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Annex 4

Recommendations for the production, control and regulation of human plasma for fractionation

1. Introduction

Human plasma is a source of important medicinal products which are obtained by a combination of large-scale processing steps known as "fractionation". It is important that these products have an appropriate quality and safety profile.

Recognizing the importance of the provision of safe blood, blood components and plasma derivatives, the 58th World Health Assembly in 2005 (WHA Resolution 58.13) (*1*) expressed its support for "the full implementation of well-organized, nationally coordinated and sustainable blood programmes with appropriate regulatory systems" and stressed the role of "voluntary, non-remunerated blood donors from low-risk populations". The provision of blood, blood components and plasma derivatives from voluntary, non remunerated donors should be the aim of all countries.

The WHO requirements for the collection, processing, and quality control of blood, blood components, and plasma derivatives were published in 1994 (*2*). Numerous developments have taken place since that time, requiring updates of both technical and regulatory guidelines to be made available at the global level. The recently published WHO guidelines on viral inactivation and removal procedures (*3*) address the measures necessary to eliminate or reduce the risk from blood-borne viruses during the processing of plasma into plasma derivatives.

The present Recommendations are intended to provide guidance on the production, control and regulation of human plasma for fractionation as a source material for plasma derived medicinal products. Such information is necessary for the manufacture of safe plasma derivatives in both developed and developing countries worldwide.

The current document, by bringing together experience and information, will serve as a guide to blood establishments in their implementation of appropriate procedures for the production and control of the starting plasma material, and will facilitate the provision of safe fractionated plasma products at the national level. It is intended to assist national (medicines) regulatory authorities in establishing the supervision necessary for assessment of the quality and safety of plasma for fractionation, either prepared locally or imported, and will therefore contribute to improved quality and safety of human plasma products worldwide. Manufacturers of plasma derivatives (fractionators) may use these guidelines when discussing the quality criteria of plasma for fractionation with representatives of blood establishments and the national regulatory authority.

This guidance document addresses only human plasma sourced for the manufacture of plasma derivatives. Plasma for clinical use is not discussed, nor is there any consideration of plasma from other species.

2. International Biological Reference Preparations

The full list of WHO Biological Reference Preparations relevant to blood products and related substances is available at: http://www.who.int/bloodproducts/ref_materials/

The biological activity of blood products should be measured by comparison with the relevant International Standard. Activity is usually expressed in International Units (IU), but may in some cases be expressed in SI units.

3. Glossary

The definitions given below apply to the terms used in these Recommendations. They may have different meanings in other contexts.

Apheresis

Procedure whereby blood is removed from the donor, separated by physical means into components and one or more of them returned to the donor.

Blood collection

Procedure whereby a single donation of blood is collected in an anticoagulant and/or stabilizing solution, under conditions designed to minimize microbiological contamination of the resulting donation.

Blood component

A constituent of blood (erythrocytes, leukocytes, platelets or plasma) that can be prepared under such conditions that it can be used either directly or after further processing for therapeutic applications.

Blood establishment

Any structure or body that is responsible for any aspect of the collection and testing of human blood or blood components, whatever their intended purpose, and for their processing, storage, and distribution when intended for transfusion.[1]

Donor

A person who gives blood or plasma used for fractionation.

Factor VIII

Blood coagulation factor VIII, deficient in patients with haemophilia A. Also called antihaemophilic factor.

[1] A blood centre is a blood establishment.

Factor IX

Blood coagulation factor IX, deficient in patients with haemophilia B.

First-time tested donor

A person whose blood or plasma is tested for the first time for infectious disease markers in a blood establishment.

Fractionation

A (large-scale) process by which plasma is separated into individual protein fractions, that are further purified for medicinal use (variously referred to as plasma derivatives, fractionated plasma products or plasma-derived medicinal products). The term fractionation is used to describe a sequence of processes, including: plasma protein separation steps (typically precipitation and/or chromatography), purification steps (typically ion-exchange or affinity chromatography) and one or more steps for the inactivation or removal of blood-borne infectious agents (most specifically viruses and, possibly, prions).

Fractionator

A company or an organization performing plasma fractionation to manufacture plasma-derived medicinal products.

Genome equivalents (GE)

The amount of nucleic acid of a particular virus assessed using nucleic acid testing.

Good Manufacturing Practice (GMP)

That part of quality assurance which ensures that products are consistently produced and controlled to the quality standards appropriate to their intended use and as required by the marketing authorization or product specification. It is concerned with both production and quality control.

Hepatitis A virus (HAV)

A non-enveloped, single-stranded RNA virus, causative agent of hepatitis A.

Hepatitis B surface antigen (HBsAg)

The antigen on the periphery of hepatitis B virus.

Hepatitis B virus (HBV)

An enveloped, double-stranded DNA virus, causative agent of hepatitis B.

Hepatitis C virus (HCV)

An enveloped, single-stranded, RNA virus, causative agent of hepatitis C.

Hepatitis E virus (HEV)

A non-enveloped, single-stranded RNA virus, causative agent of hepatitis E.

Hepatitis G virus (HGV) (or GB virus C (GBV-C))

An enveloped single-stranded RNA virus, causative agent of hepatitis G.

Human immunodeficiency virus (HIV)

An enveloped, single-stranded RNA virus, causative agent of acquired immunodeficiency syndrome (AIDS).

Incidence

The rate of newly-acquired infection identified over a specified time period in a defined population.

Inventory hold period

Period during which the plasma for fractionation is on hold pending identification and elimination of possible window-phase donations.

Intravenous immunoglobulin (IVIG)

Also known as immune globulin, intravenous.

Look-back

Procedure to be followed if it is found retrospectively that a donation from a high-risk donor should have been excluded from processing.

Manufacture

All operations of procurement of materials (including collection of plasma for fractionation) and products; production; quality control; release; storage; distribution; and quality assurance of plasma-derived medicinal products.

Nucleic acid testing (NAT)

A method to detect viral genome that uses amplification techniques such as polymerase chain reaction.

National regulatory authority

WHO terminology for referring to national medicines regulatory authorities. Such authorities promulgate medicines regulations and enforce them.

Plasma

The liquid portion remaining after separation of the cellular elements from blood collected in a receptacle containing an anticoagulant, or separated by continuous filtration or centrifugation of anticoagulated blood in an apheresis procedure.

Plasmapheresis

Procedure in which whole blood is removed from the donor, the plasma is separated from the cellular elements and at least the red blood cells are returned to the donor.

Plasma products

A range of medicinal products (as listed in Appendix 1) obtained by the process of fractionation of human plasma. Also called *plasma derivatives*, *fractionated plasma products*, or *plasma-derived medicinal products*.

Plasma for fractionation

Recovered plasma or source plasma used for the production of plasma products.

Plasma master file

A document which provides all relevant detailed information on the characteristics of the entire human plasma used by a fractionator as starting material and/or raw material for the manufacture of subintermediate or intermediate plasma fractions, constituents of the excipient and active substance(s), which are part of a medicinal product.

Prevalence

The rate of infection identified, including both past and present infections, at a specified point in time or over a specified time period in a defined population.

Prion

The infectious particle associated with transmissible spongiform encephalopathies. It is believed to consist only of protein and to contain no nucleic acid.

Production

All operations involved in the preparation of plasma-derived medicinal products, from collection of blood or plasma, through processing and packaging, to its completion as a finished product.

Recovered plasma

Plasma recovered from a whole blood donation and used for fractionation.

Repeat-tested donor

A person whose blood or plasma has been tested previously in the blood establishment for infectious disease markers.

Replacement donor

Person who gives blood upon request of a specific patient or patient's family or acquaintance, which in principle is intended to be used specifically for the treatment of that patient.

SD-plasma

Solvent/detergent-treated pooled plasma intended as a substitute for fresh frozen plasma (FFP).

Serious adverse event

Any untoward occurrence associated with the collection, testing, processing, storage and distribution of blood and blood components that might lead to death or life-threatening, disabling, or incapacitating conditions for patients or which results in, or prolongs, hospitalization or morbidity.

Serious adverse reaction

An unintended response in a donor associated with immunization that is fatal, life-threatening, disabling, incapacitating, or which results in, or prolongs, hospitalization or morbidity.

Source plasma

Plasma obtained by plasmapheresis for further fractionation into plasma products.

Traceability

Ability to trace each individual unit of blood or blood component derived thereof from the donor to its final destination, whether this is a recipient, one or more batches of medicinal product or disposal. The term is used to describe both forward tracing (donation to disposition) and reverse tracing (disposition to donation).

TT virus (TTV)

A non-enveloped, single-stranded DNA virus, causing post-transfusion hepatitis of unknown etiology.

Viral inactivation

A process of enhancing viral safety in which the virus is intentionally "killed".

Viral removal

A process of enhancing viral safety by removing or separating the virus from the protein(s) of interest.

West Nile virus (WNV)

An enveloped single-stranded RNA virus, causative agent of West Nile fever.

4 General considerations

4.1 Range of products made from human blood and plasma

Human blood is the source of a range of medicinal products. Blood products obtained from the processing of single donations of blood or plasma,

generally known as blood components, include red cell concentrates, platelet concentrates, leukocyte concentrates and plasma for transfusion. Small pools, usually of less than 10 donations, mainly for the production of platelet concentrates, can also be prepared by blood establishments. Small pool cryoprecipitate is produced in some countries. The safety of these blood components depends largely on the criteria used for selection of the donors and the screening of donations.

Other blood products are obtained by the industrial processing of plasma of a *large number* of donations (up to tens of thousands) that are pooled together. These products include pooled virally-inactivated plasma for transfusion that is not fractionated, and the purified plasma products, also known as plasma derivatives, that are obtained by a fractionation process that combines protein purification and viral inactivation and removal steps.

Table 1 summarizes the range of products made from human blood and plasma, illustrating the diversity of source material and manufacturing methods involved, and, consequently, the complex regulation needed to ensure their quality and safety, in particular with regard to the control of risks of infection.

Plasma-derived products are regarded as medicinal products worldwide and their marketing authorization, which involves the official approval of the production process and quality assurance (QA) system used as well as of product efficacy, should be the responsibility of the national regulatory authority in all Member States. The national regulatory authority has the duty to enforce regulations, to evaluate the quality and safety of products, and to conduct regular assessment and inspection of the manufacturing sites.

An important part of the evaluation of the marketing authorization for plasma products relates to the production and control of the starting plasma used for fractionation, and is the focus of these Guidelines.

4.2 Composition of human plasma

Human plasma is a complex biological material composed of hundreds of biochemical entities, some of which have not yet been fully characterized. Among these are albumin, various classes of immunoglobulins, coagulation factors, anticoagulants, protease inhibitors, and growth factors. The complexity of plasma is illustrated in the Table 2.

The concentrations of the various protein components vary from about 40 g/litre (albumin) down to a few nanograms/ml for some coagulation factors. Plasma protein molecular mass varies from several million daltons

(the von Willebrand multimer complex) to tens of thousands Daltons (for example, albumin).

Table 1
Range of blood/plasma products derived from single donor or pooled donations

Single-donor blood components ■ Whole blood ■ Red cell concentrate ■ Platelet concentrate (obtained by apheresis) ■ Leukocyte concentrate ■ Plasma for transfusion ■ Cryoprecipitate ■ Cryo-poor plasma
Small-pool blood components ■ Platelet concentrates (obtained from whole blood)[a] ■ Cryoprecipitate[b]
Large-pool, unfractionated virally inactivated plasma product ■ Plasma for transfusion, solvent-detergent (SD) treated (*4*)
Large-pool products purified by fractionation of plasma ■ See the list of products in Appendix 1

[a] Usually 4–10 platelet concentrates derived either from platelet-rich-plasma or from buffy coats.
[b] Rarely produced. Pooled cryoprecipitate should ideally be subjected to a viral inactivation treatment. Also used as a fibrinogen source for fibrin sealant (fibrin glue).

Human plasma for fractionation is the starting material for the manufacture of a range of medicinal products used for the treatment of a variety of life-threatening injuries and diseases. A list which includes the most established clinical use of these products is provided in Appendix 1.

4.3 Pathogens present in blood and plasma

A number of infectious agents can be present in human blood but not all blood-borne pathogens can be transmitted by plasma for transfusion or by plasma derivatives (*7*). Some pathogens are exclusively associated with blood cells, or are at least partially sensitive to the freeze–thaw process that takes place during the manufacture of plasma and plasma products. In addition, the multiple sterilizing filtration steps systematically included in the manufacture of plasma products, as for any other parenteral preparation, eliminate micro-organisms larger than 0.2 μm. Table 3 summarizes the major infectious risks linked to blood-borne pathogens and presents the current evidence on risks of infection from cellular components, plasma and fractionated plasma products.

Table 2
Selected proteins of human plasma

	Daltons	mg/litre
Major proteins		
• Albumin	68 000	40 000
• IgG	150 000	12 500
Protease inhibitors		
• Alpha-2-macroglobulin	815 000	2 600
• Alpha-1-antitrypsin	52 000	1 500
• C1-esterase inhibitor	104 000	170
• Antithrombin	58 000	100
• Heparin cofactor II	65 000	100
• Alpha-2-antiplasmin	69 000	70
Protease		
• ADAMTS13	190	1
Fibrinolytic proteins		
• Plasminogen	92 000	200
• Histidine-rich glycoprotein	75 000	100
Coagulation factors and anti-coagulant proteins		
• Fibrinogen	340 000	3 000
• Fibronectin	250 000	300
• Prothrombin	72 000	150
• Factor XIII	320 000	30
• Protein S	69 000	29
• Von Willebrand Factor (monomer)	220 000	10
• Factor II[a]	72 000	150
• Factor X	59 000	10
• Factor V	286 000	7
• Factor XI	80 000	5
• Factor IX	57 000	5
• Factor XII	76 000	40
• Protein C	57 000	4
• Factor VII	50 000	0.5
• Factor VIII	330 000	0.3
Cytokines[b]		
• Interleukin-2	15 000	Traces
• Granulocyte colony-stimulating factor (G-CSF)	20 000	< 30 pg/ml
• Erythropoietin	34 000	0.3 µg/litre

Source: Adapted from references *5* and *6*.

[a] Factor II is the zymogen plasma protein which upon activation generates thrombin, one of the components of fibrin sealant (fibrin glue).

[b] There are several cytokines present in traces in plasma. G-CSF and erythropoietin for therapeutic use are obtained by recombinant technology.

Some of the viruses listed in Table 3 are highly pathogenic (e.g. HIV, HCV and HBV), others are pathogenic only in certain recipient populations (e.g. cytomegalovirus (CMV) and B19) and a few are currently considered to be non-pathogenic (HGV and TTV).

Historically, clinical use of single-donor blood components and pooled plasma products (plasma derivatives) has been associated with transmission of blood-borne viruses (HBV, HCV, HIV, HAV and B19) (*3*). The

implementation of validated virus inactivation and removal steps into the manufacturing process of plasma derivatives has now virtually eliminated the risks of infection from HIV, HBV, and HCV (*3*) and has also avoided the transmission of some emerging infectious agents, such as WNV (*8*, *9*).

