1r from two individuals
R_or or R_oR_o from two individuals
r'r' from one individual
r''r'' from one individual
rr from one individual

2 If the anti-D blood grouping reagent includes polyclonal material and is recommended by the manufacturer for use by the anti-human globulin technique, rr cells with strong expression of Bg^a from three individuals.

3 Anti-D blood grouping reagents recommended by the manufacturer for the detection of D^u should be tested with a minimum of three D^u red cell samples using those techniques recommended by the manufacturer for the detection of D^u.

4 Anti-D blood grouping reagents recommended by the manufacturer for the detection of D variant should be tested with a minimum of 2 examples of the particular D variant against which reactivity is claimed, using those techniques recommended by the manufacturer for the detection of that D variant.

3.5.2 Requirements

1 Anti-D blood grouping reagents should effect a grade 5 reaction with all D positive red cell samples by all methods recommended for use by the manufacturer and negative reactions with D negative red cell samples.

2 Anti-D blood grouping reagents, if recommended for use by the anti-globulin technique should effect negative reactions with rr cells with a strong expression of the Bg^a antigen.

3 Anti-D blood grouping reagents recommended by the manufacturer for the detection of D^u should effect at least a grade 3 reaction with the three D^u red cell samples using those techniques recommended by the manufacturer for the detection of D^u.

4 Anti-D blood grouping reagent recommended by the manufacturer for the detection of D variants should effect at least a grade 3 reaction with the 2 examples of the particular D variant against which reactivity is claimed, using those techniques recommended by the manufacturer for the detection of that D variant.

3.6 Potency of anti-D blood grouping reagents for use in manual or microplate tests

3.6.1 Methods

As a minimum, R_1r red cells from 3 individuals should be used by the methods described in 2.10.

3.6.2 Requirements

1 For chemically modified anti-D blood grouping reagents recommended for use by a tube technique.

The potency titre should be grade 5 with the undiluted reagent and at least a grade 2 with the reagent diluted 1 in 4, with all the R_1r test red cell samples.

2 For other anti-D blood grouping reagents.

There should be grade 5 agglutination with the undiluted reagent and at least a grade 2 with the reagent diluted 1 in 8, with all the R_1r test red cell samples.

3.7 Avidity of anti-D blood grouping reagents for use in manual slide tests

3.7.1 Method

As a minimum, R_1r red cells from 2 individuals should be used by the methods described in 2.11.

3.7.2 Requirements

Agglutination should be grade 3 within one minute of mixing.

Chapter 4

Guidelines for Reagent Red Cells

4.1 Introduction

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

4.2 Guidelines for testing reagent red cells

4.2.1 When testing reagent red cells, in order to confirm the presence or absence of antigens listed in the antigen profile, a sample from each individual (especially from those not used previously for such reagent red cells) should be tested whenever possible, with a minimum of two different examples of blood grouping reagents corresponding to each antigen specifically listed.

4.2.2 Where such testing produces conflicting results, further testing with at least one additional example of the relevant antibody/ies should be undertaken before the antigenic status of that cell is committed to the antigen profile included within the package insert.

4.2.3 Where such testing has been performed with only one example of any blood grouping reagent, this information should be stated in the antigen profile included within the package insert.

4.2.4 Reagent red cells should be shown not to produce unwanted positive reactions by the methods recommended for use by the manufacturer.

4.2.5 Except for IgG-sensitised and C3-sensitised red cells, reagent red cells should be negative in the direct anti-human globulin technique with anti-IgG, anti-complement and polyspecific anti-human globulin reagents using the techniques recommended for use by the reagent manufacturer.

4.3 Preparation of reagent red cells

4.3.1 Red cells for use in reverse ABO grouping, in the control of blood grouping reagents, or in the detection of irregular antibodies in blood donations, may be pooled. In such instances, equal volumes of red cells from a maximum of two individuals should be used and the reagent red cells prepared by a procedure which ensures adequate mixing of the constituent red cells.

4.3.2 With the exception of umbilical cord blood, red cells used to test patient's samples for irregular antibodies should not be pooled.

4.3.3 Reagent red cells should be processed by a method consistently shown to yield a product capable of detecting, throughout its shelf-life, all antibodies directed against the antigens specified in the antigen profile included within the package insert.

4.3.4 Unless instructions are given to wash the reagent red cells before use, all reagent red cells should be free of ABH specific blood group substances and blood group antibodies, including anti-A and anti-B, demonstrable by the manufacturer's recommended methods of

use. If the reagent red cells are to be washed by the user, the package insert should give instructions on the washing procedure.

4.4 Immediate container label and package insert

In addition to the requirements of 1.6 and 1.7.

4.4.1 The final container label and package insert of reagent red cells prepared from pooled material should include the statement 'POOLED CELLS'.

4.4.2 The antigen profile of reagent red cells should accompany or be included with the package insert. Where such reagent red cells are intended for use in ABO grouping or control of ABO or Rh D blood grouping reagents, or comprise pooled umbilical cord blood, only the ABO and Rh D group need be stated.

4.4.3 When the reagent red cells are a multi-container product such as a red cell panel, the label on the immediate containers and packaging should be assigned the same identifying batch reference and carry a number or symbol to distinguish one container from another. This number or symbol should also appear in the antigen profile.

4.4.4 The date of expiry of reagent red cells should be stated in the antigen profile.

4.4.5 Where reagent red cells are provided suspended in preservative medium, the components of the medium should be stated in the package insert.

4.4.6 The concentration of the red cell suspension should be stated in the package insert and antigen profile.

4.5 Other reagent red cells

Manufacturers who wish to provide additional reagent red cells other than those described in paragraphs 4.6 to 4.12 should comply with the relevant guidelines listed herein.

4.6 Enzyme-treated reagent red cells

4.6.1 Reagent red cells pretreated with proteolytic enzymes may be provided for antibody screening and identification.

4.6.2 Enzymes used to treat red cells for manual tests should comply with the guidelines listed in Chapter 7 of this Section.

4.6.3 Enzymes used to prepare red cells for ABO or Rh D grouping using microplates may be prepared as described in Annex 2.

4.6.4 The method of enzyme treatment used by the manufacturer should be shown consistently to yield a product capable of detecting, throughout its shelf-life, those antibodies which the reagent is intended to detect.

4.7 Reagent red cells for use in ABO grouping

4.7.1 Introduction

Determination of the ABO group of donor and patient is vital in ensuring safe transfusion practice. This is primarily achieved by testing unknown red cells with standard blood grouping reagents. Confirmation of the observed ABO group can be achieved by

demonstrating ABO specific antibodies in serum or plasma - a procedure known as 'reverse' grouping. Appropriate reagent red cells are required for this purpose and for controlling ABO blood grouping reagents.

4.7.2 Reagent red cells for use in ABO grouping need only be tested for A; A_1; B and Rh D.

4.7.3 Reagent red cells for reverse grouping

Reagent red cells of groups A_1 and B should be used, although A_2 may be included.

Reagent red cells for reverse grouping should be Rh D negative.

4.7.4 Controls for ABO blood grouping reagents.

Reagent red cells of groups A_1; A_2; B and 0 should be used for the control of each batch of tests with anti-A ; anti-B ; anti-A,B ; anti-A+B or anti-A_1.

4.8 Reagent red cells for use in Rh D grouping

4.8.1 Introduction

Determination of the Rh D group of donor and patient is required to ensure safe transfusion practice. Reagent red cells which confirm the efficacy and specificity of anti-D blood grouping reagents are essential.

4.8.2 Reagent red cells for the control of Rh D blood grouping need only be ABO grouped and Rh phenotyped with anti-C; anti-D; anti-E; anti-$\bar{c}$.

4.8.3 Controls for Rh D blood grouping reagents.

Reagent red cells of group 0, R_1r and r'r should be used for the control of each batch of Rh D blood grouping tests.

4.9 Additional control red cells

Whenever possible, additional red cells intended for the control of blood group reagents should be from individuals whose zygosity for the appropriate antigen(s) is known.

4.10 Reagent red cells for use in antibody screening

4.10.1 Introduction

The detection of irregular antibodies in the serum of a patient is of greater clinical significance than if such antibodies are detected in blood donors. Consequently it is permissible to use reagent red cells of a lesser specification when performing antibody screening tests on blood donor samples.

4.10.2 General

1 Reagent red cells for use in antibody screening should be confirmed as group 0 by an ABO blood grouping procedure which is capable of demonstrating the A_x phenotype.

2 Where practicable, reagent red cells known to express low frequency antigens (that is, those antigens having a frequency of less than 1 percent in the general population of the UK) should not be included in reagent red cells for antibody screening.

3 Where practicable, red cells from individuals known consistently to effect troublesome reactions with HLA specific antibodies should not be used as reagent red cells for antibody screening.

4.10.3 Reagent red cells for use in antibody screening of patient samples

1 Introduction

Antibody screening tests on patients' serum samples provide an opportunity for the detection and subsequent identification of irregular antibodies, thereby facilitating the selection of blood for transfusion and permitting early prediction of possible haemolytic disease of the foetus and newborn.

2 General guidelines

i As a minimum the following antigens should be expressed on the reagent red cells for antibody screening;

C; $\overline{c}$; D; E; e; K; $\overline{k}$; Fy^a; Fy^b; Jk^a; Jk^b; S; $\overline{s}$; M; N; P_1; Le^a and Le^b

ii As a minimum, reagent red cells from two individuals should be provided. These red cells should not be pooled.

iii At least one of the red cell samples should express the probable Rh haplotype R_2.

iv Apparent homozygous expression of the following antigens in the stated order of priority, is desirable:

D; $\overline{c}$; Fy^a; Jk^a; Jk^b; S; $\overline{s}$ and Fy^b.

4.10.4 Reagent red cells for use in antibody screening of donor samples

1 Introduction

Antibody screening of donor samples permits identification of those donors whose plasma contains relatively potent irregular antibodies and the exclusion of those donations or their derived components, which have the potential to cause adverse reactions on transfusion, particularly to newborn infants, see Section 1, Annex 3.

2 General guidelines

i It is preferable that reagent red cells are provided, unpooled, from a minimum of two individuals but the reagent may be supplied as a pool of red cells from no more than two donors.

ii Pooled reagent red cells for antibody screening should be used only for testing samples from blood donors.

iii As a minimum the following antigens should be expressed:

C; $\overline{c}$; D; E; e; K.

iv At least one of the red cell samples should express the probable Rh haplotype R_2.

4.11 Reagent red cells for use in antibody identification

4.11.1 Introduction

Identification of the specificity of irregular antibodies detected during antibody screening is important, particularly in pretransfusion and pre-and post-natal testing programmes.

4.11.2 General guidelines

1 Reagent red cells for use in the identification of irregular antibodies should be confirmed as group 0 by an ABO blood grouping procedure which is capable of demonstrating the Ax phenotype.

2 In some instances, identification of the specificity of an irregular antibody requires the use of reagent red cells other than group 0, for example, red cells of group A_1 and A_2 are particularly useful in assigning the specificity anti-HI.

3 Where practicable, red cells from individuals known consistently to effect troublesome reactions with HLA specific antibodies should not be used in reagent red cells for antibody identification.

4 Reagent red cells for antibody identification should comprise red cells from eight or more group 0 individuals and permit the confident identification of those clinically significant alloantibodies which are most frequently encountered, for example, anti-D, anti-E, anti-$\bar{c}$, anti-K and anti-Fy^a.

