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Guidelines for Production and Quality Control of Blood Products

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Executive Board of the Health Ministers' Council for GCC States

Recommendations for the Production, Control and Regulations of Human Plasma for Fractionation

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Introduction

Human plasma is a source of important medicinal products which are obtained by a combination of large-scale processing steps called “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] supports "the full implementation of well-organized, nationally coordinated and sustainable blood programmes with appropriate regulatory systems" and stresses 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 the time that document was published, requiring that updated both technical and regulation guidelines be prepared and made public at global level. The 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 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 combination of information is necessary for the manufacture of safe plasma derivatives at global level, in both developed and developing countries.

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 national level. It is intended to assist National (Medicine) Regulatory Authorities (NRA) 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 NRA.

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

1. International Biological Reference Preparations

Rapid technological developments in the measurement of biological activity of blood and blood products has required and still require the establishment of international biological reference materials. The full list of current reference materials relevant to blood products and related substances is available at the following WHO Web site address:
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 International System of Units (SI) units.

2. List of Abbreviations and Definitions Used

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: a 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 (red cells, white cells, platelets, plasma) that can be prepared under such conditions that it can be used 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 their processing, storage, and distribution when intended for transfusion.

Donor: a person who gives blood or plasma used for fractionation.

EIS: Electronic information system

Factor VIII: Blood coagulation factor VIII, deficient in patients with haemophilia A. Also called antihæmophilic factor.

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

First time tested donor: A person whose blood/plasma is tested for the first time for infectious disease markers in a blood establishment.

Fractionation: (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 usually 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 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.

FFP: Fresh frozen plasma, used for transfusion.

GE: Genome equivalents: The amount of nucleic acid of a particular virus assessed using nucleic acid testing.

GMP. (Good Manufacturing Practice): 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.

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

HBsAg, Hepatitis B surface antigen. The antigen on the periphery of hepatitis B virus.

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

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

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

HGV, Hepatitis G virus [(or GB virus C (GBV-C)). An enveloped single-stranded RNA virus, causative agent of hepatitis G.

HIV. Human immunodeficiency virus. An enveloped, single-stranded RNA virus, causative agent of 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.

IVIG. Intravenous immunoglobulin. Also known as Immune Globulin intravenous.

Lookback: Procedure to be followed if it was 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.

NAT: Nucleic acid testing, using amplification techniques such as polymerase chain reaction.

NRA: National Regulatory Authority. WHO terminology to refer 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 Annex 1) obtained by the fractionation process of human plasma. Also called **plasma derivatives** 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 sub/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/plasma has been tested previously for infectious disease markers in the blood establishment.

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

SI: International System of Units.

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 forward tracing (donation to disposition) and reverse tracing (disposition to donation).

TSE: Transmissible spongiform encephalopathy.

TTV: TT virus, is 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.

WNV: West Nile virus is an enveloped single-stranded RNA virus, causative agent of West Nile fever.

3. General Considerations

3.1 Range of products made from human blood and plasma

A range of products can be made from human blood. Some of these products, generally known as blood components, include red cell concentrates, platelet concentrates, leukocyte concentrates, and plasma for transfusion. These components are obtained from the processing of *single* donations of blood or plasma but small pools, usually of less than 10 donations, mainly for the production of platelet concentrates[4], 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 selection criteria of the donors and the screening of the 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 regulations needed to ensure their quality and safety, in particular with regards to the control of infectious risks.

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 system used as well as of product efficacy, should be under the responsibility of the NRA in all Member States. The NRA has the duty to enforce regulations, to evaluate quality and safety of products, and to conduct regular assessment and inspection of the manufacturing sites.

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

Table 1: Range of blood / plasma products derived from single donor or pooled donations (adapted from [2]):

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) • Cryoprecipitate
LARGE- POOL, UNFRACTIONATED VIRALLY INACTIVATED PLASMA PRODUCT • Plasma for transfusion, Solvent-detergent (SD) treated
LARGE POOL PRODUCTS PURIFIED BY FRACTIONATION OF PLASMA • See the list of products in Annex 1

3.2 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 Table 2.

The concentrations of the various protein components vary from about 40g/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). 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 Annex 2.

Table 2: Selected Proteins of Human Plasma (adapted from [6,7]):

MAJOR PROTEINS	Daltons	mg/litre
• Albumin	68 000	40 000
• IgG	150 000	12 500
PROTEASE INHIBITORS	Daltons	mg/Litre
• 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	Daltons	mg/litre
• ADAMTS13	190	1
FIBRINOLYTIC PROTEINS	Daltons	mg/litre
• Plasminogen	92 000	200
• Histidine-rich glycoprotein	75 000	100
COAGULATION FACTORS and ANTI-COAGULANT PROTEINS	Daltons	mg/litre
• 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 ^{#1}	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 ^{#2}	Daltons	mg/litre
• IL-2	15 000	Traces
• G-CSF	20 000	< 30 pg/ml
• Erythropoietin	34 000	0.3µg/litre

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

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

3.3 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 plasma derivatives [8]. 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 0.2µm.

Table 3 summarizes the major infectious risks linked to blood-borne pathogens and presents the current evidence of risks of infection from cellular components, plasma and fractionated plasma products.

Some of the viruses listed in the Table are highly pathogenic (e.g. HIV, HCV, HBV), others are pathogenic only in certain recipient populations (e.g. CMV, B19) and few are currently considered to be non-pathogenic (HGV, 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 also avoided the transmission of some emerging infectious agents, such as WNV [9, 10].

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

Table 3: Evidence of Transmission of Infectious Agents by Human Blood^{#1} (adapted from [6,7]):

Infectious agents	Cellular blood components	Plasma	Plasma products
VIRUSES			
HIV I & II	+	+	+
HBV	+	+	+
HCV	+	+	+
Hepatitis Delta virus	+	+	+
HAV	+	+	+
HEV	+	+	+
HGV	+	+	+
TT virus	+	+	+
Parvovirus B19	+	+	+
Human T-cell leukemia virus I & II	+	-	-
Cytomegalovirus	+	-	-
Epstein Barr virus	+	-	-
West Nile virus	+	?	-
Dengue virus	+	?	-
Human Herpes virus-8	?	-	-
Simian foamy virus	? #2	?	-
Severe Acute Respiratory Syndrome virus	? #3	?	-
BACTERIA			
Spirochete (syphilis)	+	-	-
Parasites			
Babesia microti	+	-	-
Plasmodium (Malaria)	+	-	-
Leishmania (Leishmaniosis)	+	-	-
Trypanosoma cruzi (Chagas Disease)	+	-	-
UNCONVENTIONAL AGENTS /TSE			
Creutzfeldt Jakob Disease agent	-	-	-
Variant Creutzfeld Jakob Disease agent	+	?	- #4

+ : evidence of transmission;

- : no evidence of transmission;

? : questionable or unknown.

#1 Most viral transmissions associated to plasma products took place prior to the introduction of efficient viral inactivation or removal procedures.

#2 Transmitted by contact with animal blood but not reported by transfusion.

#3 Limited epidemiological surveys have not revealed transmission of SARS coronavirus by transfusion but further confirmation may be needed.

#4 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.

3.4. Strategies to ensure plasma products safety

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 individual blood/plasma donations,
 - 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.
- 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, 11]. Those procedures were also recently shown to provide a sufficient margin of safety against emerging lipid-enveloped viruses, like WNV [9, 10].

Although viral inactivation and removal treatments may therefore seem to offer the fractionator an ideal means to totally counter-balance occasional lapses in identification of risk donations, such an assumption would be incorrect. As powerful as the contribution of properly validated and implemented virus inactivation/removal steps 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, like parvovirus B19, where viral reduction procedures used during fractionation alone may not be sufficient to ensure safety [12, 13].

Exclusion of infectious donations, and retrospective identification of any infectious donation that would have passed through the screening and testing net, require the highest priority at

the blood establishment. The blood establishment should establish a reliable mechanism to ensure consistent identification of those donations.

Neither set of the measures listed above can, in isolation, provides 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 in order to ensure the optimal quality and margin of safety of this raw material for the manufacture of medicinal plasma products.

4. Measures to Exclude Infectious Donations

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

1. Appropriate selection of blood/plasma donors.
2. Testing of blood/plasma donations.
3. Epidemiological surveillance of the donor population.
4. Strict adherence to Good Manufacturing Practices (GMP).
5. 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 [14] used in the evaluation of the marketing authorization of plasma-derived medicinal products.

4.1 Appropriate selection of blood/plasma donors

Plasma for fractionation should be obtained from carefully selected, healthy donors who, after review of the 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 Annex 2). Local NRAs are pivotal in setting up at the national level a harmonized donor selection criteria framework appropriate to the country of plasma collection, taking into account the type of products, the relevant infectious risks, and the epidemiological situation. The local NRA 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, respectively, have published regulations and recommendations concerning the criteria for the selection of donors of whole blood and of plasma obtained by apheresis which is regularly updated. In general these regulations and recommendations can be used as reference for the collection of plasma for fractionation, although some specifications may differ from those of plasma for transfusion. Examples of donor selection criteria for the collection of plasma for fractionation are presented in Annex 3. These are not intended to constitute an absolute reference or 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. A repeat donor is someone who has donated before in the same establishment but not within the period of time considered as regular donation. Plasma fractionators may implement their own criteria of donors' eligibility to improve safety margins. Whenever possible, plasma for fractionation should be collected through a donation system involving regular and repeat donors. Obtaining plasma from regular and repeat donors plays a major contribution to ensure optimal historical medical information about the donors, and therefore for 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 upon situations - have been found [16] or not [17] to be at higher risks than regular/repeat donors to have markers of viral infections. The decision to use this plasma for fractionation is to be made jointly by the plasma fractionator and the NRAs and should be based on both a careful epidemiologic assessment and the evaluation of other safety measures in place for viral screening of donations.

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

4.2 Screening of blood/plasma donations for infectious markers

4.2.1 Screening tests

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

- an approved test for HBsAg;
- an approved test for anti-HIV; and

- an approved test for anti-HCV.

All three tests should be negative. 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. 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.

4.2.2 Other tests

The screening of plasma for fractionation for anti-HTLV is not required as the virus is cell associated and susceptible to freeze-thaw process.

In some countries, testing for anti-HBc is performed on whole blood donations as a means to reduce the exposure risks to hepatitis B positive blood components donations [18]. However, plasma for fractionation donations obtained from whole blood that are both anti-HBc positive and HBsAg negative, and which contains a sufficient titer in antibodies against hepatitis B surface antigen (anti-HBs) are usually used for fractionation. The scientific rationale is to maintain a sufficient anti-HBs antibody titre in the plasma pool to neutralize any HBV that may be present. The minimum anti-HBs titer for an anti-HBc positive/HBsAg negative plasma donation to be accepted for fractionation may be specified by the plasma fractionator and/or the NRA. Alternatively, the plasma donation may be identified by the plasma collector as being anti-HBc positive and the plasma fractionator may conduct additional testing. The setting of a minimum limit, if any, in anti-HBs antibody titre usually involves a risk assessment, considering the sensitivity of the HBsAg screening test, the testing or not of HBV by NAT, and the efficiency of the viral reduction techniques [3, 19].

Additional testing for other agents or markers may be required by the NRA, 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.

4.2.3 NAT testing

NAT testing of plasma for fractionation may be performed for the following viruses: HCV, HBV, HIV, HAV, and/or B19. If NAT testing 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.

4.2.4 Test kits

A system should exist in the country or region for approval of test 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/antibodies should be determined by the NRA. In addition, the test kits used should be agreed by the fractionator that will receive the plasma for fractionation.

4.2.5 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;
- 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, assays performance validation, test interpretation and downloading and follow-up of reactives can be found under QA and GMP in these guidelines.

4.2.6 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 was found retrospectively that a donation from a donor should have been excluded from processing, e.g. because that unit was collected from a donor that subsequently has been rejected for reactive viral marker, risk behavior, exposure to CJD/vCJD or other risks related to infectious diseases. The blood establishment should then transmit this information to the fractionator according to their agreements in place, and to the NRA. Donor notification and counseling is recommended both for purposes of donor health and for the safety of the blood supply.

4.3 Epidemiological surveillance of donor population

To ensure optimal long-term safety of plasma for fractionation, it is highly recommended to establish a continuous epidemiological surveillance of the donor population. The objective of this survey is to know, as precisely as possible, the prevalence and incidence, and their

respective trends, of infectious markers that are relevant to the safety of medicinal plasma products so that counter-measures can be made in a timely fashion.

The system should not only be able to gather epidemiological data at the national/regional level but also among donor populations which are providing blood/plasma for fractionation at individual blood establishments within a country or a region. The information from the epidemiological surveillance can furthermore be used:

- a. 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 donor selection and screening process among collection centres;
- b. 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;
- c. 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 of this specific donor group 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/deferral process [20-23].

Currently, it is advisable to collect and analyse epidemiological data at the collection sites for anti-HIV 1/2, anti-HCV, and HBsAg, since they historically represent the major pathogenic risks associated to plasma products. It is the responsibility of the local NRA to define whether the list should be modified or should include additional criteria, such as emerging infectious agents, based on local or regional epidemiology. For the current three recommended markers, only confirmed positive tests (i.e. tests which are repeatedly reactive in a screening test and positive in at least one confirmatory test) should be recorded.

4.4 Strict adherence to Good Manufacturing Practices

Because of 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 of importance to be able to trace back any quality and safety problems, in particular related to infectious risks, to individual blood/plasma donations and to take relevant measures to protect the donors as well as the patients who received the plasma derived medicinal products.

The donor selection process, the collection of blood/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 GMP principles and the implementation of a quality assurance system to address and comply with GMP requirements is crucial at all stages of the production of plasma for fractionation. See chapter on QA and GMP in these Guidelines.

4.5 Post donation events

There should be a system to ensure effective communication between the blood establishment and the fractionator so that significant post-donation events may be immediately transmitted to the fractionator and the NRA. 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 was sent for fractionation.

5. Production of Plasma for Fractionation

5.1 Methods used to obtain plasma for fractionation

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

5.1.1 Recovered plasma

Recovered plasma is plasma recovered by centrifugal separation from the cells and cellular debris of whole blood, following conditions described later.

5.1.2 Apheresis plasma (source plasma)

Apheresis Plasma obtained by a procedure in which anticoagulated blood is removed from the donor, the plasma is separated from the formed elements, and at 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 such use. The manufacturers of the equipment provide operating manuals that include instructions for installation validation, routine preventive maintenance procedures, 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. Additionally, 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 larger volume per donation, independently from the collection of whole blood, 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 micro-organisms. No antibacterial or antifungal agent is added to the plasma. The residual blood cell content of the plasma, in the absence of dedicated leucoreduction filtration, may vary with the collection method.

5.2 Characteristics of plasma for fractionation

5.2.1 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 5.6.2.1) 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). 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 [25, 26]. The content of various coagulation factors is usually higher in apheresis plasma compared to recovered plasma [26, 27], due to a combination of reasons that include rapid separation of blood cells and plasma, differing ratios of anticoagulant added, and the possibility of freezing the plasma soon after completion of collection.

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

Table 4: Characteristics of plasma for fractionation used in the manufacture of labile plasma products (adapted from [6,7]):

Characteristic	Recovered plasma	Apheresis plasma
Volume, ml	100-260	450-880
Protein content, g/l (each donation)	≥ 50 [15] (but typically greater than in apheresis plasma)	≥ 50
Factor VIII, IU/ml (average)	≥ 0.7 [28] (but typically less than in apheresis plasma)	≥ 0.7
Anticoagulant concentration	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 subject to a maximum of one donation every 2 months	Determined nationally

5.2.2 Plasma frozen after 24 hours of collection

Plasma may be available that does not fulfill 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 hr after collection;
- Plasma, separated from whole blood stored at 4°C, and frozen within 72 hr of separation but within the assigned shelf-life of the blood;
- Plasma frozen within 24 hours but stored under conditions that preclude its use for the manufacture of coagulation factors.

Provided that 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 hr of collection or separation from whole blood should not be used for fractionation.

5.2.3 Plasma not meeting the requirement for fractionation

Plasma obtained by therapeutic plasma exchange does not meet the criteria for fractionation to 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 to 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 [29].

5.2.4 Hyper-immune (antibody-specific) plasma

Detailed information regarding immunization of donors for the preparation of hyperimmune plasma is provided in Annex 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 Annex 3.

Clinically relevant antibody specific immunoglobulins include anti-D (anti-Rho), and anti-HAV, anti-HBs, anti-tetanus, anti-varicella/herpes zoster and anti-rabies immunoglobulins. For the most part, 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 (adapted from [2]):

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 usually will depend upon (a) the size and composition of the fractionation pool (which may include high-titer donations to increase the mean titer of the fractionation pool), (b) the characteristics of the immunoglobulin fractionation process, and (c) the minimum approved potency of the final IgG product.

The following general guidance may be useful for each specificity:

5.2.4.1 Anti-D (anti-Rho)

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

5.2.4.2 Anti-HAV

Antibody potency should be estimated in international units, using a quantitative assay agreed to by the fractionator.

The minimum acceptable potency in individual donation is unlikely to be less than 50 IU/ml.

5.2.4.3 Anti-HBs

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

5.2.4.4 Anti-tetanus

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

5.2.4.5 Anti-varicella/zoster

Antibody potency should be estimated using a quantitative assay (typically ELISA, immunofluorescence or complement fixation) agreed to 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.

5.2.4.6 Anti-cytomegalovirus

Antibody potency should be estimated using a quantitative assay (typically ELISA, immunofluorescence or complement fixation) agreed to 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.

5.2.4.7 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 by rabies virus.

5.3 Premises and devices for collection of plasma for fractionation

5.3.1 Premises

The collection of blood/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 NRA. 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 donor and personnel safety. 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.

5.3.2 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 reducing the content of recoverable factor VIII – they are just as likely to impact on 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 of whole blood collections are the same 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 give assurance of 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 liquid and frozen storage. 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 blood/plasma collection and storage containers 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.

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. For both, the device and the anticoagulant information should be provided to the regulatory authorities. The fractionator will need to know what anticoagulant was used, and its concentration as they may have an impact on the fractionation process.

5.3.3 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 [30-34]). One exception is when heparin is added to the anticoagulant solution. Main anticoagulant solutions currently in use for collection of either whole blood or apheresis plasma are listed in Table 6.

Table 6: Examples of anticoagulant solutions commonly used in the preparation of plasma for fractionation (adapted from [2]):

	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	X	15	(X)
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	X	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 1N (pH adjustment) pH (25°C) 5.3-5.9	X	14	(X)
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 1N (pH adjustment) pH (25°C) 5.3-5.9	X	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 1N (pH adjustment) pH (25°C) 5.3-5.9	X	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	X

(X): uncommonly used ; X, commonly used

5.4 Blood/plasma collection process

5.4.1 Procedure

A standardized and validated procedure for the preparation of the phlebotomy site should be followed using a suitable antiseptic solution, and allowed to dry depending on the type of disinfectant. The prepared area should not be touched before needle has been inserted. Prior to venipuncture the containers should be inspected for defects. Any abnormal moisture or

discolouration suggests a defect. 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 [15]). 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, at least every 90 seconds. Collection of one standard unit of blood should be achieved within 15 minutes, as longer durations may result in activation of the coagulation factors and cellular components.

In automated 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 [35, 36]. The operational parameters of the plasmapheresis equipment are defined by the manufacturers of the equipment and by requirements of NRAs. 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 harvested. For determining the number of cycles employed, the equipment requires programming by data inputs. These data elements may include such parameters as donor weight and hematocrit values. The amount of time required for the donation procedure depends on the number of cycles (and hence the volume of plasma collected) but generally falls between 35 – 70 minutes.

5.4.2 Labelling of collection bags

There should be a secure system for procurement, printing and storing of the barcode labels used to identify the main collection bags and the satellite bags, associated samples and documentation in order to ensure full traceability at each stage of plasma production. There should be a defined procedure for labelling collection bags and samples – in particular the procedure should ensure that the labels correctly identify the association between samples and donations. This 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 mislabeling. Duplicate number

sets of barcode 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.

5.4.3 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 [37] based on the specifications of the plasma for fractionation set out by the fractionator and/or the NRA. Likewise, apheresis equipment and apheresis procedures should be validated, maintained and serviced. Validation criteria with regards to 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 [27, 37-39]; such studies are usually performed by the manufacturer of the apheresis machines.

5.4.4 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.

5.4.5 Volume of plasma per unit

The volume of recovered plasma per container varies depending upon the volume of whole blood collected, the respective hematocrit 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 the collection of whole blood is in most countries 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 country's regulations.

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, container opening step, and 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, 20000 plasma donations for a pool size of 2000 litres).

5.4.6 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 needed in case there are missing numbers and leaking containers. Documentation should accompany the donations to the plasma processing site, to account for all donations in the consignment.

5.4.7 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.

5.5 Separation of plasma

5.5.1 Premises

Blood processing should be carried out in adequate facilities compliant with the intended activity. Donor area and plasma processing areas should be separated from each other whenever possible. Each area of processing and storage should be secured against the entry or intervention of unauthorised persons and should be used only for the intended purpose. Laboratory areas and plasma storage areas should be both separated from the donor and processing areas.

5.5.2 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 by ensuring packaging methods that can keep the blood/plasma within the required temperature limits. One approach to record temperature is to put portable temperature loggers for monitoring the transportation of blood/plasma to the processing site.

5.5.3 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 hr prior to separation of plasma is suitable for the production of plasma for fractionation but Factor VIII activity is reduced by an additional 15 to 20 percent if blood is stored for 24 hr [40]. Rapid cooling of whole blood to 22°C +/- 2°C immediately after collection (using e.g. cooling units with butane-1,4-diol) [41] protects Factor VIII and may allow storage of blood for 24 h [42]. 4°C transportation/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 [43-46]. Holding blood at 4°C for a period of time over 8 hr is therefore not recommended when plasma is used for fractionation in the manufacture of Factor VIII products.

5.5.4 Centrifugation of whole blood

Blood and plasma collection documentation should be checked at the processing laboratory at 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 container identification is correct. 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 NRA and the plasma fractionator are met.

It has been shown that CPD whole blood units that were centrifuged under conditions of low g force for a long time as compared to high g force for a short time yielded blood components of similar quality [47]. 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.

Blood processing methods that include removal of the buffy-coat layer have gradually shifted from manual extraction procedures to fully automated systems. This allows standardized extraction and contributes to GMP in the preparation of blood components including plasma for fractionation [48]. Blood component separation systems may be based on buffy coat extraction via the *Top & Bottom* technique [49]. 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 [49]. 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 / μ l and $0.04 \pm 0.035.6 \times 10^3$ leucocytes / μ l is obtained [50]. Blood components may be separated by initial high-speed centrifugation (4,158 g, 14 min, 22°C) 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 yields plasma containing $3 \pm 3 \times 10^6$ leucocytes and $4 \pm 3 \times 10^9$ platelets per unit [51]. The *Top & Bottom* approach allows a marked reduction in leukocyte contamination of the different blood components [41, 52], and may yield optimal plasma volume [41].

5.5.5 Impact of leucoreduction

Recently, several countries have implemented universal leucoreduction of the blood supply [53, 54] 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 leucoreduction of whole blood removes 42% of the vCJD infectivity associated with plasma [55], whereas further investigation by the same group found a ~70%

removal of infectivity. The impact of leucoreduction on plasma protein recovery and activation markers appears dependent upon the chemical nature of the filters [56, 57]. Some loss of coagulation factors and sometimes an increase in the markers of coagulation and complement activation have been found although the impact on the quality of fractionated plasma derivatives is not known [57, 58].

Therefore, until more scientific data are gathered, the benefit of leucoreduction on the quality and safety of plasma products remains debated. The decision to leuco-reduce plasma for fractionation should be assessed with the plasma fractionator and the NRA.

5.6 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 in Factor VIII. Several aspects in the freezing conditions of plasma for fractionation have been evaluated.

5.6.1 Holding time of plasma

Holding plasma, freshly harvested from CPD-whole blood, at ~ 4°C for up to 24 hr before freezing at -20°C for 4 months was shown to induce close to 25% loss of Factor VIII activity compared to plasma frozen immediately, whereas other coagulation factors were not affected [59]. Storing plasma at 22°C for 2 to 4 hours does not seem to induce a significant loss of Factor VIII activity; however, after 4 hours, some loss of activity takes place [44, 60].