The infective agents for the bacterial and parasitic infections most commonly associated with transfusions of cellular blood components are reliably removed during the processing and aseptic filtration of plasma products, as are residual blood cells.

Table 3
Evidence of transmission of infectious agents by human blood[a]

Infectious agents	Cellular blood Components	Plasma	Plasma products
Viruses			
HIV I and II	+	+	+
HBV	+	+	+
HCV	+	+	+
Hepatitis Delta virus	+	+	+
HAV	+	+	+
HEV	+	+	+
HGV	+	+	+
TT virus	+	+	+
Parvovirus B19	+	+	+
Human T-cell leukaemia virus I and II	+	–	–
Cytomegalovirus	+	–	–
Epstein–Barr virus	+	–	–
West Nile virus	+	?	–
Dengue virus	+	?	–
Human herpes virus-8	?	–	–
Simian foamy virus	?[b]	?	–
Severe acute respiratory syndrome (SARS) virus	?[c]	?	–
Bacteria			
Spirochaete (syphilis)	+	–	–
Parasites			
Babesia microti (babesiosis)	+	–	–
Plasmodium falciparum (malaria)	+	–	–
Leishmania (Leishmaniasis)	+	–	–
Trypanosoma cruzi (Chagas Disease)	+	–	–
Unconventional agents /TSE			
Creutzfeldt Jakob disease agent	–	–	–
Variant Creutzfeld Jakob disease agent	+	?	–[d]

HIV, human immunodeficiency virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HAV, hepatitis A virus; HEV, hepatitis E virus; HGV, hepatitis G virus; TSE, transmissible spongiform encephalopathies.

+, evidence of transmission; –, no evidence of transmission; ?, questionable or unknown.

[a] Most viral transmissions associated with plasma products took place prior to the introduction of efficient viral inactivation or removal procedures.

[b] Transmitted by contact with animal blood but not reported to follow transfusion.

[c] Limited epidemiological surveys have not revealed transmission of SARS coronavirus by transfusion but further confirmation may be needed.

[d] Investigational studies performed by plasma fractionators using spiked TSE agents indicate that several purification steps used in the manufacture of some plasma products are likely to remove prion agents. These data may not necessarily be extrapolated to clearance of the endogenous form of the TSE agent in human blood.

4.4 Strategies to ensure safety of plasma products

A combination of measures to exclude infectious donations, together with steps to inactivate or remove potential contaminating viruses during processing, has significantly reduced the risk of disease transmission by plasma products.

There are four distinct complementary approaches to virus risk reduction for plasma products:

- minimizing the virus content of the plasma pool by:
  - — implementing a quality system to select donors;
  - — screening blood/plasma donations; and
  - — performing plasma manufacturing pool testing.
- inactivating and removing residual viruses during plasma fractionation and processing (*3*);
- adherence to GMP at all steps of the production; and
- recognizing and responding appropriately to post-donation events affecting plasma donations that have already been processed.

In-process and finished product virus inactivation and/or removal procedures have been shown to play a powerful role in ensuring the viral safety of plasma products, in particular from HIV, HBV, and HCV risks (*3*). Those procedures have also recently been shown to provide a sufficient margin of safety against emerging lipid-enveloped viruses, such as WNV (*8*, *9*).

Although procedures for the inactivation and removal of viruses may therefore seem to offer the fractionator an ideal means for counterbalancing occasional lapses in the identification of risk donations, such an assumption would be incorrect. As powerful as the contribution of properly validated and implemented steps for virus inactivation and removal has been shown to be, it remains essential to limit the virus load at the stage of the plasma pool by avoiding, through donor selection and donation screenings, the inclusion of a high-titre infectious donation. The synergistic effects of reduced viral load in the plasma pool and validated viral inactivation and removal procedures are well illustrated for resistant non-enveloped viruses, such as parvovirus B19, for which viral reduction procedures used during fractionation alone may not be sufficient to ensure safety (*10*, *11*).

Exclusion of infectious donations, and retrospective identification of any infectious donation that would have passed undetected through the screening and testing nets, require the highest priority at the blood establishment. The blood establishment should establish a reliable mechanism to ensure consistent identification of such donations.

Neither of the sets of measures described above can, in isolation, provide sufficient assurance of safety against all potential blood-borne pathogens.

For this reason, the manufacture of plasma for fractionation according to Good Manufacturing Practices (GMP) is necessary to ensure the optimal quality and margin of safety of this raw material for the manufacture of medicinal plasma products.

5. Measures to exclude infectious donations

The safety and quality of plasma for fractionation results from the combination of several cumulative prevention measures

— appropriate selection of blood/plasma donors;
— testing of blood/plasma donations;
— epidemiological surveillance of the donor population;
— strict adherence to GMP; and
— post-donation information system.

Such information on collection and testing of plasma is requested by some regulatory authorities as part of a plasma master file (*12*) used in the evaluation of the marketing authorization of plasma-derived medicinal products. However, the plasma master file is not a universally used regulatory document.

5.1 Appropriate selection of blood/plasma donors

Plasma for fractionation should be obtained from carefully selected, healthy donors who, after review of their medical history (the donor questionnaire), medical examination and laboratory blood tests, would be considered not to present an increased risk for transmission of infectious agents by plasma-derived products (see Appendix 2). Local national regulatory authorities are pivotal in setting up at the national level a harmonized donor selection criteria framework appropriate to the country in which plasma is collected, taking into account the type of products to be manufactured, the relevant risks of infection, and the epidemiological situation. The local national regulatory authority should also be part of any decision making process intended to modify the donor selection and donation testing procedures. Specific selection criteria may be added by the plasma fractionator as part of the contractual agreement with the provider of plasma.

Regulatory agencies and a number of organizations have published regulations and recommendations concerning the criteria for the selection of donors of whole blood and of plasma obtained by apheresis (see for instance *Guide to the preparation, use and quality assurance of blood components* of the Council of Europe (*13*). In general these regulations and recommendations can be used as reference documents for the collection of plasma for fractionation, although some specifications may differ from those of plasma for transfusion. Examples of criteria for the selection

of donors for the collection of plasma for fractionation are presented in Appendix 2. These are not intended to constitute an absolute reference or an exhaustive list of requirements, but rather to provide examples and explain critical points for consideration.

A regular donor is someone who routinely donates blood or plasma in the same centre in accordance with the minimum time intervals. The period taken into account may vary from country to country. A repeat donor is someone who has donated before in the same establishment, but not within the period of time required to be considered as regular donation. Plasma fractionators may implement their own criteria for donors' eligibility to improve safety margins. Whenever possible, plasma for fractionation should be collected through a donation system that relies on regular and repeat donors. Obtaining plasma from regular and repeat donors makes a major contribution to ensuring optimal historical medical information about the donors, and therefore to detecting potential risk factors.

In some countries family or replacement donors may constitute a significant proportion of the population of blood plasma donors, and — depending on the situations — have been found (*14*) or not (*15*) to be at a higher risk than regular/repeat donors of having markers of viral infections. The decision to use this plasma for fractionation is to be made jointly by the plasma fractionator and the national regulatory authorities and should be based on both a careful epidemiological assessment and the evaluation of other safety measures in place for screening of donations for viruses.

Plasma may be collected by plasmapheresis from donors who have acquired immunity through natural infection or through active immunization. Specific information on this issue can be found in Appendix 3.

5.2 Screening of blood/plasma donations for infectious markers

5.2.1 *Screening tests*

The following tests, considered mandatory by all regulatory agencies, are relevant to the preparation of plasma for fractionation and should be performed on each blood or plasma donation:

— an approved test for HBsAg;
— an approved test for anti-HIV; and
— an approved test for anti-HCV.

The results of all three tests should be negative. Testing for HIV p24 antigen and HCV core antigens may increase the sensitivity. Initially reactive donations should be retested in duplicate by the same assay. A repeatedly reactive donation should not be used for therapeutic applications and should usually be destroyed unless useful for non-therapeutic use

or investigations. A sample of the donation should be evaluated by a confirmatory test and if confirmation is positive a system should exist to notify and counsel the donor. It is recommended that national algorithms should be developed and used to enable consistent resolution of discordant or unconfirmed results.

5.2.2 *Other tests*

The screening of plasma for fractionation for anti-human T-lymphotropic virus (HTLV) is not required as the virus is cell-associated and susceptible to inactivation by the freeze–thaw process.

In some countries, testing for anti-HBc is performed on whole blood donations as a means to reduce the risks of exposure to donations of blood components that are hepatitis B-positive (*16*). However, donations of plasma for fractionation obtained from whole blood that are both anti-HBc positive and HBsAg negative, and which contain a sufficient titre of antibodies against hepatitis B surface antigen (anti-HBs) are usually used for fractionation: the scientific rationale is to maintain a sufficient titre of anti-HBs antibody in the plasma pool to neutralize any HBV that may be present. The minimum titre of anti-HBs for an anti-HBc positive/HBsAg negative plasma donation to be accepted for fractionation may be specified by the plasma fractionator and/or the national regulatory authority. Currently, the minimum titre of anti-HBs antibodies required by some plasma fractionators ranges from 50 to 100 IU/l. Alternatively, the plasma donation may be identified by the plasma collector as being anti-HBc positive and the plasma fractionator may conduct additional tests. The setting of a minimum limit, if any, for the titre of anti-HBs antibody usually involves a risk assessment that takes into consideration the sensitivity of the HBsAg screening test, the testing or not of HBV by nucleic acid testing (NAT), and the efficiency of the viral reduction techniques (*3*, *17*).

Additional testing for other agents or markers may be required by the national regulatory authority, taking into consideration the epidemiological situation in any given area or country, or the frequency of donating blood or plasma, and at the specific request of the plasma fractionator.

5.2.3 *Nucleic acid testing*

NAT of plasma for fractionation may be performed for the following viruses: HCV, HBV, HIV, HAV, and/or B19. If NAT is performed by the fractionator, following current practice using mini-pool samples, a specific logistics system may have to be developed at the blood establishments to collect and provide labelled samples in a form suitable for the test. In addition to performing mini-pool testing, fractionators re-test the plasma manufacturing pool for the absence of various viral markers.

5.2.4 *Test kits*

A system should exist in the country or region for approval of tests kits, such as an official approval system by the national regulatory agency or a delegated laboratory. The required sensitivity of the tests for the different antigens or antibodies should be determined by the national regulatory authority. In addition, the test kits to be used should be agreed by the fractionator that will receive the plasma for fractionation.

5.2.5 *Quality control of screening*

The quality of the screening of blood/plasma donations relies on a number of measures, such as:

— validation of new techniques before implementation;
— internal control of reagents and techniques on a daily basis;
— confirmation of positive tests by an appropriate laboratory; and
— external proficiency testing which involves the testing of a panel of sera circulated to laboratories by an approved reference institution.

Details on sampling, test equipment, validation of performance of assays, test interpretation and downloading and follow-up of reactives can be found in section 7 on QA and GMP in these guidelines.

5.2.6 *Look-back*

A system should be in place to perform a look-back procedure, preferably using a computer database. A look-back is a procedure to be followed if it is found retrospectively that a donation should have been excluded from processing, e.g. because that unit was collected from a donor who was subsequently rejected because of reactive viral marker, risk behaviour, exposure to CJD/vCJD or other risks related to infectious diseases. The blood establishment should then transmit this information to the fractionator according to the agreements in place, and to the national regulatory authority. Donor notification and counselling are recommended both for purposes of donor health and for the safety of the blood supply.

5.3 Epidemiological surveillance of donor population

To ensure optimal long-term safety of plasma for fractionation, it is highly recommended to establish continuous epidemiological surveillance of the donor population This is not a requirement in all regions of the world. The objective of this survey is to know, as precisely as possible, the prevalence and incidence, and their trends, of infectious markers that are relevant to the safety of medicinal plasma products so that counter-measures can be taken in a timely fashion.

The system should not only be able to gather epidemiological data at the national and regional level but also among the donor populations that are providing blood or plasma for fractionation at individual blood establishments within a country or a region.

The information from the epidemiological surveillance can furthermore be used:

- to detect differences among donor populations of various collection centres which may be associated with objective differences in viral markers within donor populations or may reflect differences in the process of donor selection and screening among collection centres;
- to detect trends in infectious markers which may reflect either a change in the rate of viral markers in the population or a possible deviation in the donor selection or screening process at specific collection sites;
- to assess the relevance of any prevention measures such as a strengthened donor selection process, additional exclusion criteria, or implementation of additional screening tests to avoid contamination of plasma products.

When donations from first-time donors are used to prepare plasma for fractionation, epidemiological data on this specific group of donors should be included in the estimation of the risk for infectious diseases transmitted by blood. Indeed, it has been shown that first-time donors, who may occasionally include test-seeking individuals, constitute a group which in some situations is more likely to have blood-borne viral markers than regular donors group who have already gone through a selection or deferral process (*18–21*). Some plasma fractionators do not fractionate plasma from first-time donors as prevalence of infectious diseases may be higher in this donor group. Currently, it is advisable to collect and analyse epidemiological data at the collection sites for anti-HIV-1 and anti-HIV-2, anti-HCV and HBsAg, since, historically, they represent the major pathogenic risks associated with plasma products. It is the responsibility of the local national regulatory authority to determine whether the list should be modified or should include additional criteria, such as emerging infectious agents, based on local or regional epidemiology. For the three currently recommended markers, only confirmed positive tests (i.e. tests that are repeatedly reactive in a screening test and positive in at least one confirmatory test) should be recorded. When the plasma fractionator performs additional tests (such as NAT tests) on donations which gave negative results in serological tests, the results should be reported.

Recent guidelines published by the European Medicines Agency (EMEA) entitled *Guideline on epidemiological data on blood transmissible infections* (*22*) describe how to conduct epidemiological surveillance of the donor population.

5.4 Strict adherence to good manufacturing practices

Because the pooling of thousands of plasma donations is required for the manufacture of plasma derived medicinal products, it is necessary to ensure full traceability between individual blood/plasma units collected and the final plasma products manufactured. This is important to enable any quality and safety problems, in particular problems related to infectious risks, to be traced back to individual blood/plasma donations and to allow relevant measures to be taken to protect the donors as well as the patients who received the plasma-derived medicinal products.

The donor selection process, the collection of blood or plasma and the processing of the donation, in order to obtain plasma for fractionation, represent the first steps in the manufacturing of plasma-derived medicinal products, and therefore should be performed in compliance with GMP. Strict adherence to the principles of GMP and the implementation of a QA system to address and comply with the requirements of GMP is crucial at all stages of the production of plasma for fractionation (See section 7 on QA and GMP in these Guidelines).

5.5 Post-donation events

There should be a system to ensure effective communication between the blood establishment and the fractionator so that information on significant post-donation events may be immediately transmitted to the fractionator and the national regulatory authority. In particular, this procedure should allow early and effective communication of any evidence for the presence of blood-transmissible infection in a donor whose plasma has been sent for fractionation.

6. Production of plasma for fractionation

6.1 Methods used to obtain plasma for fractionation

Human plasma for fractionation may be obtained by separation of plasma from whole blood, or by apheresis.