5 Red cells from the eight or more individuals which comprise the red cell panel for antibody identification, should be tested, as a minimum, with antibodies of the following specificities:

C; C^w; $\bar{c}$; D; E; e; K; $\bar{k}$; Fy^a; Fy^b; Jk^a; Jk^b, S; $\bar{s}$; Le^a; Le^b; M; N; P_1 and Lu^a.

6 A distinct pattern of reactivity should be apparent for each of the commonly encountered alloantibodies, for example, anti-D, anti-E, anti-$\bar{c}$, anti-K and anti-Fy^a.

7 The antigenic profile of reagent red cells for antibody identification, as far as possible, should permit assignment of specificity in test sera containing more than one commonly encountered alloantibody, for example, anti-D+K.

8 For reagent red cells for antibody identification, the minimum characteristics are:

i one individual should be R_1R_1 and one $R_1{}^wR_1$. Between them, these two individuals should express the antigens:

K; $\bar{k}$; Fy^a; Fy^b; Jk^a; Jk^b; S and $\bar{s}$.

ii one individual should be R_2R_2 and one r'r.

iii A minimum of four individuals should lack the Rh antigens C and D. One of these individuals should be K positive and one should be E positive. Between them, these individuals should exhibit apparent homozygous expression of the antigens:

$\bar{c}$; $\bar{k}$; Fy^a; Fy^b; Jk^a; Jk^b; S and $\bar{s}$.

4.12 Reagent red cells for use in the control of the anti-human globulin technique.

4.12.1 Introduction

The anti-human globulin technique is the single most reliable procedure for the detection of red cell alloantibodies. Consequently, careful control of the procedure is essential. In addition, sensitised reagent red cells are required to ensure active anti-IgG is present in each negative test.

4.12.2 Reagent red cells for assuring anti-IgG activity in negative anti-human globulin tests.

1 In order to confirm that each anti-human globulin test has been conducted correctly, reagent red cells sensitised with IgG antibody should be added to all negative tests.

Strongly sensitised reagent red cells can be agglutinated by an anti-human globulin reagent that has been partially neutralised by residual human protein to an extent that weak antibodies are not detected. Whereas, in the same circumstances, weakly IgG sensitised reagent red cells are not agglutinated.

The agglutination of sensitised reagent red cells by the anti-human globulin reagent is weakened when the sensitised reagent red cells are mixed with well-washed test red cells with no bound IgG antibody in the presence of anti-human globulin reagent.

Reagent red cells for this purpose may be provided ready for use, comprising a suspension of red cells sensitised with IgG antibody.

2 Test Method

Add one volume of 2-3% well-washed unsensitised group O red cells to two tubes.

Add two volumes of the UK anti-IgG reference preparation to each tube. Mix well. Centrifuge and observe for agglutination which should not be present.

Add one volume of a 1 in 1000 dilution of human serum in saline to one tube and one volume of saline to the other.

Add one volume of the sensitised reagent red cells. Mix, incubate at 19-25°C for one minute, centrifuge and observe for agglutination.

3 Requirement

The test with the 1 in 1000 dilution of serum should be negative or give a reaction grade of 1; the other test should have a grade 2-3 reaction.

Chapter 5

Guidelines for Low Ionic Strength Solution (LISS) for Red Cell Suspensions

5.1 Introduction

By reduction of the ionic strength of the antibody/antigen reaction mixture, the use of LISS as a red cell suspending medium permits a substantial reduction in incubation time and an increase in test sensitivity with most antibody specificities. These advantages of LISS are entirely dependent on its correct preparation and use.

Test procedures which incorporate low ionic strength conditions are particularly useful in pretransfusion testing.

5.2 Provision of LISS

LISS solutes may be provided in solution ready for use by the preferred method. However, in view of transport and storage difficulties, manufacturers may provide LISS solutes as a concentrated solution to be diluted prior to use, or as a dry powder. Powders should be packaged so that the total contents are dissolved in a stated volume of water. See 1.3.3.

5.3 Shelf life

5.3.1 The shelf life of LISS is influenced by the method by which it is used; LISS which is transferred from the immediate container to a second container may become more quickly contaminated and unsuitable for use than if LISS is dispensed aseptically from a sterile immediate container that is collapsible or is fitted with a sterile airway.

5.3.2 As a guide, the following usable period is recommended for the storage of LISS at 19°C-25°C.

Ready for use:	unopened	1 year
	opened, used aseptically	2 weeks
	opened	1 week
Concentrate:	unopened	1 year
	opened, used aseptically	2 weeks
LISS prepared from packaged dry powder,		
	not sterilised:	3 days

5.4 Quality control, serological testing

5.4.1 Comparative titrations

1 Selection of anti-D

An anti-D should be selected with a potency titre of not less than 1 in 4 and not greater than 1 in 16 in tests with R_1r red cells, using a polyspecific anti-human globulin reagent by its recommended LISS technique.

2 Method

Prepare doubling dilutions of the selected anti-D in saline containing 20g/L bovine serum albumin. Prepare 1.5-2% suspensions of washed R_1r red cells from a minimum of two individuals in:

(a) the test LISS batch,
(b) a previously tested, acceptable, batch of LISS, and
(c) saline.

With each of the cell suspensions, including that in saline, and each of the doubling dilutions, perform a indirect anti-globulin test using an incubation period of 15 minutes at 37°C.

3 Requirements

The test LISS batch should give results which are comparable with the acceptable LISS batch and should effect a potency titre which at least equals that obtained with the cells suspended in saline tested in parallel as described in 5.4.1.2.

5.4.2 Physical characteristics

Measurements of pH, conductivity and osmolality should be made at 22°C±1°C. The acceptable value for these measurements should be:

pH	6.7 ± 0.2
Conductivity	3.7 ± 0.3mS/cm
Osmolality	295 ± 10 mosmol/Kg

5.5 Immediate container label and package insert

In addition to the guidelines in Chapter 1.

5.5.1 The label of LISS concentrates should include the statement 'Dilute before use - see instructions'.

5.5.2 In the event that the preparation of LISS is to be completed by the user, the package insert should give clear instructions how this is achieved together with quality control tests that are required, how these are performed and the test values which are acceptable.

5.5.3 The package insert for LISS concentrate should include instructions for checking the container for deposits of solute which, if present, should be redissolved before dilution of that concentrate.

5.5.4 The package insert should include the following additional cautionary statements:-

1 That suspension of red cells in LISS is associated with an accelerated deterioration in the expression of Fy^a, Fy^b, $\bar{s}$ and S antigens and that red cells suspended in LISS are to be discarded within 24 hours of their preparation, *see 1.7.2.21.*

2 That thorough mixing of equal volumes of serum and LISS suspended red cells is required for LISS procedures.

3 That for optimum sensitivity, the LISS indirect anti-human globulin technique requires a minimum incubation time of 15 minutes at 37°C.

4 That in order to avoid the non-specific uptake of autologous complement, red cells should be washed at least twice in normal ionic strength saline before they are finally washed and resuspended in LISS.

5 That unwanted positive reactions due to 'cold' antibodies are less likely to be encountered if the temperature of the red cell suspension, LISS or serum is 19°C-25°C before use.

6 That LISS solution is readily contaminated. Clear instructions are to be given for a reliable means of preventing bacterial and fungal contamination during the usable period of the solution.

7 Manufacturers should advise users of the most appropriate means of dispensing LISS.

Chapter 6

Guidelines for Anti-Human Globulin Reagents: Polyspecific anti-human globulin, anti-IgG and anti-complement reagents.

6.1 Introduction

6.1.1 The anti-human globulin technique is an essential test for the detection and identification of antibodies and for compatibility ('crossmatch') tests to ensure safe transfusion practice.

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

6.2 Specificity required in polyspecific anti-human globulin reagents

6.2.1 Anti-IgG is the essential component since the majority of red cell alloantibodies are non-complement binding IgG.

6.2.2 Anti-complement should be present in the reagent.

6.2.3 Anti-C4d must be avoided. It is accepted that very low titres of anti-C4c may occur in anti-complement reagents of animal polyclonal origin.

6.3 Specificity of polyspecific anti-human globulin and anti-IgG reagents for use in manual tube tests

6.3.1 Tests for IgM and IgG red cell heterospecific antibodies.

These test for heterospecific antibodies which can cause haemolysis or agglutination of unsenstised red cells in the indirect anti-globulin test.

1 Method.

Divide 12 test tubes into 2 sets of 6.

Into each of the first set of tubes, add 1 volume of washed 2-3% untreated red cells in saline from two group A_1 Rh D positive, two group B Rh D positive and two group 0 Rh D positive individuals.

Into each of the second set of tubes add 1 volume of washed 2-3% enzyme-treated red cells (papain, bromelin or ficin) in saline from the same group A_1 Rh D positive, group B Rh D positive and group 0 Rh D positive individuals.

Add 2 volumes of the anti-human globulin reagent as intended to be supplied for use, to each test tube. Mix thoroughly. Incubate the reactants for 5 minutes at 19-25°C.

Centrifuge the tubes.

Determine the reaction grade.

2 Control of enzyme treatment.

Weak IgG anti-D, known to be reactive with enzyme-treated red cells should effect a positive reaction with each washed, enzyme-treated, red cell sample by the following method:

To separate tubes, add 1 volume of the weak IgG anti-D to 1 volume of each of the washed, 2-3% suspension of enzyme-treated, Rh D positive red cell samples. Mix thoroughly. Incubate for 5 minutes at 37°C. Centrifuge the tubes. Determine the reaction grade.

3 Requirements.

Each of the enzyme-treated Rh D positive red cell samples should be agglutinated by the weak IgG anti-D.

The anti-human globulin reagent should not agglutinate or haemolyse washed unsensitised red cells from two individuals of group A_1 Rh D positive, two individuals of group B Rh D positive and two individuals of group O Rh D positive, whether or not treated with proteolytic enzyme, that is papain, bromelin or ficin.

6.3.2 Tests for unwanted positive reactions.

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

1 Preparation of the red cell suspensions from segmented bleed line samples.

Select integral segment lines from 2 packs of group A_1, two packs of group B and two packs of group 0 blood stored at 4°±2°C for at least 10 days.

Wash each of the red cell samples with saline sufficient to remove serologically reactive traces of serum.

Prepare suspensions of each red cell sample as 2-3% in saline and as 1.5-2% in LISS.

2 Incubation of red cells and fresh group-compatible serum.

Each of the six red cell samples described above is tested as a saline and a LISS suspension with a different, fresh, group - compatible serum.

For each anti-human globulin reagent to be assessed, prepare 2 sets of 6 tubes.

To the first tube of the first set of 6 tubes and the first tube of the second set of 6 tubes, add 1ml of a fresh, single donor group-compatible serum. Add 1ml of a second fresh, single donor group-compatible serum to the second tube of each set, and so on for the 6 different, fresh, group-compatible sera.

To the first tube of the first set of 6 tubes, add 0.5ml of a red cell sample as a 2-3% suspension in saline. Add 1ml of the same red cell sample as a 1.5-2% suspension in LISS to the first tube of the second set of 6 tubes. Add 0.5ml of the second red cell sample as a 2-3% suspension in saline to the second tube of the first set of tubes and lml of the same red cell sample as a 1.5-2% suspension in LISS to the second tube of the second set of tubes, and so on for each of the six different, red cell samples.

Incubate the first set of tubes (saline suspended red cell samples) for 45 minutes at 37°C. Incubate the second set of tubes (LISS suspended red cell samples) for 15 minutes at 37°C.