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

5.6.2 Freezing rate and freezing temperature

5.6.2.1 Freezing conditions

The regulatory requirements for the temperature at which plasma should be frozen follow different patterns [61], and depend upon the type of proteins fractionated. The fractionator may also wish to specify specific 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 labile protein manufacturing (e.g. production of Factor VIII concentrate) should be frozen rapidly, within 24 hours of collection, at – 30 °C or colder [28],

as this temperature has long been claimed to ensure complete solidification [62], and to be needed for optimal freezing [63]. Recovered plasma used to manufacture only stable plasma proteins (e.g. albumin and immunoglobulins) should be frozen within 72 hours of collection at -20°C or colder [28].

The US Code Federal Regulations specifies 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, again at least when coagulation factors are intended to be produced [64, 65]. Rapid plasma freezing prevents or reduces loss of factor VIII in frozen plasma either recovered or obtained by apheresis [25, 66, 67], and slow freezing of plasma was shown to influence the purity and recovery of Factor VIII in cryoprecipitate [64, 67-69]. An ice front velocity of 26 mm/hour during freezing was recently shown to preserve FVIII:C in plasma better than 9 mm/hour or less [60].

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

5.6.2.2 Impact of containers and equipment

In order to ensure optimal and consistent freezing and storage conditions, it is important to use plasma standardized containers as freezing time would be influenced by container shape, volume, and thickness [60, 67, 68].

Optimum conditions, used by some plasma collectors, to ensure reproducible freezing consist in 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. Worst case would be to place a large number of unfrozen plasma bags, close together, in a domestic (-18° to -22°C) freezer, adding more plasma bags for freezing each day, and storing the plasma under these conditions for several months are currently under debate and the wording used in the European Pharmacopeia monograph may be revised. With good practice at the time of loading (i.e. not placing too many packs in at the same time and keep them separated), a walk-in freezer at suitable temperature offers a workable compromise. The

plasma fractionator will have to specify to the plasma collector, with the approval of the NRA, which precise freezing parameters to use.

5.6.2.3 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 freezing speed will be influenced by the type of plasma container as well as by the volume of plasma [67]. Validation of the freezing process by recording the temperature of plasma donations during a freezing process allows evaluating the freezing capacity of the equipment. 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.

5.7 Storage of plasma

5.7.1 Storage conditions and validation

Plasma for fractionation should be stored at -20°C or colder. A multicenter study showed no detectable storage-related changes in 3 pools of plasma (2 recovered CPD plasma and 1 apheresis plasma) that have 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 pool during storage at -20°C for 2 years [70]. 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 impacting the fractionation process, in particular the cryoprecipitation step [63, 64, 69].

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 hours, 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.

5.7.2 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 freezing machine/electricity 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.

5.7.3 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 a secure segregation system should be used if both types of plasma are stored in a single freezer.
- 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 nonreleased plasma.
- Unacceptable plasma donations: Donations that are found to be unacceptable for fractionation should be retrieved, disinfected, and discarded using a secure system.
- Boxing: 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.
- Reconciliation: Prior to shipment, plasma boxes should be reconciled appropriately.
- Review of documentation: Prior to release of the plasma shipment to the fractionator, there should be a formal review of the documentation to ensure that 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 not complying with the specifications agreed upon with the fractionator will not be released and shipped, and that traceability of donations is ensured.

5.8 Compliance with plasma fractionator equipment

Any plasma collected and prepared for fractionation has to meet the plasma product manufacturer requirements as the specifications of plasma for fractionation are part of the marketing authorization given by the NRA for a specific plasma derivative. In addition, to the regulatory criteria related to donor selection and donation screening, the quality specifications agreed upon with the fractionator may encompass:

- The compliance with GMP during production and control,
- The residual level of blood cell (platelets, leucocytes) that should be below a certain level that may vary depending upon countries or fractionators,
- The protein content possibly including a minimal mean level of Factor VIII coagulation activity if this product is manufactured,
- The guarantee of an appropriate plasma/anticoagulant solution ratio (see Table 6) and evidence of appropriate mixing with the anticoagulant during the collection process.
- The acceptable maximum titre of ABO blood group antibodies (risks of hemolytic 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 [71]).
- Maximum haemoglobin content.
- Absence of hemolysis.
- Colour.
- Absence of opalescence (due to lipids).
- Citrate (anticoagulant) range content (usually between 15 and 25 mM).
- The minimum titre of a specific antibody when the donation is used for the production of hyperimmune IgG such as anti-rhesus, anti-HBs, anti-tetanus, or anti-rabies.

5.9 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 IT-systems.

The specifications for release of plasma for fractionation should be defined, validated, documented and approved by QA 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 product status control, 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 fails release 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 given by the 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.

5.9.1 Plasma release using electronic information systems

Special documented evidence is needed if release of plasma is subject to EIS in order 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 do not fulfill all test or donor selecting 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 permitted access to enter, amend, read or print data. Methods of preventing unauthorised entry should be in place , such as personal identity codes or passwords which are changed on a regular basis; and
- The EIS should block the release of plasma or other blood components considered not acceptable for release. There should also be a means to block the release of any future donation from a donor.

5.10 Packaging of plasma

The packaging requirements should be specified by the fractionator:

- Specification on how the plasma containers are to be packed to prevent damage during shipment.
- Plasma of different types should be kept discrete and packaged into separate cartons.
- 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.

5.11 Transportation of plasma

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

- Responsibilities for storage/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/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 refrigeration system failure are not available (as during sea-transportation of several weeks duration).

The recommendations in cold chain maintenance, as mentioned for plasma storage, should also prevail 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 of 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 in the handling of blood and plasma to optimize the recovery of labile proteins like Factor VIII in plasma. Those 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 [43, 72], possibly in part due to the loss of Factor VIII that takes place during industrial cryoprecipitation [73] as well as during purification and viral reduction procedures.

Table 7: Processing of plasma for fractionation to optimize Factor VIII stability (adapted from [2]):

Steps	Recommendations
Whole blood storage before plasma separation	• Up to 18 to 20 hrs at 22°C +/- 1°C • Not more than 8 hrs at 4°C
Freezing	• As soon as possible, within 24 hrs of blood collection or apheresis procedure^{#1}
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 (currently -30°C in the European Pharmacopoeia Monograph)
Storage temperature	• - 20° C or colder, constant
Transportation temperature	• - 20° C or colder, constant

#1 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.

5.12 Recall system

In the case of known or suspected quality defects of a plasma unit already shipped, there should be a person within the blood establishment nominated to assess the need for product recall and to initiate and co-ordinate 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 pre-defined periods of time and should include tracing all relevant components of the donation and, where applicable, should include look-back procedures.

6. QA 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 quality assurance system based on the principles of good manufacturing practice.

The quality assurance system should ensure that all critical processes such as the purchase of raw materials, starting materials, selection of donors, collection of blood/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 good manufacturing practice and comply with the appropriate regulations. The Management should review the system at regular intervals to verify the effectiveness and introduce corrective measures if deemed necessary.

Because 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 (Annex 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 QA system involving all activities, taking into account the principles of good manufacturing practice. The following items are of special relevance as part of a quality assurance system for the production of plasma for fractionation (48, 49, 50).

6.1 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 be qualified to perform their tasks. They should have appropriate qualifications and experience and should be provided with initial and continued training. Only persons that are 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.

All personnel should receive initial and continued training appropriate to their specific tasks. Training programmes should be in place, and should at least include:

- Relevant principles of plasma production and plasma characteristics,
- Quality assurance and good manufacturing practice, and
- Relevant knowledge in microbiology and hygiene.

Training should be documented and training records should be maintained. The contents of training programs 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 Givers activity.

6.2 Documentation system

Each activity, which may affect the quality of the blood and/or blood component should be documented and recorded. There should be documentation to ensure that 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 archival 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. Procedures should be designed, developed, validated and personnel trained in a consistent manner.

6.3 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 workflow in an area should be arranged 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.

6.4 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 received goods should be performed to confirm they meet specification. Inventory records should be kept for traceability. Critical materials should be released under the responsibility of QA function before use.

6.5 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.

6.6 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, transports, 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 residual cell counts (platelets, leucocyte, erythrocytes) monitoring (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 recommendations of the manufacturer of reagents and test kits. The work record should identify the test(s) employed so as 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 of that donation remain in quarantine and 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.

6.7 Virology safety testing

6.7.1 Sampling

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

- Reconciliation: There should be a reconciliation of the samples received at the virology laboratory versus expected
- Sampling machine:
 - Automatic sampling: Test samples should be taken automatically and the donation number should be read via barcode. In case of failure of the automatic system, an appropriate system for manual entry of donation should exist, and ideally should require double entry with check digit;
 - 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.

6.7.2 Test equipment

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

- Sample addition: the sample addition to the test plates should be automatic and 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 etc. should be fully validated and routinely calibrated with acceptable records.

6.7.3 Assay performance validation

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

- Independent control(s): Each test run should include an independent control.
- Analysis of positive controls.

- Analysis of data on non-repeatable reactives.
- Evidence of satisfactory participation in external proficiency schemes.

6.7.4 Test interpretation and downloading

The data 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. No transcription of results should be done as mistakes may be introduced.

6.7.5 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 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.

6.8 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 the 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 use of duplicate donation numbers or the system should be able to deal with duplication without data corruption. Hardware and software should be protected against unauthorised use or changes, e.g. by password protection of key functions. There should be procedures for each type of soft- 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 at expected and unexpected down time or function failures. Changes in computerized systems should be validated, applicable documentation revised and personnel trained, before the change is introduced into routine use. EIS should be maintained in a validated state.

6.9 Storage and transport

Storage and distribution routines should take place in a safe and controlled way in order to assure product quality during the whole storage and transport period 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.

6.10 Change control system

A formal change control system should be in place to plan, evaluate and document 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.

6.11 QA auditing

In order to monitor the implementation and compliance with the blood establishment quality management system, regular internal audits need to be in place. 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, suggestions etc.

6.12 Defect reporting system

There should be systems in place to ensure that complaints, all types of quality defects (e.g. blood bags, test kits), and adverse events or reactions are documented, carefully investigated for causative factors of the defect and, where necessary, followed by the implementation of corrective actions to prevent recurrence. This includes 'near miss events'. The corrective and preventive action system should ensure that existing product nonconformity 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 input product or quality problems into the corrective and preventive action system.

6.13 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 NRA);
- 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 out-sourced);
- frequency of donation and the system for ensuring that this is not abused;
- 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 donor and donation traceability;
- specifications of plasma to be supplied, including any arrangements for verifying compliance with specification 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;
- arrangements for notifying the fractionator of a proposed regulatory inspection, its periodicity, and of the outcome of such an inspection.

6.14 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);
- 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 paragraph 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 undertaken. 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.

7. Regulatory Control of Plasma for Fractionation

7.1 Role of national regulatory authority

According to WHO Guidelines for national regulatory authorities (NRA) on quality assurance of biological products (75,76), NRAs 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 good manufacturing practice. NRA responsibilities should also include the enforcement and implementation of effective national regulations, standard settings and controls. 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 test methods to characterize batches of the product. This requires specialized expertise by the NRA.

7.2 Establishment license and inspections

In many countries NRAs 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, (48, 49, 50), 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. Therefore, the NRA requires that the establishments involved in the collection and storage of plasma as a source material (plasmapheresis centers, blood establishments, etc.) need to have an establishment license and need to be inspected by the competent national regulatory authority. To obtain the license the establishments have to fulfill a defined set of requirements to guarantee that the collected plasma is safe and of good quality. Since each collected unit represents one single batch, a marketing authorization for the plasma as a "product" is not required in all countries. Under the latest condition, a "system control", instead of a "product control", may be more appropriate. Some countries have in addition to the establishment licensing system also introduced a product-specific approval system for blood components.

7.3 Impact of GMP

In fact, the approach of implementing the principles of GMP in the production of medicinal products is not new, and it is well 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 blood products. Therefore, taking into

account the principles of GMP and the existence of an appropriate QA system 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 it is outlined in other chapters, 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 criterias 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 collection and product.
- enables continuous improvement in collection, preparation and testing of starting material.
- supports regional co-operation networks that may result in the formation of competence centres by centralizing activities in order to reach compliance at the required level (cost benefit for implementing QA measures).
- provides suitable tools for the NRA to assess the compliance status of a plasma collection center.
- An establishment licensing system for blood establishments by the competent national regulatory authorities should therefore exist. The main requirements to obtain an establishment license may include especially:
 - Quality Assurance System and GMP has to be applied for all steps starting from donation, preparation, storage, testing, distribution etc. 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 through an appropriate labelling system,

- Requirements for selection of donors, including exclusion criteria for donors with risk behaviours, information to donors on risk situations and the donation in general, the use of a questionnaire to obtain information on donors health, etc.
- Requirements for testing of each donation.
- Requirements regarding traceability and documentation.
- Post donation information system.

7.4 Inspections

In conducting regular inspections as part of the licensing procedure, enforcement of the implementation of GMP is performed aiming to ensure the compliance of the blood establishments with the existing provisions. It is the responsibility of the inspector of the NRA for ensuring that the manufacturers, and blood and plasma establishments respectively, adhere to the approved standards of GMP and QA, including sites where plasma is collected as starting material.

The inspections and control measures should be carried out by officials, representing the competent national regulatory authority, and should involve persons which are specialized inspectors, trained in GMP inspections, and which in addition are familiar with blood bank technologies and the special features concerning 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 [incl. self inspection, change control, etc.], documentation [SOP, records, donor record files, log books, etc.], personnel and organization, qualification and process validations, error and corrective action system, look back information, recalls and complaints, product quality controls), and 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 cannot only be considered 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. critical – major – 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 the successful implementation of specific corrective actions

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

In the marketing authorization procedures of the final blood product, information on the collection and control of the starting material, human blood or plasma, has to be documented as part of the dossier.

In summary, the implementation of licensing and inspection systems for blood establishments has become an important tool through which the national regulatory authorities confirms the assurance of quality of plasma as starting material for fractionation. The use of international standards both further promotes harmonization and facilitates regional collaboration and information exchange between the NRAs.

8. Annex 1: Plasma products and clinical applications (adapted from [6]):

Products	Main Indications
Albumin	
Human Serum Albumin	Volume replacement
Blood Coagulation factors	
Factor VIII	Haemophilia A
Prothrombin complex (PCC/PPSB)	Complex liver diseases; warfarin or coumarin derivatives reversal
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 (FXI deficiency)
Fibrinogen	Fibrinogen deficiency
Factor XIII	Factor XIII deficiency
Activated PCC	Haemophilia with anti-FVIII (or FIX) inhibitors
Protease inhibitors	
Antithrombin	Antithrombin III 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 / (thrombosis)
Fibrin sealant (fibrin glue)	Topical haemostatic / healing /sealing agent (surgical adjunct)
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 the haemolytic disease of the new-born
Rabies	Prevention of rabies infection
Varicella/zoster	Prevention of chicken-pox infection
Intravenous Immunoglobulins (IVIg)	
Normal (polyvalent)	Replacement therapy in immune deficiency states immune modulation in immune disorders
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 the haemolytic disease of the new-born.
Intravenous Immunoglobulins M	septic shock; binding of endotoxins

9. Annex 2: Donor Selection

9.1 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] supports "the full implementation of well-organized, nationally coordinated and sustainable blood programmes with appropriate regulatory systems" and stresses 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.

9.2 Information to donors

Candidate donors should be explained, ideally both verbally and in writing, or any other appropriate means such as a self-administered questionnaire, that answers to questions about the medical history and personal behaviour, are necessary to define whether they are eligible for blood/plasma donations. Written information can be a leaflet explaining infectious risks associated to blood and plasma products; impact of social behaviour on infectious risks; infectious risk factors. This information is mostly given by a licensed physician, or by a person under the direct supervision of a licensed physician, who should explain 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 told to inform the blood centre in case they do not feel well after the donation or forgot to mention a possible risk factor. This is of special importance for a donation used to prepare plasma for fractionation considering that one should be able to remove at-risk donations prior to the industrial pooling stage to avoid potential needs to destroy the plasma pool or the intermediates or products derived from it.

9.3 Compliance with donor selection criteria

9.3.1 Positive identification of donors

Upon presentation at the blood/plasma collection site donors should identify themselves positively by stating their name, address, and date of birth, and a proof of a permanent place of residence in order to establish a reliable contact mean, including e.g. a telephone number where they can be contacted after donation, if needed. Evidences of identity (such as identity card, passport, driver's license) should be provided. Positive identification of donors should take place also immediately before venipuncture.

9.3.2 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 selection process so that the candidate donor will not refrain from answering questions on personal or private behaviour, which otherwise would compromise the safety of the plasma donation used for the fractionation process.

9.3.3 Questionnaire and interview

The assessment of each donor is carried out by a suitably qualified person, trained in use of donor selection criteria and involves 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, adapted to the type of 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 answering the donor information and/or the questionnaire. Such confidential self-exclusion should also be possible afterwards the donation (e.g. by phone).

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

9.3.4 Physical examination, Acceptance and deferral criteria

9.3.4.1 Physical exam

Prior to the first donation and thereafter before subsequent blood donations and in case of plasmapheresis at regular intervals, a physical exam should be carried out by a licensed physician or a physician substitute following an established procedure. Local NRAs should, usually after consultation with the blood establishment, determine the health criteria and the respective acceptable limits taken into consideration during physical examination, such as measurement of weight, blood pressure, pulse rate and temperature, or any other criteria considered to be of concern for the safety of plasma derived products or the donor.

9.3.4.2 Records and traceability

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

9.3.4.3 Selection and exclusion criteria

The following elements have been recognized to play 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 NRA and be applicable nationwide, as national requirements. Within the scope of their role to establish and implement effective national regulations, local NRAs should enforce such criteria. Based on the characteristics of the manufacturing 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 which reason. Examples of the major permanent deferral criteria found in international guidelines are presented below:

- _ 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 previous 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 last 12 months previous to donation, as blood transfusion is a risk-factor for all blood-borne infections.
- Tattooing, scarification, ear piercing, acupuncture in the last 12 months previous to donation. These practices may be a vehicle for the transmission of viral diseases unless clear evidence is provided that it was carried out under sterile conditions.
- A particular policy may have to be defined with regard to the exclusion criteria for a risk factor relevant to the safety of cellular blood components in spite of not creating 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 whereas 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 can create by itself a risk of mishandling and error at the blood collection centre.

9.3.4.4 Reinstatement

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

9.3.4.5 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, in compliance with the local NRA and fractionator's requirements. Abnormal conditions should be referred to the physician who has the responsibility of making the final decision for the donor suitability. If the physician has any doubt about the donor's suitability they should be deferred.

10. Annex 3: Donor Immunization and Plasmapheresis for Specific Immunoglobulins

There is a clinically valid need for specific immunoglobulins and plasma 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 of the desired specificity e.g. 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.

In order to ensure 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 the previous sections.

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: (a) the antibody assay; (b) the minimum level of antibody required; (c) data showing that the dose, the intervals between injections and the total dosage proposed for each

antigen are appropriate and (d) the criteria for considering a prospective donor a non-responder for a given antigen. Donor could be hyperimmunized with more than one immunizing preparation as long as the safety of the multiple procedure 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, is achieved in small groups of potential donors);
- informed that they are free to withdraw their consent at any time.

In addition, donors could also be:

- encouraged to seek advice from their family doctor, or from an independent competent counseling, before agreeing to immunization;
- 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 NRA. Special care should be taken to ensure the safety of the donor when a vaccine is administered at doses or schedules differing from those recommended for routine prophylactic immunization. Erythrocyte and other cellular antigens should be obtained from an establishment approved by the NRA. Donors should be observed for approximately 30 min following any immunization in order to determine whether an adverse reaction has taken 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.

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 Annex 2). Relevant measures should be taken to limit the risk of infectious diseases, and those could vary from country to country taking into consideration respective risks. 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, 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. Erythrocytes obtained for immunization purposes should be frozen (at least from 6 to 12 months depending upon the sensitivity and range of the tests used, e.g. the use of NAT) before use and the donor should be retested and shown negative for the above markers of infection before the stored cells are released and used for immunization. Prestorage leucoreduction 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 in vitro (80%) or in vivo (70%) cell recovery. Erythrocytes should be washed after storage to remove the cryoprotective agent (such as glycerol). Adequate sterility data to support the requested shelf-life for stored erythrocytes should be kept on file. A test for bacterial and fungal contamination should be made 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 made 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 haemolytic antibodies should include 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 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 developed after injection of erythrocytes. The plasma center should maintain records and these should be reviewed during inspection. The immunized donor should carry a card or medical alert bracelet specifying the antibodies. Those measures allow optimized care to immunized donors who may require an emergency transfusion, (e.g. road traffic accident) at some future point, 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 about 2-5 ml and given three 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, since 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 anti-D level and duration of the response. The response of each

recipient is individual, and additional injections of erythrocytes may be required at intervals of two to nine months to maintain anti-D levels [74]. 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 run the risk of: a) viral hepatitis (B and C) and HIV infection; b) other infectious diseases; c) HLA immunization; d) the production of unwanted erythrocyte antibodies that may complicate any future blood transfusion; e) a febrile haemolytic reaction if the antigen dose is too great; f) 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.

11. Annex 4: Contract Plasma Fractionation Program

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. In most countries the preparation of plasma products is governed by the same regulatory considerations that are applied to drugs. Manufacturers are required to obtain manufacturing licenses which should cover the method of preparation and product characteristics. To obtain a license, it is necessary to demonstrate adherence to good manufacturing practice (GMP). Considerable technological, pharmaceutical and scientific expertise is required to meet these demands.

Since key utilities (HVAC, refrigeration and Water for Injections) should be maintained operational even when the facility is not fractionating plasma, the investment 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 vs. labile components);
- The operating capacity of the facility; and
- Plasma availability and product demand to allow it to operate continuously at near to maximum capacity for a substantial product portfolio.

According to perception and to a set of objective parameters (including plasma cost, product portfolio, adequacy of the various plasma products vs. the plasma needed to cover those needs, and product yield) the breakpoint for minimum annual plasma throughput for economic viability may vary greatly. Therefore such projects require 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. 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, including:

- Commercial and quality agreements defining the responsibilities of both parties (the contract giver and the contract acceptor).
- Clearly defined requirements in respect of plasma quality (including the arrangements for donor selection, testing and traceability).

- Provision for audit of the plasma collection center (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 fractionator's overall free capacity and production specifics such as plasma pool and product batch size).
- Agreement on the arrangements for storage and shipment of plasma, with defined provision for monitoring and control (typically by sea, at -20°C or below).
- Agreement on the range of products to be manufactured.
- Agreement on specific aspects of plasma processing (including batch size, possible requirements for segregation of processing, agreed use or destruction of excess intermediates, yield expectations 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.

The regulatory authorities of the country where the plasma is collected may require inspection of the fractionation centre. Table 8 summarizes the responsibility and role of each party.

Table 8: Responsibilities and roles of blood establishment, plasma fractionator, and regulatory authorities (adapted from [2])

Task	Blood establishment	Plasma fractionator	Regulatory authority
Epidemiology surveillance of donor population	Collects and analyzes the data based on results of screening tests	Reviews the data	Reviews the data
Donor selection and interview	Develops and implement the criteria in selection and interview of donors	Verifies that NRA criteria 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 with the tests kits used and audits the virology laboratory	Approves test kits and inspects the blood establishment
Post-donation follow up and hemovigilance	Informs plasma fractionator (and when appropriate the regulatory authority) when relevant information is obtained	Takes appropriate measures if plasma pool or product quality is endangered	Evaluates hemovigilance/post-donation reports with regards to product quality and safety
Preparation of plasma	Collects blood plasma, prepare, freeze, and store the plasma, according to GMP	Sets the specifications and audits	Approves and inspects the blood establishment
NAT testing (mini-pool)	Prepare the NAT samples following fractionator specifications	Provide the SOP for NAT samples and perform (or sub-contract) 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 inspect fractionation facility
Preparation of plasma product regulatory files		Prepares the files	Reviews and evaluates
GMP	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

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

Blood Establishments

Reference 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

Collection process of the blood/plasma unit:

- Containers, collection sets and anticoagulants with relevant registration duration of the whole blood collection (e.g. less than 15 minutes[15] for recovered plasma).
- Guarantee of mixing of blood with the anticoagulant as soon as the collection starts, by regular manual shaking or validated automated method[15].
- Prior to freezing, plasma is clear (light opalescence may be allowed), yellow to green in colour, with no sign of hemolysis or presence of red cells [28].
- Acceptable citrate concentration range.