6.1.1 *Recovered plasma*

Recovered plasma is plasma recovered by centrifugal separation from the cells and cellular debris of whole blood under the conditions described below.

6.1.2 *Apheresis plasma (source plasma)*

Apheresis plasma is obtained by a procedure in which anticoagulant-treated blood is removed from the donor, the plasma is separated from the formed

elements, and at a minimum the red cells are returned to the donor. The separation of cellular elements and plasma may be achieved either by centrifugation or filtration. The equipment used for the collection of plasma by automated methods is designed for separating cellular elements and plasma by centrifugation or filtration. The manufacturers of the equipment provide operating manuals that include instructions for installation validation, routine preventive maintenance, periodic performance checks (e.g. weight scale checks), alert mechanisms (e.g. haemoglobin detector) and troubleshooting. Annual preventive maintenance should be performed by a qualified field service engineer. It includes e.g. visual inspection, initial operational integrity, inspection of equipment integrity, inspection of filter and/or centrifuge, calibration testing and safety testing. In addition, the manufacturers of the equipment usually provide support for the installation and train on-site technicians to maintain the equipment. Apheresis collection potentially increases the availability of plasma for fractionation, enabling higher donation frequency and a larger volume per donation, and is the preferred approach for the regular collection of plasma from hyperimmune donors who have high antibody titres against specific disorders.

In principle, the method of preparation should remove cells and cell debris as completely as possible and should be designed to prevent the introduction of microorganisms. No antibacterial or antifungal agent is added to the plasma. The residual blood cell content of the plasma, in the absence of dedicated leukoreduction filtration, may vary with the collection method.

6.2 Characteristics of plasma for fractionation

6.2.1 *Plasma frozen within 24 hours of collection*

Subject to appropriate handling (storage and transport), plasma frozen, at –20 °C or –30 °C, within 24 hours of blood collection or apheresis (see section 6.6.2.1) will normally be suitable for optimal recovery of both labile factors (factor VIII and other coagulation factors and inhibitors) and stable plasma proteins (usually albumin and immunoglobulins). Plasma meeting these quality specifications is also used for direct clinical applications; it is then referred to as fresh frozen plasma (FFP), clinical plasma or plasma for transfusion. Table 4 sets out the main characteristics of plasma prepared either from whole blood (recovered plasma) or by apheresis.

Both sources of plasma have been found by experience to be appropriate for the manufacture of the whole range of plasma products. That said, the method of collection and preparation has some impact on the characteristics and/or yield of the proteins fractionated from the plasma. Apheresis plasma collected from donors undergoing frequent plasmapheresis contains lower levels of IgG than plasma units produced by moderate serial plasmapheresis or from whole blood (*23*, *24*). The content of various coagulation factors is

usually higher in apheresis plasma than in recovered plasma (*24*, *25*), for various reasons that include rapid separation of blood cells and plasma, differing ratios of anticoagulant added, and the possibility of freezing the apheresis plasma soon after completion of collection.

Table 4
Characteristics of plasma for fractionation used in the manufacture of labile plasma products

Characteristic	Recovered plasma	Apheresis plasma
Volume, ml	100–260[a]	450–880[b]
Protein content, g/l (each donation)	≥ 50 *(13)* (but typically greater than in apheresis plasma)	≥ 50
Factor VIII, IU/ml (average)	≥ 0.7 *(26)* (but typically less than in apheresis plasma)	≥ 0.7
Concentration of anticoagulant	Variable, according to donation size (volume of anticoagulant is fixed for a given pack type; the acceptable blood volume range should be specified)	Constant (metered into donation)
Acceptable donation frequency	Determined nationally, usually a maximum of one donation every 2 months	Determined nationally

[a] Based on a standard donation size of 450 ml, with blood:anticoagulant ratio of 7:1. The maximum volume of blood to be collected during one donation procedure is determined by national authorities.
[b] With anticoagulant. The maximum volume of plasma to be removed during one plasmapheresis procedure is determined by national authorities.

Preservation of factor VIII and other labile factors depends on the collection procedure and on the subsequent handling of the blood and plasma. With good practice, an average of 0.7 IU/ml factor VIII can usually be achieved both with apheresis and recovered plasma. Units of plasma for fractionation with a lower activity may still be suitable for use in the production of coagulation factor concentrates, although the final product yield may be reduced.

The implementation of GMP in the preparation of plasma for fractionation should ensure that the plasma bioburden is controlled, labile proteins are conserved as far as possible, and minimal proteolytic activity is generated.

6.2.2 *Plasma frozen after 24 hours of collection*

Plasma may be available that does not fulfil the above-defined criteria but still has value as a source of some plasma proteins. This would include:

- plasma separated from whole blood and frozen more than 24 h but usually less than 72 h after collection;
- plasma, separated from whole blood stored at 4 °C, and frozen within 72 h of separation but within the assigned shelf-life of the blood);

- Plasma frozen within 24 h but stored under conditions that preclude its use for the manufacture of coagulation factors.

Provided the circumstances of manufacture and storage of such plasma does not result in increased bioburden, the plasma may be considered suitable for the manufacture of stable plasma proteins, but not coagulation factors.

Plasma which is not frozen within 72 h of collection or separation from whole blood should not be used for fractionation.

6.2.3 *Plasma not meeting the requirement for fractionation*

Plasma obtained by therapeutic plasma exchange does not meet the criteria for fractionation into plasma products. Indeed, plasma from individuals subjected to therapeutic plasma exchange for the treatment of a disease state may present an enhanced risk of transmitting blood-borne diseases (due to infectious risks associated with plasma) and a high risk of irregular antibodies, and should not be offered for fractionation. In addition, such plasma cannot be classified as being obtained from a voluntary donor.

Plasma from autologous blood donations is excluded from use as plasma for fractionation and may have higher prevalence of viral markers (*27*).

6.2.4 *Hyper-immune (antibody-specific) plasma*

Detailed information regarding immunization of donors for the preparation of hyperimmune plasma is provided in Appendix 3. The following are the three approaches for the preparation of plasma for the manufacture of specific immunoglobulins (antibody-specific immunoglobulins):

- Individuals selected from the normal population by screening of plasma units for antibody titres (Screening may be random, or may be informed by knowledge of history of recovery from an infectious disease — for example varicella).
- Individuals with a high titre of a specific antibody resulting from prophylactic immunization.
- Volunteers recruited to a panel for a targeted immunization programme. The clinical and ethical requirements for such a programme are considered in Appendix 3.

Clinically relevant specific immunoglobulins include anti-D (anti-Rho), and HAV, HBs, tetanus, varicella/herpes zoster and rabies immunoglobulins. Hyperimmune globulins are prepared for intramuscular administration, but products for intravenous use are also available. The typical derivation of hyperimmune plasma of each specificity is summarized in Table 5.

Table 5
Types of hyperimmune plasma

Specificity	Natural immunity	Prophylactic immunization	Targeted immunization
Anti-D (anti-Rho)	Yes	No	Yes
Anti-hepatitis A (anti-HAV)	Yes	Yes	Yes
Anti-hepatitis B (anti-HBs)	Yes	Yes	Yes
Anti-tetanus	No	Yes	Yes
Anti-varicella/herpes zoster	Yes	No	Possibly
Anti-cytomegalovirus (anti-CMV)	Yes	No	No
Anti-rabies	No	Yes	Yes

Acceptable minimum antibody potencies in individual plasma donations for fractionation should be agreed to by the fractionator. Those will usually depend upon:

- the size and composition of the fractionation pool (which may include high-titre donations to increase the mean titre of the fractionation pool);
- the characteristics of the immunoglobulin fractionation process; and
- the minimum approved potency of the final IgG product.

The following general guidance may be useful for each specificity.

6.2.4.1 *Anti-D (anti-Rho)*

- Antibody potency should be estimated in international units, using an appropriate quantitative assay (e.g. auto analyser-based assay or flow cytometry method) agreed by the fractionator.

6.2.4.2 *Anti-hepatitis A*

- Antibody potency should be estimated in international units, using a quantitative assay agreed by the fractionator.
- The minimum acceptable potency in an individual donation is unlikely to be less than 50 IU/ml.

6.2.4.3 *Anti-hepatitis B*

- Antibody potency should be estimated in international units, using a quantitative assay that detects antibody to hepatitis B surface antigen (typically radioimmunoassay (RIA) or enzyme-linked immunoassay (ELISA)) agreed to by the fractionator.
- The minimum acceptable potency in an individual donation is unlikely to be less than 10 IU/ml.

6.2.4.4 *Anti-tetanus*

- Antibody potency should be estimated using either a neutralization assay or a quantitative assay with established correlation to the neutralization assay, agreed by the fractionator.

6.2.4.5 *Anti-varicella/zoster*

- Antibody potency should be estimated using a quantitative assay (typically ELISA, immunofluorescence or complement fixation) agreed by the fractionator.
- The minimum potency should be shown to be equal to or greater than that of a control sample provided by the fractionator.

6.2.4.6 *Anti-cytomegalovirus*

- Antibody potency should be estimated using a quantitative assay (typically ELISA, immunofluorescence or complement fixation) agreed by the fractionator.
- The minimum potency should be shown to be equal to or greater than that of a control sample provided by the fractionator.

6.2.4.7 *Anti-rabies*

- Assessing plasma for rabies antibody is rarely done. A donor may be considered to have acceptable antibody titres between 1 and 3 months after a second (or booster) dose of vaccine. Plasma should not be collected from persons immunized after exposure to infection with rabies virus.

6.3 Premises and devices for collection of plasma for fractionation

6.3.1 *Premises*

The collection of blood or plasma for fractionation should be performed in licensed, or regulated, permanent premises or mobile sites which are compliant with the intended activity and comply with the GMP standards approved by the national regulatory authority. The area for blood donors should be separated from all processing and storage areas. The area for donor selection should allow confidential personal interviews with due regard for the safety of donors and personnel. Before premises are accepted for mobile donor sessions, their suitability should be assessed against the following criteria:

— the size (to allow proper operation and ensure donor privacy);
— safety for staff and donors; and
— adequate ventilation, electrical supply, lighting, hand washing facilities, blood storage and transport equipment, and reliable communication capabilities.

6.3.2 *Containers*

Because plasma is a complex and variable mix of proteins in aqueous solution, the way in which it is handled will have consequences for its safety, quality and quantity. Furthermore, the effects of mishandling will not always be as simple (or as obvious) as a reduction in the content of recoverable factor VIII — they are just as likely to affect the behaviour of the plasma when it is thawed (this is very important to the fractionator, who requires consistency from this particularly important process step).

The containers used for the collection and storage of plasma for fractionation should comply with the appropriate regulatory provisions and should be under the control of the regulatory authority. Containers should also comply with the regulatory and technical requirements of the plasma fractionator. Containers should be labelled with batch numbers traceable to individual donations. The quality of containers has a direct impact on the quality of the plasma produced and it is therefore part of GMP to control the suitability of this starting material before use.

Containers for whole blood collections are the same as for donations of whole blood from which plasma is used for fractionation. They should be plastic, and should have been manufactured in such a way as to assure internal sterility; they should be hermetically sealed to exclude contamination. If the container is not manufactured as an integral part of a blood collection set, there should be a mechanism for docking with the collection set that minimizes the risk of adventitious microbial contamination.

Validation studies will be required to confirm the suitability of the container material (and the material of any tubing or harness through which plasma should pass) during storage in contact with the plasma. Specifically, it will be necessary to establish that the plastic is physically compatible with the proposed methods for freezing and opening (or thawing) the packs and to establish the quantities of extractable materials (for example, plasticizers) during the claimed periods of storage in the liquid and frozen forms. These studies are carried out by the manufacturer of the containers. When using collection sets and containers previously established by a manufacturer as being suitable, a cross-reference to such a study may be sufficient. Validated collection and storage containers for blood/plasma are available from several manufacturers worldwide.

The choice of the containers (e.g. type of plastic bags for recovered plasma or plastic bags or bottles for apheresis collection) has a direct impact on the design of the container opening machine that is used at the plasma fractionation plant at the plasma pooling stage.

6.3.3 *Anticoagulants*

Most anticoagulant solutions developed and introduced for the collection of blood cellular components and plasma for transfusion are compatible with

the preparation of plasma for fractionation and with the manufacture of plasma products (although some influence on factor VIII content in plasma has been described (*28–32*)). One exception is when heparin is added to the anticoagulant solution. The main anticoagulant solutions currently in use for collection of either whole blood or apheresis plasma are listed in Table 6.

Anticoagulant solutions should comply with the appropriate regulatory provisions. They can be already present in the collection container (e.g. plastic containers used for whole blood collection) or added to the blood flow during apheresis procedures. In both cases, information on the device and the anticoagulant should be provided to the regulatory authorities. The fractionator will need to know what anticoagulant has been used, and its concentration as these may have an impact on the fractionation process.

6.4 Blood/plasma collection process

6.4.1 *Procedure*

A standardized and validated procedure for the preparation of the phlebotomy site should be followed using a suitable antiseptic solution, and should be allowed to dry (depending on the type of disinfectant). The prepared area should not be touched before insertion of the needle. Prior to venipuncture the containers should be inspected for defects. Any abnormal moisture or discoloration suggests a defect. A careful check of the identity of the donor should be performed immediately before venipuncture.

The collection of a whole blood unit used to prepare plasma for fractionation should be performed following already established recommendations (for instance as described in the Council of Europe Guide (*13*)). In particular, good mixing of the blood with the anticoagulant solution should be ensured as soon as the collection process starts to avoid risks of activation of the coagulation cascade. The mixing can be done manually, every 30 to 45 seconds, and at least every 90 seconds. Collection of one standard unit of blood should be achieved within 15 minutes, as longer collection periods may result in activation of the coagulation factors and cellular components.

In automated apheresis procedures, whole blood is collected from the donor, mixed with anticoagulant, and passed through an automated cell separator. The plasma for fractionation is separated from the cellular components of the blood, which are returned to the donor in a series of collection/separation and return cycles. The plasma is separated from the red blood cells by centrifugation or filtration, or a combination of both (*33*, *34*). The operational parameters of the plasmapheresis equipment are defined by the manufacturers of the equipment and by the requirements of national regulatory authorities. In general, the anticoagulant (often 4% sodium citrate)

is delivered at a rate to yield a specified ratio of anticoagulant to blood. The volume of plasma collected from the donor during one procedure and over a period of time is regulated. The number of collection/separation and return cycles for each donor depends on the total volume of plasma that is to be collected. For determining the number of cycles employed, the equipment requires programming by input of data. These data elements may include such parameters as donor weight and haematocrit values. The amount of time required for the donation procedure depends on the number of cycles (and hence the volume of plasma collected) but is generally between 35 and 70 minutes.