Wash the red cell samples with saline sufficient to remove serologically reactive traces of serum. Resuspend the red cells to 2-3% in saline.

3 Tests with anti-human globulin reagents.

For each anti-human globulin reagent, prepare 2 sets of 6 tubes. To each of the first set of 6 tubes, add in sequence I volume of the 2-3% suspension of washed red cells from the saline test above.

To each of the second set of 6 tubes, add in sequence 1 volume of the washed 2-3% suspension of washed red cells from the LISS tests above.

Add 2 volumes of undiluted anti-human globulin, that is as intended to be supplied for use, to each of the 12 tubes. Mix thoroughly.

Centrifuge the tubes.

Determine the reaction grade.

4 Requirements.

All reactions should be negative on macroscopic examination. A few small clumps of red cells seen microscopically, although not desirable, are acceptable.

6.4 Anti-IgG potency: polyspecific anti-human globulin and anti-IgG reagents for use in manual tube tests

The UK anti-IgG reference reagent should be tested in parallel with the test reagent.

6.4.1 The anti-IgG potency titre with red cells heavily sensitised with IgG antibody should be determined as follows.

1 Test cells

A 2-3% suspension in saline of washed pooled group O R_1r red cells is prepared from 4 individuals.

2 Determination of the anti-IgG potency titre of a test anti-human globulin preparation using red cells strongly sensitised with IgG anti-D.

Anti-D suitable for use in this application should have a potency titre of greater than 512.

To 4ml of the potent IgG anti-D add 2ml of the 2-3% suspension of pooled group 0 R_1r red cells.

Mix and incubate at 37°C for 45 minutes.

Wash the red cell sample with saline sufficient to remove serologically reactive traces of serum. Prepare suspensions of each red cell sample as 2-3% in saline.

3 Test method

Prepare 1ml volumes of twofold serial dilutions of the test anti-human globulin reagent and anti-IgG reference preparation from 1 in 8 to 1 in 4096 (10 tubes).

Prepare a set of 10 tubes for each anti-human globulin reagent to be assessed.

Place 2 volumes of each dilution into each of the series of 10 tubes.

Add 1 volume of the 2-3% suspension of pooled sensitised R_1r red cells to each tube, mix and centrifuge.

Determine the potency titre.

4 Controls

The washed, strongly sensitised 2-3% suspension of R_1r red cells gives a negative result when centrifuged and gives negative results using the direct anti-human globulin technique with anti-complement (anti-C3c, anti-C3d, anti-C4c and anti-C4d) reagents and with anti-human globulin diluent in place of the anti-human globulin reagent. (The anti-complement specificities may be present as mixtures in one or more reagents).

5 Requirements.

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

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

1 Selection of weak, single-donor IgG antibodies.

An IgG anti-D is selected to give an anti-human globulin potency titre of 1 in 8 to 1 in 16 using a pool of group 0 R_1r red cells from 4 individuals.

An IgG anti-K containing a final concentration of 0.014M neutralised EDTA is selected to give an anti-human globulin potency titre of 1 in 8 to 1 in 16 using $K\overline{k}$ red cells.

An IgG anti-Fy^a containing a final concentration of 0.014M neutralised EDTA is selected to give an anti-human globulin potency titre of 1 in 8 to 1 in 16 using Fy(a+b+) red cells.

EDTA treatment

To 1ml serum, add 0.14ml of 0.11m diamino ethanetetra acetic acid dipotassium salt (4.45% K_2 EDTA. $2H_2O$). Incubate for 15-20 minutes at 19°C-25°C.

2 Test cells

Prepare 10ml of a 2-3% suspension of washed R_1r red cells pooled in equal proportions from 4 individuals. Similarly, prepare 10ml of a 2-3% suspension of washed $K\overline{k}$ red cells and 10ml of a 2-3% suspension of washed Fy(a+b+) red cells.

3 Sensitisation of test cells

(i) Anti-D

Using a set of 5 containers each of 20 to 25ml volume, prepare 4ml volumes of serial twofold dilutions of the anti-D from undiluted to 1 in 16.

Add 2ml of the 2-3% suspension of pooled R_1r red cells in saline to each container. Mix and incubate at 37°C for 45 minutes.

Wash the red cells 4 times with 20ml volumes of saline at each wash and remove the last supernatant.

Add 2ml of saline to the packed washed red cells to prepare the 2-3% suspensions of sensitised red cells.

(ii) Anti-K

As above, but using the anti-K with the $K\overline{k}$ red cells.

(iii) Anti-Fy^a

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

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

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

5 Test method for anti-IgG or anti-globulin potency by chequerboard titration.

(i) Anti-D sensitised red cells

Prepare 5 sets of 5 tubes for each anti-human globulin reagent under test and the anti-IgG reference reagent.

Place 2 volumes of the anti-human globulin reagent, undiluted to 1 in 16 in the appropriate tubes for each of the 5 sets of 5 tubes.

Using the 2-3% suspension of red cells sensitised with the undiluted anti-D for the first set of 5 tubes, the 2-3% suspension of red cells sensitised with the anti-D

diluted 1 in 2 for the second set of 5 tubes, and so on, finishing with the 2-3% suspension of red cells sensitised using the anti-D diluted 1 in 16 for the fifth set of 5 tubes, add 1 volume of the washed red cells to each of the sets of anti-human globulin dilutions, See below:

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

Mix thoroughly. Centrifuge the tubes.

Determine the reaction grade

(ii) Anti-K sensitised red cells

As above, but using the anti-K sensitised K $\overline{k}$ cells.

(iii) Anti-Fy^a sensitised red cells

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

6 Controls.

The 2-3% red cell suspensions sensitised with the undiluted anti-D, anti-K and anti-Fy^a give negative results when centrifuged and give negative results using the direct anti-human globulin technique with anti-complement (anti-C3c, anti-C3d, anti-C4c and anti-C4d) reagents and with anti-human globulin diluent in place of the anti-human globulin reagent. (The anti-complement specificities may be present as mixtures in one or more reagents).

7 Requirements.

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

6.5 Anti-complement potency; polyspecific anti-human globulin reagents for use in manual tube tests.

Although complement binding antibodies such as anti-Le^a and anti-Jk^a are scarce, manufacturers should validate the anti-complement activity by tests with complement-fixing antibodies.

6.5.1 Preparation of the complement sensitised red cells.

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

The C3 and C4 activation states produced on red cells by the various methods are as follows:

Method of Preparation	Initial State	State after trypsin treatment
Very low ionic strength medium* 37°C	iC3b/C4b	iC3d/C4d
Cold acquired haemolytic anaemia (alpha 2D, CHAD)	C3dg	C3d
Very low ionic strength medium* 37°C with EDTA	C4b	C4d

**These media are not to be confused with low ionic stength solution (LISS).*

See Annex 1 for suggested methods of preparation of these sensitised red cells. As a minimum, red cell samples from 2 individuals are to be prepared and tested as described below.

6.5.2 Anti-C4c potency

1 Method.

Prepare a set of 3 tubes for each anti-human globulin reagent under test.

Prepare doubling dilutions of the anti-human globulin reagent from undiluted to 1 in 4.

Place 2 volumes of each anti-human globulin dilution in the appropriate tubes.

Add 1 volume of 2-3% EC4b red cells to each tube. Mix thoroughly. Centrifuge the tubes.

Determine the reaction grade.

2 Controls.

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

3 Requirements

The anti-human globulin reagent should have an anti-C4c titre of 1 in 2 or less.

6.5.3 Anti-C4d potency

1 Method.

Place 2 volumes of undiluted anti-human globulin in a tube.

Add 1 volume of 2-3% EC4d red cells. Mix thoroughly. Incubate for 5 minutes at 19-25°C.

Centrifuge the tubes. Determine the reaction grade.

2 Controls.

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

3 Requirements.

The anti-human globulin reagent should not effect a macroscopic reaction with EC4d red cells.

6.5.4 Anti-C3c potency

The term anti-C3b is not used as C3b has several C3 determinants.

The presence of traces of anti-C4c in conventional anti-human globulin reagents does not obscure the reactions with EiC3b red cells which also have C4b. Therefore, EiC3b/C4b red cells may be used for these tests.

1 Method.

Prepare a set of 8 tubes for each anti-human globulin reagent under test and the UK anti-C3c reference preparation which is tested in parallel.

Prepare doubling dilutions of the anti-human globulin reagent from undiluted to 1 in 128.

Place 2 volumes of each anti-human globulin dilution in the appropriate tube.

Add 1 volume of 2-3% EiC3b/C4b red cells to each tube. Mix thoroughly. Centrifuge the tubes. Determine the reaction grade.

2 Requirements.

The anti-human globulin reagent should attain or exceed the potency titre of the appropriate UK anti-C3 reference reagent.

6.5.5 Anti-C3d potency

1 Polyspecific anti-human globulin with IgM monoclonal anti-C3d.

(i) Method

Prepare a set of 5 tubes for each anti-human globulin under test and the UK anti-C3d reference reagent which is tested in parallel, at the dilution for the 'immediate test' stated in its accompanying package insert.

Place 2 volumes of each anti-human globulin dilution in each of the tubes (undiluted, that is as intended to be supplied for use, to 1 in 16).

Add 1 volume of the 2-3% EiC3d/EC4d red cells to each tube. Mix thoroughly and centrifuge the tubes.

Determine the reaction grade.

(ii) Controls

The EiC3d/EC4d cells do not react with anti-C3c, anti-C4c, anti-IgG, saline or anti-human globulin diluent using the direct anti-human globulin technique. They do react with anti-C3d.

(iii) Requirements.

The reagent should attain or exceed the potency titre of the UK anti-C3d reference reagent.

2 Conventional (polyclonal) anti-human globulin or anti-human globulin containing monoclonal IgG anti-C3d.

Polyspecific anti-human globulin reagents that achieve the requirements of 6.5.5.1.iii need not be tested by 6.5.5.2.

(i) Use the method described in 6.5.5.1 above but with an incubation period of 5 minutes at 19°-25°C and using the anti-C3d reference preparation at the dilution for the '5 minute test' stated in its accompanying package insert.

(ii) Requirements.

The reagent attains or exceeds the potency titre of the UK anti-C3d reference reagent.

Anti-human globulin reagents that do not achieve the requirements of 6.5.5.1.iii but which do achieve the requirements of 6.5.5.2.ii should state in the package insert that a 5 minute incubation period is required for the optimum detection of red cell bound C3d complement components.

6.6 Requirements for polyspecific anti-human globulin, anti-IgG and anti-complement for use in microplates

6.6.1 Tests for IgM and IgG red cell heterospecific antibodies.

Using the microplate methods recommended by the manufacturer of the anti-human globulin reagent, the requirements of 6.3.1.3 apply

6.6.2 Tests for unwanted positive reactions.

Using the microplate methods recommended by the manufacturer of the anti-human globulin reagent and the six red cell samples as described in 6.3.2.1 each red cell sample is incubated with one different fresh group-compatible serum. All tests should be macroscopically negative.

6.6.3 Tests for anti-IgG potency.

Using anti-D, anti-Fy^a and anti-K selected as described in 6.4.2.1, red cell samples selected and sensitised as described in 6.4.2.2 and 6.4.2.3, chequerboard titrations as described in 6.4.2.5 and 6.4.2.6 but performed using the microplate methods recommended by the manufacturer of the anti-human globulin reagent, the requirements of 6.4.2.7 applies.