Infectious markers:

- Test kits used to be of acceptable sensitivity and to be agreed with manufacturer.
- Absence of anti-HIV 1 and 2, anti-HCV, HBsAg, laboratory evidence of Syphilis.
- When applicable: specific handling of anti-HBc positive donations (e.g. accepted only if anti-HBs antibody titer > 0.050 IU/ml and HBsAg negative).
- HCV NAT and HIV tests must be negative.

Immuno-hematologic markers

- Anti-A and anti-B titer < 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.
- Statistical records of hemoglobin contamination showing that the relevant specifications are met.

Protein quality control

- Protein content ≥ 50 g/l after mixture with the anticoagulant.
- Minimum Factor VIII content.

Other criteria

- Minimal acceptable volume of plasma per container.
- Plasma freezing conditions: core temperature, time to freeze, and absence of folding to avoid thin plasma layer that would be more susceptible of 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.
- Maximum duration between donation and shipment to the fractionator.

Standard Plasma

Plasma types

o Examples of plasma categories:

- 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

- 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 international units 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 following an agreed procedure.
- Shipping documentation should include:
 - o Dated shipping document signed by responsible person.
 - o Certificate of origin and control of the plasma, mentioning for each donation collection date, carton number, results of virology and immuno-hematology

screening, test kits used and their batch number. Should be signed by the director or an authorized person.

- Password protected electronic file of the plasma donations and samples sent, mentioning for each donation collection date, carton number, results of virology and immuno-hematology 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. on a yearly basis.

Shipment

Plasma donations

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

Containers for shipment specification.

Auditing Program

- Obligation of the blood establishment to be subjected to auditing by the fractionator.
- Routine auditing performed by the fractionator follows an internally approved and regularly revised procedure with an established list of questions and check-points.
- Special auditing performed on an annual/bi-annual frequency based on a program previously communicated to the blood establishment director.
- Audit reports are communicated to the blood establishment director.
- List of reference documents (such as plasma for fractionation internal acceptance criteria).

Notification Obligations

Obligations:

- 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, syphilis, etc. has been sent by mistake.
 - A deviation is subsequently discovered in any of the screening tests performed on supplied plasma units.
 - A regular donor is found to be positive for a marker while the previous donation was found to be negative.
 - The blood establishment is informed that a donor, previously contributing to plasma for fractionation, 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:
 - has developed CJD or vCJD. In such case the report with pathological findings should be provided if available.
 - has risk factors for vCJD.
 - is identified as having risk behaviour or other factors impacting 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 fractionator may request additional data on previous donations when thought necessary.
 - A communication procedure must be in place indicating which information must be provided:
 - 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/her delegate.

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1. Introduction

In 1976, a WHO Working Group on the Standardization of Human Blood Products and Related Substances (1) considered the need for international requirements for the processing and control of whole human blood and blood products. It emphasized that, as the quality of the source material played an important part in determining the quality of the final products, such requirements should cover all the stages in the process, from the collection of the source materials to the quality control of the final product. In response to the Working Group's recommendations, the Requirements for the Collection, Processing and Quality Control of Human Blood and Blood Products were published in 1978 (2). These Requirements were updated and revised in 1988(3), and WHO recommendations concerning testing for antibodies to human immunodeficiency virus (HIV, 4) were taken into account. This Annex contains a further revision of the Requirements, applicable to the quality control of blood, blood components and plasma derivatives.

2. General considerations

The setting up of an organization for the collection and fractionation of human blood and blood components calls for a great deal of expertise and considerable investment. Any country contemplating the establishment of such an organization should carry out a careful cost—benefit analysis to determine whether the investment is justified. A logical developmental sequence for a comprehensive organization starts with the collection and distribution of whole blood, progressing later to the separation of whole blood into components and then the fractionation of plasma pools. It is not always possible to be specific about the details of the procedures employed, the in-process controls or the tests applied at each stage of production, in particular for whole blood and component cells. In addition, although the general principle of fractionation of plasma is well established, there are in practice numerous variations in the details of the various production steps. Therefore, any country wishing to begin the collection and fractionation of blood and blood components should send personnel for training to a plant that is operating successfully. WHO may be able to help in arranging such training.

One of the basic questions to be answered by a country considering whether to start fractionation of plasma is whether there is a suitable donor population of sufficient size to guarantee an adequate supply of source material. It is not possible to set a lower limit for the quantity of source material that would be necessary to make such an operation economic because too many factors are involved. However, in order to maintain competence in production and to avoid certain contamination risks, it is important to have sufficient source material to maintain the fractionation facility in continuous operation.

In a comprehensive organization, the greatest expense is that involved in setting up the fractionation plant, but it is also possible to regard the collection of source material and its fractionation as quite separate operations. A country may wish to establish collection centers for separating the cell components and then send the plasma to an established fractionation plant in another country, from where the products could be returned to the original country. The costs of such an operation might be less than those involved in establishing and operating a fractionation plant.

The general prevalence of certain infectious diseases, such as various forms of hepatitis and parasitic diseases, and of HIV infection differs so markedly in different geographical regions that each national authority must decide for itself whether it is cost-effective to apply the most sensitive test to each blood donation and whether it is feasible to collect suitable source material. Great emphasis should be placed on the production of fractions by a process that experience has shown results in the least risk of contamination. For example, immunoglobulin is prepared by the cold ethanol fractionation method of Cohn has a well established clinical record of being free from contamination with HIV and hepatitis B virus (HBV), as have albumin products prepared by the same method, stabilized and heated for 10 hours at 60 °C (5). Nevertheless, extreme care is required in manufacture to ensure that these products are free from infectious viruses, and it cannot be assumed that different fractionation methods will be equally effective. When a fractionation process is introduced or significant modifications are made to an existing production process, the process or the modifications should be validated or revalidated by appropriate procedures, including the use of marker viruses and, where applicable, special *in vitro* and *in vivo* testing.

Blood can harbor a number of different viruses, and the use of medicinal products derived from human blood has led to transmission of viruses such as HCV, HBV and HIV. The risk of virus transmission by blood and blood products can be diminished by the testing of all individual donations. Policies for mandatory testing shall be determined by the national control authority, and should be reviewed regularly and modified according to the current state of knowledge.

Special care and appropriate measures approved by the national control authority must be taken to protect the health of the staff of blood collection and fractionation facilities.

The transport of source materials from blood collecting centers and hospitals to fractionation facilities requires special consideration. Refrigeration at the temperature range appropriate for the product must be efficient and reliable and proved to be so by monitoring. Thermal insulation must provide an adequate safeguard against a temporary failure of refrigeration. Containers of liquid source material should be filled so as to minimize frothing due to shaking. Because of the potentially infective nature of these biological materials, suitable protection should be provided against breakage, spillage and leakage of containers.

In these Requirements, the word “human” has been omitted from the names of products derived from human blood. Products of animal origin are immunogenic, and their administration to humans should be avoided whenever equivalent products of human origin can be used instead. The proper name of any blood product of non-human origin should include the species of origin.

These Requirements consist of four parts:

Part A. Requirements for the collection of source materials

Part B. Requirements for single-donor and small-pool products

Part C. Requirements for large-pool products

Part D. National control requirements

Each deals with a separate aspect of collection, processing and quality control but all the parts are intended to be taken together to constitute a single document. It will not be possible to rely on any blood product unless the relevant requirements for each step are complied with, and any attempt to make them less stringent may have serious consequences for the safety of the final product.

Parts (A-D) are divided into sections, each of which constitutes a recommendation. The parts of each section printed in normal type have been written in the form of requirements, SO that, if a health administration so desires, they may be adopted as they stand as definitive national requirements. The parts of each section printed in small type are comments or recommendations for guidance.

Should individual countries wish to adopt these Requirements as the basis for their national regulations concerning blood products and related substances, it is recommended that modifications be made only on condition that the modified requirements ensure at least an equal degree of safety and potency of the products. It is desirable that the World Health Organization should be informed of any such changes.

Increasing demand for blood products is resulting in the extensive movement of such products from one country to another. Internationally accepted requirements are therefore necessary so that countries without any regulations on blood products and related substances may refer to them when importing such products.

3. International Biological Standards and Reference Reagents

Rapid technological developments in the measurement of the biological activity of blood products and related substances require the establishment of international biological reference materials. The first two such materials (for anti-A and anti-B blood-typing sera) were established in 1950, and further reference materials have been established since. A number of materials are currently under investigation for use in the preparation of new standards.

The activity of blood products must be expressed in International Units where an International Standard exists. WHO publishes a list of such standards (revised from time to time and most recently in 1990) under the title *Biological substances: International Standards and Reference Reagents*.

4. Definitions:

The following definitions are intended for use in this document and are not necessarily valid for other purposes.

Blood collection: a procedure whereby a single donation of blood is collected in an anticoagulant and/or stabilizing solution.

Processing: any procedure that takes place after the blood is collected.

Plasmapheresis, apheresis and cytappheresis: procedures whereby whole blood is separated by physical means into components and one or more of them returned to the donor.

Closed blood-collection and processing system: a system for collecting and processing blood in containers that have been connected together by the manufacturer before sterilization, so that there is no possibility of bacterial or viral contamination from outside after collection of blood from the donor.

Donor: a person who gives blood or one of its components.

Single-donor materials

Whole blood (sometimes referred to as “blood”): blood collected in an anticoagulant solution with or without the addition of nutrients such as glucose or adenine. Whole blood is collected in units of 450 ml.

Blood component: any part of blood separated from the rest by means of physical procedures.

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.

Plasma, frozen: a plasma separated more than 8 h after collection of the blood and stored below - 20 °C.

Plasma, fresh-frozen: Plasma separated within 8h of donation, frozen rapidly and stored below -20 °C (and preferably below -30 °C).

Plasma, platelet-rich: plasma containing at least 70% of the platelets of the original whole blood.

Plasma, freeze-dried: any one of the above forms of plasma that has been freeze-dried for preservation.

Plasma, recovered: plasma recovered from a whole blood donation.

Cyoprecipitated factorVIII: crude preparation containing factor VIII that is obtained from single units (or small pools) of plasma derived either from whole blood or by plasmapheresis, by means of a process involving freezing, thawing and precipitation.

Serum: the liquid part of coagulated blood or plasma.

Red cells: whole blood from which most of the plasma has been removed and having an erythrocyte volume fraction greater than 0.7.

Red cells suspended in additive solution: red cells to which a preservative solution, for example containing adenine, glucose and mannitol, is added to permit storage for longer periods; the resulting suspension has an erythrocyte volume fraction of approximately 0.6-0.7.

Red cells, washed: red cells from which most of the plasma has been removed by one or more stages of washing with an isotonic solution.

Red cells, leukocyte-depleted: a unit of a red-cell preparation containing less than 1.2×10^9 leukocytes.

Red cells, leukocyte -poor: a unit of a red-cell preparation containing fewer than 5×10^6 leukocytes.

Red cells, frozen: red cells that have been stored continuously at -65 °C or below, and to which a cryoprotective agent such as glycerol has been added before freezing.

Red cells deglycerolized: frozen red cells that have been thawed and from which glycerol has been removed by washing.

Platelets: platelets obtained either by separation of whole blood, buffy coat or platelet-rich plasma or by apheresis and suspended in a small volume of plasma from the same donation.

Leukocytes: *leukocytes* obtained either by the separation of whole blood or by apheresis and suspended in a small volume of plasma from the same donation.

Large-pool products

Bulk material: plasma, powder, paste or liquid material prepared by the fractionation of pooled plasma.

Final bulk: a sterile solution prepared from bulk material and bearing the corresponding batch number. It is used to fill the final containers.

In some countries, the final bulk is distributed into containers through a sterilizing filter. If the total final bulk is not distributed into containers in one session, each of the filling lots is given a sub-batch number.

Filling lot (final lot): a collection of sealed final containers that are homogeneous with respect to composition and the risk of contamination during filling and (where appropriate) drying or other further processing such as heat treatment. A filling lot must therefore have been filled and (where appropriate) dried in one working session.

Part A. Requirements for the collection of source materials

1. Premises

The premises shall be of suitable size, construction and location to facilitate their proper operation, cleaning and maintenance in accordance with accepted rules of hygiene. They shall

comply with the requirements of Good Manufacturing Practices for Pharmaceutical (7) and Biological (8) Products and in addition provide adequate space, lighting and ventilation for the following activities where applicable:

- The medical examination of individuals in private to determine their fitness as donors of blood and/or blood components and to provide an opportunity for the confidential self-exclusion of unsuitable potential donors.
- The withdrawal of blood from donors and, where applicable, the re-infusion of blood components with minimum risk of contamination and errors.
- The care of donors, including the treatment of those who suffer adverse reactions.
- The storage of whole blood and blood components in quarantine pending completion of processing and testing.
- The laboratory testing of blood and blood components.

The processing and distribution of whole blood and blood components in a manner that prevents contamination and loss of potency.

- The performance of all steps in apheresis procedures, if applicable.
- The performance of labelling, packaging and other finishing operations in a manner that prevents errors.
- The storage of equipment.
- The separate storage of quarantined and finished products.
- The documentation, recording and storage of data on the donor, the donated blood and the ultimate recipient.

Mobile teams can be used for the collection of blood. Although the premises said by such teams may not comply with the more stringent requirements for centers built specially for the purpose, they must be adequate to ensure the safety of the donor, the collected blood or blood components and the staff participating in blood collection. The safety of the subsequent users of the premises should also not be forgotten.

2. Equipment

The equipment used in the collection, processing, storage and distribution of blood and blood components shall be calibrated, tested and validated before initial use, and shall be kept clean and

maintained and checked regularly. The requirements of Good Manufacturing Practices for Pharmaceutical (7) and Biological (8) Products shall apply in every particular.

The equipment employed to sterilize materials used in the collection of blood or blood components or for the disposal of contaminated products shall ensure that contaminating microorganisms are destroyed and shall be validated for this purpose. The effectiveness of the sterilization procedure shall be not less than that achieved by a temperature of 121.5 °C maintained for 20 min by means of saturated steam at a pressure of 103 kPa (1.05 kgf/cm² or 15 lbf/in²) or by a temperature of 170 °C maintained for 2 h with dry heat.

All contaminated material should be made safe before disposal. Disposal should comply with the relevant local laws

3. Personnel

An organization for the collection of blood or blood components shall be under the direction of a designated and appropriately qualified person who shall be responsible for ensuring that all operations are carried out properly and competently. The director shall have adequate knowledge and experience of the scientific and medical principles involved in the procurement of blood and, if applicable, the separation of blood components and the collection of such components by apheresis.

The director shall be responsible for ensuring that employees are adequately trained and acquire practical experience and that they are aware of the application of accepted good practice to their respective functions.

The director should have the authority to enforce or to delegate the enforcement of discipline among relevant employees.

The persons responsible for the collection of the blood and blood components shall be supervised by licensed physicians who shall be responsible for all medical decisions, for review of the procedures manual and for the quality-control programme, including techniques, equipment, procedures and staff.

The personnel responsible for the processing, storage, distribution and quality control of blood, blood components and plasma shall be adequate in number and each member of the personnel shall have a suitable educational background and training or experience that will ensure competent performance of assigned functions so that the final product has the required safety purity, potency and efficacy.

4. Donors

4.1 Donor selection

The provision of blood, blood components and plasma derivatives from voluntary, non-remunerated donors should be the aim of all countries.

In selecting individuals for blood donation, it is most important to determine whether the person is in good health, in order to protect the donor against damage to his or her own health and to protect the recipient against exposure to diseases or to medicinal products from the blood or blood products. It should be recognized that the donor selection process contributes significantly to the safety of blood products derived from large plasma pools. The following provisions apply to donations of blood or blood components not intended for autologous use.

The health of a donor shall be determined by a licensed physician or a person under the direct supervision of a licensed physician, and the donor shall be free from any disease transmissible by blood transfusion in so far as can be determined by history-taking and examination (see section 4.3). Donors shall be healthy persons of either sex between the ages of 18 and 65 years.

In some countries, there is no upper limit to the age of the donor. With parental consent the minimum age may be lowered to 16 years.

A donor should be considered for plasmapheresis only where the procedures involved result in products or services shown to serve accepted medical purposes, including prophylaxis, therapy and diagnosis, as verified by valid scientific evidence. All donors should be certified as acceptable, at the time of each plasmapheresis procedure, by a registered physician or by trained personnel under the direct supervision of the physician.

Those eligible for apheresis donation include: (a) healthy persons who fulfill the general criteria for blood donors; (b) persons with antibody levels that have been increased, either naturally or by

immunization; (c) subject to (a) above, persons with plasma that is of value for diagnostic or reference purposes; and (d) persons whose blood may be used in the preparation of certain vaccines.

When a potential donor does not fulfill the general criteria for blood donation, the acceptance of her or him as a donor for a specific component of blood should be at the discretion of the responsible physician. Where appropriate, the physician should have access to an ethical committee.

Donor education and selection programmes are intended to prevent potentially infectious units of blood and plasma from being collected. It is essential that such programmes are comprehensible and readily accessible to all potential donors.

To reduce the likelihood of transmitting infections, all potential donors should be informed of factors in their history or behaviour that may increase their risk of being infected. The national control authority must determine the appropriate exclusion criteria for the country concerned.

Persons in the following categories shall be excluded from acting as donors:

- those with clinical or laboratory evidence of infectious disease, e.g. infection with hepatitis viruses, HIV-1 or HIV-2;
- past or present intravenous drug abusers;
- men who have had a sexual relationship with another man;
- men and women who have engaged in prostitution;
- those with haemophilia or other clotting-factor defects who have received clotting-factor preparations;
- Sexual partners of any of the above.

Persons who have received blood transfusions should be excluded from acting as donors for at least one year.

Donors should be made aware before donating blood that it will be tested for the presence of serological markers of infection. It is advisable that the right to test donations and the legal implications of testing donations should be clarified by the appropriate authority.

4.2 *Donation frequency and volume*

4.2.1 *Whole blood*

The frequency of whole-blood donations shall not exceed once every two months, with a maximum volume in any consecutive 12-month period of 3 L.

A standard donation should not be collected from persons weighing less than 50 kg.

A *standard donation* is 450 ml; an optimum blood/anticoagulant ratio is 7 to 1. The frequency of donation may have to be modified on an individual basis. In general, premenopausal women should not donate blood as frequently as men.

4.2.2 *Plasma*

Plasma donors can be divided into three groups: those who donate at a frequency comparable to that allowed for whole-blood donations; those who donate two to three times as frequently as whole-blood donors; and those who donate at a maximum of twice a week. The first group shall be accepted on the basis of the general criteria for blood donors.

The maximum volume of plasma that may be removed from a donor during one plasmapheresis procedure shall be determined by the national health authority, and shall depend on whether the plasma is obtained by manual or automated plasmapheresis.

In some countries, the volume of plasma collected during a manual procedure is the quantity obtained from 1.0—1.2 L of whole blood. The volume of plasma collected during an automated procedure depends on the equipment used.

It is difficult to specify the maximum volumes of plasma that can be safely collected from donors until more definitive data are available on the effects of plasmapheresis on donors. The limits imposed in different countries vary and depend on the nutritional status of the donor.

If a plasma donor donates a unit of whole blood or if the red blood cells are not returned in an apheresis procedure, the next donation shall be deferred by eight weeks unless special circumstances warrant approval by the responsible physician of plasmapheresis at an earlier date.

In general, plasma collected by therapeutic plasmapheresis shall not be used for fractionation.

4.3 *Medical history*

4.3.1 *General*

Before each donation, questions shall be asked so as to ensure that the donor is in normal health and has not suffered, or is not suffering, from any serious illness.

A donor who appears to be suffering from symptoms of acute or chronic disease or who is receiving oral or parenteral medication, with the exception of vitamins, postmenopausal hormone therapy or oral contraceptives, shall not be accepted unless approved by a physician.

A donor who appears to be under the influence of any drug including alcohol or who does not appear to be providing reliable answers to medical history questions shall not be accepted.

4.3.2 *Infectious diseases*

Potential donors with a history that places them at increased risk of transmitting infection shall not donate blood or plasma for an appropriate time period. A donor shall be permanently excluded if one of his or her previous blood donations was believed to be responsible for transmitting disease.

In most countries, questions concerning the signs and symptoms of HIV infection will be part of the routine assessment of medical history and appropriate monitoring for HIV as defined by the national control authority, will be included. As a result of this assessment, a potential donor may be disqualified.

Donors shall not have a history of positive laboratory test results for hepatitis or corresponding symptoms and signs; close contact with an individual with hepatitis within the previous year; receipt within the previous year of human blood or any blood component or fraction that might be a source of transmission of infectious agents; or tattooing, scarification or ear piercing (unless performed under sterile conditions) within the previous year.

Acupuncture within the previous year may also present a risk if not carried out under sterile conditions.

Potential donors with a history of viral hepatitis or of a positive test for hepatitis B surface antigen (HBsAg) or antibodies to hepatitis C virus (anti-HCV) are permanently excluded.

The requirements concerning viral hepatitis may be varied, at the discretion of the national control authority, according to the local epidemiological circumstances.

In areas with a low incidence of transfusion-transmitted disease, whole blood or blood components should not be used for transfusion if obtained from source material collected in an area where there is a high incidence of blood-borne infectious disease.

Blood and plasma shall be tested for the presence of HBsAg, anti-HIV and anti-HCV by the methods described in Part B, section 7.2; the tests used should be approved by the NRA or other appropriate authority.

Anyone whose blood has been shown to be reactive for infectious disease markers by approved screening tests shall be excluded as a donor. Selection as a donor may later be permitted if sufficient data are available from tests approved by the national control authority to indicate that the original results were non-specific.

National health authorities shall develop policies designed to prevent the transmission of infectious diseases based on the prevalence of these diseases in the donor population and the susceptibility of recipients to them.

In countries where malaria is not endemic, donors of cellular blood products should have a negative history of malaria exposure during the previous six months and a negative history of clinical malaria, or a history of malaria prophylaxis if they have resided in, or visited, an endemic area within the three years preceding the donation. Such restrictions may be less important in countries where the prevalence of endemic malaria is high among both donors and recipients, except when blood products are required by visitors from non-endemic areas. Malaria history is not pertinent to plasma donation for source material that will be fractionated.

Particular attention should be paid to skin decontamination procedures before blood collection.

Many parasitic, bacterial and viral diseases, including trypanosomiasis, toxoplasmosis, syphilis and brucellosis, can be transmitted by blood. Precautions should be taken to avoid blood collection during the viraemia phase of viral diseases like measles and rubella. Potential donors

who have lived in or recently traveled to areas where human T-cell lymphotropic virus infections and haemorrhagic fever are endemic should be investigated for evidence of such infections.

Anyone who has received pituitary hormones of human origin should be permanently excluded as a donor because of possible infection with the agent causing Creutzfeldt-Jakob disease, although transmission of this agent through blood products has not been proved.

4.3.3 *Minor surgery*

Donors shall not have undergone tooth extraction or other minor surgery during a period of 72 h before donation.

4.3.4 *Pregnancy and lactation*

Pregnant women shall be excluded from blood donation. In general, mothers shall also be excluded during lactation and for at Least six months after full-term delivery.

The interval before blood donation is permissible after pregnancy may be shorter in some cases, e.g. six weeks after an abortion during the first trimester.

In some countries, donors are accepted when pregnant or during the period of lactation if their blood contains certain blood-group antibodies or is needed for autologous transfusion. The volume to be taken should be determined by the physician responsible.

4.3.5 *Prophylactic immunization*

Symptom-free donors who have recently been immunized may be accepted with the following exceptions:

- Those receiving attenuated vaccines for measles, mumps, yellow fever or poliomyelitis shall be excluded until two weeks after the last immunization or injection.
- Those receiving attenuated rubella (German measles) vaccine shall be excluded until four weeks after the last injection.