Table 6

Examples of anticoagulant solutions commonly used in the preparation of plasma for fractionation

	Composition	Recovered plasma	Ratio per 100ml blood	Apheresis plasma
ACD-A	Sodium citrate dihydrate 22.0 g/l Citric acid hydrous 8.0 g/l Dextrose monohydrate 25.38 g/l pH (25 °C) 4.7–5.3	×	15	(×)
ACD-B	Sodium citrate dihydrate 13.2 g/l Citric acid hydrous 8.0 g/l Dextrose monohydrate 15.18 g/l pH (25 °C) 4.7–5.3	×	25	
CPD	Sodium citrate dihydrate 26.3 g/l Citric acid hydrous 3.7 g/l Dextrose monohydrate 25.5 g/l Sodium biphosphate 2.22 g/l Sodium hydroxide 1 N (pH adjustment) pH (25 °C) 5.3–5.9	×	14	(×)
CPD-A	Sodium citrate dihydrate 26.3 g/l Citric acid hydrous 2.99 g/l Dextrose monohydrate 29 g/l Sodium biphosphate 2.22 g/l Adenine 0.27 g/l Sodium hydroxide 1 N (pH adjustment) pH (25 °C) 5.3–5.9	×	14	
CP2D	Sodium citrate dihydrate 26.3 g/l Citric acid hydrous 3.7 g/l Dextrose monohydrate 50.95 g/l Sodium biphosphate 2.22 g/l Sodium hydroxide 1 N (pH adjustment) pH (25 °C) 5.3–5.9	×	14	
4% Citrate	Sodium citrate dihydrate 40 g/l Citric acid hydrous: as required for pH adjustment pH (25 °C) 6.4–7.5		6.25	×

(×), seldom used; ×, commonly used.

6.4.2 *Labelling of collection bags*

There should be a secure system for procurement, printing and storing of the bar code labels used to identify the main collection bags and the satellite bags, associated samples and documentation to ensure full traceability at each stage of plasma production. There should be a defined procedure for labelling collection bags and samples — in particular to ensure that the labels correctly identify the association between samples and donations. Labelling should be performed in a secure manner, e.g. at the donor couch, prior to collection, or immediately after the start of collection, to avoid mislabelling. Duplicate number sets of bar code donation numbers should not be used. Information on the label of the donation should include: official name of the product; volume or weight; unique donor identification; name of the blood establishment; shelf-life or shelf term; shelf temperature; and name, content and volume of anticoagulant.

6.4.3 *Equipment*

Equipment used for the collection and further separation of blood should be maintained and calibrated regularly, and the collection and separation process needs to be validated. When validating the quality of the recovered plasma, a set of quality control tests, including measurement of total proteins, residual blood cells, haemoglobin, and relevant coagulation factors, such as Factor VIII, should be included. In addition, markers of activation of the coagulation and fibrinolytic systems may, if necessary, be performed with the support of the plasma fractionator based on the specifications of the plasma for fractionation set out by the fractionator and/or the national regulatory authority.

Likewise, apheresis equipment and apheresis procedures should be validated, maintained and serviced. Validation criteria for assessing the quality of plasma for fractionation also include protein recovery, residual content of blood cell and haemoglobin, and relevant coagulation factors. Validation studies of new apheresis procedures should also evaluate possible risks of activation of the coagulation, fibrinolysis, and complement systems potentially induced by the material in contact with blood (*25*, *35, 36*); such studies are usually performed by the manufacturer of the apheresis machines.

6.4.4 *Laboratory samples*

Laboratory samples should be taken at the time of blood/plasma collection. Procedures should be designed to avoid any mix-up of samples and samples awaiting testing should be stored at an appropriate temperature, as specified in the operating instructions of the test kits.

6.4.5 *Volume of plasma per unit*

The volume of recovered plasma per container varies depending upon the volume of whole blood collected, the respective haematocrit of the donor, and

the volume of the anticoagulant solution. The volume of apheresis plasma per container depends directly upon the volume collected during the apheresis session and the volume of anticoagulant. The range of volume of blood and plasma collected per donor is usually defined in national regulations taking into consideration criteria such as the weight of the donor.

Although in most countries the volume of whole blood collected is close to 400–450 ml per donor, in some it may be as low as 200 ml (under those circumstances, the volume of anticoagulant solution is reduced so that the plasma:anticoagulant ratio is constant). As a result the volume of recovered plasma per unit (including anticoagulant) may vary from about 100 to 260 ml per container. In the case of plasmapheresis plasma, the volume may range from about 450 to 880 ml per container, depending upon the regulations in the country of collection.

The volume of plasma per container has direct practical impact on the fractionation process and manufacture of plasma products. Small-volume donations (e.g. 100 ml) will require more handling by the plasma fractionation operators at the stage of plasma preparation, at the container opening step, and during plasma thawing. The overall container opening process will take longer, requiring additional care to control bacterial contamination. Another consequence is that the number of donations contributing to a plasma pool will be higher (for instance, 20 000 plasma donations for a pool size of 2000 litres).

6.4.6 *Secure holding and reconciliation*

When the collection process is finished, it should be ensured that blood/plasma donations are held at the donation site using a secure system to avoid mishandling.

Prior to dispatching the collected donations to the blood/plasma processing site, reconciliation of the collected donations should be performed according to a standardized procedure. The procedure should also specify the actions to be taken if there are found to be missing numbers or leaking containers. Documentation should accompany the donations to the plasma processing site, to account for all donations in the consignment.

6.4.7 *Donor call-back system*

A system should be in place in the blood establishment which allows recall of a donor if further analysis or investigation is necessary.

6.5 Separation of plasma

6.5.1 *Premises*

Blood processing should be carried out in adequate facilities suitable for the needs of the intended activity. The donor area and plasma processing areas

should be separated whenever possible. Each area used for processing and storage should be secured against the entry or intervention of unauthorized persons and should be used only for the intended purpose. Laboratory areas and plasma storage areas should be separate from the donor and processing areas.

6.5.2 *Intermediate storage and transport*

Transport of the donations and samples to the processing site should be done according to procedures that ensure both constant approved temperature and secure confinement. This is especially important when blood/plasma is transported from distant blood drive sessions.

Temperature monitoring is important to ensure optimal compliance and quality. One way is to use packaging methods that can keep the blood/plasma within the required temperature limits. Portable temperature loggers can be used to monitor and record temperatures during the transportation of blood/plasma to the processing site.

6.5.3 *Impact of whole-blood holding period*

It has been shown that whole blood anticoagulated with CPD, transported and stored at 22 °C for up to 8 h prior to separation of plasma is suitable for the production of plasma for fractionation, but factor VIII activity is reduced by an additional 15–20% if blood is stored for 24 h (*37*). Rapid cooling of whole blood to 22 °C +/– 2 °C immediately after collection (e.g. using cooling units with butane-1,4-diol) (*38*) protects factor VIII and may allow storage of blood for 24 h (*39*). A temperature of 4 °C during transportation or storage of blood collected with either ACD, ACD-adenine, or CPD anticoagulants consistently appears to reduce the factor VIII content, but not necessarily that of other proteins, especially after 8 hrs of holding time (*40–43*). Holding blood at 4 °C for longer than 8 h is therefore not recommended when plasma is used for fractionation in the manufacture of factor VIII products**.**

6.5.4 *Centrifugation of whole blood*

Documentation on blood and plasma collection should be checked at the processing laboratory on receipt of the donations; reconciliation between consignment and documentation received should be performed. Blood separation procedures should be performed using a closed system and should be validated, documented and proven to ensure that containers are correctly identified.

Reproducible production characteristics of the plasma for fractionation, following a validated procedure, should ensure consistency in the residual blood cell count and protein content and quality to meet the specifications

set out by the blood establishment or the national regulatory authority and the plasma fractionator.

Comparisons have shown that CPD whole blood units that were centrifuged under conditions of low g force for a long time and those subjected to high g force for a short time yielded blood components of similar quality (*44*). Blood separation classically starts with the isolation of the platelet-rich plasma (PRP) fraction from whole blood by low-speed centrifugation. Subsequent high-speed centrifugation of PRP in turn yields the corresponding platelet concentrate and the plasma.

Fully automated systems for blood processing including removal of the buffy-coat layer have replaced manual extraction procedures. This allows standardized extraction and contributes to compliance with GMP in the preparation of blood components including plasma for fractionation (*45*). Blood component separation systems may be based on buffy coat extraction by the "top and bottom" technique (*46*). Its efficacy in terms of yield, purity, and standardization of blood components has been well established.

Several technical approaches have been developed to separate blood components. The process may involve normal centrifugation to separate the blood components, which are subsequently squeezed out from the top and bottom simultaneously under control of a photocell. This primary separation step results in three components: a leukocyte-poor red-cell suspension, plasma, and a buffy-coat preparation (*46*). A multiple-bag system with top and bottom drainage of the primary bag allows automatic separation of blood components; plasma containing $14.6 \pm 5.6 \times 10^3$ platelets/µl and $0.04 \pm 0.035.6 \times 10^3$ leukocytes/µl is obtained (*47*). Blood components may be separated by initial high-speed centrifugation (4158 g, 14 min, 22 ºC) of whole blood in sealed triple or quadruple bag systems, followed by simultaneous extraction of fresh plasma at the top, and the red blood cell concentrate at the bottom, of the respective satellite bags that constitute the blood extraction bag system — keeping the leukocyte-platelet buffy coat layer stable throughout the process within the original extraction bag. The buffy coat component yields the platelet concentrate after low-speed centrifugation and removal of the plasma from the PRP. Automatic separators that subsequently express the various components into their respective satellite bags in top and bottom systems yield plasma containing $3 \pm 3 \times 10^6$ leukocytes and $4 \pm 3 \times 10^9$ platelets per unit (*48*). The "top and bottom" approach allows a marked reduction in leukocyte contamination of the different blood components (*38*, *49*), and may yield optimal plasma volume (*38*).

6.5.5 *Impact of leukoreduction*

Recently, several countries have implemented universal leukoreduction of the blood supply (*50*, *51*) to avoid cell-mediated adverse events or improve viral

safety of blood components. It has also been considered as a precautionary measure against the risk of transmission of variant Creutzfeldt-Jakob disease (vCJD). A recent study in an endogenous animal infectivity model reports that leukoreduction of whole blood removes 42% of the vCJD infectivity associated with plasma (*52*), whereas further investigation by the same group found a ~70% removal of infectivity (R. Rohwer, unpublished data). The impact of leukoreduction on plasma protein recovery and activation markers appears to depend upon the chemical nature of the filters (*53*, *54*). Some loss of coagulation factors and sometimes an increase in the markers of coagulation and complement activation has been found, although the impact on the quality of fractionated plasma derivatives is not known (*54*, *55*).

Therefore, until more scientific data are gathered, the benefits of leukoreduction for the quality and safety of plasma products remains debated. The decision to leukoreduce plasma for fractionation should be assessed with the plasma fractionator and the national regulatory authority.

6.6 Freezing of plasma

Freezing is an important processing step that has an impact on some aspects of the quality of plasma for fractionation, in particular with regard to the content of factor VIII.

Several aspects of the freezing conditions of plasma for fractionation have been evaluated.

6.6.1 *Holding time of plasma*

Holding plasma, freshly harvested from CPD-whole blood, at ~ 4 °C for up to 24 h before freezing at –20 °C for 4 months was shown to induce almost 25% loss of factor VIII activity compared to that in plasma frozen immediately, whereas other coagulation factors were not affected (*56*). Storing plasma at 22 °C for 2–4 h does not seem to induce a significant loss of factor VIII activity; however, after 4 h, some loss of activity takes place (*41*, *57*).

Therefore, placing recovered plasma in a freezer as soon as possible, or at least within 4 h, after separation from cellular elements, would be favourable to the recovery of factor VIII. Similarly apheresis plasma should be frozen as soon as possible after completion of the collection procedure.

6.6.2 *Freezing rate and freezing temperature*

6.6.2.1 *Freezing conditions*

The regulatory requirements for the temperature at which plasma should be frozen vary (*58*), and depend upon the type of proteins fractionated.

The fractionator may also wish to specify freezing conditions depending on the intended use of the plasma.

The *European pharmacopoeia* currently states that recovered or apheresis plasma for fractionation to be used for the manufacturing of labile proteins (e.g. production of factor VIII concentrate) should be frozen rapidly, within 24 hours of collection, at –30 °C or colder (*26*), as this temperature has long been claimed to ensure complete solidification (*59*), and to be needed for optimal freezing (*60*). However, freezing conditions are currently under debate and the wording used in the *European pharmacopoeia* monograph may be revised. Recovered plasma used to manufacture only stable plasma proteins (e.g. albumin and immunoglobulins) should be frozen within 72 h of collection at –20 °C or colder.

The US Code of Federal Regulations specify that plasma collected by apheresis and intended as source material for further manufacturing should be stored at –20 °C or colder immediately after collection.

The rate at which freezing proceeds is considered to be an important quality factor, at least when coagulation factors are intended to be produced (*61*, *62*). Rapid freezing of plasma prevents or reduces loss of factor VIII in frozen plasma either recovered or obtained by apheresis (*23*, *63*, *64*), whereas slow freezing of plasma has been shown to influence the purity and recovery of factor VIII in cryoprecipitate (*61*, *64–66*). An ice front velocity of 26 mm/h during freezing was recently shown to preserve factor VIII:C in plasma better than 9 mm/h or less (*57*).

Therefore, freezing plasma rapidly (typically in less than 2 h, so as to ensure a high ice front velocity) down to a core temperature of at least –20 °C, and preferably colder, appears to be the best approach for the preservation of labile proteins.

6.6.2.2 *Impact of containers and equipment*

To ensure optimal and consistent freezing and storage conditions, it is important to use standardized plasma containers as freezing time is influenced by container shape, volume and thickness (*57*, *64*, *65*).

Optimum conditions used by some plasma collectors to ensure reproducible freezing are achieved by freezing "well separated" plasma packs in a stream of moving cold air at the lowest temperature tolerable to the plastic of the pack (a so-called "blast freezer"), and then to store the frozen packs "close-packed" in a storage freezer at the agreed upon storage temperature. The worst case would be to place a large number of unfrozen plasma bags, close together, in a domestic (–18 °C to –22 °C) freezer, adding more plasma bags for freezing each day, and storing the plasma under these conditions for several months. With good practice at the time of loading (i.e. not putting too many packs in at the same time and keeping them separated), a walk-in freezer at a suitable temperature offers a workable compromise.

The plasma fractionator should specify precisely to the plasma collector, with the approval of the national regulatory authority, which precise freezing parameters to use.

6.6.2.3 *Validation of the freezing process*

Recovered plasma and apheresis plasma should be shown to be frozen in a consistent manner at the required temperature. A system should be in place for ensuring that plasma is frozen to the correct core temperature within the time limit agreed upon with the plasma fractionator, keeping in mind that the speed of freezing will be influenced by the type of plasma container as well as by the volume of plasma (*64*). Validation of the freezing process by recording the temperature of plasma donations during a freezing process allows evaluating the freezing capacity of the equipment to be evaluated. Validation studies should be available, and should demonstrate that the temperature of a frozen pack reaches the proposed storage temperature following the specifications agreed upon with the manufacturer.

As indicated above, the aim should be to achieve rapid freezing, and thereafter to minimize temperature changes to the frozen plasma.