6.6.4 Tests for anti-complement potency; polyspecific anti-human globulin reagents.

Using red cells coated with complement components as described in Annex 1 and the microplate methods recommended by the manufacturer of the anti-human globulin reagent with the controls descibed in 6.5.2.2, 6.5.3.2, the requirements of 6.5.2.3, 6.5.3.3, 6.5.4.2, 6.5.5.1.iii and 6.5.5.2.ii apply.

6.7 Package insert.

In addition to the requirements of Chapter 1, the package insert should include a statement that:

6.7.1 Inadequate washing of red cells in the anti-human globulin test may result in neutralization of the anti-human globulin reagent.

6.7.2 Following completion of the wash phase in the anti-human globulin test, excess residual saline may dilute the anti-human globulin reagent, when added, beyond that in the manufacturer's assessment.

6.7.3 No single test is capable of detecting all clinically significant antibodies.

Chapter 7

Serological Guidelines for Enzyme Reagents: Papain and Bromelin

7.1 Introduction

7.1.1 Proteolytic enzymes, predominantly papain or bromelin, are used in red cell serology to modify the red cell surface to enhance or destroy the reactivity of many red cell antigens as an adjunct to grouping, antibody screening or antibody identification procedures.

7.1.2 Purified bromelin does not require activation to achieve a high specific activity (enzyme activity per unit weight) whereas purified papain does require the presence of an activator (such as a cysteine or activated cysteine) which itself may be unstable, to achieve a high specific activity. However, papain can be formulated without such an activator if a high concentration of enzyme is used to achieve a satisfactory activity. The activity of bromelin and particularly papain does show a dependence on the pH of the reactants.

7.1.3 Enzyme preparations may be formulated with EDTA to chelate heavy metal ion contaminants which can inhibit activity. Enzyme preparations autolyse unless this is prevented by frozen storage at an appropriate temperature, by the presence of a displaceable temporary inhibitor or by freeze-drying.

7.2 Tests for unwanted positive reactions.

7.2.1 Method

Select integral segment lines from 2 packs of group A_1, 2 packs of group B, two packs of group O blood stored at 4°±2°C to within 5 days of their recommended expiry date.

Using recommended methods for use with the enzyme preparation, test each red cell sample with fresh, single donor, group-compatible serum, using a different serum for each red cell sample.

7.2.2 Requirements.

All reactions should be negative macroscopically.

7.3 Tests for potency

7.3.1 Papain and bromelin reagents; manual tube and microplate procedures.

1 Method

Each batch of enzyme reagent should potentiate, by all methods recommended for use, the reaction between IgG anti-D and group O R_1r red cells from three individuals. IgG anti-D should have a maximum concentration of 0.3 IU/ml which may be achieved by diluting a more potent anti-D with inert, group-compatible, serum. An antiserum with an anti-D content in excess of 10 IU/ml should not be used for this purpose.

2 Requirements

The enzyme reagent should effect, as a minimum and with all methods recommended for use, a grade 4 reaction with the three R_1r samples tested.

7.3.2 Papain and bromelin reagents for use in automated procedures

1 Method

The ability of each batch of enzyme reagent to potentiate the reaction between IgG anti-D and R_1r red cells from three individuals should be tested as follows.

Using the Automated procedure(s) recommended for use, test the three unpooled group O R_1r red cell samples with IgG anti-D at a concentration, when sampled by the automated system, of 0.5 IU/ml.

The IgG anti-D may be obtained by diluting a more potent anti-D with inert, group compatible, serum. An antiserum with an anti-D content in excess of 10 IU/ml should not be used for this purpose.

2 Requirements.

The enzyme reagent should effect with all methods recommended for use, the unequivocal detection of the reaction between the IgG anti-D and the three unpooled group O R_1r samples tested.

7.4 Package insert

The package insert should comply with the requirements in Chapter 1 and, in addition, should include the following.

7.4.1 A statement that microscopic reading of the results of enzyme tests is not recommended.

7.4.2 One-stage mix techniques in which enzyme, serum and red cells are mixed and incubated together are not recommended for use in the screening of patients' sera for atypical antibodies or in the compatibility testing of patients' sera with donors' red cells.

7.4.3 One stage procedures where there is:

a defined pre-incubation of cell suspension and enzyme followed by a defined incubation of the cell/enzyme mixture with serum, or,

a layer technique, in which serum is overlaid with enzyme that in turn is overlaid with the red cell sample in a 7mm (diameter) x 50mm glass tube, or,

an inhibitor technique, in which red cells are treated with enzyme that is inactivated before the subsequent addition of the serum to the mixture,

may be recommended for use in the screening of patients' sera for atypical antibodies or in compatibility tests

7.4.4 A list of those red cell antigens that can stimulate clinically significant antibodies (*see 4.10.3.2.i*), whose reactivity is removed or measurably diminished by the methods recommended for use of the product.

7.4.5 A statement that enzyme tests do not detect all antibodies of probable clinical significance.

7.4.6 A statement that care should be taken to maintain sterility of enzyme preparations since they readily become contaminated with micro-organisms which can result in false negative or false positive reactions.

7.4.7 If stored frozen prior to use, a statement that on thawing the reagent is to be stored at 4±2°C and is to be discarded within 24 hours of thawing.

7.4.8 If stored as a liquid prior to use in the absence of any preservative, a statement that the reagent is to be discarded within 24 hours of opening the container when stored as recommended.

7.4.9 If stored as a liquid prior to use with a preservative shown to be effective against common contaminants, a statement that the reagent is to be discarded within 48 hours of opening when stored as recommended.

7.4.10 Where the method(s) recommended for use involve the pre-treatment of red cells prior to incubation with serum, the recommended method of pre-treatment should be described in addition to the recommended method(s) of use of the treated red cells.

7.4.11 A statement that deviation from the recommended methods of use may result in false positive or false negative results. This includes very slight changes in buffers or in solutions which may result in sub-optimal pH for enzyme treatment.

7.4.12 A statement that procedures involving enzymes should include procedures to ensure the adequate enzyme-treatment of red cells. For example, in the use of enzyme-treated rr red cells for antibody screening procedures, the use of anti-$\bar{c}$ known to react weakly with adequately treated red cells would be appropriate.

7.4.13 A statement that no single test is capable of detecting all clinically significant antibodies.

Chapter 8

Guidelines for Bovine Serum Albumin

8.1 Introduction

Bovine serum albumin (BSA) has various applications in blood group serology. It is generally provided as a 300g/L solution. Some preparations may be deliberately polymerised or contain additives to enhance the potentiating properties. It is used to:

- potentiate the reactions of blood group antibodies that do not directly agglutinate red cells suspended in saline or LISS. For this purpose, it is generally added at a concentration of 200 g/L (20% w/v);

-potentiate the haemagglutination reaction in automated blood grouping equipment;

-potentiate the haemagglutination reaction in microplate test procedures;

-stabilise blood grouping reagents, in which its final concentration is generally less than 80g/L.

8.2 General considerations.

8.2.1 Where BSA is used in the formulation of a reagent control, it is essential that the reagent control is formulated in the same way as the blood grouping reagent it is to control, but without the specific blood group reactivity of the reagent.

8.2.2 Albumin sources other than bovine may be used provided the requirements of this chapter are satisfied.

8.2.3 BSA should be obtained from animals taken from a closed herd in the female line since 1980, in which no animal has been clinically suspected of having bovine spongiform encephelopathy, and which has not been fed rations containing ruminant derived protein during that period.

8.3 Tests for potentiating ability.

An albumin previously found to be satisfactory should be selected as a reference albumin. Neither this nor the test albumin should be deliberately further polymerised or potentiated.

8.3.1 Selection of test antibodies.

Examples should be selected from IgG antibodies with the following specificities and indirect antiglobulin potency titres when tested with red cells from a pool of 4, group O R_1r individuals.

Specificity Potency titre

Anti-D 1 in 64 to 1 in 128
Anti-D 1 in 8 to 1 in 16
Anti-$\bar{c}$ 1 in 16 to 1 in 32

Unless the albumin is intended to be used as provided, dilute the test albumin reagent to 200g/L in saline.

8.3.2 Method

Prepare doubling dilutions of the antibodies in saline (not containing BSA) to two dilutions beyond their potency titre.

Prepare a 2-3% suspension of washed pooled group 0 R_1r red cells in saline.

Each antibody preparation and its dilutions is tested in parallel using the test and reference albumin and saline, in place of albumin, using the method described below.

Those albumin preparations recommended for use by methods other than albumin displacement should be tested using the method recommended for use.

Three sets of tubes are required, add one volume of each dilution of the reagent to a separate tube.

Add one volume of the test cell suspension to each tube.

Mix thoroughly and incubate for 45 minutes at 37°C.

Add one volume of each of the test albumin, reference albumin or saline to each tube in the appropriate row and allow it to run down the inner wall of the tube. The albumin will displace the saline and lie over the sedimented red cells. Do not mix.

Incubate for 15 minutes at 37°C.

Determine the reaction grade.

8.3.3 Requirements.

The albumin reagent is satisfactory if:

1 the potency titre is equal to or attains that of a previously satisfactory albumin preparation.

2 with each dilution and the undiluted antisera, the reaction grades using the test albumin are at least equal to those with a previously satisfactory albumin preparation.

3 there is no significant prozone.

8.3.4 Albumin preparations recommended for use in AutoAnalyzers.

Albumin preparations recommended for use in AutoAnalyzers, should be tested to ensure their suitability for use by that procedure. The testing should include comparative quantitation of the UK anti-D Working Standard for AutoAnalyzers with the test albumin preparations.

8.4 Tests for unwanted positive reactions.

Albumin preparations which contain low concentrations of fatty acids in the presence of significant amounts of polymerised albumin (for example, more than 7 percent of the albumin as trimer or more than 8 percent of the albumin as oligomer), are likely to cause unwanted positive reactions.

If an albumin preparation is used as a component in a reagent formulation, manufacturers should test the albumin at the final concentration to be used to ensure the absence of unwanted positive reactions, haemolysis and rouleaux.

8.5 Tests for IgG protein

The absence of IgG protein is particularly important if the albumin preparation is used to stabilise anti-human globulin reagents. Standard electrophoretic or nephelometric assays may be used in addition to the following serological assessment.

8.5.1 Method

Prepare red cells sensitised with a weak IgG anti-D, see 6.4.2. Prepare doubling dilutions of polyspecific anti-human globulin reagent from undiluted to 1 in 32 using saline containing no BSA.

Prepare 3 sets of 6 tubes. Add one volume of the undiluted anti-human globulin to the first tube in each of the 3 sets. Add 1 volume of the anti-human globulin reagent diluted 1 in 2 to the second tube of each set, and so on until adding 1 volume of the anti-human globulin reagent diluted 1 in 32 to the sixth tube of each set.

To the tubes of the first set, add 1 volume of the undiluted test albumin. To the second set, add 1 volume of saline. To the third set, add 1 volume of pooled normal human serum diluted 1 in 20 in saline containing no BSA.

Add 1 volume of the 2-3% suspension of red cells weakly sensitised with IgG anti-D.

Mix and centrifuge the tubes. Determine the reaction grade.

8.5.2 Requirements.

The potency titre with the first set of tubes should be at least equal to that with the second set of tubes. The potency titre of the third set of tubes should be clearly reduced to indicate inhibition of the anti-human IgG by the diluted human serum.