- Those receiving rabies vaccines for post-exposure treatment shall be excluded until one year after the last injection.
- Those receiving passive immunization with animal serum products shall be excluded until four weeks after the last injection.
- Those receiving hepatitis B vaccine need not be excluded unless the vaccine is being given because of exposure to a specific risk, in which case the donor shall be disqualified for at least 12 months after the last such exposure. If hepatitis B immunoglobulin has been administered, the period of deferral shall be at least 12 months because disease onset may be delayed.

4.4 *Physical examination*

As determined by the regulatory authority, physical examination of donors may include measurement of weight, blood pressure, pulse rate and temperature. If these are measured and the results lie outside the ranges recommended below, the donor concerned shall be accepted only if approved by the licensed physician in charge.

- *Blood pressure:* systolic blood pressure between 12 and 24 kPa (90 and 180 mm/Hg); diastolic blood pressure between 6.67 and 13.3 kPa (50 and 100 mm/Hg).
- *Pulse:* between 50 and 110 beats per minute and regular. Lower values may be accepted in healthy athletes with endurance training.
- *Temperature:* oral temperature not exceeding 37.5 °C.
- *Weight:* donors weighing less than 50 kg may donate a volume of blood proportionally less than 450 ml in an appropriate volume of anticoagulant, provided that all other donor requirements are met.

Donors shall be free from any infectious skin disease at the venepuncture site and of skin punctures or scars indicative of abuse of intravenous drugs.

4.5 *Additional requirements applicable to donors for plasmapheresis*

All phases of apheresis, including explaining to donors what is involved in the process and obtaining their informed consent, should be performed under the direct supervision of a licensed physician or by trained personnel reporting to such a physician.

4.5.1 *First-time plasma donors*

When prospective plasma donors present themselves to a centre for the first time, initial screening shall begin only after the procedure of plasmapheresis has been explained and the donor has given consent.

The following information shall be permanently recorded:

- Personal information and identification. If the donor is to participate in an ongoing programme, an effective means of identification is especially important. The use of identity numbers, photographs or other equally effective measures should be considered.
- A preliminary medical history as required for blood donors, covering infectious diseases and the donor's general state of health.

If there are no contraindications to plasmapheresis, preliminary laboratory tests shall be carried out, namely reading of the erythrocyte volume fraction or hemoglobin concentration, determination of total serum protein and screening for protein and sugar in the urine. The hemoglobin concentration or erythrocyte volume fraction of the donor's blood shall be within normal limits, as defined by the national control authority or the national blood transfusion authority.

If normal values are also obtained in the other laboratory tests, evaluation of the potential donor by the physician begins.

Donors participating in a programme in which plasmapheresis is more frequent than is blood donation for those eligible for whole-blood collection shall be examined by a licensed physician on the day of the first donation, or not more than one week before that donation. This examination shall include measurement of temperature and blood pressure, auscultation of the heart and Lungs, palpation of the abdomen, assessment of neurological signs, urine analysis and blood sampling for tests required by the national control authority. Liver function tests (e.g. for alanine aminotransferase), tests for HBsAg, anti-HIV and anti-HCV, and quantification of plasma proteins by electrophoresis or another suitable method shall also be included. The physician shall obtain informed consent after explaining the procedure of plasmapheresis and describing the

hazards and adverse reactions that may occur. At this stage, donors shall be given an opportunity to refuse participation. If they consent, it must be on the condition that their legal rights to recover damages are not waived.

In some countries, the first plasmapheresis procedure may be performed before the results are available for the liver function tests, the serological tests for syphilis (it required by the national control authority) and the tests for HBsAg, anti-HCV and anti-HIV. The results of the tests for quantifying plasma proteins should be reviewed by the physician before subsequent plasmapheresis procedures.

4.5.2 Donors who have undergone plasmapheresis previously in the same programme

For donors who have already taken part in a plasmapheresis programme:

- The receptionist shall note the date of the last donation (at least two days must have elapsed since that time). No more than two donations shall be permitted within a seven-day period.
- The medical history and weight of the donor shall be recorded; blood pressure, temperature, pulse rate and haemoglobin concentration shall be measured by trained personnel. On the day of each donation, in addition to meeting the general requirements for donors, plasma donors shall be shown to have a total serum protein concentration of not less than 60 g/l.

The medical evaluation of plasma donors shall be repeated at regular intervals, as specified by the national control authority, and tests carried out as specified in section 4.5.3.

Whenever the result of a laboratory test is found to be outside the established normal limits or a donor exhibits any important abnormalities of history or on physical examination, the donor shall be excluded from the programme. The donor shall not be readmitted to the programme until the results of relevant tests have returned to normal and the responsible physician has given approval in writing. It is the responsibility of national health authorities to define normal ranges and standard deviations of test results on the basis of data from a sufficiently large sample of healthy individuals not undergoing plasmapheresis.

4.5.3 Tests for plasma donors

The following tests shall be performed at each donation:

- Measurement of haemoglobin concentration or erythrocyte volume fraction.
- Determination of total serum protein concentration, which shall be at least 60 g/l.
- An approved test for HBsAg, which shall be negative.
- An approved test for anti-HIV which shall be negative.
- An approved test for anti-HCV; which shall be negative.

The following tests shall be performed initially and then every four months or after every 10 donations, whichever time interval is longer:

- If required by the national control authority, a serological test for syphilis, which shall be negative.
- Urine analysis for glucose and protein, which shall be negative.
- Serum protein electrophoresis: this shall be normal (unusual changes in a donor's results may be more significant than absolute values). The albumin and globulin concentrations may be calculated from the known total protein value, and shall be: albumin, minimum 35 g/l; 1gM, minimum 0.5 g/l; IgG, between 5 and 20 g/l.
- Liver function tests.

When determination of serum alanine aminotransferase is required, the enzyme concentration measured photometrically using approved reagents shall be no more than two standard deviations above an established normal mean.

4.6 Donors for platelet and Leukocyte apheresis

In general, platelet and leukocyte donors shall meet the general criteria for donors and the specific criteria for plasma donors (sections 4.1-4.5). In addition, platelet donors should not have taken aspirin or other platelet-active drugs for at least 72 h before donation.

The requirements to be satisfied in the performance of plateletpheresis and leukopheresis in order to ensure that there is no danger to donors and that the products obtained are of satisfactory

quality are under active investigation in many countries. The following recommendations may be useful as guidance.

On the day of each donation, donors for plateletpheresis should have an absolute platelet number concentration (count) of not less than $200 \times 10^9/L$ and donors for leukapheresis should have an absolute granulocyte number concentration of not less than $3 \times 10^9/L$. Both types of donor should have a normal differential Leukocyte count and haemoglobin level.

Although levels of circulating platelets and leukocytes recover promptly in donors, data are not at present available from which the maximum numbers of platelets and leukocytes that can be safely collected from donors can be defined. The long-term effects of the repeated removal of cellular elements are not known.

Leukapheresis may entail the administration of drugs to donors and their exposure to colloidal agents to enhance the yield of granulocytes. Appropriate precautions should be taken to protect donors, such as investigation for latent diabetes by means of a glucose tolerance test if a donor is to be given corticosteroids.

Leukapheresis should be performed as part of the treatment of a patient with chronic myeloid leukaemia only if approved by the patient's attending physician. It is inadvisable to use the leukocytes from such patients.

4.7 Donor immunization and plasma for special purposes

4.7.1 Plasmapheresis in donors with naturally acquired antibodies and other types of medically useful plasma

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 and from donors with plasma useful for diagnostic purposes as a result of acquired or congenital underlying conditions.

Donors with medically useful plasma may be identified by screening whole blood donations and by examining patients convalescing from specific diseases or vaccinated individuals, e.g.

veterinary students who have received rabies vaccine or military recruits who have been immunized with tetanus toxoid. Unnecessary immunizations can be avoided by this approach.

The following are examples of medically useful plasma:

- Antibody-rich plasma for control reagents in diagnostic tests, such as those for anti-HIV, hepatitis A and B, cytomegalovirus, rubella, measles and uncommon infectious agents; plasma should be collected in appropriately isolated premises when products are being prepared that are known to be capable of transmitting infection.
- Plasma containing antibodies to human cellular and serum antigens of diagnostic use, for example in HLA (human Leukocyte antigen) typing reagents, erythrocyte typing reagents and immunoglobulin allotyping reagents.
- Plasma containing reagents useful for diagnostic tests, such as rheumatoid factors, heterophile antibody and C-reactive protein.
- Factor-deficient plasma for specific assays, such as factor-VIII-deficient plasma. Donors who have received factor VIII are at increased risk of transmitting hepatitis B, hepatitis C and HIV; their plasma should therefore be collected in appropriately isolated premises.

4.7.2 Precautions to be taken when handling blood or blood products containing infectious agents

All blood and plasma may contain unknown infectious agents and must be handled accordingly. In addition, special precautions must be taken when handling infected donors and blood products known to contain infectious agents. The precautions to be taken might include:

- isolation by means of the appropriate timing or location of the procedures, special labelling and quarantine of the products collected, use of protective packaging with double wrapping in impervious plastic;
- disinfection of all work surfaces and equipment with a disinfectant of known efficacy, such as freshly prepared 0.25% sodium hypochlorite solution;
- protection of staff by means of adequate training, avoidance of aerosols and use of gloves, gowns, masks and eye protection; it is strongly recommended that such staff also be protected by immunization with hepatitis B vaccine;

- Fulfillment of the labelling, shipping and waste-disposal requirements appropriate to the etiological agents in question.

4.7.3 Immunization of donors

There is a clinically valid need for specific immunoglobulin and plasma for therapeutic, prophylactic and diagnostic uses. 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 shall take into consideration all the requirements of the previous sections.

Donors shall be immunized with antigens only when sufficient supplies of material of suitable quality cannot be obtained from other appropriate donors, from donations selected by screening, or in the form of safe and efficacious licensed monoclonal antibodies. Donors must be fully informed of the risk of any proposed immunization procedure, and pressure shall not be brought to bear on a donor to agree to immunization. Women capable of child-bearing shall not be immunized with erythrocytes or other antigens that may produce antibodies harmful to the fetus. Donors of blood and those undergoing plasmapheresis shall, if necessary, undergo investigations that can reveal hypersensitivity to a proposed antigen (see also Part B, section 6).

An approved schedule of immunization shall be used. Every effort shall be made to use the minimum dose of antigen and number of injections. In any immunization programme, the following shall be taken into consideration as a minimum: (a) the antibody assay; (b) the minimum level of antibody required; (c) data showing that the dose, the intervals between injections and the total dosage proposed for each antigen are appropriate; and (d) the criteria for considering a prospective donor a non-responder for a given antigen. No donor shall be hyperimmunized with more than one immunizing preparation unless the safety of the multiple procedures 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, is achieved in small groups of potential donors);

- encouraged to seek advice from their family doctor before agreeing to immunization;
- informed that any licensed physician of their choice will be sent all the information about the proposed immunization procedure;
- Informed that they are free to withdraw consent at any time.

All vaccines used for immunizing donors shall be registered or recognized by the national health authority but may be administered at doses and with schedules differing from those recommended for routine prophylactic immunization. Erythrocyte and other cellular antigens shall be obtained from an establishment approved by the national control authority.

Donors shall be observed for approximately 30 min following any immunization in order to determine whether an adverse reaction has taken place. Because reactions often occur 2-3 h after immunization, donors shall 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.

4.7.4 Immunization with human erythrocytes

Erythrocyte donors A donor of erythrocytes for the purposes of immunization shall meet all the general health criteria for donors (see sections 4.3 and 4.4). In addition, the donor shall not have had a blood transfusion at any time.

The volume of erythrocytes drawn from a donor should not exceed 450-500 ml of whole blood in any eight-week period.

At each donation the donor shall be found to be negative for syphilis, HBsAg, anti-HIV antibody to hepatitis 13 core antigen (anti-HBc), anti-HCV and antibodies to human T-cell lymphotropic viruses (anti-HTLV). The serum level of aminotransferases should be within normal limits as established by the national control authority.

Erythrocyte phenotyping shall be done for ABO as well as for C, D, E, c, e, Kell and Fya. Phenotyping for other specificities is often desirable and is recommended especially for JK^a, JK^b, Fyb, S and s.

Ideally erythrocytes obtained for immunization purposes should be frozen for at least 12 months before use and the donor should be recalled and retested for anti-HIV, anti-HCV, anti-HBc, HBsAg and anti-HTLV before the stored cells are used for immunization.

Where suitable facilities for freezing erythrocytes are not available, national control authorities may authorize the use of cells from a single donor to immunize no more than three persons (preferably who have not previously had a blood transfusion) in an initial 12-month period, during which monthly determinations of anti-HIV, anti-HCV, anti-HBc, HBsAg and serum alanine aminotransferase should be made in both the donor and the recipients.

If, after 12 months, the initial three recipients show no clinical or laboratory evidence of hepatitis, HIV infection or other blood-transmissible diseases, the donor may be considered acceptable for providing erythrocytes for immunization. As small a number of donors of erythrocytes should be used as possible.

Collection and storage of erythrocytes. Erythrocytes shall 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 shall be validated.

Erythrocytes should be stored frozen for at least 12 months to permit retesting of donors for disease markers. The method selected should have been validated such that there is 70% cell recovery *in vivo*. Erythrocytes should be washed after storage to remove the cryoprotective agent.

Adequate sterility data to support the requested shelf-life for stored erythrocytes should be submitted by the manufacturer to the national control authority. A test for bacterial and fungal contamination should be made on all blood dispensed in aliquots in an open system (9). 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 made on the eighth day after

collection and again on the expiry date. Cultures for the sterility test should be maintained for at least 14 days, with sub culturing on day 3, 4 or 5.

Erythrocyte recipients The following additional testing of erythrocyte recipients is necessary:

The recipient should be phenotyped for ABO, Rh, Kell and Duffy antigens before immunization. Kdll-negative and/or Fy(a-) persons should not receive Kell-positive or Fy(a+) cells except for the specific purpose of producing anti-Kell or anti-Fya. Only ABO-compatible erythrocytes may be transfused. Matching of Jk^a, Jk^b, Fya, S and s phenotypes is also desirable.

Screening for unexpected antibodies by methods that demonstrate coating and haemolytic antibodies should include 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 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 developed after injection of erythrocytes. The national control authority should be notified annually in writing of unexpected antibodies induced by immunization, and the immunized donor should carry a card specifying the antibodies.

Immunization schedules. Erythrocytes used for immunization purposes shall 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.

For primary immunization two injections of erythrocytes, each of about 1-2 ml and given three months apart, elicit antibody formation within three months of the second injection in approximately 50% of volunteers; the result is not improved by injecting larger amounts or giving more frequent injections.

It is advantageous to choose as donors of anti-D (anti-Rh0) volunteers who are already immunized, since useful levels of anti-D are then usually attained within a few weeks of reimmunization. In some people, the level of antibody reaches its maximum within the first three weeks and will not increase after further immunization. In others, antibody levels may continue to rise for more than 12 months when injections of 0.5-1 ml of erythrocytes are given at intervals of five to eight weeks. About 70% of immunized volunteers eventually produce antibody levels well above 100 IU/mL. Once attained, such levels can be maintained by injections of 0.1-0.5 ml of erythrocytes at intervals of two to nine months, as required. If injections of erythrocytes are discontinued, antibody levels usually fall appreciably within 6-12 months.

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.

Erythrocytes to be used for immunization purposes should be selected, for each recipient, by a licensed physician.

Risks to recipients. Recipients of erythrocytes for immunization purposes may run the 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 reaction if the antigen dose is too great;
- the production of antibodies that may interfere with future organ transplantation if it is needed.

Record-keeping Records of erythrocyte donors and of the recipients of their erythrocytes should be maintained and cross-referenced.

5. Collection of blood and plasma

A number of precautions must be taken in the collection of blood and plasma, as described in the following sections.

5.1 *Blood collection and aphaeresis procedures*

The skin of the donor at the site of venepuncture shall be prepared by a method that has been shown to give reasonable assurance that the blood collected will be sterile, Blood shall be collected into a container by means of an aseptic method. The equipment for collecting the sterile blood may be closed or vented provided that the vent is designed to protect the blood against microbial contamination.

With aphaeresis procedures, care shall be taken to ensure that the maximum volume of erythrocytes is returned to the donor by intravenous infusion, If the red cells cannot be returned to the donor, no further collection should be made until the donor has been re-evaluated. Several checks shall be made to ensure that donors receive their own erythrocytes, including identification of the containers of erythrocytes by donors before re-infusion. Hemolytic transfusion reactions are avoidable, since they are caused by the accidental infusion of incompatible erythrocytes. Personnel involved in re-infusion procedures should be adequately trained to prevent them. The signs and symptoms are hypotension, shortness of breath, stomach and/or flank pain, apprehension, cyanosis and haemoglobinuria.

If a hemolytic transfusion reaction occurs, the infusion of cells to all donors at the centre concerned should be discontinued until the identity of all containers of erythrocytes has been checked. Automated plasmapheresis is preferred to manual plasmapheresis in some institutions because of its greater safety.

5.1.1 Summary of minimum general requirements for apheresis

Equipment This must be electrically safe and non-destructive for blood elements; disposable tubing must be used wherever there is blood contact. In addition, equipment must be accessible to detailed inspection and servicing and its decommissioning should not significantly interrupt the programme. It should also be provided with suitable automatic alarms.

Procedure this must be non-destructive for blood elements and aseptic; there must be adequate safeguards against air embolism.

Disposables these must be pyrogen-free, sterile and biocompatible (e.g. there must be no activation of enzyme systems).

5.1.2 Adverse reactions

Provision must be made to prevent and treat any adverse reactions in donors. As with any medical procedure involving the treatment of individuals, adverse reactions may occur with blood collection and plasmapheresis. Almost all such reactions are mild and transient, but an occasional serious reaction may occur. The possibility of adverse reactions, though remote, should be anticipated and adequate provision should be made to ensure that care is available to donors. Initial and continuing training in emergency care is mandatory for personnel. If any serious adverse reaction occurs, a physician should be called.

5.1.3 Types of adverse reaction

Vasovagal syncope. This is most likely to occur with new donors. The signs and symptoms are hypotension, bradycardia, syncope, sweating and (rarely) convulsions.

Local infection, inflammation and haematoma at the phlebotomy site. Reactions of this type are best prevented by adequate preparation of the venepuncture site and by training phlebotomists in proper methods of initiating blood flow. The symptoms are localized pain and redness and swelling at the phlebotomy site.

Allergic and anaphylactic reactions. These may occur during the introduction of saline into the donor while red cells are being processed, or during re-infusion of red cells. The signs and symptoms are urticaria, burning in the throat, tightness of the chest, wheezing, pain in the abdomen and hypotension.

Systemic infection. Care should be taken at all stages of plasmapheresis to avoid the transmission of infectious organisms to the donor.

5.2. Containers

The original blood container or a satellite attached in an integral manner shall be the final container for whole blood and red cells, with the exception of modified red cells, for which the storage period after processing should be as short as possible and certainly not longer than 24 h. Containers shall be uncolored and translucent and the labelling shall be placed in such a position as to allow visual inspection of the contents. They shall be sterilized and hermetically sealed by means of suitable closures so that contamination of the contents is prevented. The container

material shall not interact adversely with the contents under the prescribed conditions of storage and use.

The specifications for containers should be approved by the regulatory authority (10, 11).

If sterile docking devices are not available, closed blood-collection and processing systems should be used to prepare blood components.

5.3. *Anticoagulants*

The anticoagulant solution shall be sterile, pyrogen-free and of a composition such as to ensure that the whole blood and separate blood components are of satisfactory safety and efficacy.

Commonly used anticoagulant solutions are acid-citrate-glucose, citrate-phosphate-glucose and citrate-phosphate-glucose-adenine; the amount of adenine used varies in different countries. Solutions of adenine, glucose and mannitol used for red cell preservation may be added after removal of the plasma. For plasmapheresis. Sodium citrate as a 40 g/l solution is widely used as an anticoagulant.

5.4 *Pilot samples*

Pilot samples are blood samples provided with each unit of whole blood or of red blood cells. They shall be collected at the time of donation by the person who collects the whole blood. The containers for pilot samples shall be marked at the collection site before the samples are collected, and the marking used must be such that the sample can be identified with the corresponding unit of whole blood. Pilot samples must be collected by a technique that does not compromise the sterility of the blood product.

Pilot samples should be attached to the final container in a manner such that it will later be clear whether they have been removed and reattached.

5.5 *Identification of samples*

Each container of blood, blood components and pilot and laboratory samples shall be identified by a unique number or symbol so that it can be traced back to the donor and from the donor to the

recipient. The identity of each donor shall be established both when donor fitness is determined and at the time of blood collection.

When blood-derived materials are transferred to a fractionation plant, the following details shall be provided by the supplier:

- Name and address of collecting centre,
- type of material,
- Donor identification,
- date of collection
- results of mandatory tests,
- conditions of storage,
- Other details required by the fractionators,
- name and signature of responsible person,
- date.

Part B. Requirements for single-donor and small-pool products

6. General considerations

These requirements for single-donor and small-pool products cover the methods used to prepare products directly from units of whole blood or of components collected by apheresis, starting with the testing of the units and proceeding to the separation of the various cell and plasma protein components. Among the products that may be prepared in small pools (12 donors or fewer) are cryoprecipitated factor VIII and platelets. In addition to tests on the units of whole blood that provide information on the safety, efficacy and labelling of the components, specific tests are included, where applicable, to ensure the quality of various components.

It is important to note that single-donor and small-pool products have certain specialized uses other than therapeutic application to correct deficits in patients. Although not dealt with further in these Requirements, these uses include the stimulation of plasma donors with red blood cells in order to raise antibody levels for the preparation of anti-D (anti-Rh0) immunoglobulin (12) and

special blood-grouping reagents. It is of the utmost importance that the donors of cells and plasma for such purposes be carefully studied both initially and on a continuing basis to minimize the likelihood of the transmission of infectious diseases to recipients. The use of red cells, stored frozen, that have been demonstrated to be free from infectious agents by retesting the donor 12 months after the initial collection reduces the risk of such transmission to volunteers for immunization.

Plasma donors may also be immunized with viral or bacterial antigens for the preparation of specific immunoglobulin products. All donor immunization procedures must be planned and carried out under the supervision of a physician who is familiar with the antigens being used and especially with the reactions or complications that may occur. Donors being immunized shall have been fully informed of all known hazards and shall have given their written informed consent to the procedures.

Donor immunization practices are considered in more detail in Part A, section 4.7.

Minimum general requirements for apheresis are summarized in Part A, section 5.1.1.

7. Production and control

7.1 *General requirements*

Single-donor and small-pool products shall comply with any specifications established by the national control authority. Cellular blood components and certain plasma components may deteriorate during separation or storage. Whatever the method of separation (sedimentation, centrifugation, washing or filtration) used for the preparation of cell components, therefore, it is important that a portion of plasma protein sufficient to ensure optimum cell preservation be left with the cells, except when a cryoprotective substance is added to enable them to be stored for long periods in the frozen state, or additive solutions (for example containing adenine, glucose and mannitol) are used for the same purpose for liquid storage.

The methods employed for component separation should be checked before they are introduced. The characteristics assessed might include yield of the component, purity, *in vivo* recovery, biological half-life, functional behaviour and sterility.

The nature and number of such checks should be determined by the national control authority.

Immediately before issue for transfusion or for other purposes, blood components shall be inspected visually. They shall not be issued for transfusion if abnormalities of colour are observed or if there is any other indication of microbial contamination or of defects in the container.

Blood components shall be stored and transported at the appropriate temperature. Refrigerator or freezer compartments in which components are stored shall contain only whole blood and blood components. Reagents required for use in testing may be stored in a separate section of the same refrigerator or freezer provided that they have been properly isolated and are in suitable containers.

7.2 *Testing of whole blood and plasma*

7.2.1 *Sterility*

Each donation of whole blood intended for transfusion and each preparation of component cells constitutes a single batch. Single batches shall not be tested for sterility by any method that entails breaching the final container before the blood is transfused.

The national control authority may require tests for sterility to be carried out at regular intervals on final containers chosen at random and at the end of the storage period. The purpose of such tests is to check on the aseptic technique used for taking and processing the blood and on the conditions of storage.

7.2.2 *Laboratory tests*

Laboratory tests shall be made on laboratory samples taken either at the time of collection or from the pilot samples accompanying the final container, labelled as required in Part A, section 5.