6.7 Storage of plasma

6.7.1 *Storage conditions and validation*

Plasma for fractionation should be stored at –20 °C or colder.

A multicentre study showed no detectable storage-related changes in three pools of plasma (2 recovered CPD plasma and 1 apheresis plasma) that had been quick-frozen at –30 °C, or colder, and stored over a period of 36 months at –20 °C, -25 °C, -30 °C, or –40 °C. An 11% reduction in factor IX was found in one of the recovered plasma pools during storage at –20 °C for 2 years (*67*). The authors concluded that plasma may be stored at –20 °C for 2 years, or at –25 °C, –30 °C, or –40 °C for 3 years.

By keeping the average storage temperature of the frozen plasma as constant as possible, at or below –20 °C, the original quality of the plasma is maintained, without having any impact on the fractionation process, in particular the cryoprecipitation step (*60*, *61*, *66*).

The *European pharmacopoeia* has a provision stating that if the temperature of the plasma is between –20 °C and –15 °C for a maximum of 72 h, or if it is above –15 °C (but colder than –5 °C) in no more than one occurrence, the plasma can still be used for fractionation. Therefore, maintaining a constant storage temperature of –20 °C or colder is a recommended approach to ensure a consistent and optimal plasma quality.

6.7.2 *Premises and equipment*

Storage conditions should be controlled, monitored and checked. Temperature records should be available to prove that the full plasma containment is stored at the temperature agreed upon with the plasma fractionator throughout the storage area. Appropriate alarms should be present and regularly checked; the checks should be recorded. Appropriate actions on alarms should be defined. Areas for storage should be secured against the entry of unauthorised persons and should be used only for the intended purpose. Storage areas should provide effective segregation of quarantined and released materials or components. There should be a separate area for rejected components and material.

If a temporary breakdown of the freezing machine or failure of the electricity supply occurs (e.g. electricity used for the stored plasma), examination of the temperature records should be made together with the plasma fractionator to evaluate the impact on plasma quality.

6.7.3 *Segregation procedures*

The following should be taken into account in the storage and boxing of plasma for fractionation.

- Untested plasma and released plasma should be stored in separate freezers, or if both types of plasma are stored in a single freezer a secure segregation system should be used.
- Initially reactive plasma donations should be stored in a separate quarantine freezer or a secure system (e.g. validated computer hold system) should be used to prevent boxing of non-released plasma.
- Donations that are found to be unacceptable for fractionation should be retrieved, disinfected and discarded using a secure system.
- Plasma donations for shipment to the plasma fractionator should be boxed in a secure manner and an effective procedure (such as a computerized system) should exist to make sure that only fully tested and released plasma donations are boxed.
- Prior to shipment, plasma boxes should be reconciled appropriately.
- Prior to release of the plasma shipment to the fractionator, there should be a formal review of the documentation to ensure that the plasma shipped complies fully with the specifications agreed upon with the plasma fractionator.

The goal of the above-mentioned measures is to make sure that donations that do not comply with the specifications agreed upon with the fractionator will not be released and shipped, and that traceability of donations is ensured.

6.8 Compliance with plasma fractionator requirements

Any plasma collected and prepared for fractionation should meet the plasma product manufacturer requirements as the specifications of plasma

for fractionation are part of the marketing authorization granted by the national regulatory authority for a specific plasma derivative. In addition, to the regulatory criteria related to donor selection and screening of donations, the quality specifications agreed upon with the fractionator may encompass:

— compliance with GMP during production and control;
— residual level of blood cells (platelets, leukocytes) that should be below a certain level that may vary depending upon the requirements of different countries or fractionators;
— protein content possibly including a minimal mean level of Factor VIII coagulation activity if this product is manufactured;
— guarantee of an appropriate ratio of plasma:anticoagulant solution (see Table 6) and evidence of appropriate mixing with the anticoagulant during the collection process (for instance, clots should be absent);
— acceptable maximum titre of ABO blood group antibodies (risks of haemolytic reactions due to the presence of ABO antibodies, or antibodies to other blood group systems, in intravenous IgG and low-purity factor VIII preparations have been described (*68*)). The *European pharmacopoeia* requires an ABO titre of less than 1:64 for the release of plasma products for intravenous use.
— maximum haemoglobin content;
— absence of haemolysis;
— colour;
— absence of opalescence (due to lipids);
— citrate (anticoagulant) range content (usually between 15 and 25 mM); and
— minimum titre of a specific antibody when the donation is used for the production of hyperimmune IgG such as anti-Rho, anti-HBs, anti-tetanus or anti-rabies.

6.9 Release of plasma for fractionation

Each blood establishment should be able to demonstrate that each unit of plasma has been formally approved for release by an authorized person preferably assisted by validated information technology (IT)-systems. The specifications for release of plasma for fractionation should be defined, validated, documented and approved by quality assurance and the fractionator.

There should be a system of administrative and physical quarantine for plasma units to ensure that they cannot be released until all mandatory requirements have been satisfied. In the absence of a computerized system for control of product status, the label of the plasma unit should identify the product status and should clearly distinguish released from non-released

(quarantined) plasma. Records should demonstrate that before a plasma unit is released, all current declaration forms, relevant medical records and test results have been verified by an authorized person.

Before final product release, if plasma has been prepared from a donor who has donated on previous occasions, a comparison with previous records should be made to ensure that current records accurately reflect the donor history.

In the event that the final product is not released due to potential impact on plasma quality or safety, all other implicated components from the same donation should be identified. A check should be made to ensure that (if relevant) other components from the same donation(s) and plasma units or other components prepared from previous donations from the same donor(s) are identified. There should be an immediate update of the donor record(s) to ensure that the donor(s) cannot make a further donation, if appropriate.

6.9.1 *Plasma release using electronic information systems*

Special documented evidence is needed if release of plasma is subject to use of electronic information systems (EIS) to ensure that the system correctly releases plasma units only if all requirements are met. The following points should be checked:

- The EIS should be validated to be fully secure against the possibility of plasma which does not fulfil all test or donor selection criteria, being released.
- The manual entry of critical data, such as laboratory test results, should require independent verification by a second authorized person.
- There should be a hierarchy of personnel permitted access to enter, amend, read or print data. Methods of preventing unauthorized entry should be in place, such as personal identity codes or passwords which are changed regularly.
- The EIS should block the release of plasma or other blood components considered not to be acceptable for release. There should also be a means to block the release of any future donation from a donor.

6.10 Packaging of plasma

The packaging requirements should be specified by the fractionator. The specification should include the following information:

— how the plasma containers are to be packed to prevent damage during shipment;
— that plasma of different types should be kept discrete and packaged into separate cartons; and

— that each carton should have a unique identification number or a bar code which should be clearly displayed on the carton and recorded in the shipping documentation.

6.11 Transportation of plasma

Although it is possible to think of transport as an extension of storage, some additional qualification is appropriate. This need arises because of the additional requirements for risk management during transport. Plasma is at increased risk when:

- Responsibilities for storage and transportation conditions change (especially when handling is the responsibility of individuals with little understanding of the consequences of temperature elevation, as will often be the case with contract shippers).
- Plasma is moved from one freezer or container to another (especially if this involves even temporary exposure to ambient temperatures, as on the loading dock of a blood establishment or a fractionation facility).
- The usual provisions for backup in the event of failure of the refrigeration system are not available (as during sea-transportation of several weeks duration).

The recommendations for cold chain maintenance, as mentioned for plasma storage, should also apply during transportation of plasma. The arrangements for temperature control and monitoring during shipping should be clearly defined and documented. The requirements for number and location of temperature logging devices during shipping should be based on a documented assessment of risk throughout the process. The temperature to be maintained during transportation should be defined by the fractionator in accordance with relevant regulations.

The responsibilities of organizations and individuals during shipping should be identified; in particular any requirements for documented hand-over checks should be specified. The final responsibility for acceptance of quality as compliant with specification lies with the quality department of the fractionation facility.

Table 7 summarizes some recommendations on the handling of blood and plasma to optimize the recovery of labile proteins such as factor VIII in plasma. These recommendations should be examined keeping in mind that the relationship between the content of factor VIII in the starting plasma and its recovery in factor VIII concentrates is unclear (*40*, *69*), possibly in part due to the loss of factor VIII that takes place during industrial cryoprecipitation (*70*) as well as during purification and virus reduction procedures.

Table 7
Processing of plasma for fractionation to optimize factor VIII stability

Steps	Recommendations
Whole blood storage before plasma separation	• Up to 18 to 20 h at 22 °C ± 1 °C • Not more than 8 h at 4 °C
Freezing	• As soon as possible, within 24 hrs of blood collection or apheresis procedure[a]
Freezing rate and temperature	• As specified by plasma fractionator, following relevant regulations pertaining to the countries where plasma will be fractionated and products will be marketed • < –20 °C or colder
Storage temperature	• –20 °C or colder, constant
Transportation temperature	• –20 °C or colder, constant

[a] Collection of plasma by apheresis makes it possible to freeze plasma immediately after the end of the collection procedure by contrast to whole blood processing.

6.12 Recall system

In the case of known or suspected quality defects of a plasma unit that has already been shipped, a person within the blood establishment should be nominated to assess the need for product recall and to initiate and coordinate the necessary actions. An effective recall procedure should be in place, including a description of the responsibilities and actions to be taken. Actions should be taken within predefined periods of time and should include tracing all relevant components of the donation and, where applicable, should include look-back procedures.

7. Quality assurance system and Good Manufacturing Practices

Human plasma for fractionation is the single most critical raw material in the manufacture of plasma derivatives. Fractionators should only use plasma for fractionation from blood establishments that are subject to inspection and approved by a national regulatory authority. When the mandatory safety testing is outsourced, the laboratories need to be inspected and approved. The safety and quality of plasma for fractionation should be assured by implementation of standards at the blood establishment where plasma is prepared. These standards should be assured by implementation, at the blood establishment, of an effective QA system based on the principles of GMP.

The QA system should ensure that all critical processes such as the purchase of raw materials, starting materials, selection of donors, collection of blood and plasma, production of plasma, storage, laboratory testing, dispatch and associated quality control measures, are specified in appropriate instructions and are performed in accordance with the principles of GMP and comply with the appropriate regulations. The management should review the system regularly to verify the effectiveness and introduce corrective measures if deemed necessary.

Because the quality standards implemented at the blood establishment have such a profound impact on the quality of plasma, it is a requirement that their implementation be agreed between the blood establishment and the fractionator, under the terms of the contract for plasma supply (Appendix 5). Medicines regulatory authorities will verify that such a contract is in place and that it complies with the regulations in force.

A blood establishment should establish and maintain an active and operational quality assurance system covering all activities, and taking into account the principles of GMP. The following items are of special relevance as part of a QA system for the production of plasma for fractionation (*71*).

7.1 Organization and personnel

There should be an organization chart showing the hierarchical structure of the blood establishment and clear delineation of lines of responsibilities. All personnel should have appropriate qualifications and experience to enable them to perform their tasks and should be provided with initial and continued training. Only persons authorized by defined procedures and documented as such should be involved in the production and control of plasma. The tasks and responsibilities should be clearly documented and understood. All personnel should have clear, documented and up to date job descriptions.

Training programmes appropriate to the specific tasks of staff members should be in place, and should include at least:

— relevant principles of plasma production and plasma characteristics;
— quality assurance and GMP; and
— relevant knowledge of microbiology and hygiene.

Training should be documented and training records should be maintained. The contents of training programmes should be periodically assessed.

If certain tasks, such as separation of blood or viral safety testing, are performed externally, these should be subject to a specific written contract. The contract should ensure that the contract acceptor meets good practice requirements in all disciplines relevant to the contract giver's activity.

7.2 Documentation system

Every activity that may affect the quality of the blood and/or blood component should be documented and recorded. The documentation should be designed to ensure that the work performed is standardized and that there is traceability of all steps in the process. The documentation should allow all steps and all data to be checked. All documentation should be traceable and reliable. A document control procedure should be established for review, revision history and archiving of documents. It should include a distribution list. All changes to documents should be acted upon promptly and should be reviewed, dated and signed by an authorized person. Documentation procedures should be designed, developed, validated and personnel trained in a consistent manner.

7.3 Premises and equipment

Premises should be located, constructed, adapted and maintained to suit the operations to be carried out. Premises should be designed to permit effective cleaning and maintenance to minimize risk of contamination. The tasks in each area should take place in a logical sequence to minimize the risk of errors.

All critical equipment should be designed, validated and maintained to suit its intended purpose and should not present any hazard to donors or operators. Maintenance, cleaning and calibration should be performed regularly and recorded. Instructions for use, maintenance, service, cleaning and sanitation should be available. There should be procedures for each type of equipment, detailing the action to be taken when malfunctions or failures occur. New and repaired equipment should meet qualification requirements when installed and authorized before use. Qualification results should be documented.

7.4 Materials

Only reagents and materials from approved suppliers that meet the documented requirements and specifications should be used. Where relevant, materials, reagents and equipment should meet the requirements of other local legislation for medical devices. Appropriate checks on goods received should be performed to confirm that they meet specifications. Inventory records should be kept for traceability. Critical materials should be released under the responsibility of quality assurance function before use.

7.5 Validation programme

All processes and equipment involved in the production and control of plasma for fractionation should be validated. Data should be available to ensure that the final product will be able to meet specifications.

7.6 Quality monitoring data

Quality control of plasma should be carried out according to a defined sampling plan taking into account different collection and production sites, modes of transport, methods of preparation and equipment used. Acceptance criteria should be based on a defined specification for each type of plasma for fractionation. These data may include monitoring of factor VIII or any other protein quality criteria determined by the plasma fractionator, and monitoring of residual cell counts (platelets, leukocytes, erythrocytes) when requested by the plasma fractionator. All quality control procedures should be validated before use.

The viral safety testing should be performed in accordance with the recommendations of the manufacturers of the reagents and test kits. The work record should identify the test(s) employed to ensure that entries, such as the calculation of results, are available for review. The results of quality control testing should be subject to periodic review.

Test results that do not satisfy the specified acceptance criteria should be clearly identified to ensure that plasma from that donation remains in quarantine and that the relevant samples are kept for further testing. Where possible the performance of the testing procedures should be regularly assessed by participation in a formal system of proficiency testing.

7.7 Virology safety testing

7.7.1 *Sampling*

The following are practical points to consider in ensuring that sampling is performed appropriately:

- Sampling machine:
  - *Automatic sampling*: Test samples should be taken automatically and the donation number should be read from the barcode. In case of failure of the automatic system, an appropriate system for manual entry of records of donations should exist, and should require double entry with digit checks;
  - *Sampler validation*: The sampling machine should be validated and a validation report should be available; and
  - *Calibration*: The sampling machine should be calibrated on schedule and records available.
- Reconciliation: There should be a reconciliation of the samples received at the virology laboratory versus expected.

7.7.2 *Test equipment*

The following are practical points to consider in ensuring that the equipment used for the virology testing performs appropriately:

- Sample addition**.** The process of addition of samples to the test plates should be automatic and should include identification of the barcode of the plates.
- Test processing. Ideally, the test processing should be automated. If addition of reagents is done manually, full documentation should be available
- Equipment. Pipettes, incubators and other items of equipment should be fully validated and routinely calibrated and appropriate records maintained.