8.6 Tests for blood group substances.

Albumin solutions may contain substances similar to human blood group substances A or B. The absence of such activity is important if the albumin is to be used to stabilise ABO blood grouping reagents. The presence of substances similar to other soluble blood group antigens should be excluded if the albumin is used to stabilise other blood grouping reagents, for example, anti-Le^a or anti-Le^b.

8.6.1 Method.

Example for anti-A blood grouping reagents.

Prepare doubling dilutions of anti-A blood grouping reagent, in saline not containing BSA.

Prepare three sets of tubes. Add one volume of the undiluted blood grouping reagent to the first tube in each of the 3 sets. Add 1 volume of the blood grouping reagent diluted 1 in 2 to the second tube of each set, and so on.

To the tubes of the first set, add 1 volume of the test albumin as intended to be supplied for use. To the second set of tubes, add 1 volume of saline containing no BSA. To the third set of tubes, add one volume of group A blood group substance at a concentration known to be inhibitory in this procedure.

Mix and add 1 volume of a 2-3% suspension of group A_2B red cells in saline containing no BSA. For other reagents, red cells with a weak expression of the corresponding antigen are used. For example, for anti-B, A_1B red cells should be used.

Mix and incubate at 19-25°C for 15 minutes. For other reagents, use the temperature and for the time appropriate for that reagent.

Centrifuge. For other reagents, centrifuge if appropriate for that reagent.

Determine the reaction grade.

8.6.2 Requirements.

The potency titre with the first set of tubes should be at least equal to that with the second set of tubes. The potency titre of the third set of tubes should be clearly reduced to indicate inhibition of the antibody by the blood group substance.

8.7 Tests for freedom from neuraminidase-like activity

Albumin preparations may contain neuraminidase-like substances capable of exposing the red cell cryptantigen T. Anti-T is a naturally occurring antibody found in human sera.

8.7.1 Method.

Obtain integral segment lines from 3 packs of group 0 CPDA-1 blood stored at 4±2°C to within 5 days of their recommended expiry date. Wash each red cell sample 4 times with saline and make a 3% suspension in saline (containing no BSA).

Prepare two sets of 3 tubes. To the tubes of one set add lml of test albumin preparation. To the tubes of the second set add 1ml of saline.

Add 1ml of the 2-3% suspension of the first cell sample to the first tube of each set, of the second cell sample to the second tube of each set, and of the third cell sample to the third tube of each set.

Mix and incubate for 3 hours at 37°C. Wash the cells four times in saline (containing no BSA) and resuspend to a 2-3% suspension in saline.

Prepare doubling dilutions of anti-T reagent in saline containing no BSA, from undiluted to 1 in 8.

Test each red cell suspension with each dilution of anti-T reagent using the method described below. Use a 2-3% suspension of neuraminidase-treated red cells as a control of the anti-T reagent dilutions.

Add one volume of each dilution of the reagent to a separate tube.

Add one volume of the test cell suspension to each tube.

Mix thoroughly and centrifuge the tubes immediately.

Determine the reaction grade.

8.7.2 Requirements.

The albumin reagent is satisfactory if no agglutination is observed with the untreated red cells samples.

8.8 Tests for inhibitors of erythrocyte agglutination.

Although tests for the potentiating ability of an albumin preparation will detect the presence of an inhibitor of erythrocyte agglutination, this phenomenon is more pronounced in automated systems, particularly where bromelin is incorporated as an added reagent rather than used to pretreat the red cells.

8.8.1 Method

A previously accepted preparation of albumin and the test albumin should be tested in parallel in the automated system.

Assess the British Working Standard anti-D for AutoAnalyzer use or other reference anti-D preparation.

Only the albumin preparations under test are to be present within the automated system.

8.8.2 Requirements.

The albumin preparation is satisfactory if its performance is at least as good as the acceptable preparation. Attention is paid to the performance of the automated system when no antibody is being assessed as well as when antibody is being assessed.

8.9 Immediate container label

In addition to the guidelines in 1.6 the immediate label should contain the following.

8.9.1 A statement that the albumin is for use in blood group serology.

8.9.2 The concentration of the albumin.

8.9.3 Whether the albumin has been deliberately polymerised.

8.9.4 Whether any other potentiators of haemagglutination have been added.

8.9.5 If the albumin is to be diluted prior to use, the statement 'dilute for use'. If no dilution is required, the statement 'do not dilute'.

8.10 Package insert

In addition to the guidelines in 1.7, the package insert should contain the following.

8.10.1 The concentration of trimeric and oligomeric albumin expressed as a percentage of the total albumin.

8.10.2 A statement that agglutinins to albumin are found in a small proportion of serum samples.

8.10.3 A statement that the efficacy of albumin reagents is to be controlled throughout their use.

8.10.4 A statement that albumin preparations are not to be used as a negative control for potentiated IgG blood grouping reagents.

8.10.5 If the albumin is to be diluted by the user prior to use, the recommended dilution, diluent, method of dilution and the concentration of albumin produced by the dilution; together with the storage conditions and usable period of the diluted albumin.

8.10.6 The osmolality of the albumin preparation as recommended for use.

8.10.7 A statement that the BSA has been obtained from a closed herd in the female line since 1980, in which no animal has been clinically suspected of having bovine spongiform encephelapathy, and which has not been fed rations containing ruminant derived protein during that period.

Chapter 9

Serological Guidelines for Blood Grouping Reagents of Specificity Other than ABO and RhD

9.1 Introduction

This chapter gives specific guidelines on the selection of grouping reagents of specificity other than ABO and RhD, additional to the general guidelines given in Chapter 2.

9.2 Anti-C; anti-c̄; anti-E; anti-e; anti-CDE and anti-C^w

9.2.1 Specificity

1 Method

In addition to the guidelines in 2.9, as a minimum, the antisera should be tested with test red cells of the following Rh genotypes (or presumptive genotypes) by all the methods recommended for use by the manufacturer. Numbers in parentheses represent the minimum number of each presumptive genotype to be tested.

For anti-C (or anti-C^w, or anti-C^x) blood grouping reagents, the package insert should specify the reactivity of the reagent against C^w and C^x (or C and C^x, or C and C^w) postive red cells by the recommended methods of use.

See table:

Antibody Specificity	Positive Reactors		Negative Reactors	
Anti-C	R_1R_2 R_2R_z or r^yr r'r r'^sr	(2) (1) (1) (1)	R_2R_2 r''r'' rr	(1) (1) (1)
In addition the reagent should be tested with the following positive reactors: one $R_1{}^xr$ or $R_1{}^xR_2$, when reactivity with C^x red cells is claimed one $R_1{}^wr$ or $R_1{}^wR_2$ or r'^wr, when reactivity against C^w is claimed				
Anti-c̄	R_1R_2 R_1r r'r	(1) (2) (1)	R_1R_1 R_1R_z	(1) (2)
Anti-E	R_1R_2 R_1R_z r''r	(2) (1) (1)	R_1R_1 r'r rr	(1) (1) (1)
Anti-e	R_2r R_1R_2 r''r	(2) (2) (1)	R_2R_2 R_2R_z	(1) (2)
Anti-CDE	R_or or R_oR_o r'r r''r	(2) (2) (2)	rr	(1)
Anti-C^w	$R_1{}^wr$ or $R_1{}^wR_2$ r'^wr	(2) (1)	R_1R_1 r'r rr	(1) (1) (1)

Red cells of Rh genotype R_zr or r^yr may be used in place of R_2R_z, red cells in the testing of anti-C or in place of R_1R_z, red cells in the testing of anti-E.

Red cells of Rh genotype R_zR_z or r^yr^y may be used in place of R_1R_z red cells in the testing of anti-$\bar{c}$ or in place of R_2R_z red cells in the testing of anti-e.

2 Requirements.

(i) The reagent should produce a reaction grade of not less than 4 when tested with any red cell sample expressing the corresponding antigen, including those listed at 9.2.1 by any method recommended for use by the manufacturer.

(ii) A blended reagent comprising anti-D with anti-C and/or anti-E activity, recommended for the detection of G antigen, should produce a reaction grade of not less than 4 when tested with red cells of the presumptive Rh genotype r^Gr or, for a blood grouping reagent not containing anti-E, r''^Gr by any method of use recommended by the manufacturer for the detection of G antigen.

9.2.2 Potency testing

1 Method

(i) As a minimum, the manufacturer should determine the potency of the test reagent with red cells of the following presumptive Rh genotypes. Numbers in parentheses represent the minimum number of each presumptive genotype to be tested.

(ii) The methods described in 2.10 which correspond to those recommended by the manufacturer should be used.

Anti-C	R_1R_2 r'r	(2) (1)
Anti-$\bar{c}$	R_1R_2 R_1r	(2) (2)
Anti-E	R_1R_2 r''r	(2) (1)
Anti-e	R_1R_2 R_2r	(2) (2)
Anti-CDE	R_or or R_oR_o r'r r''r	(2) (2) (2)
Anti-C^w	$R_1{}^wr$ or $R_1{}^wR_2$ or r'^wr	(2)

2 Requirements

(i) Blood grouping reagents recommended for use with red cells suspended in saline or LISS by tube or slide direct agglutination technique.

The reagent should produce with all red cell samples tested, as a minimum, grade 4 agglutination when undiluted and grade 2 agglutination when diluted 1 in 4

(ii) Blood grouping reagents recommended for use with other techniques.

Reagents recommended for use by a tube or slide technique should produce with all red cell samples tested by the methods of use recommended by the

manufacturer, as a minimum, grade 4 agglutination when undiluted and grade 2 agglutination when diluted 1 in 4.

9.2.3 Avidity testing

1 Method

The avidity of reagents recommended for use by slide techniques should be determined, using the method described in 2.11.

2 Requirements

Agglutination should be grade 3 within 1 minute of mixing.

9.3 Anti-K; anti-$\overline{k}$; anti-Fy^a; anti-Fy^b; anti-Jk^a; anti-Jk^b; anti-S and anti-$\overline{s}$.

9.3.1 Specificity

1 Method

(i) In addition to the guidelines in 2.9, as a minimum the manufacturer should test the blood grouping reagent with red cells from a minimum of 4 individuals known to exhibit heterozygous (or weak) expression of the antigen corresponding to the antibody under test, by all methods of use recommended by the manufacturer.

(ii) Anti-$\overline{k}$ reagents should additionally be tested by all methods of use recommended by the manufacturer with red cells from a minimum of 2 individuals with an heterozygous expression of K, $\overline{k}$ and Kp^a antigens.

2 Requirements

(i) Anti-K, anti-Fy^a, anti-Fy^b, anti-Jk^a, anti-Jk^b, anti-$\overline{s}$ and anti-S should produce a reaction grade of not less than 4 when tested by any method of use recommended by the manufacturer with any red cell sample expressing the corresponding antigen, including those listed at 9.3.1.1.i.

(ii) Anti-$\overline{k}$ should produce, when tested by any method of use recommended by the manufacturer, a reaction grade of not less than 4 with red cells from 2 K $\overline{k}$ Kp(a-) individuals and not less than 3 with red cells from 2 K $\overline{k}$ Kp(a+) individuals.

9.3.2 Potency

1 Method

The methods described in 2.10 corresponding to the methods recommended for use by the manufacturer should be used, using red cells from a minimum of two individuals known to exhibit heterozygous expression of the antigen corresponding to the antibody under test.

2 Requirements

(i) Anti-K, anti-Fy^a and anti-S should produce, as a minimum and with all red cell samples tested, grade 4 agglutination when undiluted and grade 2 agglutination when diluted 1 in 8.