In some countries, test reagents, in particular those used for blood-grouping and for detecting anti-HIV anti-HCV and HBsAg, must be approved by the national control authority.

The results of the tests shall be used for ensuring the safety and proper labelling of all components prepared from units of whole blood.

7.2.3 *Tests for infectious agents*

Syphilis. Each donation of whole blood shall, if required by the national control authority, be subjected to a serological test for syphilis. If so tested, only units giving negative results shall be used for transfusion or component preparation.

Viral hepatitis. Each unit of blood or plasma collected shall be tested for HBsAg and anti-HCV by a method approved by the national control authority and only those giving a negative result shall be used (13). Units giving a positive result shall be so marked, segregated and disposed of by a method approved by the national control authority, unless designated for the production of a reagent or experimental vaccine in an area designed and segregated for such production.

- In some countries plasma pools are also tested.
- The label on the container or the record accompanying the container should indicate the geographical source of the blood or plasma as well as whether and how the material has been tested for HBsAg and anti-HCV
- Liver function tests, such as serum transaminase determinations, are used in some countries to detect liver damage that may be associated with hepatitis.

Anti-HIV-1 and anti-HI V-2. Blood for transfusion and for use in the preparation of blood components must be tested by a method approved by the national control authority for antibodies to HIV-1 and HIV-2 and be found negative. However, when other important factors outweigh the benefits of such testing (e.g. in emergencies) formal arrangements, approved in advance by the national control authority, should be in place that enable the prescribing physician to have access to an untested product. In all such cases, retrospective testing of the pilot sample shall be performed.

Other infectious agents. It is important for the national control authority to reassess testing requirements from time to time in the light of current knowledge, the prevalence of infectious agents in different populations and the availability of tests for serological markers of infection. For example, human retroviruses other than HTLV have been described (HTLV types 1 and 2) and more may be identified in the future.

7.3 Blood-grouping

Each unit of blood collected shall be classified according to its ABO blood group by testing the red blood cells with anti-A and anti-B sera and by testing the serum or plasma with pooled known group A (or single subtype A1) cells and known group B cells. The unit shall not be labelled as to ABO group unless the results of the two tests (cell and serum grouping) are in agreement. Where discrepancies are found in the testing or the donor's records, they shall be resolved before the units are labelled.

In countries where polymorphism for the D (Rho) antigen is present each unit of blood shall be classified according to Rh blood type on the basis of the results of testing for D (Rh0) red cell antigen. The D (Rh0) type shall be determined with anti-D (anti-Rh0) reagents.

With the high-strength antisera and sensitive techniques now available, it is usually considered unnecessary to use the D test if the cells are found to be D-negative in routine testing.

7.4 Red cells

Whole blood for the preparation of all components shall be collected as described in Part A, section 5, and tested as described in Part B, section 7.2.

Red cells shall be processed under aseptic conditions and whenever possible in a closed system. The sterility of all components shall be maintained during processing by the use of aseptic techniques and sterile pyrogen-free equipment. The methods shall be approved by the national control authority, and a written description of the procedures shall be prepared for each product, covering each step in production and testing. Proposals for any procedural modifications shall be submitted to the national control authority for approval before they are implemented.

The following may be prepared for therapeutic purposes (see definitions):

- red cells;
- red cells suspended in additive solution;
- modified red cells:
 - red cells, leukocyte-depleted;
 - red cells, leukocyte-poor;
 - red cells, washed;

- red cells, frozen;
- red cells, deglycerolized.

7.4.1 *Methods and timing of separation*

Red cells shall be prepared from whole blood collected in plastic bags or in glass bottles.

Multiple-plastic-bag systems with sterile docking devices are preferable because they minimize the risk of microbial contamination by providing completely closed systems. They are easy to handle and are disposable. The use of glass bottles is cheaper but has the disadvantage that the system is then an open or vented one. so that separation must be carried out under strictly aseptic conditions in sterile rooms or laminar-flow cabinets and microbiological monitoring is necessary. The same conditions also apply to the separation procedure when plasma is transferred from disposable single plastic bags to separate containers.

All surfaces that come into contact with the blood cells shall be sterile, biocompatible and pyrogen-free. If an open plastic-bag system is used, i.e. the transfer container is not integrally attached to the blood container and the blood container is opened after blood collection, the plasma shall be separated from the cells under conditions such that the original container is kept under positive pressure until it has been sealed. If the separation procedure involves a vented system, i.e. if an airway is inserted into the container for withdrawal of the plasma, the airway and vent shall be sterile and constructed so as to exclude microorganisms.

In some countries, the sterility of products prepared in open systems is monitored by testing a sample of at least 2% of the units. The national control authority should approve the system used.

The final containers for red cells (but not necessarily modified red cells) shall be the containers in which the blood was originally collected or satellite containers attached in an integral manner. If pilot samples are detached from the blood container during removal of any component, such samples shall be reattached to the container of red cells. The removal and reattachment of the pilot samples shall be recorded conspicuously (with a signature) on the label of the unit. The final containers for all other components shall meet the requirements for blood containers given in Part A, section 5.2. If the final container differs from the container in which the blood was originally

collected, it shall be given a number or other symbol to identify the donor(s) of the source blood. Whenever appropriate, the secondary final container shall be similarly labelled while attached to the primary final container.

The timing and the method of separation (centrifugation, undisturbed sedimentation or a combination of the two) will depend on the components to be prepared from the donation. When platelets and coagulation factors are being prepared from the same donation, the components shall be separated as soon as possible after withdrawal of the blood from the donor.

Separation should preferably be effected within 8 h of blood donation. When platelets and coagulation factors are to be prepared, it is especially important that the venepuncture be performed in such a way as to cause minimal tissue damage so as to prevent the initiation of coagulation. The blood should flow freely without interruption and as rapidly as possible, and be mixed thoroughly with the anticoagulant

If platelets are to be prepared from a unit of whole blood, the blood shall be kept at a temperature of 20-24 °C for up to 8 h until the platelet-rich plasma has been separated from the red blood cells.

Red cells may be prepared either by centrifugation or by undisturbed sedimentation before the expiry date of the original whole blood. Blood cells shall be separated by centrifugation in a manner that will not increase the temperature of the blood.

7.4.2 *Expiry date*

The expiry date of whole blood and red cells prepared in a closed system from blood collected in acid-citrate-glucose or citrate –phosphate-glucose is generally 21 days after collection. The time of removal of plasma is not relevant to the expiry date of the red cells when the integrity of the container is not compromised.

The shelf-life of stored blood has been extended to 35 days by collecting the blood in acid-citrate-glucose supplemented with 0.5 mmol/l adenine or in a mixture of 0.5 mmol/l adenine and 0.25 mmol/L guanosine with extra glucose, and to 42 days by adding a solution containing adenine, glucose and mannitol. recent studies indicate that it may also be possible to extend the

shelf-life of stored blood to 35 days by collecting it in citrate—phosphate—glucose supplemented with 0.25 mmol/L adenine and extra glucose.

When red cells are prepared with very high erythrocyte volume fractions, an expiry date 14 days after collection is recommended in some countries because the cells may become glucose-deficient after this time. The erythrocyte volume fraction of red cells collected in citrate—phosphate—glucose—adenine should not exceed 0.9 if the expiry date is more than 21 days after collection.

The usefulness of acid—citrate—glucose is limited by the significant reduction in cell viability when the volume of cells collected is small, which is unavoidable for some donations.

Provided that sterility is maintained, the shelf-life of red cells is not influenced by the method of separation used. However, if an open system is used that does not maintain sterility, the expiry date shall be 24 h after separation and the cells should be used as soon as possible. Red cells and whole blood should be stored at $5 \pm 3^{\circ}\text{C}$ and transported with wet ice in insulated boxes 'at $5 \pm 3^{\circ}\text{C}$. Care should be taken not to place containers directly on ice.

Refrigerated whole blood and red cells will warm up rapidly when placed at room temperature. Every effort should be made to limit the periods during which the products are handled at ambient temperatures in order to prevent the temperature from rising above 10°C until they are used.

7.4.3 Modified red cells

Red cells, leukocyte-depleted and red cells, leukocyte-poor

Because of the possibility of reactions, some countries require that red cells contain less than 2% of the leukocytes of the original whole blood.

Leukocyte depletion may be achieved by buffy-coat removal, freezing and washing, or by washing alone.

Leucocytes-poor red-cell concentrates are prepared by filtration.

Red cells, washed. Red cells can be washed by means of interrupted or continuous-flow centrifugation. If the first of these methods is used, the washing procedure shall be repeated three times.

Centrifugation should be carried out in refrigerated centrifuges. If such equipment is not available, the washing solution should have a temperature of $5 \pm 3^{\circ}\text{C}$.

Red cells can also be washed by means of reversible agglomeration and sedimentation using sugar solutions.

Requirements for pilot samples, labels and storage and transport temperatures are the same as those for unmodified red cells.

Red cells, frozen and red cells deglycerolized. Red cells less than six days old are usually selected for freezing in order to minimize loss of yield due to haemolysis during processing.

Frozen red cells are red cells that have been stored continuously at low temperatures (-65°C or below) in the presence of a cryoprotective agent. The red cells must be washed to remove the cryoprotective agent before use for transfusion. The methods of preparation, storage, thawing and washing used should be such as to ensure that at least 70% of the transfused cells are viable 24 h after transfusion, storage at temperatures below -65°C is usually necessary to achieve 70% recovery.

The cryoprotective agent in most common use is glycerol. The temperature of storage should be between -65°C and -160°C , depending on the glycerol concentration used.

The shelf-life of frozen cells below -65°C is at least three years and may be much longer under certain circumstances, but the reconstituted (thawed and washed) red cells should be used as soon as possible and not later than 24 h after thawing unless a closed system is used.

Frozen cells are usually shipped in solid carbon dioxide ("dry ice) or liquid nitrogen, depending upon the glycerol concentration used. Deglycerolized red cells should be stored at a temperature of $1-6^{\circ}\text{C}$ and shipped at $5 \pm 3^{\circ}\text{C}$.

Requirements for pilot samples and labels are the same as those for unmodified red cells.

7.5 Plasma

Single-donor plasma shall be obtained by plasmapheresis or from units of whole blood that comply with the requirements of Part A, section 5, and Part B, section 7.2.

Fresh-frozen plasma and frozen plasma should be stored in carefully monitored freezers equipped with recording thermometers and audio and visual alarms to give warning of mechanical or electrical failure. If refrigeration is interrupted for longer than 72 h and the temperature rises above -5 °C, the product may no longer be considered as fresh-frozen plasma, although testing may indicate that reasonable amounts of factor VIII remain if the plasma has not become liquid. Repeated thawing and freezing may cause denaturation of plasma constituents and cause prekallikrein activation.

7.5.1 Plasma, fresh-frozen

Fresh-frozen plasma shall be prepared by separating plasma from whole blood and freezing it rapidly within 8 h of collection.

Ideally, fresh-frozen plasma should be prepared by rapid freezing using a combination of solid carbon dioxide and an organic solvent such as ethanol. If this procedure is used, it should have been shown that the container cannot be penetrated by the solvent or substances leached from the container into the contents. Fresh frozen plasma should be stored at or below -20 °C, and below -30°C if to be used for transfusion purposes

Before use for infusion, fresh-frozen plasma should be thawed rapidly at 30-70 °C. Agitation of the container and/or circulation of water at a temperature of 37 °C during the thaw cycle will speed thawing. Once thawed, fresh-frozen plasma must not be refrozen. It can be stored at ambient temperature and should be used within 2 h of completion of thawing.

Fresh-frozen plasma shall have an expiry date one year from the date of collection.

Before its expiry date, fresh-frozen plasma may be used for preparing cryoprecipitated factor VIII. It may be used for the preparation of other pooled plasma fractions (e.g. factors 1,11, VII, VIII, IX and X) at any time, even after its expiry date.

7.5.2 Plasma, frozen

Frozen plasma is, by definition, plasma separated from whole blood more than 8 h after the latter has been collected, but the delay should be as short as possible. Frozen plasma may be used directly for transfusion or fractionation, or it may be freeze-dried as single-donor units. Plasma may be combined in small pools before freezing if it is to be used to prepare freeze-dried plasma.

The national control authority should determine the specific requirements for frozen plasma

If frozen or freeze-dried plasma is intended to be used directly in patients without further processing, the blood shall be collected in such a manner and in containers of such a type as to allow aseptic handling, e.g. by means of closed systems.

In some countries, frozen plasma is given an expiry date five years from the date of collection.

Whenever the container of frozen plasma is opened in an open procedure, the method of handling shall avoid microbial contamination; as an additional precaution, sterile rooms or laminar-flow cabinets can be used.

Delay in processing shall be avoided, and the ambient conditions shall be regulated so as to minimize the risk of contamination.

Plasma may be pooled at any time after collection.

7.5.3 Plasma, freeze -dried

Freeze-dried plasma shall be made from single units or small pools of fresh-frozen plasma or frozen plasma.

The storage conditions and expiry dates of different forms of freeze-dried plasma shall be approved by the national control authority. The product normally has a shelf-life of five years

when stored at $5\pm 3^{\circ}\text{C}$, but this will depend on the source material, storage conditions and residual moisture in the product. Pooled freeze-dried plasma has a significant potential for the transmission of infectious diseases. This is likely to be substantially diminished by the introduction of viral inactivation procedures applicable to plasma.

7.5.4 Plasma, *recovered*

Recovered plasma intended to be pooled for fractionation shall not be used directly for transfusion; a preservative shall not be added.

Plasma may be separated from whole blood at any time up to five days after the expiry date of the blood. The method used for separation shall avoid microbial contamination. As an additional precaution, sterile rooms or laminar-flow cabinets can be used.

If the plasma has been pooled, it shall be stored and transported frozen at or below -20°C .

7.5.5 Plasma, *platelet-rich*

Platelet-rich plasma is a preparation containing at least 70% of the platelets of the original whole blood.

The preparation shall be separated by centrifugation, preferably within 8h of collection of the whole blood. The temperature and time of processing and storage shall be consistent with platelet survival and maintenance of function.

To achieve the desired haemostatic effect, platelet-rich plasma shall be transfused as soon as possible after collection, and not later than 72 h afterwards, unless stored at $22\pm 2^{\circ}\text{C}$ in containers approved for a longer storage period.

7.6 Platelets

Platelets shall be obtained by cytappheresis or from whole blood, buffy coat or platelet-rich plasma that complies with the requirements of Part A, section 5, and Part B, section 7.2. Aspirin ingestion within the previous three days precludes a donor from serving as a source of platelets.

Whole blood or platelet-rich plasma from which platelets are derived shall be maintained at 22 ± 2 °C until the platelets have been separated.

The separation shall preferably be performed within 8 h of collection of the whole blood. Blood shall be obtained from the donor by means of a single venepuncture giving an uninterrupted flow of blood with minimum damage to the tissue. It must have been demonstrated that the time and speed of centrifugation used to separate the platelets will produce a suspension without visible aggregation or haemolysis.

The national control authority shall determine the minimum acceptable number of platelets that should be present in the products prepared (e.g. 5.5×10^{10}).

A pH of 6.5-7.4 shall be maintained throughout storage of platelets. The volume of plasma used to resuspend platelets will be governed by the required pH of the platelet suspension at the end of its shelf-life, but shall be no less than 50 ± 10 ml.

Licensed artificial suspension media may be used to replace plasma.

Platelets stored at 5 °C are inferior to the same product stored at 22 ± 2 °C. Cold storage should be avoided where possible.

When stored at 22 ± 2 °C, platelet products shall be gently agitated throughout the storage period.

The material of which the final container used for platelets is made shall not interact with the contents under normal conditions of storage in such a manner as to have an adverse effect on the product.

The requirements for labelling the final container are given in section 7.9. In addition to the customary data, the label shall bear: (a) the recommended storage temperature; (b) the statement

that, when stored at $22 \pm 2^{\circ}\text{C}$, the platelets should be gently agitated throughout storage to obtain maximum haemostatic effectiveness; and (c) a statement to the effect that the contents should be used as soon as possible, and preferably within 4 h once the containers have been opened for pooling.

7.6.1 *Monitoring the quality of platelets*

Units randomly selected at the end of their shelf-life shall be tested on a regular basis. They shall be shown to have: (a) plasma volumes appropriate to the storage temperature; and (b) a pH between 6.5 and 7.4.

The number of units and of platelets to be tested shall be specified by the national control authority.

7.6.2 *Expiry date*

The expiry date of platelets processed in a closed system shall be 72 h after the original whole blood was collected, unless they are stored in a plastic container approved by the national control authority for a longer storage period.

7.7 *Leukocytes*

Leukocytes are obtained by the separation of whole blood or by apheresis, and may contain a large number of platelets and red blood cells, depending on the method of preparation. When leukocytes are obtained from units of whole blood, such units shall comply with the requirements of Part A, section 5, and Part B, section 7.2.

The methods used to process leukocytes shall comply with the requirements and recommendations given in section 7.4.1 for the separation of red cells.

The label on the final container shall bear, in addition to customary data, instructions to use the leukocytes as soon as possible and in any case not more than 4 h after the container has been opened for pooling. The temperature of storage and transport shall be $22 \pm 2^{\circ}\text{C}$.

The large number of red cells present in products prepared by some methods makes compatibility testing before transfusion necessary.

7.7.1 *Testing of Leukocytes*

The number of units to be tested and the leukocyte yield (number) required shall be specified by the national control authority.

7.7.2 *Expiry date*

The expiry date of leukocytes shall be 24 h after collection of the original whole blood.

7.8 *Cryoprecipitated factor VIII*

Cryoprecipitated factor VIII is a crude preparation of factor VIII. It shall be obtained from single units or small pools of plasma derived either from units of whole blood that comply with the requirements of Part A, section 5, and Part B, section 7.2. or by plasmapheresis.

The product may be prepared as a pool from a small number of donations, usually four to six but not exceeding ten. It may be freeze-dried. However, preparations of cryoprecipitated factor VIII carry the risk of viral transmission unless they have undergone specific virucidal procedures during manufacture.

The method of thawing and harvesting the cryoprecipitate shall have been shown to yield a product containing an adequate activity of factor VIII (see section 7.8.1).

In procuring source material for coagulation factors, the following technical considerations should be borne in mind:

- In order to prevent coagulation, venepuncture should perform in such a way that tissue damage is minimal. The blood should flow freely without interruption, and be mixed thoroughly with anticoagulant during collection.
- Microbial contamination should be avoided during separation of the plasma by using multiple-plastic-bag closed systems or laminar-flow cabinets if an open procedure is used.
- The recovery of factor VIII depends on the interval between venepuncture and freezing of the plasma, the temperature at which the plasma is held and the freezing method. While a useful product may be obtained with plasma frozen as late as 18-24 h after phlebotomy, freezing the plasma as early and as rapidly as possible is strongly recommended.

- Ideally, fresh-frozen plasma should be prepared by rapid freezing using a combination of solid carbon dioxide and an organic solvent such as ethanol. Fresh-frozen plasma should be stored at or below -20°C . Contamination of the plasma by the solvent or leaching of substances from the container into the plasma should be avoided.
- If the temperature of the thawed plasma exceeds 2°C , a high proportion of the factor VIII is lost in the supernatant. During thawing or separation of the supernatant plasma, therefore, the temperature should not be allowed to exceed 2°C . The plasma may be separated while there is still a small quantity of the ice present in the plasma container. Increasing the speed of thawing by circulating air or water at a temperature of 0°C is believed to increase the yield of factor VIII.

7.8.1 Testing of cryoprecipitated factor VIII

Randomly selected units shall be tested for potency and sterility on a regular basis. The number of units to be tested shall be specified by the national control authority- The freeze-dried preparation shall dissolve without any signs of precipitation in the solvent recommended by the manufacturer within 30 min when held at a temperature not exceeding 37°C .

The potency of cryoprecipitated factor VIII shall be compared with that of an appropriate plasma or intermediate-purity standard, by measuring its ability to correct the prolonged activated partial thromboplastin time of haemophilia A plasma or by another suitable method.

When cryoprecipitated factor VIII is produced from fresh-frozen plasma (frozen within 8 h of donation) the yield should be greater than 400 IU/l of starting plasma. Plasma frozen after this time will yield less cryoprecipitated factor VIII.

In many laboratories, the average yield of factor VIII is 400 IU/l of starting plasma. The average yield of factor VIII as freeze-dried cryoprecipitate is then at least 300 IU/l of starting plasma. Whether this yield can be obtained elsewhere will depend on local technical possibilities. In some countries, the yields will be much lower, and the national control authority should decide as to the yield that is acceptable.

7.8.2 Expiry date

The frozen product shall be stored at or below -20 °C (if possible below -30 °C) and shall have an expiry date one year from the date of collection. The freeze-dried product shall be stored at 5±3 °C and shall also have an expiry date one year from the date of collection. After thawing or reconstitution, cryoprecipitated factor VIII should be kept at 20-24 °C. It shall be used as soon as possible and in any case not more than 4 h after its container has been opened for pooling or reconstitution.

7.9 Labelling

After having been tested and before being issued for transfusion, units of single-donor and small-pool products shall be identified by means of container labels that clearly state at least the following information:

- The proper name of the product;
- The unique number or symbol identifying the donor(s),
- The expiry date, and when appropriate, the expiry time after reconstitution
- Any special storage conditions or handling precautions those are necessary;
- A reference to a package insert containing instructions for use, warnings and precautions;
- The name and address of the blood donor centre and, where applicable, the manufacturer and distributor;

The average content in International Units of activity, where appropriate.

The results of red cell grouping shall be stated on the label of whole blood, red cells, fresh-frozen plasma (for clinical use), platelets and leukocytes but not necessarily on that of cryoprecipitated factor VIII.

Part C. Requirements for large-pool products

8. Introduction

A number of requirements common to albumin, plasma protein fraction, immunoglobulin preparations and coagulation-factor concentrates are given in Parts A and B, sections 3-7. However, for clarity, it has proved convenient to bring together in Part C certain specific requirements applicable to these products when manufactured on a large scale.

The source material for the large-scale preparation of blood products should comply with the relevant provisions of Parts A and B,

9. Buildings

The buildings used for the fractionation of plasma shall be of suitable size, construction and location to facilitate their proper operation, cleaning and maintenance in accordance with the requirements of Good Manufacturing Practices for Pharmaceutical and Biological Products, and in addition provide adequate space, lighting and ventilation for the activities listed below.

9.1 Storage of whole blood and plasma

Whole human blood and plasma shall be stored frozen or refrigerated in separate facilities that are used only for this purpose. The source materials shall remain in quarantine until the results of testing show that they are suitable for introduction into the fractionation premises.

9.2 Separation of cells and fractionation of plasma

Cells shall be separated and plasma fractionated in a building isolated from those where non-human proteins or microbiological materials, such as vaccines, are manufactured or processed and separate from the animal house.

9.3 Supply and recovery of ancillary materials

Adequate facilities shall be provided for the supply of ancillary materials, such as ethanol, water, salts and polyethylene glycol.

Facilities for the recovery of organic solvents used in fractionation may also be provided.

9.4 Viral inactivation

A separate area shall be provided for all processing subsequent to the completion of viral inactivation procedures when these are carried out at a stage in production before aseptic dispensing and filling (see section 9.5).

9.5 Freeze-drying, filling, packaging, labelling and storage

Separate facilities shall be used for the freeze-drying, filling, labelling and packaging of containers. A separate area shall be provided for the storage of labels, package inserts and packages. Another separate area shall be used for the storage of final containers before dispatch.

9.6 Keeping of records

Adequate provision shall be made for keeping records of all donors, materials, fractionation steps, quality-control procedures and results, of the distribution of the final products and of the disposal of potentially infectious materials. Records should be retained for at least two years beyond the expiry date of the products to which they relate.

Some manufacturers might wish to extend this period to cover any future legal disputes.

9.7 *Quality control*

Separate facilities shall be provided for quality control, including hematological, biochemical, physicochemical, microbiological, pyrogen and safety testing.

9.8 *Disposal of Infective material*

Provision shall be made for the suitable disposal of potentially infectious materials by autoclaving or incineration according to good manufacturing practices.

The disposal of these materials should comply with local legislation.