7.7.3 *Assay performance validation*

The objective of validation of assay performance is to make sure that the performance of the virology assays, as carried out by the entity responsible for plasma collection, is satisfactory. Points to consider include:

- Each test run should include an independent control.
- Analysis of positive controls.
- Analysis of data on non-repeatable reactives (see 7.7.5 below).
- Evidence of satisfactory participation in external proficiency schemes.

7.7.4 *Test interpretation and downloading*

The data from virology safety testing should be examined by the supervisor before being officially accepted. Accepted data should be downloaded directly to the mainframe computer, or there should be a secure system for manual download which ensures positive release of the samples. No transcription of results should be done as mistakes may be introduced.

7.7.5 *Follow-up of reactives*

The following should be given special attention:

- *Identification of initial reactives*. They should be identified using a secure system.
- *Repeat reactives*. An acceptable system should be in place to confirm repeat reactives, including sampling, labelling, testing, and entry of results.
- *Editing of repeat reactive*. A computer algorithm should edit reactive status to repeat reactive, or the editing should be performed by two staff members.
- *Deferral system*. An appropriate deferral system should exist for repeat reactives.
- *Re-entry of deferred donors*. Appropriate documentation should be in place.

7.8 Electronic information system

Importance should be given to the introduction of an EIS for blood establishments involved in the preparation of plasma for fractionation and

when possible linked to other establishments to facilitate and speed tracing of individual plasma donations. This will allow timely identification of the location of donations in the chain of production of plasma products.

All software, hardware and backup procedures should be validated before use and checked at least once a year to ensure reliability. The system should prevent the use of duplicate donation numbers, or else the system should be able to deal with duplication without data corruption.

Hardware and software should be protected against unauthorized use or changes (e.g. by password protection of key functions). There should be procedures for each type of software and hardware, detailing the action to be taken when malfunctions or failures occur.

A backup procedure should be in place to prevent loss of records during expected and unexpected downtime or function failures. Changes in computerized systems should be validated, applicable documentation revised and personnel trained, before the change is introduced into routine use. The EIS should be maintained in a validated state.

7.9 Storage and transport

Storage and distribution routines should take place in a safe and controlled way to assure product quality throughout storage and transport and to exclude identification errors of plasma units. Intermediate storage and transport should be carried out under defined conditions to ensure that set requirements are met.

7.10 Change control system

A formal change control system should be in place for planning, evaluating and documenting all changes that may affect the quality, traceability, availability or effect of components or safety of components, donors or patients. The potential impact of the proposed change should be evaluated. The need for additional testing and validation should be determined.

7.11 Quality assurance auditing

In order to monitor the implementation and compliance with the blood establishment quality management system, regular internal audits are needed. These should be conducted independently by trained and competent persons from within the organization, according to approved protocols. Inter-institutional audits should be actively promoted.

All audit results should be documented and reported to management. Appropriate corrective actions should be taken. Preventive and corrective actions should be documented and assessed for effectiveness after

implementation. In general the blood establishment should have procedures for systematic improvement. Input for this process can come from complaints, errors, inspections, audits and suggestions.

7.12 Defect reporting system

There should be systems in place to ensure that complaints, all types of quality defects (e.g. in blood bags or test kits), and adverse events or reactions are documented, carefully investigated for causative factors and, where necessary, followed by the implementation of corrective actions to prevent recurrence. This also applies to "near miss events". The corrective and preventive action system should ensure that nonconformity of the product or quality problems are corrected, that recurrence of the problem is prevented, and that the plasma fractionator is notified according to the agreed procedure. The blood establishment should have methods and procedures in place to channel product or quality problems into the corrective and preventive action system.

7.13 Quality agreement between blood establishment and fractionator

The important elements of a blood establishment quality system with critical implications for plasma quality, will normally be addressed in a quality agreement — an addendum to the contract for plasma supply. The quality agreement should address at least the following areas of concern:

- agreement on specific donor selection criteria (with approval of the national regulatory authority);
- schedule of requirements for exclusion or acceptance of donors, including the arrangements for establishing donor identity and the provision for possibility of self-exclusion;
- arrangements for monitoring and reporting the epidemiology of the donor population;
- location of blood establishments (and of any facility to which a quality-critical function, for example donation testing, has been outsourced);
- frequency of donation and the system for ensuring that donations are not taken more frequently than allowed;
- requirements for donor screening and for donation testing, including any provision for the preparation and testing of mini-pools;
- procedure for validation and approval of relevant test reagents and kits;
- record-keeping, including the arrangements for traceability of donors and donations;
- specifications of plasma to be supplied, including any arrangements for verifying compliance with specifications and documentation of compliance;

- specifications of containers to be used for blood/plasma collection and supply;
- detailed requirements for labelling of individual plasma units (the adhesive used for the labels should not compromise the quality of the plasma products);
- arrangements for freezing, storage and shipment of plasma;
- notifiable events, including the arrangements for post-donation notification;
- procedure for review and approval of any proposal for procedural change;
- procedure and agreed frequency for audit of blood establishments by the fractionator; and
- arrangements for notifying the fractionator of a proposed regulatory inspection, its periodicity, and of the outcome of such an inspection.

7.14 Blood/plasma establishment audit and inspection

It is a requirement of GMP that the regulatory authorities and the plasma fractionator should establish the basis of confidence in the quality of critical raw materials. In the case of plasma, this is achieved by four basic provisions:

- maintenance of a list of blood establishments approved (by the fractionator and the regulatory authorities) for supply of plasma;
- agreement in a contract, or in the technical agreement to a contract of supply, of the quality arrangements made at each blood establishment approved for supply of plasma;
- regular audit of blood establishments to confirm satisfactory implementation of the quality arrangements (these audits should be reported in writing to the blood/plasma establishment and any remedial actions confirmed); and
- monitoring of the quality of plasma supplied, with trending of quality-critical parameters.

There will normally be a requirement for independent inspection and approval of each blood establishment by the relevant regulatory authority (see below). Such inspections should be provided for in any contract between the plasma supplier and the fractionator, and will normally be undertaken by the responsible authority in the country where plasma preparation is performed. Written reports of such inspections should be made available to the blood establishment and a remediation plan agreed upon. Reports of regulatory inspections and associated remediation plans should be made available to the fractionator under the terms of the contract for plasma supply.

8. Regulatory control of plasma for fractionation

8.1 Role of national regulatory authority

According to the WHO Guidelines for national regulatory authorities on quality assurance of biological products (*72*, *73*), national regulatory authorities have the duty to ensure that available biological products, whether imported or manufactured locally, are of good quality, safe and efficacious, and should thus ensure that manufacturers adhere to approved standards of quality assurance and GMP. The responsibilities of the national regulatory authority should also include the enforcement and implementation of effective national regulations, and the setting of appropriate standards and control measures. The evaluation and control of the quality, safety and consistency of production of blood products involve the evaluation of the starting material, production processes and the test methods to characterize batches of the product. This requires specialist expertise by the national regulatory authority.

8.2 Establishment licence and inspections

In many countries, national regulatory authorities have implemented a control system based on licensing the establishments, inspecting them regularly, and enforcing the implementation of the legal requirements and applicable standards.

According to international GMP standards for the manufacturing of blood products, the following two main principles are important for the control of plasma as starting material:

- Quality assurance should cover all stages leading to the finished product, from collection (including donor selection) to storage, transport, processing, quality control and delivery of the finished product.
- Blood or plasma used as a source material for the manufacture of medicinal products should be collected by establishments and be tested in laboratories which are subject to inspection and approved by a national regulatory authority.

These two points in the GMP requirements summarize an important basic principle which is relevant for the manufacture of plasma derivatives and the control of plasma as starting material. Most national regulations therefore require that the establishments involved in the collection and storage of plasma as a source material (e.g. plasmapheresis centres and blood establishments) need to have an establishment licence and need to be inspected by the competent national regulatory authority. To obtain the licence the establishments have to fulfil a defined set of requirements to guarantee that the plasma collected is safe and of good quality. Since each unit collected represents a single batch, a marketing authorization for the plasma

as a "product" is not required in all countries. Under the latter condition, a "system control", instead of a "product control", may be more appropriate. In addition to the establishment licensing system some countries have also introduced a product-specific approval system for blood components.

8.3 Impact of good manufacturing practices

The approach of implementing the principles of GMP in the production of medicinal products is not new, and it is widely acknowledged that it is essential in assuring the quality and safety of medicinal products. For blood products, GMP becomes even more important and more complex due to the biological nature of the products. Therefore, taking into account the principles of GMP and the existence of an appropriate system of quality assurance to address and implement these requirements in the manufacturing steps of blood products should be a pivotal element of the preparation of plasma for fractionation. As outlined in the previous sections, implementation of GMP in the manufacture of blood products is essential, and quality assurance and GMP should cover all stages, including the collection of plasma as starting material. The implementation and enforcement of GMP in blood establishments therefore has the following impact:

— introduces the application of quality assurance principles in all steps involved in the collection, preparation and testing of blood components;
— supports systematic application of donor selection criteria for each donation;
— reduces errors and technical problems in collection, preparation, testing, and distribution;
— contributes to the release of products which comply with safety and quality requirements;
— ensures adequate documentation and full traceability for each donation and product;
— enables continuous improvement in collection, preparation and testing of starting material;
— supports regional cooperation networks that may result in the formation of centres of competence by centralizing activities in order to reach compliance at the required level (cost-benefit for implementing quality assurance measures);
— provides suitable tools for the national regulatory authority to assess the compliance of a plasma collection centre.

An establishment licensing system for blood establishments by the competent national regulatory authorities should therefore exist. The main requirements for obtaining an establishment licence may include:

- Application of quality assurance system and GMP to all steps from donation, to preparation, storage, testing and distribution of plasma.

- Personnel directly involved in the collection, testing, processing, storage and distribution of plasma need to be appropriately qualified and provided with timely and relevant training.
- Adequate premises and equipment should be available.
- An adequate system to ensure traceability of plasma should be established; traceability should be enforced through accurate donor, donation, product and laboratory sample identification procedures, through record maintenance and use of an appropriate labelling system.
- Requirements for selection of donors, including exclusion criteria for donors with risk behaviours; provision of information to donors on risk situations and the donation in general; and the use of a questionnaire to obtain information on donor's health.
- Requirements for testing of each donation.
- Requirements regarding traceability and documentation.
- Post-donation information system.

8.4 Inspections

In conducting regular inspections as part of the licensing procedure, enforcement of the implementation of GMP is required aiming to ensure the compliance of the blood establishments with the existing provisions. It is the responsibility of the inspector from the national regulatory authority to ensure that the manufacturers and the blood and plasma establishments adhere to the approved standards of GMP and quality assurance, including at sites where plasma is collected as starting material.

The inspections should be carried out by officials representing the competent national regulatory authority. These officials should be specialized inspectors, trained in GMP inspections, and they should be familiar with blood bank technologies and the special features of quality assurance in the collection of plasma. Inspections may follow common inspection procedures, including:

- an opening meeting;
- a blood establishment tour;
- inspection of main areas and activities;
  - — donor acceptance and identification
  - — donor suitability
  - — collection process
  - — processing and sampling
  - — plasma freezing
  - — testing and availability of test results
  - — release of plasma units
  - — storage, transportation and shipment
  - — quality assurance (including self inspection and change control)
  - — documentation (standard operating procedures, records, donor record files and log books)

  - personnel and organization
  - qualification and process validations
  - error and corrective action system
  - look-back information, recalls and complaints
  - product quality controls
- a final meeting summarizing the inspection outcome.

A thorough inspection includes the observation of staff during performance operation and comparison with defined written procedures. In a "system control", the inspection can be considered not only as a GMP inspection, but also as an indirect product quality assessment by checking product-specific validation and quality control data.

A written report should summarize the main aspects of the inspection including its scope, a description of the company, the deficiencies listed, specified and classified (e.g. as critical, major or minor), and a conclusion. The written report will be sent to the company. The companies are requested to notify the national regulatory authority about the specific steps which are taken or planned to correct the failures and to prevent their recurrence. If necessary, follow-up inspections should be performed e.g. to check that specific corrective actions have been implementd.

The national regulatory authority should have the authority to withdraw an establishment licence in a case where inspection results showed critical non-compliance with the requirements or product specifications.

Information on the collection and control of the starting material, human blood or plasma, and on the procedures conducted during the preparation of the final blood derived medicinal product have to be documented as part of the dossier in the marketing authorization.

In summary, the implementation of licensing and inspection systems for blood establishments has become an important tool through which the national regulatory authorities confirm the assurance of quality of plasma as starting material for fractionation. The use of international standards not only further promotes harmonization, but also facilitates regional collaboration and information exchange between the national regulatory authorities.

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Appendix 1

Plasma products and clinical applications[1]

Products	Main indications
Albumin	
Human serum albumin	Volume replacement
Blood coagulation factors	
Factor VIII[a]	Haemophilia A
Prothrombin complex	Complex liver diseases; warfarin or coumarin derivatives reversal[c]
Factor IX	Haemophilia B
Factor VII	Factor VII deficiency
Von Willebrand factor	Von Willebrand factor deficiency (type 3 and severe forms of type 2)
Factor XI	Haemophilia C (factor XI deficiency)
Fibrinogen	Fibrinogen deficiency
Factor XIII	Factor XIII deficiency
Activated PCC	Haemophilia with anti-factor VIII (or factor IX) inhibitors
Protease inhibitors	
Antithrombin	Antithrombin deficiency
Alpha 1 antitrypsin	Congenital deficiency of alpha 1 antitrypsin with clinically demonstrable panacinar emphysema
C1-inhibitor	Hereditary angioedema
Anticoagulants	
Protein C	Protein C deficiency
Fibrin sealant (fibrin glue)[d]	Topical haemostatic/healing/sealing agent (surgical adjunct)

[a] Some factor VIII concentrates containing von Willebrand factor are effective for the treatment of von Willebrand disease

[b] Prothrombin complex contains factor II, factor VII, factor IX, and factor X. The content of factor VII may vary depending upon products.

[c] May be used, in the absence of purified plasma products, for substitutive therapy in factor VII, factor X, or protein C deficiency. Whenever available, purified factor IX should be used to treat haemophilia B.

[d] Product obtained by mixing a concentrate rich in fibrinogen and a concentrate rich in thrombin.

[1] Adapted from: Ala, F, Burnouf T, El-Nageh M. *Plasma fractionation programmes for developing economies. Technical aspects and organizational requirements*. Cairo, WHO Regional Publications, 1999 (Eastern Mediterranean Series).