(ii) Anti-$\overline{k}$, anti-Fy^b, anti-Jk^a, anti-Jk^b and anti-$\overline{s}$ should produce with all red cell samples tested, as a minimum, grade 4 agglutination when undiluted and grade 2 agglutination when diluted 1 in 4.

9.4 Anti-A_1; anti-M; anti-N; anti-Le^a and anti-Le^b.

9.4.1 Specificity testing

1 Method

(i) In addition to the guidelines in 2.9, as a minimum the blood grouping reagents should be tested by all methods of use recommended by the manufacturer with red cells from 4 individuals known to express the antigen under test, where relevant, in the heterozygous form.

(ii) Anti-A_1 blood grouping reagents should be tested with red cells from a minimum of 2 individuals each of blood groups A_1; A_2; A_1B; A_2B; B and O by all methods of use recommended by the manufacturer.

(iii) Anti-M blood grouping reagents should be tested with red cells from a minimum of 3 individuals of phenotype NN by all methods of use recommended by the manufacturer.

(iv) Anti-N blood grouping reagents should be tested with red cells from a minimum of 3 individuals of phenotype MMS by all methods of use recommended by the manufacturer.

(v) Anti-Le^{bL} blood grouping reagents should be tested with red cells from a minimum of 3 A_1 Le(a- b+) individuals by all methods recommended for use by the manufacturer.

2 Requirements

(i) The blood grouping reagent should produce a reaction grade of not less than 4 when tested by any method of use recommended by the manufacturer with any red cell sample expressing the corresponding antigen, including those listed at 9.4.1.1.

(ii) Anti-M and anti-N should not agglutinate red cells of phenotype NN and MMS, respectively, by any method of use recommended by the manufacturer.

9.4.2 Potency

1 Method

The methods described in 2.10 corresponding to the methods recommended for use by the manufacturer should be used, using red cells from a minimum of 2 individuals known, where relevant, to exhibit heterozygous expression of the antigen corresponding to the antibody under test.

2 Requirements

The blood grouping reagents should produce, as a minimum and with all red cell samples tested, grade 4 agglutination when undiluted and grade 2 agglutination when diluted 1 in 4.

9.5 Anti-P_1

9.5.1 Specificity testing

1 Method

In addition to the guidelines in 2.9, as a minimum the blood grouping reagent should be tested by all methods of use recommended by the manufacturer with red cells from 4 individuals known to express the P_1 antigen in the strong form and from 4 individuals known to express the P_1 antigen in the weakened form.

2 Requirements

(i) The blood grouping reagent should produce a reaction grade of not less than 4 when tested by any method of use recommended by the manufacturer with any red cell sample expressing the P_1 antigen in the strong form.

(ii) The blood grouping reagent should produce an unequivocal positive reaction when tested by any method of use recommended by the manufacturer with any red cell sample expressing the P_1 antigen in the weakened form.

9.5.2 Potency

1 Method

The methods described in 2.10 corresponding to the methods recommended for use by the manufacturer should be used, using red cells from a minimum of 2 individuals known to express the P_1 antigen in the strong form and a minimum of 2 individuals known to express the P_1 antigen in the weakened form.

2 Requirements

(i) The blood grouping reagents should produce, as a minimum and with all red cell samples tested having a strong expression of the P_1 antigen, a grade 4 agglutination when undiluted and grade 2 agglutination when diluted 1 in 4.

(ii) The blood grouping reagent should produce, as a minimum, an unequivocal positive reaction with all red cell samples tested having a weakened expression of the P_1 antigen.

9.6 Blood grouping reagents of other specificities

9.6.1 Specificity and potency

1 Method

Blood grouping reagents of specificity not mentioned in these guidelines should be tested for potency and specificity as described in 2.10 using the methods corresponding to those recommended for use by the manufacturer.

2 Requirements

(i) These reagents should produce a reaction grade of not less than 3 with all red cell samples tested which express the corresponding antigen and by all methods of use recommended by the manufacturer, using red cells from a minimum of 2 individuals known, where relevant, to exhibit heterozygous or weakened expression of the antigen specific to the antibody under test.

(ii) In some circumstances, it may not be possible to ensure that blood grouping reagents of a rare specificity are tested as required by these Guidelines. In such instances, the package insert should contain a statement to this effect.

Chapter 10

Guidelines for Reagents used in Automated Systems

10.1 Introduction

In automated systems, blood grouping reagents produce optimal reactions over a narrower range of dilutions than reagents characterised for manual use, e.g. anti-A reagent used undiluted may react too strongly with A_1 cells causing the instrument to misread the result. However, the same anti-A diluted 1 in 2 may fail to react reliably with A_2B cells.

10.2 Specificity

10.2.1 Method

1 The specificity of the undiluted reagent should be assessed by preparing red cells as described in Annex 2 and testing these with the candidate automated system reagents by the 5 minute incubation agglutination technique.

2 Reagents to be used at 19°C-25°C should be pre-warmed and tested at that temperature. Reagents to be used at 37°C should be pre-warmed and tested at that temperature.

3 For the selection of red cells to be tested see 2.9.1.4. In addition, for ABO and Rh D blood grouping reagents see 3.2 and 3.5. For other Rh specific blood grouping reagents see 9.2.1. For blood grouping reagents of specificity other than ABO and Rh, *see Chapter 9, 9.3 and 9.4*

10.2.2 Requirements.

1 Blood grouping reagents intended for use in automated systems should not produce a positive reaction when tested with red cells lacking the antigen corresponding to the antibody specificity under test by the procedure described in 10.2.1.1 (*see also 2.9*).

In addition:

- for ABO blood grouping reagents *see 3.2.2.1 to 3.2.2.4.*
- for Rh D blood grouping reagents *see 3.5.2.1 and 3.5.2.3.*
- for other Rh specific blood grouping reagents *see 9.2.1.2.*
- for blood grouping reagents of specificity other than ABO and Rh, *see Chapter 9, 9.3 and 9.4.*

2 Reagents of specificity other than ABO or Rh should be tested with red cell samples from at least 4 individuals known to lack the antigen corresponding to the antibody under test.

10.3 Selection of working dilution

The optimum dilution of the reagent is that which maximises the correct detection whilst minimising the false reading rate due to either excessively dilute or potent reagent.

The optimum dilution for the reagent should be determined by the following procedure.

10.3.1 Method

1 Using the diluent recommended by the manufacturer of the automated system, prepare a series of dilutions of the candidate reagent, e.g. 1 in 10, 1 in 20, 1 in 30, 1 in 40.

2 (i) For ABO and Rh reagents, using the method recommended by the manufacturer of the automated system, test each of these reagent dilutions with the following numbers of red cell samples.

	A_1	A_2	A_1B	A_2B	B	A_x
Anti-A	3			3		
Anti-B			3		3	
Anti-A+B	2	2			3	
Anti-A,B	2	2			3	3
	R_1r	R_2r	R_o	r'r	r"r	
Anti-D	3		2			
Anti-C	3			3		
Anti-$\bar{c}$	3			2		
Anti-E		3			3	
Anti-e		3			2	
Anti-C+D	2		2	2		
Anti-D+E		2	2		2	
Anti-CDE			2	2	2	

(ii) Reagents of specificity other than ABO or Rh should be tested with red cells from a minimum of 2 individuals known, where relevant, to exhibit heterozygous or weakened expression of the antigen corresponding to the antibody under test.

3 That reagent dilution which provides optimal results with the test red cells should be selected for repeat testing. In addition, dilutions 25% less and 25% greater than that initially selected as optimal should be tested. For example, for a reagent which initially produced optimal results at a dilution of 1 in 20, repeat testing should be performed with the reagent diluted 1 in 15, 1 in 20 and 1 in 25.

4 Using the method recommended by the manufacturer of the automated system, test each of the 3 reagent dilutions with the following numbers of red cell samples.

	A_1	B	0
Anti-A	0	3	3
Anti-B	3	0	3
Anti-A+B	0	0	5
Anti-A,B	0	0	5
	rr	r'r	r"
Anti-D	2	2	2
Anti-C	2	0	2
Anti-E	2	2	0
Anti-C+D	2	0	2
Anti-D+E	2	2	0
Anti-CDE	2	0	0

10.3.2 Requirements.

The dilutions prepared or recommended by the manufacturer of reagents for use in automated systems should produce, qualitatively, the same result at dilutions ±25% of the optimal dilution.

Chapter 11

Guidelines for HLA Typing Reagents

11.1 General Guidelines

11.1.1 Nomenclature

The nomenclature for HLA antigens and corresponding antibodies should conform to that of the World Health Organisation (WHO) Committee on HLA nomenclature.

11.1.2 Human materials

1 HLA serological reagents should comply with the guidelines in 1.5.

2 Exceptionally, reagents not tested at source as required in 1.5, and for which no alternative exists may be supplied for use with the expressed approval of the user and with the understanding that the reagent must be regarded as potentially infectious (HN(86)25).

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

The package insert of these reagents should indicate that the reagent(s) has not been tested at source as required in 1.5, and that the reagents are to be considered as potentially infectious. In addition the package insert should give information on the safe disposal of the material and the container, multi-well tray or reservoir.

11.1.3 Immediate container label

1 HLA typing reagents issued separately

The minimum labelling requirement for HLA typing reagents issued separately in individual immediate containers is the reagent specificity, the batch (or sub-batch) reference and the name or unequivocal logo of the manufacturer or distributor.

2 Multi-well tray label

The minimum labelling requirement for multi-well trays or reservoirs is a unique designation on the reservoir or body (not the lid) of the tray from which the HLA locus (or loci), for which the HLA typing set is intended, can be identified, the batch (or sub-batch) reference and the name or unequivocal logo of the manufacturer or distributor.

If the HLA typing set contains more than one tray or reservoir, the tray or reservoir number is included in the requirement.

11.1.4 Package insert

1 In addition to the information required by 1.6.3.2 to 1.6.3.15 and the requirements of 1.7.1 to 1.7.2.2, 1.7.2.4 or 11.1.2.2, 1.7.2.5 to 1.7.2.16, 1.7.2.25 and 1.7.2.26 the accompanying package insert should include the following information on each individual HLA typing reagent or HLA typing set.

2 The claimed HLA specificity(ies) of the reagent, the percentage of specific reactions giving a cytotoxicity score of 80% to 100% cell death (termed %8), the values of the correlation coefficient (r) obtained by the pre-testing of the reagent against a well-characterised cell panel, and the reaction score. The reaction score is expressed as:

++ the number of positive serum reactions against lymphocyte samples bearing the antigen(s) corresponding to the claimed antibody specificity(s),

+- the number of positive serum reactions against lymphocyte samples not bearing the antigen(s) corresponding to the claimed antibody specificity,

-+ the number of negative serum reactions against lymphocyte samples bearing the antigen(s) corresponding to the claimed antibody specificity,

-- the number of negative serum reactions against lymphocyte samples not bearing the antigen(s) corresponding to the claimed antibody specificity.

For the purpose of this section, a negative reaction is defined as a cytotoxicity score less than 20% above background; a positive reaction is defined as a cytotoxicity score greater than 60% above background. Cytotoxicity scores of 20% to 60% above background are termed equivocal.

The manufacturer should provide information of the incidence of equivocal cytotoxicity scores within the package insert.

3 For HLA typing sets the claimed specificities may take the form of a listing of the details required in 11.1.4.2 of the individual sera comprising the set.

HLA typing sets should include a map or diagram of the multi-well tray or reservoir layout indicating the position, HLA specificity(ies) and batch (or sub-batch) reference of the HLA typing reagent contained in each well.