10. Equipment

Equipment used for the collection, processing, storage and distribution of source materials and large-pool blood products shall comply with the requirements of *Good Manufacturing Practices for Pharmaceutical and Biological Products Guideline*.

Particular attention shall be paid to:

- The maintenance, monitoring and recording of the operation of continuously operating equipment, the validation of its reliability and the provision of stand-by equipment.
- The suitability and compatibility of the surfaces of all materials (e.g. filter medium, glass, stainless steel, plastic and rubber) that come into contact with the products.

Metal surfaces that come into contact with proteins should be resistant to scratching. The surfaces of some materials can denature certain proteins or activate certain coagulation factors.

- The ease and efficiency with which equipment can be cleaned and, where necessary, sterilized. Any bactericidal agent used shall be capable of being completely eliminated before the equipment is used.
- Caution should be exercised in the use of detergents because of their possible effects on the final product; tests should be made to ensure that they do not have any adverse effect on it.
- The provision of suitable facilities for decontamination and for the disposal of potentially infective materials and equipment.

11. Provision at support services

A number of support services are essential for the fractionation of source materials.

11.1 *Water supply*

An adequate supply of suitable pyrogen-free water shall be provided for use during the fractionation process and for the reconstitution and/or dilution of the plasma fractions before filling and freeze-drying.

The two most commonly used types of water are pyrogen-free distilled water and pyrogen-free deionized water, each of which should be maintained at 80°C. Water preparation and delivery systems should be tested at regular intervals for end toxin content and conductance. The water system should be a continuously circulating one and should have no dead ends.

Water for injections is generally used for the preparation of final products (14).

11.2 *Steam supply*

An adequate supply of steam shall be provided for the operation of sterilizing and cleaning equipment. The steam shall be clean and have the quality of water for injections.

11.3 *Other support facilities*

Other support facilities required are:

A supply of electrical and thermal energy.

A means of refrigeration for:

- storing various source materials and fractions;
- keeping the various fractionation areas at the correct temperature;
- keeping the process equipment at the correct temperature;
- storing final products under test;
- Storing final products awaiting dispatch.
- A system of ventilation providing the following two grades of filtered air:

- air filtered to remove particles of 5 μm or greater in diameter, which shall be supplied to the entire work area; and
- Air passed through a filter with a *retention* capacity of more than 99.95% for particles greater than 0.5 μm in diameter, which shall be supplied at a positive pressure to areas where aseptic dispensing is to take place.

Other support facilities may include solvent recovery and a sewage disposal service. Sewage disposal must be carried out in accordance with the sanitary standards of the competent health authority

Proteinaceous sewage from a plasma processing plant is highly nitrogenous and has a high biological oxygen demand; it should therefore not be discharged untreated.

These support facilities shall be located separately from the main process areas and in a place where the conditions (light, physical access, etc.) are conducive to the establishment of effective and routine preventive maintenance programmes. The equipment shall incorporate devices capable of monitoring and recording its operation so as to ensure the safety both of the material being processed and of the process operators. In this way a proper record of the operations of support facilities can be kept and, where necessary, entered into the process record of the product batches.

The equipment should be such as to ensure that both the fractionation process and the proteins are protected if the support services are interrupted. To this end, adequate spare equipment *and* emergency reserve systems should be available, serviced by engineering staff skilled in the maintenance and repair of such equipment

12. Personnel

The plasma fractionation plant shall be under the direction of a designated qualified *person* who shall be *responsible* for ensuring that all operations are carried out properly and competently. The director shall have a good working knowledge of the scientific principles involved and shall be responsible for ensuring that employees are adequately trained, have adequate practical experience and are aware that accepted good practices should be applied in their work.

The personnel involved in quality-control functions shall be separate from those involved in production. The head of the quality-control department shall be responsible only to the director.

Where appropriate, personnel shall wear gowns, masks, boots, gloves and eye protectors.

Personnel should be medically examined at regular intervals. Those known to be carriers of specific pathogenic organisms that may adversely affect the product shall be excluded from the production area.

Vaccination against hepatitis B is strongly recommended for employees routinely exposed to blood or blood products.

13. Production control

13.1 *Fractionation of source materials*

The general conditions for the large-scale fractionation of source materials to prepare prophylactic or therapeutic blood products shall comply with Good Manufacturing Practices for Pharmaceutical and Biological Products Guideline, and shall be approved by the national control authority.

Most physical and chemical techniques of protein separation may be used for the preparation of plasma fractions, provided that they yield protein preparations that have previously been shown to be safe and effective.

The fractionation procedures used shall give a good yield of products meeting the quality requirements of international or national authorities. Fractionation shall be carried out in such a manner that the risk of microbiological contamination and protein denaturation is minimized.

The safety of fractionation steps may be increased by using protected or closed systems. Reproducibility may be increased by the use of automation.

The biological characteristics of the products (such as antibody activity, biological half-life and *in vivo* recovery of the proteins) should not be affected by the fractionation procedures to the extent that they are unacceptable for clinical use.

Methods shall be used that exclude or inactivate pathogenic organisms, in particular hepatitis viruses and human retroviruses, from the final products intended for clinical use. Manufacturers shall validate the ability of their manufacturing processes to inactivate and/or remove potential contaminating viruses by the use of relevant model viruses.

There is increasing evidence that certain manufacturing procedures, coupled with strict control to ensure that the final product complies with precise specifications, result in a product free from HIV, hepatitis B and hepatitis C infectivity.

For coagulation products, viral inactivation and removal methods such as chromatography or treatment with dry heat, wet heat, steam under pressure, heated organic solvents or solvents/detergents shall be used, in combination with other methods that have been shown to be successful in reducing or eliminating the risk of HIV and hepatitis virus transmission.

Donor screening and viral inactivation procedures used in manufacturing plasma coagulation concentrates have significantly improved the safety of these products.

Fibrinogen prepared from plasma pools continues to carry a risk of infection unless it is treated to remove or inactivate viruses. Where large-pool, virally inactivated fibrinogen concentrates are not available, cryoprecipitated factor VIII prepared from individual units or small pools of plasma is preferred as a source of fibrinogen. Approximately 150 mg of fibrinogen is contained in the cryoprecipitate from one unit of plasma (200 ml) frozen within 8 h of collection from the donor.

The operating manual for the fractionation procedure shall specify the times of sampling of the products and the volumes to be taken at each stage of the process as well as the tests to be made on the samples.

Where appropriate, all materials used for fractionation shall be tested for microbiological contamination, identity, purity, endotoxin content and toxicity in accordance with *The international pharmacopoeia* (14,15) .

Certain procedures, equipment and materials may introduce contaminants into the final product that can induce allergenic or immunogenic responses in recipients. The quantities of such

contaminants in the final product shall be minimized. For example, where monoclonal antibodies are used for product purification, the residual concentration in the final product must be below clinically reactive levels.

It is advisable to use air filtration under positive pressure during fractionation, to exclude airborne allergenic dust.

13.1.1 Preservatives and stabilizers

No preservatives shall be added to albumin, plasma protein fraction, intravenous immunoglobulin or coagulation-factor concentrates either during fractionation or at the stage of the final bulk solution. Antibiotics shall not be used as preservatives or for any other purpose in the fractionation of plasma.

To prevent protein denaturation, stabilizers may be added. Such substances shall have been shown to the satisfaction of the national control authority not to have any deleterious effect on the final product in the amounts present and to cause no untoward reactions in humans.

Stable solutions of immunoglobulin may be prepared in approximately 0.3 mol/L glycine or 0.15 mol/L sodium chloride. In some countries, thiomersal and sodium timerfonate are not permitted as preservatives in intramuscular immunoglobulins.

13.2 Storage and control of source materials

At all stages of the manufacturing process, the source materials and resulting fractions shall be stored at temperatures and under conditions shown to prevent further contamination and the growth of microorganisms, to protect the identity and the integrity of the proteins and to preserve the biological activity and safety of the products.

If similar materials are stored together, the places allocated to them shall be clearly demarcated.

All source materials and resulting fractions shall be fully identified at all times; such identification shall include the batch number of all in-process fractions and final containers waiting labelling.

13.2.1 In-process control

Source materials are subject to biological variability and the products resulting from protein separation will contain various amounts of other protein components of plasma. It is essential, therefore, to establish a monitoring system such that the safe operating limits of each process are maintained.

The main information collected is on variations in physical conditions (temperature, pH, ionic strength, timing, etc.) and in the number and species of contaminating organisms.

Owing to the numerous and interdependent factors involved, there are no universally accepted specifications for such in-process quality-assurance systems. For This reason, the information collected should be combined with data from previous experience with the same manufacturing process to ensure production control appropriate to the quality requirements of the final product.

13.2.2 Record-keeping

Records shall be kept of the performance of all steps in the manufacture, quality control and distribution of large-pool blood products and related substances (7, 8)

These records shall:

- be original (not a transcription), indelible, legible and dated;
- be made at the time that the specific operations and tests are performed;
- identify the person recording the data as well as the person checking them or authorizing the continuation of processing;
- be detailed enough to allow all the relevant procedures performed to be clearly reconstructed and understood;
- permit the tracing of all successive steps and identify the relationships between dependent procedures, products and waste materials;
- be maintained in an orderly fashion that will permit the retrieval of data for a period consistent with shelf-lives and the legal requirements of the national control authority and, if necessary; allow a prompt and complete recall of any particular lot;
- show the lot numbers of the materials used for specified lots of products;

- Indicate that processing and testing were carried out in accordance with procedures established and approved by the designated responsible authority.

14. Control of albumin and plasma Protein fraction

Source materials should be processed in such a manner that the albumin in the solutions manufactured will be changed as little as possible and will not cause undesirable reactions in the recipients. Source materials may contain either vasoactive substances or substances capable of generating or releasing endogenous vasoactive substances. Such substances may also be formed in the course of fractionation, and consequently contaminate the albumin and plasma protein fraction. To guard against this possibility, adequate in-process controls and the testing before release for prekallikrein activator activity are mandatory for albumin solutions of purity less than 95% (such as plasma protein fraction) containing 35-50 g of protein per litre. Such testing is also recommended for highly purified albumin products (purity greater than 95%).

Within 24 h of the start of filling, albumin and plasma protein fraction in solution shall be heated in the final container to 60 ± 0.5 °C and maintained at that temperature for not less than 10 h but not more than 11 h by a method that ensures uniform heat distribution throughout the batch. Although pasteurization at the final bulk stage may be possible, this approach requires careful validation before use.

Special attention should be given to microbial contamination of source material and intermediates, since soluble microbial substances, especially endotoxins, may accumulate in the finished albumin solution. In addition, it is possible that small amounts of endotoxin, present even in products for which satisfactory results have been obtained in tests for pyrogens, may have a cumulative effect in recipients receiving large product volumes in relatively short periods of time, as, for example, in therapeutic plasma exchange.

The in-process controls should be capable of detecting contamination with bacteria and moulds. In addition, care should be taken to ensure, by a method that shall be validated, that all equipment and reagents used in the manufacturing process are scrupulously clean and free from toxic materials.

14.1 *Stability of albumin solutions*

The stability of solutions of albumin and plasma protein fraction (that have been heated for 10-11 h at 60 °C) shall be tested by heating adequate samples at 57 °C for 50 h. The test solutions shall remain visually unchanged when compared to control samples that have been heated for only 10—11 h at 60°C.

The thermal stability of albumin solutions shall be taken into consideration by the national control authority in determining the expiry dates.

The physicochemical quality of stored albumin solutions, as measured by the formation of dimers and particularly polymers, is influenced by:

- the quality of the starting plasma
- the quality of the fractionation, particularly with respect to the degree of purity achieved and the number of reprecipitation and reheating procedures involved; and
- the storage conditions with respect not only to temperature and time but also to the physical state and concentration of the solutions.

With regard to the thermal stability of albumin solutions, the following general statements may be made:

- The addition of stabilizing chemicals is necessary. Commonly used products are sodium octanoate and sodium acetyltryptophanate.
- Albumin prepared From aged liquid or dried plasma is less stable than albumin made from fresh-frozen plasma
- Reprocessing steps, such as reprecipitation and reheating, may reduce the stability of albumin solutions.
- On long-term storage, albumin solutions are more stable at 5 ± 3 °C than at 32—35°C. Long-term storage above 30°C should be avoided.

14.2 Control of bulk material

14.2.1 Tests on bulk material

Tests on the bulk powder or solution shall be made if the manufacturer sends the material to another institution for further processing. Samples for these tests shall be taken under conditions that do not impair the quality of the bulk material. Tests shall be carried out on a specially

dissolved sample processed to a stage equivalent to the final product, after sterilization by filtration. The tests shall be those listed in sections 14.3.2 to 14.3.7 inclusive.

14.2.2 Storage

The bulk material shall be stored as liquid or powder in sealed containers under conditions that minimize denaturation and the multiplication of microbial agents.

14.3 *Control of the final bulk solution*

14.3.1 *Preparation*

The final bulk solution shall be prepared from bulk powder or by the dilution of concentrates by a method approved by the national control authority. It shall meet all of the requirements of sections 14.3.2 to 14.3.7 inclusive.

14.3.2 *Concentration and purity*

The albumin concentration in final bulk albumin solutions shall be between 35 and 265 g/l. Not less than 95% of the proteins present shall be albumin, as determined by a suitable electrophoretic method after the sample has been heated for 10 h at 60 °C.

The protein concentration in final bulk solutions of plasma protein fraction shall be at least 35 g/l. Plasma protein fraction shall contain at least 83% albumin and not more than 17% globulins. Not more than 1% of the protein in plasma protein fraction shall be γ -globulin.

14.3.3 *Hydrogen ion concentration*

The final bulk solution, diluted with 0.15 mol/l sodium chloride to give a protein concentration of 10 g/l, shall, when measured at a temperature of 20-27 °C, have a pH of 6.9 ± 0.5 (albumin) or 7.0 ± 0.3 (plasma protein fraction).

In some countries, different ranges of pH values and temperatures are permitted.

14.3.4 *Sterility and safety*

The final bulk shall be sterile. If required by the national control authority, it shall be tested for sterility; samples shall be taken for such testing in a manner that does not compromise the sterility of the bulk material.

14.3.5 Sodium content

The final bulk solutions of albumin and plasma protein fraction shall have a maximum sodium concentration of 160 mmol/l.

14.3.6 Potassium content

The final bulk solutions of albumin and plasma protein fraction shall have a maximum potassium concentration of 2.0 mol/l.

14.3.7 Aluminium content

The final bulk solutions or albumin and plasma protein fraction shall have a maximum aluminium concentration of 7.5 µmol/l (200 µg/l).

14.4 *filling and containers*

The requirements concerning filling and containers given in Good Manufacturing Practices for Biological Products Guideline, shall apply

Special attention shall be paid to the requirement that solutions of albumin and plasma protein fraction in the closed final containers shall be heated to inactivate any infectious agents that may be present (see section 14, paragraph 2). In order to prevent protein denaturation, a stabilizer shall be added to albumin solution before heating (see section 13.1.1).

14.5 *Control tests on the final product*

The tests specified below shall be performed on representative samples from every filling lot. If the product is processed further after filling, e.g. by freeze-drying, the tests shall be performed on samples from each drying chamber.

14.5.1 Identity test

An identity test shall be performed on at least one labelled container from each filling lot to verify that the preparation is of human origin. The test shall be one approved by the national control authority. Additional tests shall be made to determine that the protein is predominantly albumin or plasma protein fraction as appropriate. The tests mentioned in section 14.3.2 shall be used.

14.5.2 *Protein concentration and purity*

The protein concentration and purity of each filling lot shall be within the limits prescribed in section 14.3.2.

Tests to determine the concentration of additives (such as polyethylene glycol, porcine enzymes and reducing and alkylating agents) used during production shall be carried out if required by the national control authority.

14.5.3 *Sterility test*

Each Filling lot shall be tested for sterility. Part A, section 5, of the revised Requirements for Biological Substances No.6 (General Requirements for the Sterility of Biological Substances) (9) shall apply. Samples for sterility testing shall be taken from final containers selected at random after heating at 60 °C for 10-11 h.

In one country the sterility test is carried out at least 10 days after heating at 60°C for 10 h. In some countries, the sterility test is carried out both before and after heating at 60°C for 10 h.

14.5.4 *General safety test*

General safety test shall be required, whereby each filling lot is tested for extraneous toxic contaminants by appropriate tests involving injection into mice and guinea-pigs. The injection shall cause neither significant untoward reactions nor death within an observation period of seven days. The tests shall be approved by the national control authority

14.5.5 *Freedom from pyrogenicity*

Each filling lot shall be tested for pyrogenicity by the intravenous injection of the test dose into three or more rabbits that have not previously received blood products. In general, the dose shall be at least equivalent proportionally, on a rabbit body-weight basis, to the maximum single

human dose recommended, but not more than 10 mL/kg of body weight. For albumin at concentrations of 200 mL and 250 g/L, the test dose for each rabbit shall be at least 3 mL/kg of body weight, and for albumin at concentrations of 35 g/L and 50 g/L and plasma protein fraction, 10 mL/kg of body weight.

A filling lot shall pass the test if it satisfies the requirements specified by the national control authority.

14.5.6 *Moisture content*

The residual moisture content shall, where appropriate, be determined by a method approved by the national control authority.

The acceptable moisture content shall be determined by the national control authority.

14.5.7 *Prekallikrein activator*

An assay shall be performed for prekallikrein activator. The product shall contain not more than 35 IU of prekallikrein activator per mL.

14.5.8 *Hydrogen ion concentration*

The final product, reconstituted if necessary and diluted with 0.15 mol/L sodium chloride to give a protein concentration of 10 g/L, shall, when measured at a temperature of 20-27 °C, have a pH of 6.9 ± 0.5 (albumin) or 7.0 ± 0.3 (plasma protein fraction).

In some countries, different ranges of pH values are permitted.

14.5.9 *Absorbance*

A sample taken from the final solutions of albumin and plasma protein fraction, when diluted with water to a concentration of 10 g/L of protein and placed in a cell with a 1-cm light path, shall have an absorbance not exceeding 0.25 when measured in a spectrophotometer set at 403 nm.

14.5.10 *inspection of filled containers*

All final containers shall be inspected for abnormalities, such as non-uniform colour, turbidity, microbial contamination and the presence of atypical particles, after storage at 20-35 °C for at least 14 days following heat treatment at 60 °C for 10 h. Containers showing abnormalities shall not be distributed.

The normal colour of albumin solutions may range from colourless to yellow or green to brown.

When turbidity or non-uniform colour raises the possibility of microbial contamination, testing should be done to isolate and identify the microorganisms.

14.6. Records

The requirements of Good Manufacturing Practices for pharmaceutical Products shall apply.

14.7. Samples

The requirements of Good Manufacturing Practices for pharmaceutical Products shall apply.

14.8 Labelling

The requirements of Good Manufacturing Practices for pharmaceutical Products and the national control requirements for parenteral solutions shall apply.

In addition, the label on the container should state:

- the type of source material,
- the protein concentration,
- the oncotic equivalent in terms of plasma, that preservatives are absent
- the warning “Do not use if turbid”
- The sodium and potassium concentrators.

14.9 Distribution and shipping

The requirements of Good Manufacturing Practices for pharmaceutical Products shall apply.

14.10 Storage and shelf-life

The requirements of Good Manufacturing Practices for pharmaceutical Products shall apply.

Final containers of albumin solution shall have a maximum shelf-life of three years if they are stored at or below 30 °C, and of five years if they are stored at 5 ± 3 °C.

Final containers of plasma protein fraction solution shall have a maximum shelf-life of three years if they are stored at or below 30 °C, and of five years if they are stored at 5 ± 3 °C.

14.11. Testing for viral Marker

All final products shall be tested for viral marker as HCV, HBsAg, and HIV1 & 2 by screening and confirmatory ELISA and PCR assay and final products shall be free from these pathogens.

15. Control of Immunoglobulin

The final bulk solution of normal immunoglobulin shall be made from material from at least 1000 donors. If normal immunoglobulin is to be used for preventing or treating a particular infection, the titer of specific antibody should be measured.

For normal immunoglobulin, a large number of donors are needed if the final product is to contain adequate amounts of the various desired antibodies.

For specific immunoglobulin, whether intended for intravenous or intramuscular injection, the number of donors represented is less important because the requirement for specific antibody in the final product will be defined.

The immunoglobulin concentration in the final bulk of normal and specific immunoglobulin preparations for intramuscular use shall be 100-180 g/l. Concentrations lower than 100 g/l shall require the approval of the national control authority.

The immunoglobulin concentration in the final bulk of intravenous immunoglobulin shall be at least 30 g/l. If, in a specific immunoglobulin preparation, the concentration is lower than 30 g/l, it shall require the approval of the national control authority.

The immunoglobulin preparation shall be composed of not less than 90% of immunoglobulin, as determined by a method approved by the national control authority.

Tests shall be conducted on each filling lot of immunoglobulin solution to determine the proportion of aggregated and fragmented immunoglobulin. The recommended distribution shall be that at least 90% of the protein, other than proteins added as stabilizers to intravenous immunoglobulins, shall have the molecular size of immunoglobulin monomer and dimer. Not more than 10% shall consist of split products together with aggregates (oligomers of relative molecular mass equal to or greater than that of immunoglobulin trimer). This requirement shall not apply to products deliberately fragmented

For intravenous immunoglobulin, the following tests shall be performed on a sample from each filling lot:

- A test for hypotensive activity.
- A test for anticomplement activity.
- A test for haemagglutinins by the antiglobulin (Coombs) technique.

15.1 *Potency of normal Immunoglobulins*

A 160 g/l solution of normal immunoglobulin shall be prepared from final bulk solution by a method that has been shown to be capable of concentrating, by a factor of 10 from source material, at least two different antibodies, one viral and one bacterial, for which an international standard or reference preparation is available (16) (e.g. antibodies against poliomyelitis virus, measles virus, streptolysin O, diphtheria toxin, tetanus toxin, staphylococcal a-toxin).

For immunoglobulin formulated at an immunoglobulin concentration lower than 16%, the concentrating factor for antibodies from source material may be proportionally lower.

The immunoglobulin solution shall be tested for potency at the concentration at which it will be present in the final container.

15.2 *Potency of specific immunoglobulin*

The potency of each final lot of specific immunoglobulin shall be tested with respect to the particular antibody that the preparation has been specified to contain. For intramuscular immunoglobulin, the following levels shall apply:

- For tetanus immunoglobulin, at least 100 IU/ml of tetanus antitoxin, as determined by a neutralization protection test in animals or by a method shown to be equivalent.
- For rabies immunoglobulin, at least 100 IU/mL of anti-rabies antibody, as determined by an appropriate neutralization test in animals or by a method shown to be equivalent.
- For hepatitis B immunoglobulin, at least 100 IU/mL of anti-hepatitis antibody.
- For varicella zoster immunoglobulin, at least 100 IU/ml of antivariella zoster antibody, as measured by a comparative enzyme- linked immunosorbent assay or by a method shown to be equivalent.
- For anti-D (anti-Rh0) immunoglobulin, the estimated potency shall be expressed in International Units and shall be not less than 90% and not more than 120% of the stated potency, and the fiducial limits of error shall be within 80% and 125% of the estimated potency.

15.3 *Sterility and safety*

Each filling lot shall be tested for sterility. (General Requirements for the Sterility of Biological Substances) (9) Shall apply.

General safety test shall be required, whereby each filling lot is tested for extraneous toxic contaminants by appropriate tests involving injection into mice and guinea-pigs. The injection shall cause neither significant toxic reactions nor death within an observation period of seven days.

15.4 *Identity test*

An identity test shall be performed on at least one labelled container from each filling lot to verify that the preparation is of human origin. The test shall be one approved by the national control authority.

Additional tests shall be made to determine that the protein is predominantly immunoglobulin.

15.5 *Freedom from pyrogenicity*

Each filling lot shall be tested for pyrogenicity by the intravenous injection of the test dose into three or more rabbits that have not previously received blood products. In general the dose shall be at least equivalent proportionally, on a rabbit body-weight basis, to the maximum single human dose recommended, but not more than 10 mL/kg of body weight.

The recommended test doses are 1 mL/kg and 10 mL/kg of body weight for intramuscular and intravenous preparations, respectively.

A filling lot shall pass the test if it satisfies the requirements specified by the national control authority.