Products	Main indications
Intramuscular immunoglobulins (IMIG)	
Normal (polyvalent)	Prevention of hepatitis A (also rubella, and other specific infections)
Hepatitis B	Prevention of hepatitis B
Tetanus	Treatment or prevention of tetanus infection
Anti-Rho (D)	Prevention of haemolytic disease of the newborn
Rabies	Prevention of rabies infection
Varicella/zoster	Prevention of chickenpox infection
Intravenous immunoglobulins (IVIG)	
Normal (polyvalent)	Replacement therapy in immune deficiency states;
Cytomegalovirus (CMV)	Prevention of CMV infection (e.g. after bone marrow transplantation)
Hepatitis B	Prevention of HBV infection (e.g. liver transplant)
Rho (D)	Prevention of haemolytic disease of the newborn

Appendix 2

Donor selection

1. Preamble

Recognizing the importance of the provision of safe blood, blood components and plasma derivatives, the 58th World Health Assembly in 2005 (WHA Resolution 58.13) (*1*) expressed its support for "the full implementation of well-organized, nationally coordinated and sustainable blood programmes with appropriate regulatory systems" and stressed the role of "voluntary, non-remunerated blood donors from low-risk populations". The provision of blood, blood components and plasma derivatives from voluntary, non remunerated donors should be the aim of all countries.

2. Information to donors

Candidate donors should receive an explanation, ideally both verbally and in writing, or by any other appropriate means such as a self-administered questionnaire, that answers to questions about their medical history and personal behaviour are necessary to determine whether they are eligible to donate blood or plasma. Written information can be in the form of a leaflet explaining the risks of infection associated with blood and plasma products; impact of social behaviour on risks of infection and risk factors for infection. This information is generally given by a licensed physician, or by a person under the direct supervision of a licensed physician, who should explain the exclusion criteria for donating blood and plasma. A convenient communication system should ensure that risk factors are well understood by the candidate donor.

Additionally, the donor should be asked to inform the blood centre if he or she feels unwell after the donation or if he or she forgot to mention a possible risk factor. This is of special importance for a donation used to prepare plasma for fractionation as it is important to be able to remove at-risk donations prior to the industrial pooling stage to avoid the potential need to destroy the plasma pool or the intermediates or products derived from it.

3. Compliance with donor selection criteria

3.1 Positive identification of donors

Upon presentation at the blood/plasma collection site, donors should be asked to identify themselves by stating their name, address and date of birth, and to supply proof of a permanent place of residence to establish a reliable means of contact, including, for example, a telephone number where they can be contacted after donation, if needed. Proof of identity (such as identity card, passport or driving licence) should be provided. Identification of donors should also take place immediately before venipuncture.

3.2 Confidentiality

The premises and setting of the blood/plasma collection centre (or the mobile collection unit) should allow for adequate confidentiality during the donor's interview and the selection process so that the candidate donor will not avoid answering questions on his or her personal or private behaviour, which otherwise would compromise the safety of the plasma donation used for the fractionation process**.**

3.3 Questionnaire and interview

The assessment of each donor should be carried out by a suitably qualified person, trained in use of donor selection criteria and will involve an interview, a questionnaire and further direct questions if necessary. In order to obtain relevant and consistent information about the donor's medical history and general health, it is recommended that the donor can review, complete and sign a pre-printed questionnaire (computer-assisted self-administered interviewing (CASI) is being developed in some regions), adapted to the type of donor (for instance, first-time donor versus regular donor). The questionnaire should be drafted in such a way that donors may easily identify whether they are in good health.

Candidate donors who are at risk of carrying a disease transmissible by blood/plasma derived products should be able to exclude themselves voluntarily after reading and responding to the donor information and/or the questionnaire. Such confidential self-exclusion should also be possible after the donation (e.g. by telephone).

The candidate donor should be asked to sign an informed consent to give blood/plasma in which he or she acknowledges an understanding of the moral responsibility behind the donation of blood/plasma.

3.4 Physical examination, acceptance and deferral criteria

3.4.1 *Physical examination*

Prior to the first donation and before subsequent blood donations and in case of plasmapheresis at regular intervals, a physical examination should

be carried out by a licensed physician or a physician substitute following an established procedure. Local national regulatory authorities, usually after consultation with the blood establishment, should determine the health criteria and the respective acceptable limits to be taken into consideration during physical examination, such as measurement of weight, blood pressure, pulse rate and temperature, or any other criteria considered to affect the safety of plasma-derived products or the donor.

3.4.2 *Records and traceability*

An appropriate computerized or, if not available, manual system should exist to keep records of the donors, of their medical history and health status, and to ensure efficient traceability of their donations. Such information provides historical perspective on the health status of the donors, including previous temporary deferrals (should they exist), and contributes to reinforcing the judgement as to whether the donation would present a risk to the quality and safety of plasma for fractionation.

3.4.3 *Selection and exclusion criteria*

The following elements have been recognized as playing a role in selecting the safest donors:

Establishment of exclusion criteria: Relevant acceptance, deferral and exclusion criteria for the donation of blood/plasma used for fractionation should be formulated by the national regulatory authority and be applicable nationwide, as national requirements. Within the scope of their role to establish and implement effective national regulations, local national regulatory authorities should enforce such criteria. Based on the characteristics of the production process used to manufacture plasma-derived products, the plasma fractionator may suggest additional or alternative exclusion criteria. For instance, in some countries, the plasma from first-time donors is not used.

*Deferral***:** A defined list of permanent or temporary deferral criteria used for candidate donors from which the plasma would be used for fractionation, should be clearly stated, made public, and incorporated in the donor educational material. The physician performing the physical examination should be able to identify whether the donor has been previously deferred and, if so, for what reason. Examples of the major permanent deferral criteria found in international guidelines include:

- clinical or laboratory evidence of blood-borne infectious diseases, e.g. infection with HIV, HBV or HCV;
- past or present intravenous drug use.

Other exclusion criteria, either permanent or temporary, may include:

- sexual relationship between men;
- men or women who are engaged in prostitution;

- subjects with haemophilia or other clotting-factor defects, in particular if treated with clotting factors;
- sexual partners of any of the above or of someone the donor suspects may carry the above risk factors;
- jaundice within the 12 months prior to donation, as it may be a clinical sign of hepatitis A, B or C;
- transfusion with blood, blood components, or plasma products in the 12 months previous to donation, as blood transfusion is a risk factor for all blood-borne infections;
- tattooing, scarification, ear piercing, acupuncture in the 12 months prior to donation. These practices may be a vehicle for the transmission of viral diseases unless clear evidence is provided that the procedure was carried out under sterile conditions;
- a particular policy may be required with regard to the exclusion criteria for a risk factor relevant to the safety of cellular blood components although it does not create safety issues for the preparation of plasma for fractionation and plasma derived products. For instance, risk factors for HTLV infection (e.g. due to travel in countries where the prevalence is high) may be an exclusion criterion for the donation of blood components, but this virus cannot be transmitted by plasma products. It is however not advisable to introduce two screening and quality standards for products separated from a whole blood unit (e.g. red cell concentrates and plasma for fractionation) as this may in itself create a risk of mishandling and error at the blood collection centre.

3.4.4 *Reinstatement*

When temporary deferral criteria are applied, a specific procedure conducted by trained personnel should be in place for reinstatement of donors. Some exclusion criteria are temporary (e.g. as long as a risk factor has been identified) and can be waived once additional checks on the donor have been made, or the time period for exclusion has passed.

3.4.5 *Procedures*

Based on such criteria, a written procedure should be in place at the blood/plasma collection centre to control donor acceptance and deferral criteria. The procedure should comply with the requirements of the national regulatory authority and fractionator. Abnormal conditions should be referred to the physician who has the responsibility of making the final decision on the donor suitability. If the physician has any doubt about the donor's suitability, donation should be deferred.

Reference

1. Resolution WHA58.13. Blood safety: proposal to establish World Blood Donor Day. In: *Fifty-eighth World Health Assembly*. Geneva, World Health Organization, 2005.

Appendix 3

Donor immunization and plasmapheresis for the manufacture of specific immunoglobulins

There is a need for hyperimmune plasma for the manufacture of specific immunoglobulins that are clinically valid for therapeutic and prophylactic uses.

Donors with acquired antibodies

Plasma may be collected by plasmapheresis from donors who have acquired immunity through natural infection or through active immunization with approved vaccines for their own protection. Donors with medically useful plasma may be identified by screening whole blood donations or by testing the plasma of convalescent patients or vaccinated individuals who have produced high-titre antibodies with the desired specificity, for example, patients recovering from varicella-zoster infection or donors who have been immunized with rabies vaccine. Unnecessary primary immunizations can be avoided by this approach. Donation of plasma following natural infection should be deferred until the potential donor is asymptomatic, and non-viraemic.

Donors who require immunization

To ensure a sufficient supply of life-saving immunoglobulins to treat patients, deliberate immunization of healthy volunteers may be necessary in addition to collection of plasma from convalescent patients and donors selected by screening for high levels of specific antibodies. The immunization of donors requires informed consent in writing and should take into consideration all the requirements of this Annex.

Donors should be immunized with antigens only when sufficient supplies of material of suitable quality cannot be obtained from other appropriate donors, or from donations selected by screening. Donors should be fully informed of the risk of any proposed immunization procedure, and pressure should not be brought to bear on a donor to agree to immunization. Women capable of child-bearing should not be immunized with erythrocytes or other antigens that may produce antibodies harmful to the fetus. Donors with known allergies should preferably not be recruited.

Every effort should be made to use the minimum dose of antigen and number of injections. In any immunization programme, the following should be taken into consideration as a minimum:

— the antibody assay;
— the minimum level of antibody required;

— data showing that the dose, the intervals between injections and the total dosage proposed for each antigen are appropriate; and
— the criteria for considering a prospective donor a non-responder for a given antigen.

A donor could be hyperimmunized with more than one immunizing preparation as long as the safety of the procedure of multiple immunizations is demonstrated.

Potential donors should be:

- informed by a licensed physician of the procedures, risks and possible sequelae and how to report any adverse effects, and encouraged to take part in a free discussion (which, in some countries, takes place with small groups of potential donors);
- informed that they are free to withdraw their consent at any time.

In addition, donors may also be:

- encouraged to seek advice from their family doctor, or from an independent competent counsellor, before agreeing to immunization; and
- informed that any licensed physician of their choice will be sent all the information about the proposed immunization procedure.

All vaccines used for immunizing donors should be approved by the national regulatory authority. Special care should be taken to ensure the safety of the donor when a vaccine is administered at doses or according to schedules that differ from those recommended for routine prophylactic immunization. Erythrocyte and other cellular antigens should be obtained from an establishment approved by the national regulatory authority. Donors should be observed for approximately 30 min following any immunization in order to determine whether an adverse reaction takes place. Because reactions often occur 2–3 h after immunization, donors should be advised of this possibility and instructed to contact the facility's physician if a reaction is suspected in the first 12 h after immunization. Reactions may be local or systemic. Local reactions, which may be immediate or delayed, take the form of redness, swelling or pain at the injection site. Systemic reactions may include fever, chills, malaise, arthralgia, anorexia, shortness of breath and wheezing. An insurance system should be in place to compensate for side-effects to the donor.

Immunization with human erythrocytes

Erythrocyte donors

A donor of erythrocytes for the purposes of immunization should meet all the general health criteria for donors (see Appendix 2). Relevant measures should be taken to limit the risk of infectious diseases; these may vary from

country to country taking into consideration the relevant risks. For instance, in some countries, the donor should never have had a blood transfusion in order to reduce risks of vCJD. Prior to the first donation, the donor should be found to be negative for relevant markers, which may include the following: syphilis, HBsAg, anti-HIV, antibody to hepatitis B core antigen (anti-HBc), anti-HCV and antibodies to human T-cell lymphotropic viruses (anti-HTLV), and the serum level of aminotransferases should be within normal limits as established by the national control authority. Erythrocyte phenotyping should be done for ABO as well as for C, D, E, c and e. It is advantageous to select red cells expressing high amounts of RhD antigen, e.g. homozygous D or Rho, for immunization. Phenotyping for other clinically relevant specificities is also required , especially for Kell, Fya/Fyb, Jka/Jkb and S/s. The volume of erythrocytes drawn from a donor should not exceed 450–500 ml of whole blood in any 12 week period. Shorter intervals may induce iron deficiency and, possibly, anaemia. Erythrocytes obtained for immunization purposes should be frozen (at least for 6–12 months depending upon the sensitivity and range of the tests performed, e.g. the use of NAT) before use and the donor should be retested and shown to be negative for the above markers of infection before the stored cells are released and used for immunization. Pre-storage leukoreduction of donations is considered desirable, and NAT testing for HBV, HCV and HIV would give an additional level of safety.

Collection and storage of erythrocytes

Erythrocytes should be collected under aseptic conditions into sterile pyrogen-free containers in an appropriate proportion of an approved anticoagulant. They may then be dispensed in aliquots under aseptic conditions into single-dose sterile, pyrogen-free containers for storage. The microbiological safety of the dispensing environment should be validated. The method selected should have been shown to provide acceptable cell recovery in vitro (80%) or in vivo (70%). Erythrocytes should be washed after storage to remove the cryoprotective agent (e.g. glycerol). Adequate sterility data to support the shelf-life for stored erythrocytes should be kept on file. A test for bacterial and fungal contamination should be done on all blood dispensed in aliquots in an open system. The test should also be performed on at least one single-dose vial from each lot of whole blood that has been stored unfrozen for more than seven days. The test should be done on the eighth day after collection and again on the expiry date. Sterility tests should be performed following an approved procedure.

Erythrocyte recipients

The following additional testing of erythrocyte recipients is necessary:

- The recipient should be phenotyped for ABO, Rh, Kell Fya/Fyb, Jka/Jkb and S/s antigens before immunization. The red cell donor and the recipient

should be matched as far as possible for major blood group antigens other than RhD. Only ABO-compatible erythrocytes may be transfused. Whereas mismatching within the Rh system for C and or E is acceptable, mismatching in the Kell, Fy, Jk and S/s systems is unacceptable.
- Screening for unexpected antibodies by methods that demonstrate coating and haemolvtic antibodies should use the antiglobulin method or a procedure of equivalent sensitivity.

Prospective erythrocyte recipients in whom antibody screening tests demonstrate the presence of erythrocyte antibodies (other than those deliberately stimulated through immunization by the plasmapheresis centre) should be asked whether they have ever been pregnant or had a blood transfusion, a tissue graft or an injection of erythrocytes for any reason. This history should form part of the permanent record and should identify the cause of immunization as clearly as possible. Recipients should be notified in writing of any specific antibodies they have developed after injection of erythrocytes. The plasma centre should maintain records, which should be reviewed during inspection. The immunized donor should carry a card or medicalert bracelet specifying the antibodies. These measures allow optimal care of immunized donors who may require an emergency transfusion, (e.g. following a road traffic accident) at some future time, and for whom knowledge of the antibody status, especially mixtures of antibodies, is important.

Immunization schedules

Erythrocytes used for immunization purposes should not be administered as part of any plasmapheresis procedure. Such immunization may be performed on the same day as plasmapheresis, but only after it and as a separate procedure.

To minimize the risk of infection to the donor, the immunization schedule should involve as few doses of erythrocytes as possible. Wherever possible, the same red cell donor should be used throughout the immunization programme of an individual plasma donor.