4 Monoclonal antibodies should be identified to alert the user to the possibility of false positive reactions caused by the carry over of traces of a potent reagent into adjacent wells.

5 An instruction that thawing and re-freezing of the HLA typing reagents should be kept to the absolute minimum from the date of manufacture to the date of use. HLA typing sera frozen in micro-well trays should be used within 1 hour of thawing. Sera supplied freeze dried in micro-well trays should be used within 1 hour of their re-constitution; unused trays should not be re-frozen for later use.

6 In addition to the above, to avoid delay when HLA serological reagents are freely exchanged as a gift between different countries of the European Community, each consignment is to be accompanied by a customs certificate as specified in the annex of the European Agreement on the exchange of tissue typing reagents, revised Protocol no. 84.

7 When reagents are supplied as a HLA typing set for the detection of a single antigen (e.g. HLA B27), the package insert should indicate which controls are appropriate to demonstrate specificity and cross-reactivity.

11.1.5 Preservation

1 HLA typing reagents may be preserved in the liquid or in the dried state. Human serum reagents should be kept at a temperature not above -30°C. Freeze-dried reagents and monoclonal reagents may be stored at 4 ± 2°C.

2 Freeze-dried reagents should be kept in an atmosphere of inert gas or in vacuo in the container in which they were dried and which should be closed or sealed to exclude moisture.

3 HLA typing reagents, after being thawed or reconstituted should be transparent and should not contain any sediment, gel or particles visible on microscopy (x200).

11.1.6 Stability and expiry date

1 HLA typing reagents, when despatched and stored as recommended by the manufacturer, should retain the requisite properties for at least one year.

2 The expiry date of an HLA typing reagent issued in an individual immediate container should be given in the package insert.

3 The manufacturer may permit an extension of the expiry date for further periods of one year, of an HLA typing reagent issued in an individual immediate container, or of an HLA typing set issued in the liquid state in a multi-well tray or reservoir, dependant on the user undertaking relevant tests. Full details of the tests to be performed and of the minimum performance for an extension to the expiry date to be valid, should be

included in the package insert. The user should maintain full records of the tests performed to extend the expiry date, and their results.

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

11.2 Serological requirements

11.2.1 Specificity guidelines for HLA typing reagents to be used in typing sets for cytotoxicity techniques with lymphocytes.

1 HLA typing reagents should, when used by all methods recommended by the producer, react with all lymphocyte samples with the corresponding antigen(s) when tested against a panel of lymphocyte samples bearing those antigen(s) collected from at least 25 individuals.

2 HLA typing reagents should not react with any lymphocyte samples when tested against a panel of lymphocyte samples known not to bear the corresponding antigen(s) collected from at least 100 individuals.

3 Two reagents each complying with the requirements of 11.2.1.1 and 11.2.1.2 and prepared from different donors, should be used as individual HLA typing reagents for a given antigen.

4 For the HLA specificities listed below, if two reagents do not each satisfy the conditions of 11.2.1.1 and 11.2.1.2, at least three HLA typing reagents, prepared from different donors, should be used as individual HLA typing reagents for that antigen.

HLA-A 1,2,3,9,10,11,29.

HLA-B 5,7,8,12,13,14,15,16,17,18,21,22,27,40

HLA-DR 1,2,3,4,5,7

5 If a combination of three or more HLA typing reagents is used to detect an HLA antigen all of them should have been shown to react with at least 80% of samples bearing the antigen in question.

6 Not more than half of the HLA typing reagents used in combination to detect an antigen should have been shown to react with the same additional HLA specificity.

7 None of the HLA typing reagents used in combination should have been shown to react with more than 5% of the separate samples of a lymphocyte panel which do not express any of the antigen(s) which the reagent is claimed to detect.

8 Rarer specificities: whenever possible, at least two antisera should be employed to define antigens not listed in 11.2.1.4. The use of several multispecific sera is permissible.

11.3 Rabbit complement for use in HLA serology

11.3.1 General guidelines

1 Rabbit complement supplied for use in HLA serology should be collected from healthy rabbits.

2 Rabbit complement should be dispensed into immediate containers in a volume convenient for a small batch of HLA tests (1ml-5ml).

3 If supplied in the liquid state, rabbit complement should be stored at a temperature below -70°C. If freeze-dried, it may be stored at 4 ± 2°C.

4 Rabbit complement after being thawed or reconstituted should not be turbid and should not contain any sediment, gel or particulate matter visible on microscopy (x200).

11.3.2 Immediate container label

1 The label of the immediate container of rabbit complement for use in HLA serology should conform to the specifications in 1.6.

11.3.3 Package insert

1 The package insert supplied with rabbit complement for use in HLA serology should conform to the specifications in 1.7.

2 The package insert should contain an instruction on the method of thawing, for example, 'Thaw rapidly at 37°C with gentle agitation until a vestige of ice remains.'. In addition, the package insert should contain an instruction that the complement, once thawed from the immediate container or reconstituted from the freeze-dried state, should not be re-frozen.

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

11.3.4 Potency tests on rabbit complement for use in HLA serology

1 Rabbit complement for HLA class I serology

The following UK reference reagents are available for use in assessing rabbit complement for HLA class I serology:

Rabbit complement (HLA class I serology),
Anti HLA-A2 serum.

(i) Method

Manufacturers should determine the potency titre of the UK reference reagent for anti-HLA-A2 employing the UK reference reagent for rabbit complement (HLA class I serology) in tests against a normal lymphocyte preparation known to be positive for HLA-A2, and test their own rabbit complement for use in HLA class I serology, in parallel as detailed in these guidelines.

Each batch of rabbit complement recommended for use in HLA class I serology should be tested by the following procedure.

Reconstitute the UK reference reagent for anti-HLA-A2 in 0.5ml water, see 1.3.3.

Prepare successive dilutions of the UK reference reagent for anti-HLA-A2 of Undiluted (N), 1 in 2, 1 in 3, 1 in 4, 1 in 8, 1 in 16 and 1 in 32 in TC199 or similar tissue culture medium supplemented with 200ml/L of inactivated foetal bovine serum.

Row	Dilution of UK reference reagent for anti-HLA A2						
	N	1 in 2	1 in 3	1 in 4	1 in 8	1 in 16	1 in 32
	col1	col2	col3	col4	col5	col6	col7
Ref row 1 row 2 row 3							
Test row 4 row 5 row 6							

Add 1µl of each dilution to six wells of an oiled microwell tray as shown above.

Add 1µl of a suspension of 1-2 x 10^6 ml^{-1} of normal peripheral lymphocytes, separated from an individual known to possess the HLA-A2 antigen, to all the wells taking care to avoid carry-over.

Incubate at 20°-22°C for 30 min.

Five minutes before the complement is required to be added, reconstitute the UK reference reagent for rabbit complement (HLA class I serology) as described in the corresponding package insert. Thaw or reconstitute the complement for HLA class I serology under test. Keep on melting ice until required.

Add 5µl of UK reference reagent for rabbit complement (HLA class I serology) to each well in rows 1 to 3 and 5 µl of test complement for class I serology to each well in rows 4 to 6, avoiding carry-over at all times. Re-incubate at 20°-22°C for 60 minutes.

Stain the dead cells with eosin. After staining, fix with formalin solution for 3 minutes, apply coverglass, examine with xl0 eyepiece and x20 phase contrast optics.

Alternatively, flick off the excess serum and stain with trypan blue, examine immediately with xl0 eyepiece and x20 objective.

A technique using single or double fluorescence to assess cell death is also acceptable.

Record percentage cell death in each well. Compare the degree of cell death observed in the 3 replicate tests of each dilution of the UK reference reagent for anti-HLA-A2 obtained with the UK reference reagent for rabbit complement (HLA class I serology) and the complement for HLA class I serology under test.

(ii) Requirements

Rabbit complement for use in HLA class I serology should enable all dilutions of the UK reference reagent of anti HLA-A2 to attain or exceed the percentage cell death obtained with the UK reference reagent for rabbit complement (HLA class I serology).

2 Rabbit complement for HLA class II serology

The following UK reference reagents are being prepared for use in assessing rabbit complement for HLA class II serology:

Rabbit complement for HLA class II serology,
Anti-B lymphocyte (monoclonal) preparation.

11.3.5 Toxicity tests on rabbit complement for use in HLA serology.

1 Rabbit complement for HLA class I serology.

(i) Rabbit complement recommended for use in HLA class I serology should not cause cell death in the absence of HLA antibody when tested against serum collected from 5 non-transfused male volunteers using samples of unfractionated lymphocytes from 10 random donors in the standard one and one half hour incubation NIH cytoxicity test.

(ii) Rabbit complement for HLA class I serology recommended for use with monoclonal HLA typing reagents should not cause cell death in the absence of human serum when tested against TC199 or similar tissue culture medium supplemented with 200ml/L foetal bovine serum, using samples of unfractionated lymphocytes from 10 random donors in the standard one and one half hour incubation NIH cytotoxicity test.

2 Rabbit complement for HLA class II serology.

(i) Rabbit complement recommended for use in HLA class II serology, in addition to the requirements of 11.3.5.1.i, should not cause cell death in the absence of HLA antibody when tested against serum collected from 5 non-transfused male volunteers using samples of B lymphocytes from 10 random donors in the extended 3 hour incubation cytotoxicity test.

(ii) Rabbit complement for HLA class II serology recommended for use with monoclonal HLA typing reagents should not cause cell death in the absence of human serum when tested against TC199 or similar tissue culture medium supplemented with 200ml/L foetal bovine serum, using samples of B lymphocytes from 10 random donors in the extended 3 hour incubation cytotoxicity test.

11.4 Guidelines for HLA reagent lymphocytes

11.4.1 Introduction

HLA reagent lymphocytes have wide use in the detection and identification of HLA antibodies in patients with febrile reactions to blood transfusion, in patients refractory to platelet transfusions, in the regular screening of patients' serum pre and post transplantation, and in the screening of donors for new sources of HLA antibodies.

11.4.2 Tests required

HLA reagent lymphocytes for use in HLA antibody detection should detect common and some rarer HLA A, B, Cw, DR and DQ serologically definable specificities. The manufacturer should specify in the package insert those antigens known to be present or absent, and those for which no testing has been performed.

11.4.3 Lymphocyte quality

Freshly isolated reagent lymphocytes or previously frozen reagent lymphocytes recovered according to the manufacturer's instructions, for use in antibody detection and identification by a dye exclusion or fluorescence technique should have a viability of at least 80% as judged by dye exclusion, phase contrast or fluorescence microscopy and should contain not more than 1% of platelets or granular cells.

1 Reagent lymphocytes supplied previously frozen in test trays should be dispensed such that each well contains between 1000 and 2000 cells when the lymphocytes have been recovered according to the manufacturer's instructions.

2 Reagent B lymphocytes isolated from peripheral blood for use in the identification of class II antibodies should contain not more than 20% of T cells when assessed by rosetting with neuraminidase-treated sheep RBC or with T cell specific magnetic microspheres.

3 The background incidence of spontaneous cell death of reagent lymphocytes should be assessed in the cytotoxicity test with a negative control of serum known not to contain HLA antibody. Reagent lymphocytes with 30% or more dead cells observed in the negative control at the completion of the test are not suitable for use by dye exclusion or fluorescence cytotoxicity tests.