15.6 *Moisture content*

The residual moisture content of a sample from each filling lot shall, where appropriate, be determined by a method approved by the national control authority.

The acceptable moisture content shall be determined by the national control authority.

15.7 *Hydrogen ion concentration*

The final product, reconstituted if necessary and diluted with 0.15 mol/l sodium chloride to give a protein concentration of 10 g/l, should, when measured at a temperature of 20—27 °C, have a pH of 6.9 ± 0.5 .

15.8 *Stability*

For immunoglobulin solutions, a stability test shall be performed on each filling lot by heating an adequate sample at 37 °C for four weeks. No gelation or flocculation shall occur.

15.9 *Records*

The requirements of Good Manufacturing Practices for Biological Products shall apply.

15.10 *Samples*

The requirements of Good Manufacturing Practices for Biological Products shall apply.

15.11 Labelling

The requirements of Good Manufacturing Practices for Biological Products shall apply.

In addition, the label on the container shall state:

- The type of source material;
- The protein concentration;
- The concentration of preservative, if any;
- “For intramuscular use only” (if the immunoglobulin are not specially prepared for intravenous use);
- “For intravenous use”, when appropriate;
- For specific immunoglobulin, the content of specific antibody expressed in International Units or equivalent national units;
- For freeze-dried preparations, the name and volume of reconstituting liquid to be added.

The label on the package or the package insert shall show:

- the approximate concentration of electrolytes and excipients and, for intravenous preparations, the approximate osmolality;
- the buffering capacity when the pH of the diluted product is lower than that specified in section 15.7;
- the concentration of preservative, if any;
- the recommended dose for each particular disease or condition;
- the warning “Do not use if turbid”;
- The sodium and potassium concentrations (if the immunoglobulin is intended for intravenous use).

15.12 Distribution and shipping

The requirements of Good Manufacturing Practices for Biological Products shall apply.

15.13 *Storage and shelf-life*

The requirements of Good Manufacturing Practices for Biological Products shall apply.

Liquid immunoglobulin shall be stored at $5 \pm 30^{\circ}\text{C}$ and shall have a shelf-life of not more than three years. Freeze-dried preparations shall be stored below 25°C and shall have a shelf-life of not more than five years.

15.14. *Testing for viral Marker*

All final products shall be tested for viral marker as HCV, HBsAg, and HIV1 & 2 by screening and confirmatory ELISA and PCR assay and final products shall be free from these pathogens.

16. Control of preparations of coagulation-factor concentrates (factor VIII, factor IX and fibrinogen)

Factor VIII preparations are available as both frozen products and freeze-dried concentrates. The frozen products are usually derived from a single donation and consists of the cryoprecipitated factor VIII from the donor concerned prepared in a closed separation system. The control of this product and the freeze-dried product from fewer than 10 plasma donations is covered in Part B. section 7.8.1.

Generally, the small-pool product undergoes little or no purification and is handled and subdivided in such a way that many control tests are inappropriate. However, freeze-dried factor VIII concentrates prepared from more than 10 donations may be purified.

Source material for factor VIII preparations shall meet the general criteria for donor selection and testing for disease markers as specified in Parts A and B. It shall preferably be plasma frozen within 8 h of collection or frozen cryoprecipitate. Such material shall be kept frozen at such a temperature that the activity of the factor VIII is maintained.

16.1 *Tests on final containers*

16.1.1 *Sterility and safety*

Each filling lot shall be tested for sterility. (General Requirements for the Sterility of Biological Substances) (9) shall apply.

General safety test shall be required, whereby each filling lot is tested for extraneous toxic contaminants by appropriate tests involving injection into mice and Guinea pigs. The injection shall cause neither significant toxic reactions nor death within an observation period of seven days. The tests shall be approved by the national control authority.

16.1.2 *Freedom from pyrogenicity*

Each filling lot shall be tested for pyrogenicity by the intravenous injection of the test dose into three or more rabbits that have not previously received blood products. In general, the dose shall be at least equivalent proportionally, on a rabbit body-weight basis, to the maximum single human dose recommended, but not more than 10 ml/kg of body weight.

The following test doses are suggested: factor VIII, 10 IU/kg of body weight; factor IX, 50 IU/kg of body weight; and fibrinogen, 30 mg/kg of body weight.

16.1.3 *Solubility and clarity*

Factor VIII preparations shall dissolve in the solvent recommended by the manufacturer within 30 min when held at a temperature not exceeding 37 °C. Factor IX preparations shall dissolve in the solvent recommended by the manufacturer within 15 min when held at 20-25 °C. The solutions, when kept at room temperature, shall not show any sign of precipitation or gel formation within 3 h of dissolution of the coagulation factors.

16.1.4 *Protein content*

The amount of protein in a final container shall be determined by a method approved by the national control authority.

16.1.5 *Additives*

Tests to determine the concentration of additives (such as heparin, polyethylene glycol, sodium citrate and glycine) used during production shall be carried out if required by the national control authority.

16.1.6 *Moisture content*

The residual moisture content shall be determined by a method approved by the national control authority. The acceptable moisture content shall be determined by the national control authority.

16.1.7 *Hydrogen ion concentration*

When the product is dissolved in a volume of water equal to the volume stated on the label, the pH of the resulting solution shall be 7.2 ± 0.4 .

16.2 *Test applicable to factor VIII concentrates*

Each filling lot shall be assayed for factor VIII activity by a test approved by the national control authority, using a standard calibrated against the International Standard for Blood Coagulation Factor VIII: Concentrate.

The specific activity shall be at least 500 IU/g of protein. The estimated potency shall be not less than 80% and not more than 125% of the stated potency. The confidence limits of error shall be not less than 64% and not more than 156% of the estimated potency.

16.3 *Tests applicable to factor IX concentrate*

16.3.1 *Potency*

Each filling lot shall be assayed for factor IX activity by a test approved by the national control authority using a standard calibrated against the International Standard for Human Blood Coagulation Factors II, IX, and X in Concentrates.

Other coagulation factors may also be present in the final product, depending on the method of production, and products shall be assayed for all coagulation factors claimed to be present at a therapeutic level, including factors II, VII and X. The assay methods used for these factors shall be approved by the national control authority.

16.3.2 *Presence at activated coagulation factors*

A test for the presence of activated coagulation factors shall be carried out by a method approved by the national control authority.

16.3.3 *Alloantibodies*

A test shall be made for the presence of all antibodies A and B by a method approved by the national control authority.

It's not possible to or specific about the tests for all antibodies or to specify an upper limit for the titre.

16.4 *Test applicable to fibrinogen*

Each filling lot shall be assayed for clottable protein by a test approved by the national control authority.

Not less than 70% of the total protein should be clottable by thrombin.

16.5 *Identity test*

An identity test shall be performed on at least one labelled container from each filling lot of coagulation-factor concentrate to verify that the preparation is of human origin. The test shall be one approved by the national control authority.

For albumin and plasma protein fraction, additional tests shall be made to determine that the protein is predominantly albumin.

16.6 *Records*

The requirements of Good Manufacturing Practices for Biological Products shall apply

16.7 *Samples*

The requirements of Good Manufacturing Practices for Biological Products shall apply.

16.8 *Labelling*

The requirements of Good Manufacturing Practices for Biological Products shall apply.

In addition, the label on the container shall state:

- the content of the coagulation factor expressed in International Units, where they exist;
- the amount of protein in the container;
- the volume of diluents needed for reconstitution;
- a reference to a package insert giving instructions for use, warnings about the possible transmission of infectious agents and precautions.

16.9 *Distribution and shipping*

The requirements of Good Manufacturing Practices for Biological Products (8) shall apply.

16.10 *Storage and shelf-life*

The requirements of Good Manufacturing Practices for Biological Products (8) shall apply.

Final containers of freeze-dried preparations of factor VIII and factor IX shall have a maximum shelf-life of two years if they are stored at 5 ± 3 °C. Final containers of fibrinogen shall have a maximum shelf-life of five years if they are stored at ± 30 °C.

Other storage conditions and shelf-lives may be approved by the national control authority provided that they are consistent with the data on the stability of the products.

Part D. National control requirements

17. General

The general requirements for control laboratories in the Guidelines for National Authorities on Quality Assurance for Biological Products (6) shall apply.

The national control authority shall provide the standards and reference preparations necessary for the quality control of human blood and blood products. Where appropriate, these standards should be calibrated against the relevant International Standard.

The national control authority shall have authority to approve the production and control methods used and settle all matters left for its decision or approval in Parts A, B and C.

The national control authority shall also have authority to approve the use of materials that carry potential risk and shall approve any new method of production and the preparation of any new product.

New products or products prepared by new production methods may be monitored to confirm their efficacy and safety.

18. Release and certification

Human blood and blood products shall be released only if they satisfy the requirements of Parts A, B and C, wherever applicable.

A certificate signed by the appropriate official of the national control authority shall be provided at the request of the manufacturing establishment and shall state whether the product in question meets all national requirements as well as Parts A, B and C (whichever is relevant) of the present Requirements. The certificate shall also state the date of the last satisfactory potency test performed by the manufacturer, if applicable, the number under which the lot is released, and the number appearing on the labels of the containers. In addition, a copy of the official national release document shall be attached.

The purpose of this certificate is to facilitate the exchange of human blood and blood products between countries.

4. References

1. Report of a WHO Working Group on the Standardization of Human Blood Products and Related Substances. In: *WHO Expert Committee on Biological Standardization. Twenty-eighth Report* Geneva, World Health Organization, 1977, Annex 1 (WHO Technical Report Series, No. 610).
2. Requirements for the Collection, Processing and Quality Control of Human Blood and Blood Products (Requirements for Biological Substances No. 27). In: *WHO Expert Committee on Biological Standardization. Twenty-ninth Report*. Geneva, World Health Organization, 1978, Annex 1 (WHO Technical Report Series, No. 626).
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Guidelines on Transmissible Spongiform Encephalopathies in Relation to Biological and Pharmaceutical Products

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1. Introduction

The appearance of a variant form of human Creutzfeldt-Jakob Disease (vCJD) in the mid- 1990s, as a result of the bovine spongiform encephalopathy (BSE) epidemic in the United Kingdom (UK), has increased the profile of transmissible spongiform encephalopathies (TSEs) as a risk to human health and has already affected public health policy worldwide. It is assumed that variant CJD (vCJD) results from the consumption of meat products from cattle infected with the BSE agent. Policies developed to reduce the risks resulting from the hazard of vCJD and its potential for human-to-human transmission are based on three main factors:

- a) An unknown number of individuals might be infected with the BSE agent;
- b) The pathological misfolded prion protein and infectivity (detected by bioassay) is present in some peripheral tissues of patients who died of vCJD;
- c) Blood of rodents, monkeys and sheep infected with TSE agents has transmitted disease experimentally.

New scientific information regarding the distribution of infectivity in various tissues from different species affected with a transmissible spongiform encephalopathy (TSE or prion disease) has emerged during the last three years. Some of these findings have challenged the current understanding of the tissue distribution of the pathological misfolded prion protein (PrP^{TSE}) and tissue infectivity. An example of these is the finding of PrP^{TSE} in sheep muscle. This is the first evidence of PrP^{TSE} in muscle from an animal species that entered the human food chain.

Also of concern has been the identification of three cases of probable transfusion-transmitted vCJD, suggesting that second transmission of vCJD from human to human has occurred. The demonstration of vCJD transmission via blood creates special concern for those countries that have no human TSE surveillance system in place. Blood donors subsequently diagnosed as suffering from vCJD have been identified in the UK, France, Ireland, Saudi Arabia and Spain. It is clear that the blood of donors incubating vCJD might contribute to an unrecognized spread of the disease, especially in countries where surveillance and reporting system are not established. This new concern about human-to-human transmission should not distract from efforts to estimate and reduce the well-established risk of food borne BSE agent leading to vCJD cases. Each country should evaluate the risk of vCJD from both sources.

The development of reliable diagnostic procedures to detect asymptomatic subjects during the long period of incubation of CJD and vCJD is of vital importance. However, test methods must be appropriately validated, and validation requires that appropriate blood reference materials be developed, characterized and made available both to qualified test developers and to regulatory authorities.

There is increasing concern about the possibility that vaccines, blood products and other pharmaceutical products—products that contain or were manufactured using bovine-derived or human-derived materials—might spread the agent of vCJD worldwide, even in countries where BSE has not yet been reported. There is, therefore, a need to ensure that regulatory authorities worldwide have reliable information to assess risk and evaluate product safety, so that steps can be taken to prevent the transmission of TSE to humans via biological and other pharmaceutical products. In order to update the preventive measures proposed by the World Health Organization (WHO) in 2003 to minimize the risks associated with the use of vaccines, blood products and other pharmaceutical products containing bovine-derived and human-derived materials, a meeting of international experts was convened at WHO in Geneva on 14-16 September 2005. The purpose was to review the latest available data on the epidemiology, antemortem and postmortem diagnosis, detection of the infectious agents, and distribution of infectivity in tissues or body fluids of relevant species with TSEs.

2. Review of Scientific Developments

Major scientific developments have occurred in the field of TSEs during the last 7 years. These include a better knowledge of the epidemiology of sporadic CJD (sCJD) and vCJD and an improved diagnostic criteria for sCJD and vCJD. Changes in the distribution and size of the BSE epidemic in Europe and elsewhere have been observed. The distribution of infectivity in tissues and body fluids in sCJD, vCJD, BSE and scrapie has been better established and the detection of the pathological misfolded prion protein (PrP^{TSE}) in different tissues has improved.

2.1 Epidemiology, clinical features and diagnostic criteria of CJD

CJD is a rare and fatal human neurodegenerative condition. Like other TSEs, CJD is experimentally transmissible to animals, and a characteristic spongiform change is seen on microscopic examination of the brain. Epidemiological studies indicate a worldwide occurrence

of sporadic disease of approximately 1-2 cases per million people per year. Globally, over 80% of cases of CJD occur as a sporadic disease (sCJD). Familial, iatrogenic, and variant forms of CJD show much lower and variable incidence in different countries. Most cases of vCJD have been found in the UK. The origin of sCJD remains unknown despite extensive study and, in particular, there is no evidence of a causal link with scrapie, a naturally occurring TSE of sheep and goats, or with BSE. Most sCJD cases occur in persons between the age of 60 and 80 years with an average age at death of about 67 years. Characteristically the patient with sCJD develops a rapidly progressive dementia associated with multifocal neurological signs, ataxia, and myoclonus. Although, in the correct clinical context, a characteristic EEG recording and/or the detection of 14-3-3 protein in the cerebrospinal fluid are considered diagnostic, confirmation of the diagnosis of CJD still relies on neuropathological examination. The clinical and histopathological features of sCJD are variable, and are influenced by a naturally occurring polymorphism at codon 129 of the gene encoding the prion protein (*PRNP* gene). A novel test based upon the detection of PrP^{TSE} in the nasal olfactory mucosa may improve the diagnosis of sCJD, but this test has not yet been evaluated in living patients.

Familial CJD, also experimentally transmissible, is expressed as an autosomal dominant trait associated with an abnormality of the *PRNP* gene. Gerstmann-Sträussler-Scheinker syndrome (GSS) and fatal familial insomnia (FFI) are similarly inherited transmissible neurodegenerative disorders linked to mutations in the *PRNP* gene, have also been transmitted to animals.

There have now been at least 362 recognized cases of iatrogenic CJD following use of following products: contaminated human pituitary-derived growth hormone (180 cases) and gonadotropin (4 cases), human dura mater grafts (168 cases), corneal transplants (at least 3 possible cases), neurosurgical instruments (4 or 5 cases) and a stereotactic cortical-probe EEG electrode (2 cases). Except for the three transfusion-transmitted vCJD infection described above, no new class of products causing iatrogenic CJD has been identified during the last 9 years, although the number of cases resulting from past exposure to known products continues to increase.

Since first reported in 1996, up to June 2006, there have been 161 cases of vCJD in the United Kingdom, 17 in France, four in Ireland, two in the USA and in the Netherlands and single cases in Canada, Italy, Japan, Portugal, Saudi Arabia and Spain. Cases of BSE and vCJD have been decreasing in the United Kingdom in recent years, but both diseases have appeared in other

countries and the notification rate for new cases of vCJD has increased in France during the past two years. The US, Canadian and two of the Irish patients had spent several years in the UK between 1980 and 1996, and were probably exposed to the BSE agent there, while the Italian, the Dutch cases and 16 of the 17 French cases had no history of significant travel outside their home countries. The median age at onset of vCJD is 26 years (range 12-74 years) with a median duration of illness of 14 months (range 6-39 months). Although a definite diagnosis of vCJD requires neuropathological examination, clinical and laboratory criteria have been established for the diagnosis of probable vCJD in living patients. All tested vCJD cases (191 worldwide) were homozygous for methionine at codon 129 of the *PRNP* gene. A distinctive feature of vCJD—in contrast to sCJD—is the frequent occurrence of PrP^{TSE} in lymphoid tissues (tonsil, spleen, lymph node, and appendix). In 3 patients who had undergone appendectomy before onset of vCJD, two had immunoreactive PrP^{TSE} in lymphoid follicles of the appendix at 8 months and 2 years prior to death. One appendix was negative for PrP^{TSE} 9 years prior to death. An anonymous survey of surgically removed tonsils and appendices in the UK revealed three out of 12,674 cases which stained positively for PrP^{TSE}. All three were appendices. Genetic studies on DNA extracted from two of the three positive appendices found that they were valine homozygous at codon 129 in the *PRNP* gene, unlike any of the clinical case of vCJD encountered so far. The causal link between vCJD and BSE is based on epidemiological, biochemical and transmission studies. The Joint WHO/FAO/OIE Technical Consultation on BSE (2001) reached a scientific consensus that BSE-contaminated food is the main avenue of exposure. Bovines, bovine products and by-products potentially carrying the BSE agent have been traded worldwide, giving this risk a global dimension. Epidemiological analysis does not indicate that medicinal products, blood and blood-derived products, or occupational exposure have been sources of infection in vCJD cases identified to date. Three cases of vCJD infection presumptively transmitted by transfusion of red blood cell concentrates are described above.

2.2 Bovine spongiform encephalopathy (BSE) and scrapie

BSE was first identified in British cattle in November 1986. Current evidence suggests that the disease originated from the use of feed supplements containing meat-and-bone meal (MBM) contaminated with a TSE agent (probably from scrapie-infected carcasses). By the end of 2005, over 184,370 confirmed cases of BSE had been reported in the UK. Smaller outbreaks have been reported in native-born cattle in most other Western and Central European countries and in Israel

and Japan (totaling 5,428 confirmed cases reported to the OIE as of December 2005). Most recent cases were in clinically unremarkable animals recognized at the abattoir following the introduction of a statutory test for PrP^{TSE} applied after 1999 by member States of the European Union (EU) and by Switzerland to the brainstems of healthy cattle older than 30 months and suspect cattle at increased risk of BSE (fallen stock and other animals older than 24 months sent for casualty slaughter). The increase in recognized cases presumably resulted from better detection of infected animals during the pre-clinical and early clinical stages of illness rather than a true “second wave” of BSE. In the UK the incidence of BSE has continued to decline rapidly since 1992, almost certainly in response to a statutory ruminant feed ban introduced in 1988. This is consistent with the hypothesis that cases arose by infection from contaminated feed. Although epidemics of BSE in other European countries have been recognized more recently than that of the UK, most are also in decline, and, so far, no single country except the UK has recognized more than 1500 cases.

BSE infectivity has been demonstrated in the brain, spinal cord and retina of naturally affected cattle and also in the dorsal root ganglia, trigeminal ganglia, distal ileum (during incubation) and bone marrow (during clinical illness only) of those infected experimentally by the oral route. A wide range of other tissues (including most lymphoreticular tissues) from cattle sick with BSE—both naturally and experimentally acquired—showed no detectable infectivity using the mouse bioassay; parallel bioassays in cattle so far support the conclusion that there is a limited distribution of BSE infectivity in bovine tissues. BSE has been experimentally transmitted via the oral route to sheep and goats, and there is recent evidence that one goat has been naturally infected. However, concern over the possibility has led to increased efforts at active and passive surveillance of scrapie in the EU, based on the observation that experimental BSE in small ruminants resembles scrapie. Recently, infectivity was found in blood of sheep with natural scrapie and in blood of sheep with experimental BSE during both clinical illness and pre-symptomatic periods.

2.3 Diagnosis

The accumulation of PrP^{TSE} occurs only in TSEs and therefore its detection serves as a surrogate for detection of infectivity in biological samples. After experimental inoculation of rodents with TSE agents, PrP^{TSE} is, like infectivity, usually detectable in the central nervous system (CNS) many weeks before the appearance of overt disease, and its level increases during clinical illness.

While the increase in PrP^{TSE} generally parallels that of infectivity, the precise relationship between PrP^{TSE} and infectivity is unclear. However, there are exceptions; under specific experimental conditions, the brain of TSE-affected rodents may be infectious (by bioassay) while PrP^{TSE} remains undetected. From the perspective of pre-clinical diagnosis, both the sensitivity of diagnostic methods and procedures to concentrate PrP^{TSE} are crucial, because the amount of PrP^{TSE} outside the CNS is likely to be extremely small, particularly in circulating blood. Concentration of PrP^{TSE} can be realized by chemicophysical precipitation, affinity chromatography or affinity precipitation techniques. Moreover, it has been reported that the amount of PrP^{TSE} in dilute solutions can be increased considerably by at least 10 to 20-fold by the “protein misfolding cyclic amplification” (PMCA) technique, potentially allowing improved detection of extremely small amounts of infected material. In recent reports, the test developer described an improved PMCA that detected PrP^{TSE} in the blood of most hamsters with scrapie and not in the blood of uninfected hamsters. In addition to blood, other readily accessible tissues might offer the possibility for diagnosis of clinical TSE. Tonsil biopsy has been used to diagnose vCJD in a minority of patients after the onset of clinical signs and symptoms. Also, a study that demonstrated PrP^{TSE} and infectivity in the skeletal muscle of mice experimentally infected with laboratory strains of TSE has been at least partially confirmed by the detection of PrP^{TSE} in skeletal muscles of small ruminants with TSEs and humans with both sporadic CJD and vCJD. These findings are under intense study by a number of laboratories. Among immunological methods for PrP^{TSE} detection, western blotting is the most thoroughly characterized and widely used method. It offers the advantage of recognizing different forms of PrP^{TSE} through the analysis of the molecular mass and the relative abundance of di-, mono and non-glycosylated bands. These parameters characterize the so-called PrP glycotype, a kind of “PrP signature” that varies among different forms of TSE. PrP^{TSE} glycotyping has been proposed for distinguishing various forms of TSE (e.g., scrapie from BSE and sCJD from vCJD) and for improving the classification of human TSEs.

The latest generation of immunoassays are claimed to detect PrP^{TSE} in samples containing less than 1 LD50 of BSE infectivity as measured by bioassay in transgenic mice expressing bovine PrP. Several ELISA and western blot methods are commercially available as ready-to-use kits, and have been validated in a study by the European Commission as screening tests for BSE in slaughtered cattle (so-called “rapid tests”). EC-approved immunoassays have also detected

PrP^{TSE} in the brains of BSE-infected cattle at least 3 months before onset of clinical illness. However, no immunological method has yet proven sufficiently sensitive to detect PrP^{TSE} in the blood of infected animals or humans.

2.4 Risk of transmitting Creutzfeldt-Jakob disease (CJD) and variant CJD (vCJD) by human blood and blood products:

Since the last WHO Consultation on this issue in 2003, new evidence relevant to risk assessments for the transmission of vCJD by human blood has accrued. Salient information is summarized here:

- a) It has been known for more than 20 years that the blood of rodents experimentally infected with agents of several TSEs contains infectivity. Most recently, infectivity has been found in the blood of mice experimentally infected with the agent of vCJD.
- b) There is convincing evidence that both scrapie and BSE can be transmitted from sheep to sheep by blood transfusions with either whole blood or buffy coat. Transfusions of blood from animals in the incubation period and clinical phase of illness have transmitted disease. Transfusion appears to be a relatively efficient mechanism for transmitting infection from sheep to sheep.
- c) Epidemiological evidence, reviewed above, indicates that vCJD infection has been transmitted to three recipients of blood transfusion. These three infected recipients demonstrate that blood contained infectivity during the latter part of the incubation period of vCJD, from 18 months to 3.5 years before the donors showed signs of neurological illness. The finding of three transfusion-transmitted vCJD cases among a relatively small number of persons transfused with blood components from vCJD donors, only about 18 of whom survived for more than five years, suggests that the transfusion of a human blood component has transmitted vCJD efficiently, an observation consistent with experimental animal studies. The first recipient developed vCJD 6.5 years after transfusion—considerably shorter than the probable minimum incubation periods of presumed food-borne cases of vCJD. The second recipient died without signs of neurological disease five years after transfusion but already had detectable PrP^{TSE} in spleen and lymph nodes, though not in appendix, tonsil, or brain. The third patient, who developed clinically typical probable vCJD almost eight years after

transfusion of red cells from a different donor, was still alive at the time of the Consultation in 2005 but has subsequently died.