For primary immunization two injections of erythrocytes, each of a volume of about 2–5 ml and given 3 months apart, elicit antibody formation within three months of the second injection. Different schedules may be used for de novo immunization. It is advantageous to choose as donors of anti-D (anti-Rho) volunteers who are already immunized, because useful levels of anti-D are then usually attained within a few weeks of reimmunization with 2–5 ml of erythrocytes. About 70% of immunized volunteers eventually produce antibody levels well above 100 IU/ml. The baseline antibody titre of every recipient of erythrocytes should be established, and the antibody response, including both type and titre, should be monitored monthly to

establish the peak level of anti-D and duration of the response. The response of each recipient is individual, and additional injections of erythrocytes may be required at intervals of 2–9 months to maintain anti-D levels (*1*). If injections of erythrocytes are discontinued, antibody levels usually fall appreciably within 6-12 months. Erythrocytes to be used for immunization purposes should be selected, for each recipient, by a licensed physician or a suitably trained and qualified person**.**

Donors undergoing primary immunization who have not responded to a total of up to 150 ml erythrocytes are likely to be 'non-responders' and should be removed from the panel.

Plasmapheresis schedules

Donors should comply with the requirements for health screening and maximum plasma donation allowed by their national authorities.

Risks to recipients

Recipients of erythrocytes for immunization purposes may be at risk of:

— viral hepatitis (B and C) and HIV infection;
— other infectious diseases;
— HLA immunization;
— the production of unwanted erythrocyte antibodies that may complicate any future blood transfusion;
— a febrile haemolytic reaction if the antigen dose is too high;
— vCJD in countries where this is endemic.

Record-keeping

Records of erythrocyte donors and of the recipients of their erythrocytes should be maintained and cross-referenced and stored at least for the minimum time required for blood transfusion recipients by the national authorities.

Reference

1. **Cook I et al.** Frozen red cells in Rhesus immunization. *British Journal of Haematology,* 1980, **44**:627.

Appendix 4

Contract plasma fractionation programme

The fractionation of plasma requires specialized facilities, with provision for large-scale protein separation, purification, virus inactivation and formulation, as well as for aseptic finishing and freeze-drying. The preparation of plasma-derived products should be governed by the same regulatory considerations that are applied to medicines. Manufacturers are required to obtain manufacturing licences which should cover the method of preparation and product characteristics. To obtain a licence, it is necessary to demonstrate adherence to GMP. Considerable technological, pharmaceutical and scientific expertise is required to meet these demands. Since key utilities (such as heating, ventilation and air-conditioning (HVAC), refrigeration and water for injection) should be maintained operational even when the facility is not fractionating plasma, the investment in and running costs of fractionation are substantial. The economic viability of a fractionation facility will be determined by:

- the cost of the plasma for fractionation (in particular cost-allocation of the whole blood collection system on plasma versus labile components);
- the operating capacity of the facility; and
- plasma availability and product demand to allow the facility to operate continuously at near to maximum capacity.

The break-even point for minimum annual plasma throughput for economic viability may vary greatly according to a set of parameters, these including plasma cost, product portfolio, adequacy of the various plasma products versus the plasma needed to cover those needs, and product yield. Therefore such projects require a careful feasibility study.

Countries which cannot justify building and operating a fractionation facility, may opt to have plasma collected locally and shipped for processing in an independent facility—so-called contract or toll fractionation. Plasma-derived products are then returned to the originating country on payment of a fee (toll). Such arrangements can work well, subject to specific provisions being made and adhered to. These include:

- commercial and quality agreements defining the responsibilities of both parties (the contract giver and the contract acceptor);
- clearly defined requirements for plasma quality (including the arrangements for donor selection, testing and traceability);
- provision for audit of the plasma collection centre (by the fractionator) and inspection by an appropriate regulatory body;
- formal approval of the contract plasma fractionation activities by the regulatory authority of the fractionator;

- a contractual commitment to supply agreed quantities of plasma. The annual minimal volume is dependent upon the fractionator's overall free capacity and specific aspects of production such as plasma pool and product batch size;
- agreement on the arrangements for storage and shipment of plasma, with defined provisions for monitoring and control (typically transport by sea, at –20 °C or below);
- agreement on the range of products to be manufactured; and
- agreement on specific aspects of plasma processing (including batch size, possible requirements for segregation of processing, agreed use or destruction of excess intermediates, expected yield and toll fees).

Plasma products made from local plasma need to receive a specific registration, even if the same products made from foreign plasma are already licensed in the country of origin.

The regulatory authorities of the country where the plasma is collected may require inspection of the fractionation centre. Table 1 summarizes the responsibilities and roles of each party.

Table 1
Responsibilities and roles of blood establishment, plasma fractionator, and regulatory authorities

Task	Blood establishment	Plasma fractionator	Regulatory authority
Epidemiology surveillance of donor population	Collects and analyses the data based on results of screening tests	Reviews the data	Reviews the data
Donor selection and interview	Develops and implements the criteria in selection and interview of donors	Verifies that criteria set by national regulatory authority are met; may provide additional selection criteria	Sets the criteria and inspects the blood establishment
Serological testing of donation	Performs validated tests (or the tests may be sub-contracted)	Agrees on the tests kits used and audits the virology laboratory	Approves test kits and inspects the blood establishment
Post-donation follow-up and haemovigilance	Informs plasma fractionator (and when appropriate the regulatory authority) when relevant information is obtained	Takes appropriate measures if plasma pool or product quality is compromised	Evaluates haemovigilance/post-donation reports with regards to product quality and safety

Task	Blood establishment	Plasma fractionator	Regulatory authority
Preparation of plasma	Collects blood plasma, prepares, freezes, and stores the plasma, according to good manufacturing practice (GMP)	Sets the specifications and audits	Approves and inspects the blood establishment
Nucleic acid testing (NAT) (mini-pool)	Prepares the NAT samples following fractionator's specifications	Provides the standard operating procedure for NAT samples and performs (or sub-contracts) the validated testing	Approves the procedure and inspects the plasma fractionator
Fractionation methods including viral reduction		Applies the fractionation methods following GMPs and processes described in marketing authorization	Evaluates the data presented in the dossiers prepared by the fractionator, and inspects fractionation facility
Preparation of plasma product regulatory files		Prepares the files	Reviews and evaluates
GMP[a]	Implements GMP	Audits the blood establishment	Inspects blood establishment and enforces GMP
Granting of marketing authorization			Grants the marketing authorization
Plasma product pharmacovigilance		Does pharmacovigilance studies and informs regulatory authorities and blood establishment when relevant side-effects are found	Evaluates pharmacovigilance reports with regards to product quality and safety

[a] See sections 7 and 8 of this annex.

Appendix 5

Technical points to consider in establishing plasma specifications criteria and obligations between blood establishment and plasma fractionator

The purpose of the contract is to have a "legally binding" document between the plasma supplier and the fractionator.

The following is an example of the quality control and documentation required by a plasma fractionator to acquire plasma for fractionation from a blood establishment. It is not meant to represent the only possible way to define plasma specifications criteria and obligations between a blood establishment and a plasma fractionator. Depending upon the prevalence of blood-borne diseases in a country, additional safety requirements on donor selection and testing should be considered.

General specifications

Donors

Reference should be made to local regulations pertaining to the selection, eligibility, and exclusion criteria for donors of blood or plasma used for the manufacture of blood components and plasma derivatives. Newly introduced criteria may be spelled out (such as travel restrictions related to vCJD).

Blood establishments

Reference should be made to the official legislation of blood establishments in the country of origin and to relevant legislation relating to plasma fractionation.

Donation process and plasma unit specifications

The contract should cover the following aspects of the donation process and plasma unit specifications.

- *Collection process of the blood/plasma units:*
  - — containers, collection sets and anticoagulants with relevant registration;
  - — duration of the whole blood collection (e.g. less than 15 minutes (*1*) for recovered plasma);
  - — guarantee that blood will be mixed with the anticoagulant as soon as the collection starts, by regular manual shaking or using a validated automated method (*1*);

— prior to freezing, plasma is clear (light opalescence may be allowed), yellow to — green in colour, with no sign of haemolysis or presence of red cells (*2*); and
— acceptable citrate concentration range.

- *Infectious markers:*
  — test kits used should be of acceptable sensitivity and be agreed with manufacturer;
  — anti-HIV 1 and 2, anti-HCV and HBsAg should be absent, and there should be no laboratory evidence of syphilis;
  — when applicable: specific handling of anti-HBc positive donations (e.g. accepted only if anti-HBs antibody titre > 0.050 IU/ml and HBsAg negative); and
  — HCV NAT and HIV tests must be negative (i.e. when a blood establishment organization performs NAT for HCV and HIV for blood components).
- *Immunohaematological markers*
  — anti-A and anti-B titre < 1/64 using a validated assay;
  — special requirements relative to the absence of irregular antibodies.
- *Cellular content and haemoglobin*
  — statistical records of blood cell contamination showing that the relevant specifications are met. Some countries/fractionators have set specific limits on the residual leukocyte content of plasma for fractionation;
  — statistical records of haemoglobin contamination showing that the relevant specifications are met.
- *Protein quality control*
  — protein content ≥ 50 g/l after mixture with the anticoagulant;
  — when plasma is used for production of factor VIII concentrate; minimum factor VIII content to be specified for a pool sample of a defined number of donations
- *Other criteria*
  — minimal acceptable volume of plasma per container;
  — plasma freezing conditions: core temperature, time taken to freeze, and absence of folding to avoid a thin plasma layer that would be more susceptible to thawing during subsequent handling;
  — maximum acceptable thickness of plasma containers;
  — positioning of the donation identification label (number and bar code);
  — plasma storage temperature;
  — plasma density (used to determine the volume of plasma shipped to/ received by fractionator);
  — maximum time elapsed between donation and shipment to the fractionator.

Standard plasma

Plasma types

Plasma categories vary depending upon fractionator and local regulations. For instance, some fractionators may classify as plasma, either from whole blood or from apheresis, based on the time interval between the collection procedure and freezing.

Examples of plasma categories include:

- *Category A*: apheresis plasma frozen within 6 hours, with a factor VIII content ≥ 0.7 IU/ml;
- *Category B*: Recovered plasma with a factor VIII content ≥ 0.7 IU/ml, obtained from whole blood kept at 20–22 °C and frozen within 6 hours (in the absence of devices to maintain blood temperature), or frozen within 20 hours (if devices to maintain blood temperature are used);
- *Category C*: Plasma frozen within 24 hours after collection, or plasma initially categorized as A or B but containing ≤ 0.7 IU factor VIII/ml. This plasma is used to produce immunoglobulins and albumin only.

Hyperimmune plasma

Quality criteria

Acceptable criteria include:

- protein content, factor VIII, haemoglobin: usually the same as for standard plasma;
- a minimum potency level will be set for each antibody type. Where possible, the required potency will be specified in IU per ml when assayed using an agreed method which includes an agreed reference control calibrated in IU/ml. Examples of limits are as follows:
  - — anti-tetanus: 10 IU/ml;
  - — anti-varicella/zoster: 10 IU/ml;
  - — anti-HBs: 25 IU/ml;
- Indication of the assay procedure, procurement of standards, test laboratory and communication procedure of the data.

Documentation

Each blood establishment delivering plasma should have an approved organizational chart, and changes should be communicated to the plasma fractionation centre according to an agreed procedure.

Shipping documentation should include:

- dated shipping document signed by responsible person;

- certificate of origin and control of the plasma, stating for each donation the:
  - collection date;
  - carton number;
  - results of virology and immunohaematology screening;
  - test kits used and their batch number;
  - signature of the director or an authorized person;
- password-protected electronic file of the plasma donations and samples sent, stating for each donation collection date (this needs to be agreed with the fractionator):
  - carton number;
  - results of virology and immunohaematology screening;
  - test kits used and their batch number;
- upon request, additional information on viral screening tests and confirmatory assays can be provided to the fractionator;
- epidemiology data should be made available as appropriate, e.g. annually.

Shipment

Specifications relating to shipment include the following:

Plasma donations

- Broken plasma containers are not acceptable.
- When applicable, specifications of "pig tail" used for additional screening tests by the fractionator (e.g. length of 10–20 cm, attached to the plasma donation, and ideally, identified with the donation number).
- Specification of the plasma container identification (labels and barcode).
- Specification on potential additional samples sent with the shipment for additional screening tests such as NAT or for the look-back procedure.
- Statement on minimal number of plasma containers per shipping box or carton, and positioning.

Containers for shipment

Auditing programme

The contract should cover the following aspects of the auditing programme:

- obligation of the blood establishment to be subjected to auditing by the fractionator;
- routine auditing performed by the fractionator should follow an internally approved and regularly revised procedure with an established list of questions and check-points;
- special auditing performed annually/biannually based on a programme previously communicated to the director of the blood establishment;

- audit reports are communicated to the director of the blood establishment;
- list of reference documents (such as internal acceptance criteria for the preparation of plasma for fractionation).

Notification obligations

Notification obligations cover the following:

- obligation to notify the fractionator each time the safety of a previous donation may be questionable;
- obligation to notify the fractionator when:
  - a unit positive for viral markers such as HBsAg, HIV-1 and HIV-2 antibodies, HCV antibody or syphilis has been sent by mistake;
  - a deviation is subsequently discovered in any of the screening tests performed on the plasma units supplied. In this situation, the blood establishment should attempt to retest the implicated units if suitable library samples are available;
  - a regular donor is found to be positive for a marker although the previous donation was found to be negative;
  - the blood establishment is informed that a donor, previously contributing to plasma for fractionator, has developed an infectious disease potentially transmissible by plasma;
  - a donation is found to have transmitted an infectious disease, or there is strong evidence implicating a donation in disease transmission;
  - the blood establishment is informed that a donor previously contributing to plasma for fractionation: (a) has developed CJD or vCJD (in such a case the report with the pathological findings should be provided if available); (b) has risk factors for vCJD; or (c) is identified as exhibiting risk behaviour or other factors that affect the safety of the plasma;
  - the blood establishment is informed that a patient has developed post-transfusion infection following transfusion of blood component(s) obtained from a donor who has also donated one or more units of plasma for fractionation.

Notifications should provide the list of all donations made within a 6-month period prior to the last donation found to be negative. The period of time depends on local regulations and the type of disease. The fractionator may request additional data on previous donations when thought necessary.

A communication procedure must be in place indicating information that must be provided. This should include:

- name of qualified person at the fractionator to be contacted;
- reasons and description of the problem (under confidentiality clauses);

- the time period between information being known and communication to the fractionator;
- if the problem is related to an infectious disease, a list of all plasma for fractionation donations made in the defined period prior to the last donation found negative;
- name of the blood establishment, director, donation number, carton number as indicated on the electronic file sent with the shipment, date of shipment, date of notification and signature of the responsible person or his or her delegate.

References

1. Anonymous. *Guide to the preparation, use and quality assurance of blood components*. 13th ed. Strasbourg, Council of Europe Publishing, 2007.
2. Anonymous. Monograph of human plasma for fractionation 01/2005:0853 corrected. *European Pharmacopoeia*, Strasbourg, 2005.