11.4.4 Immediate container label

1 Immediate container label for HLA reagent lymphocytes issued in separate containers.

Because of the small volumes in which HLA reagent lymphocytes are supplied, the minimum labelling requirement for HLA reagent lymphocytes issued separately in individual immediate containers is a unique combination of numbers and/or letters to identify the donation, the reagent batch number or batch reference and the name of the manufacturer or distributor.

2 Multi-well tray label.

The minimum labelling requirement for multi-well trays or reservoirs is a unique designation on the reservoir or body, not the lid, of the tray from which it is possible to identify the HLA reagent lymphocytes set (or sub-set) reference and the name or unequivocal logo of the manufacturer or distributor.

If the complete set of HLA reagent lymphocytes comprises more than one tray or reservoir, the tray or reservoir number is included in the requirement.

11.4.5 Package insert

In addition to the information required in 11.1.4.1, the package insert should include the following information on each individual preparation of HLA reagent lymphocytes or set of HLA reagent lymphocytes.

1 The HLA phenotype of the reagent lymphocytes.

2 The nature of the HLA reagent lymphocytes (e.g. Normal peripheral lymphocytes, separated peripheral B lymphocytes, separated peripheral T lymphocytes, CLL cells, splenic lymphocytes, lymph node lymphocytes, lymphoblastoid cell line, etc).

3 When the HLA reagent lymphocytes are supplied as a multi container product such as a lymphocyte panel, the label on the containers and packaging should be assigned the same batch reference and carry a number or symbol to distinguish one container from another, this batch reference should also be on the package insert. The phenotype information on these multi container products may take the form of the phenotypes of each of the individual donations of the batch listed against the numbers or symbols distinguishing one donation from another.

4 The concentration of the lymphocyte suspension should be stated on the package insert of HLA reagent lymphocytes issued in individual containers and also on the phenotype listing of batches issued as multi container products.

5 HLA reagent lymphocyte sets issued in multi well trays should include a map or diagram of the tray or reservoir layout indicating the location of the various HLA reagent lymphocytes in the wells of the tray.

6 For HLA reagent lymphocyte sets issued in multi well trays or reservoirs the phenotype information may take the form of a listing of the phenotypes of each of the individual donations comprising the set.

7 The shelf life of the HLA reagent lymphocytes following recovery from long term storage and subsequent storage in conditions recommended by the manufacturer should be stated in the package insert.

8 When HLA reagent lymphocytes are provided suspended in preservative or medium, the components of the preservative or the name of the medium should be stated in the package insert.

Annex 1

Methods for the Preparation of Complement Coated Red Cells

1.1 Introduction

The use of the term 'very low ionic strength medium' in this annex is to avoid any confusion with low ionic strength solutions (LISS).

Water is as described in 1.3.3.

Buffered saline is where the total molarity does not exceed 0.15M, for example 0.1M NaCl, 0.05M phosphate buffer pH 7.0-7.2.

pH is determined at 22 ± 1°C

Solution A Dipotassium hydrogen orthophosphate 1.0M

$K_2HPO_4.3H_20$ 228.2g
water to 1 litre
Store at 4 ± 2°C

Solution B Potassium dihydrogen orthophosphate 1.0M

KH_2PO_4 136.1g
water to 1 litre
Store at 4 ± 2°C

1.2 Method for EiC3b/C4b produced by a warm, very low ionic strength medium technique

1.2.1 Reagents

1 Solution C

Stock Solution A 0.5ml

water to 100ml

2 Solution D

Stock Solution B 5.0ml

water to 1000ml

3 Solution E

Place Solution C in 1 litre beaker. Using a magnetic mixer and pH meter, add Solution D until pH is 6.1.

Dissolve 92.4g sucrose in approximately 500ml of the pH6.1 buffer prepared above, and make up to 1 litre with the same pH6.1 buffer. Dispense in 10-20ml aliquots and store at -20°C or below.

1.2.2 Method

Place 8.5ml of Solution E into a 20-25ml container.

Add 0.5ml of fresh inert group 0 serum.

Mix and immediately add 1.0ml of 50% group 0 red cells, washed three times and suspended in buffered saline.

Mix thoroughly. Incubate at 37°C for 30 minutes with occasional further mixing.

Centrifuge the container. Remove the supernate and wash the red cells 4 times with 20ml of buffered saline for each wash. Remove the last wash.

Add 14.5ml of buffered saline to the packed red cells to prepare a 2-3% suspension of EiC3b/C4b red cells.

Store at 4° ± 2°C. These cells should be prepared on the day of use.

1.3 Method for EC4b produced by a warm very low ionic strength medium technique

1.3.1 Reagents

1 Solution K

Sucrose 25g
water to 250ml

Store frozen at -20°C or below, or use within 24 hours if stored at 4° ± 2°C.

2 Solution L

$Na_2EDTA.2H_20$ 7.45g
Water to 100ml

3 Solution M

$Na_4EDTA.2H_20$ 8.32g (or $Na_4EDTA.4H_20$ 9.05g)
water to 100ml

4 Solution N

Add equal volumes of solution L and solution M to prepare 0.2 M Na_3EDTA. Store at 4° ± 2°C.

1.3.2 Method

Place 10ml of Solution K into a 20-25ml container.

Add

(a) 0.06ml of Solution N
(b) 0.5ml of packed group 0 red cells, washed three times in buffered saline
(c) 0.5m of fresh, inert serum

Mix thoroughly and incubate at 37°C for 15 min.

Centrifuge the container. Remove the supernate and wash the red cells 4 times with 20ml of buffered saline at 4° ± 2°C. Remove the last wash.

Add 15ml of buffered saline to the packed red cells to prepare the 2-3% EC4b red cells.

Store at 4° ± 2°C. These cells should be used within 3 days of preparation.

NOTE: This method has less EDTA than that recommended by the FDA in docket 845-0182, and produces a greater degree of EC4 coating.

1.4 Method for EC3d/C4d or EC4d prepared by trypsin treatment of EiC3b/C4b or EC4b respectively

1.4.1 Reagents

1 Solution P

1N HCl 2.5ml
water to 50ml

2 Solution Q

Trypsin* 0.1g
Solution P to 10ml

*twice crystalised trypsin (e.g. SIGMA CHEMICAL COMPANY, Catalogue 1987 No. T8253).

Dispense in 0.1ml aliquots and store frozen at -20°C or below.

3 Solution R

Solution A 5.0ml
water to 50ml

4 Solution S
Solution B 1.0ml
water to 10ml

5 Solution T

Using a magnetic stirrer, add Solution S to Solution R until the pH of the mixture is 7.7.

Dispense in convenient volumes and store at -20°C.

6 EiC3b/C4b or EC4b red cells, as required.

1.4.2 Method

Prepare 1.0ml of 0.1% trypsin solution by the addition of 0.9ml of Stock Solution T to 0.1ml Stock Solution Q.

Add 1ml of 0.1% trypsin to the 0.5ml of the washed packed EC3b/C4b or EC4b red cells in a 20-25ml container. Mix and incubate at 37°C for 30 minutes, with occasional further mixing.

Wash 4 times with 20ml of buffered saline at each wash. Remove the last wash.

Add 14.5ml of buffered saline to the packed red cells to prepare the 2-3% EC3d/C4d or EC4d red cells respectively.

Store at 4° ± 2°C. These cells should be used within 3 days of preparation.

Annex 2

1 Preparation of BROMELIN treated red cells for microplate use.

Bromelin ("Bromelain")

To 1g of bromelin powder add 5ml saline. Mix to form a thin homogenous paste. Add 100 ml saline and mix thoroughly for 5 minutes. Centrifuge the suspension and remove any insoluble material. Add saline to the supernate to a final volume of 1000 ml.

Test red cells.

Test red cells, washed at least once in saline, are suspended in the bromelin solution to a suspension of 2-3%.

Incubate at 19°C-25°C for 2 minutes before use. Cell suspensions not used within one hour are washed, re-suspended to a 2-3% suspension in saline and used within 24 hours of preparation or discarded.

Control of enzyme treatment.

A pool of R_1r red cells from 3 individuals are treated with bromelin as above and tested using the microplate method described in Annex 2, 3.3, with an inert group-compatible serum and anti-D (0.3 IU/ml).

If a grade 4-5 reaction is not observed with the anti-D (0.3 IU/ml), the use of a more potent bromelin preparation should be investigated.

The group-compatible, inert serum must effect a negative reaction with the bromelin-treated R_1r red cell samples.

2 Potency titrations.

The use of a semi-automatic pipette is recommended; one volume being in the order of 40µl.

A separate pipette tip or Pasteur pipette should be used for each reagent.

If the reagent is formulated with a medium to enhance its reactivity then the same formulation but with no blood group activity is used as the diluent for the determination of the potency titre. Otherwise, dilutions should be prepared in saline containing a final concentration of 20g/L bovine serum albumin that has not been deliberately polymerised or otherwise potentiated.

Beginning with the undiluted blood grouping reagent, separate doubling dilutions (1 in 2, 1 in 4, 1 in 8, etc) should be prepared.

When preparing doubling dilutions, after the addition of the reagent or diluted reagent to an equal volume of the diluent, the tip of the pipette is emptied and blotted before the dilution is mixed and a volume transferred to prepare the subsequent dilution.

The potency titre is the reciprocal of the highest dilution of the reagent that effects a grade 2 reaction using the required technique.

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

3 Potency test methods for manual and microplate blood grouping reagents.

3.1 Manual method. Direct Test.

Add one volume of each dilution of the reagent to a separate tube.

Add one volume of 2-3 percent test red cell suspension to each tube.

Mix thoroughly and incubate for the appropriate temperature and duration.

Centrifuge and determine the reaction grade.

3.2 Manual method. Indirect anti-human globulin Test

Add 2 volumes of each dilution of the reagent to a separate tube.

Add 1 volume of 2-3 percent test red cell suspension in saline , or 2 volumes of 1.5-2 percent test red cell suspension in LISS.

Mix thoroughly and incubate at 37°C for 45 minutes if the red cells are suspended in saline, or for 15 minutes if suspended in LISS.

Wash the red cells four times.

Add two volumes of anti-human globulin reagent to the button of test red cells. Mix. Centrifuge and determine the reaction grade.

3.3 Microplate method.

Equipment:

(a) Rigid polystyrene microplates with 'U' shaped wells.
(b) Centrifuge with microplate carriers having a radius of at least 10cm.
(c) Microplate shaker.
(d) Concave microplate reading mirror or automated plate reader.
(e) Red cells for microplate use, bromelin-treated if required, see Annex 2, section 1

Method.

Using a microplate, add 1 volume (25-50µl) of each dilution of the reagent to 1 volume of 2-3% test red cells.

Mix the contents of the wells using a microplate shaker. Incubate at 19°-25°C for 15 minutes.

Centrifuge the microplate at 100g for 40 seconds. Gently dislodge the red cells from the bottom of the wells using a microplate shaker.

Determine the reaction grade using a concave mirror or automatic plate reader.

4 Avidity determination

Mix over an oval area of approximately 20mm x 40mm on a glass slide, one volume of the undiluted reagent and one volume of a 30-45 percent red cell suspension in allogenic serum or ABO group-compatible plasma.

Maintain the slide at the recommended temperature for a slide test. If a range of incubation temperatures is given, for those blood grouping reagents where the antibody-antigen reaction is favoured by a colder temperature, the higher temperature should be used; for other blood grouping reagents, the lower temperature should be used.

Determine the time from mixing at which macroscopic agglutination first appears and the reaction grade at 1 minute.

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