- d) To date, all cases of vCJD tested have been in persons homozygous for methionine at codon 129 of the prion-protein-encoding gene (PRNP gene). However, the transfusion recipient with pre-clinical or sub-clinical vCJD infection was heterozygous, having methionine and valine at PRNP codon 129, indicating that vCJD infection can occur in persons of this genotype, as can other forms of CJD. Furthermore, a study of anonymous tonsil and appendix specimens in the UK identified three instances in which appendix samples stained positively for PrP^{TSE} using immunohistochemical techniques—although the staining pattern was described as being slightly different from that in lymphoid tissues of known vCJD cases. Genetic studies on DNA extracted from two of these three appendices found them to be valine homozygous at codon 129 in the PRNP gene; no DNA was extractable from the other specimen. Taken together, these findings suggest that, if exposed to a sufficient dose, most people are probably susceptible to infection with the BSE agent. In the UK, approximately 30% of Caucasian populations are homozygous for methionine at codon 129 of the PRNP gene, about 50% are heterozygous for methionine/valine, and the rest are homozygous for valine. The PRNP codon-129-valine allele is rarely found in East Asian populations.
- e) The same tonsil-appendix survey results also suggest that a substantial number of individuals in the UK might be incubating vCJD—a mathematical analysis predicting that as many as 5,000 individuals in the total UK population (a rate of 237/million) might be infected. Some proportion of healthy individuals with sub-clinical or pre-clinical vCJD would presumably be blood donors. The possible prevalence of asymptomatic vCJD infections in other countries is not known.
- f) A probabilistic risk assessment model concluded that transmissions of infection by blood transfusion had a potential to increase the eventual size and duration of the current vCJD outbreak in the UK significantly. Deferral of transfusion recipients as blood donors was implemented in the UK in 2005; this step is anticipated to reduce substantially the risk of recycling vCJD infections. The same measure has been in place in France since 1998 and more recently in several other European countries such as Ireland, the Netherlands and

Switzerland. In some other countries, like Canada, Australia, Italy and the US, blood donors previously transfused in the UK have been deferred.

- g) A risk assessment estimated that some derivatives prepared from pools of UK plasma were likely to have included one or more donations from persons incubating vCJD. That poses a small and uncertain risk of transmitting infection to some recipients of the products. The relative risk depends on the type of plasma product, the specific manufacturing process used and the year of production. The UK has no longer fractionated plasma of UK residents since 1999 and has imported all plasma for manufacture—most from the USA and some from Germany. Assessments suggested that vCJD risk from derivatives of plasma collected and manufactured in other countries was low or negligible.
- h) A few vCJD cases in the UK were in people previously transfused with blood components from donors not diagnosed with vCJD, and a risk assessment concluded that some of these recipients might conceivably have been infected through the transfusion. For two cases, the time between blood transfusion and onset of vCJD was so short that transfusion transmission seems highly improbable. Authorities have not concluded that any of the donors involved is sub-clinically infected with vCJD agent. Nevertheless, the implicated donors were informed that, as a precaution, they should no longer donate blood.

In conclusion, it is probable that vCJD has been transmitted through blood transfusion, with important implications for public health. Several vCJD cases have occurred outside the UK in persons who previously donated blood (in France, Ireland, Saudi Arabia and Spain). Authorities in those countries are aware of the potential risk. To date there is no evidence that vCJD has been transmitted by human plasma derivatives, in spite of intensive use of some products manufactured from plasma of UK donors during and after peak years of the UK BSE outbreak. But the incubation periods of TSEs can be very long, and possible transmission of vCJD by plasma derivatives, while not recognized to date, cannot be confidently excluded yet. Cumulative epidemiological evidence, including follow-up studies in Europe and USA of more than a hundred long-term survivors transfused with blood components from donors who later developed other forms of CJD, suggests that the infectious agents associated with sporadic, familial and iatrogenic CJD have probably not been transmitted through blood or blood products, at least not with a frequency detectable by epidemiological surveys.

3. Recommendations of the Consultation

3.1 Tissue infectivity

The foundation of any attempt to construct a rational analysis of TSE risk from biological and pharmaceutical products must begin with an evaluation of infectivity in the human or animal tissues from which these products are derived. Although straightforward in principle, the task is complicated by differences in the timing of first appearance and final tissue distribution of infectivity in different species and diseases, by differences in the sensitivity of bioassay methods, and by incomplete data about infectivity levels in various tissues of interest. Tables IA, IB and IC in the Annex summarize current data about the distribution of infectivity and PrP^{TSE} in humans with vCJD and other human forms of TSE, in cattle with BSE, and in sheep and goats with scrapie. In general, it can be said that, paradoxically, whereas infectivity (PrP^{TSE}) in cattle with BSE has a more limited tissue distribution than in any other animal or human form of TSE, infectivity (PrP^{TSE}) in vCJD has a wider distribution than in other forms of human CJD. In using the tables, it is important to note that two classes of material were intentionally excluded: (1) materials like bile of ruminants and humans that have never been studied, and (2) highly processed chemically pure reagents like tallow derivatives and bovine bone gelatin produced by the alkaline process that have been studied and found to pose a negligible risk if any for transmitting infection.

Several new methods attempting to detect PrP^{TSE} using novel techniques, if successfully developed, might eventually offer sufficient sensitivity to demonstrate amounts of agent below the level of detection of currently validated tests. It has been speculated that such methods might find small amounts of agent in some tissues currently thought to be free of infectivity. It remains unknown whether tissues containing such very small amounts of infectious material would transmit infection to humans.

3.2 Measures to minimize risks to humans from biological and pharmaceutical products in which bovine, ovine or caprine materials are used during manufacture

On the basis of current scientific knowledge about the agents causing BSE and other animal TSEs, the group stressed that the ideal situation would be to avoid the use of bovine materials in the manufacture of any biological or pharmaceutical product, as well as the use of materials from other animal species in which TSEs naturally occur. In practice, this is not always feasible, in which case a risk assessment should be performed. The risk assessment should take into account:

- a) The source of starting materials used as active substances, excipients or reagents and their potential infectivity;
- b) The possibility of cross contamination where starting materials are collected and processed; and
- c) The production processes for seed and other materials. The TSE risk assessment contributes to the overall risk-benefit analysis of biological and other pharmaceutical products.

3.2.1 Source of starting materials

Careful selection of the source of ruminant starting materials to be used to manufacture active substances, excipients and reagents is an important criterion in the TSE risk assessment. It was agreed that the most satisfactory source of materials is from countries where the risk of BSE in cattle is absent or low. Countries are encouraged to assess geographic BSE risk, and the Office International des Epizooties (OIE)—the world organization for animal health (oie@oie.int)—[offers](#) guidance in that process. The Geographical BSE Risk (GBR) categorization (risk of BSE for cattle in countries submitting requests) has been expanded and updated continuously by the Scientific Steering Committee (European Commission, Health and Consumer Protection Directorate General): http://europa.eu.int/comm/food/fs/sc/ssc/outcome_en.html; that important activity will be continued by its successor, the European Food Safety Authority (EFSA).

The use of starting materials from countries with a high risk of BSE is usually not acceptable. However, even in those countries, the collection of starting materials for the manufacture of specific products from well-monitored herds may sometimes be allowed. This may be done after evidence is provided that the herds have had no cases of BSE, have an active surveillance programme, have never been fed mammalian-derived protein (other than milk), have a fully documented breeding history, and have introduced new genetic material only from herds with the same BSE-free status.

The age of an animal from which tissues or fluids are collected as starting materials may also be taken into account, since data are now available on the infectivity of tissues collected during the incubation period of experimental BSE. In general, tissues of younger animals contain less infectivity than those of older animals.

3.2.2 Tissue removal and processing

It is recognized that potential TSE risks might be influenced by circumstances under which tissues are removed. For example, slaughter of infected animals by penetrative brain stunning or sawing the skull or spinal column increase the risk that tissues containing little or no infectivity will be contaminated with higher-risk tissues. Body fluids should be collected with minimal damage to tissue, and cellular components should be removed. Fetal blood should be collected without contamination from other maternal or fetal tissues including placenta, amniotic fluid and allantoic fluid. Whenever possible, single-use instruments should be used for collection of tissues and fluids. When potential cross contamination of a source tissue with a tissue of higher-risk cannot be reasonably excluded, a higher risk of infection must be assumed for purposes of risk evaluation.

Facilities that provide starting materials for medicinal products should have an appropriate quality system to document the process used and provide a record for each batch of starting material collected. They should either have, or work towards, official accreditation of the quality system. Procedures should also be in place to reduce the risks of adulteration of batches.

The sources and types of starting materials, while important, are not necessarily the only determinant of risk of potential TSE transmission. Some manufacturing processes—for example those used to produce bovine serum albumin and tallow derivatives—have substantial ability to eliminate infectivity that might be present in the starting material. Processes that inactivate infectivity or remove infectivity from starting materials augment the safety provided by sourcing. Manufacturers should consider including such procedures in their manufacturing processes for starting materials. Claims that a production process contributes significantly to the safety of a product should be validated.

3.2.3 Production systems

3.2.3.1 Vaccines

The consistent production of safe and effective vaccines poses special problems, because many of them are prepared from organisms that cannot be treated with harsh methods of extraction or purification without reducing or destroying their antigenicity. Additional difficulties are inherent in the cell bank and seed lot systems employed to produce many vaccines.

Production systems impact the TSE risk assessment. Concerns with respect to TSE may arise from an animal used for *in vivo* production or as a source of cells for production *in vitro*, from components of any medium used in production, or from the cell banks or seeds used to initiate production. Where vaccines are grown in animals, careful selection of source materials and, in some cases, postmortem testing of each production animal can greatly reduce the TSE risk. Some vaccines are produced in primary cell cultures, usually derived from animals not known to have TSEs; such cultures are very unlikely to be infected or contaminated with TSE agents. However, any culture medium used in the growth of bacterial, yeast, mammalian or other cells *in vitro* may contain components of animal origin. Such media should be evaluated for TSE risk.

The most complex TSE issues are raised by banked eukaryotic or bacterial cells and viral vaccine seeds. The group strongly emphasized that, by virtue of the level of characterization possible, the overall risk-benefit assessment overwhelmingly favors the use of banked cells and the seed lot system for viral vaccine production. However the TSE risk assessments of banked cells and viral vaccine seed stocks should take into account the possible carryover of any potentially infectious material from the seed into the final product as a contaminant. There is also a theoretical possibility that production cells might be infected with a TSE agent, although none of the very few cell lines that are known to support replication of a TSE agent has been used to produce any vaccine.

When evaluating the possibility of potentially infectious material in a seed, not all relevant information may be available; since seeds often have a lifetime of decades, tracing their origin and full production history may be impossible. Where information is incomplete, it is recommended that working seeds be replaced as a precautionary measure, taking into account the need to maintain adequate supplies of vaccines with public health benefits during the replacement. For existing products, master seed materials and original experimental preparations from which master seeds are derived (“pre-master” seeds) may not need replacement, since the biological phenotype of the vaccine depends on these materials, and they may be difficult or impossible to recreate. However, the goal of eventually replacing all seeds with those of impeccable provenance for all reagents should not be abandoned. The origin of newly developed products should be documented as completely as possible, recognizing that this may also be difficult. For new products made using old seed lots, any existing risk assessment for the seed and history of prior use of the seed should be taken into account.

Initiation of production of seed materials for new vaccines (either new cell banks or viral or bacterial seeds) should also take into account all guidance on TSE risk in force at the time that laboratory work begins. However, it is recognized that research and development for new vaccines often takes years; complete information on TSE risk for older seed materials may not meet requirements in force later, when a candidate new product must be assessed for final licensure. The principles outlined above, in the paragraph on seed materials, should be applied in such cases.

3.2.3.2 Recombinant DNA products

Medicines produced by recombinant DNA technology use a cell banking system similar to that used for many vaccines. Similar considerations with respect to production media, carryover of contaminants and the theoretical possibility of infection of the cells therefore apply. Risk assessments should be based on the same approaches used for vaccines.

3.2.3.3 Other medicinal products

A number of bovine derived materials are commonly used to manufacture both biological and pharmaceutical products. These include milk and milk derivatives, meat extracts, bovine serum including fetal bovine serum, bovine bone gelatin and beef tallow derivatives. Materials originating from other ruminant species are less commonly used. Milk and certain milk derivatives, such as lactose, are generally considered non-infectious, regardless of geographic origin, provided that the milk is from healthy cows fit for human consumption and no other potentially infectious ruminant-derived materials were used in the manufacturing process.

Extracts prepared from tissues like bovine muscle, in which TSE infectivity was detected during the clinical phase of BSE in one cow (Table 1B), are unlikely to present any risk of TSE contamination, provided that the manufacturer has scrupulously complied with procedures designed to avoid cross contamination during preparation of the source material. If assurances of compliance are not available, then it is recommended to collect meat extracts from animals in countries where risk of BSE is remote.

TSE infectivity has been detected in transfused blood of sheep with natural scrapie and with experimental BSE infections. Similar experiments have not been conducted in bovines nor has the effect of blood clot formation on TSE infectivity in serum been established. Studies using smaller amounts of blood components and spleen of cattle with BSE assayed in mice and cattle

have failed to detect infectivity. A conservative regulatory approach would assume that bovine serum might potentially contain TSE infectivity—presumably in small amounts. Blood for the preparation of donor calf serum is most often collected from well controlled living animals, reducing the risk of cross contamination of blood with higher risk materials attendant to the stunning and slaughtering process. Thus the sourcing of bovine serum (country/herd/animal) combined with appropriate precautions to avoid cross contamination during collection is important in the risk analysis.

Gelatin may be extracted from the skin and/or bones of cattle. Gelatin extracted from skins has a lower risk than does gelatin extracted from bones—especially bones from which skulls and vertebral columns have not been carefully excluded—because hide gelatin offers little opportunity for cross contamination with potentially infective tissue (for example brain, spinal cord and ganglia). Thus, it is recommended to collect bovine bones for processing into gelatin only from BSE-free countries or from countries with a low prevalence of BSE; it is preferable to exclude skull and vertebral columns from bones used for gelatin. The use of bone gelatin produced by alkaline hydrolysis (augmented, whenever possible, by additional approved processes) rather than by acid treatment alone further reduces the risk of contamination with TSE agents. Compliance with these precautions provides assurance that gelatin used in the manufacture of medicinal products is unlikely to be contaminated. Amino acids derived from gelatin are further highly processed, so their risk may be even lower.

Materials derived from ruminant tallow (for example, triglycerides, glycerol, sorbitan esters, polysorbate etc.) or amino acids of ruminant origin (even if higher-risk tissues were not completely eliminated) are considered highly unlikely to remain contaminated by the time the final reagent has been produced, so long as they were prepared by processes of extraction and purification at high temperatures and if good manufacturing practices (GMP) were rigorously controlled.

Safety is further assured when specified risk materials are excluded from starting materials, when raw materials are pressure cooked according to the OIE Code (particle size ≤ 50 mm, temperature $> 133^{\circ}\text{C}$, pressure ≥ 3 bar, exposure time ≥ 20 min) and when proteins have been removed from tallow to meet OIE specifications. The OIE Code chapter on BSE provides specific guidance on

requirements for safe trading in commodities used to manufacture biologicals and other medicinal products.

3.3 Measures to minimize risks to humans from human-derived materials

3.3.1 The risk of transmission of CJD and vCJD by blood and blood products

For many years it has been known that infectivity can be detected in the blood of rodents experimentally infected with certain TSE agents, including BSE agent. In the rodent models, infectivity is detected in both plasma and cellular components. Infectivity has also been detected in buffy coat of primates experimentally infected with BSE and in whole blood and buffy coat of sheep with experimental BSE. Most recently, infectivity has been detected in blood of sheep with scrapie—the first evidence of infectivity in blood in a naturally occurring TSE.

No credible instance of transmission of CJD by human blood, blood components or plasma derivatives has been reported, and an increasing number of epidemiological investigations over many years have failed to find any evidence that a history of treatment with blood components or plasma products is associated with increased risk of CJD. Among hemophiliacs treated with clotting factor concentrates derived from plasma there have been no reports of CJD to date, and postmortem examination of brain tissue from patients with hemophilia have not detected evidence of CJD. No transmission of vCJD by blood, blood components or plasma derivatives has been observed. Experience with vCJD—first recognized less than ten years ago—is limited, and epidemiological surveillance of surviving recipients of those blood products is underway. Attempts to transmit disease to monkeys and mice from blood of patients with vCJD have also been negative to date.

In spite of these reassuring results, the documented occurrence of infectivity in one natural and several experimental TSEs of animals continues to generate concern about risk from the blood of humans with TSE. Unfortunately, there is still no diagnostic test available that can be used to screen donors at risk of transmitting CJD or vCJD. In the absence of such tests, precautionary exclusion of donors at increased risk of CJD remains the only available measure to reduce the theoretical risk of transmitting CJD by blood products, and a number of countries have instituted such a measure.

Experimental studies from several research groups have consistently shown substantial reduction of spiked TSE infectivity during plasma fractionation steps, and there is growing evidence that the risk from plasma derivatives is negligible. It has also been shown that nanofiltration can reduce or eliminate TSE infectivity spiked into blood, but the method has so far been applied only to a limited number of plasma products. Precautionary leucodepletion of blood components has been introduced in a number of countries to reduce non-haemolytic febrile reactions and infections with some cell-associated viruses, but it has not been shown to reduce TSE infectivity.

Human albumin is used as a growth medium or stabilizer in a number of biological products, including vaccines. Some countries have implemented a policy to withdraw products containing plasma-derived medicinal products, including products containing albumin as an excipient, when post-donation information reveals that a donor who contributed to the plasma pool has developed vCJD. Withdrawal of batches of medicinal products containing albumin as an excipient might cause shortages of needed products, such as vaccines. To avoid such a situation, the group encouraged developing alternatives to plasma-derived albumin for excipient use.

3.3.2 Donor deferral policies:

Classical CJD and other human TSEs except vCJD:

The current internationally recognized donor selection criteria should be maintained. This implies that the following donors should be excluded because of increased CJD risk:

- Donors who have been treated with extracts derived from human pituitary glands (growth hormone, gonadotropin);
- Donors who have a family history of CJD or a similar TSE; and
- Donors who have received a human dura mater graft.

The G generally recommend discarding blood components (red cell concentrates, platelets and plasma) still in inventory when post-donation information reveals that a donor has CJD or a risk factor for CJD.

Although there is a significant probability that any large plasma pool contains a donation from a person who will eventually develop CJD (the estimated lifetime risk of CJD in the general population being approximately one per ten thousand), epidemiological studies have consistently failed to find any evidence that plasma derivatives have transmitted CJD. As noted, some plasma

fractionation steps were effective in clearing substantial amounts of spiked infectivity, reducing the risk of transmission. Furthermore, when precautionary withdrawals of plasma derivatives were attempted after post-donation reports of CJD or CJD risk factors in a donor, those withdrawals contributed to severe shortages of certain derivatives. Considering the reassuring epidemiological data and the overall adverse effect that shortages would cause, there has been general agreement that products prepared from large pools of plasma need not be withdrawn from the market when post-donation information reveals that a donor has CJD or a risk factor for CJD.

vCJD

The primary risk for acquiring vCJD appears to be the presence of BSE agent in beef products entering the human food chain. Potential human exposure depends on both “internal” and “external” factors. Internal factors are the geographical risk—the probability that BSE infectivity occurs in cattle of a region and domestic human consumption patterns of bovine-derived products in that region. External factors relate to human exposure to the BSE agent through importation of infected animals or animal products or through exposure while travelling in areas where cattle have BSE or where the agent enters the food supply through imports. The introduction of precautionary measures should take these factors into account.

Because experience with vCJD is comparatively recent, conclusions about the absence of vCJD infectivity in the blood are less secure than for sporadic disease. The possibility that blood of a person incubating vCJD might transmit the infection has led to policies aimed to minimize the risk, and many countries have instituted precautionary deferral of donors who traveled or lived in countries with a potential risk for BSE and vCJD. The periods of time that a suitable donor may have spent in various BSE countries were adjusted for the probable intensity of exposure to the BSE agent in comparison with the UK. After a country has implemented effective food chain controls and other protective measures, the risk of human exposure should be markedly reduced, and time spent there by donors after that is of little concern. Policies on donor suitability should also take into account the estimated effect that various deferral criteria might have on the blood supply. It may be reassuring to note that for those countries unlikely to have BSE in cattle or the BSE agent in food and from which few citizens have traveled to countries with BSE there is a negligible risk of blood-borne vCJD.

Plasma Product

To date, there is no evidence that vCJD has been transmitted by human plasma derivatives, even though, at least in the UK, some patients incubating vCJD have contributed to the pools of plasma from which derivatives were manufactured. Several analyses of the risk from plasma derivatives have been conducted, yielding somewhat discordant results, mainly resulting from difference in key assumptions used for the risk models. Several steps in the processes used to manufacture plasma derivatives are likely to reduce the amounts of infectivity, if any, in end products. As noted above, measures to minimize the risk from plasma derivatives have been implemented in the UK, the country reporting the largest number of vCJD cases and highest estimated prevalence of infected persons.

Despite reassuring evidence that plasma fractionation reduces the risk of transmission by plasma derivatives, as a prudent measure until more experience is gained with this new disease, it is advocated to recall batches of plasma-derived products if a donor with vCJD is recognized after donation. Withdrawal of plasma derivatives is not recommended when a donor is recognized post donation to have any form of CJD other than vCJD or to have had a risk factor for any form of CJD, or when post-donation information reveals that a donor was unsuitable due to travel or residence in BSE countries.

3.3.3 Human cells, tissues and tissue derived products

In addition to theoretical transmission of TSE through blood products, transmission by human tissues is possible, either because of inherent cellular infectivity or contamination by residual blood or plasma. In principle, although the same precautions should be applied to the transfer of cells and tissues as to blood, the risk-benefit assessment of TSE transmission versus therapeutic need should determine the use of such products. For example, the need for a rare type of bone marrow matched only from a UK resident would outweigh the remote possibility of TSE transmission. That policy might not be appropriate for another human tissue, like cornea, that is more readily available and that need not be carefully matched. Thus, the risk-benefit estimate for therapeutic administration of cells and tissues is probably best decided on a case-by-case basis.

4. Conclusions

The potential risks associated with a given medicinal product administered to humans should be considered on a case-by-case basis, taking into account all the foregoing factors and the potential benefits to patients. Recommendations are intended to apply to all medicinal products for which active substances, excipients or reagents derived from bovine tissues are used during their production processes. The recommendations relate particularly to materials of bovine origin, but the same principles should also be applied to materials used in the manufacture of medicinal products when these are obtained from sheep, goats and other species naturally affected with TSEs.

The development of substitutes for bovine, ovine or caprine materials used to manufacture medicinal products is encouraged. However it is recognized that this may not always be feasible, given the current level of scientific development for some products. This goal is sufficiently important to justify taking a long-term approach to reach it. It is emphasized that, when considering precautionary measures to maintain the safety of blood products, authorities should take into account their possible effect on the supply of blood. In that regard, it seems premature to recommend a global uniform policy, and every country should decide if precautionary measures are indicated to reduce the theoretical risk of transmitting CJD and vCJD by blood products.

Participants felt it important to stress that eliminating inappropriate use of blood and blood products would substantially reduce the risk of transfusion-related adverse events including the theoretical risk of blood-borne transmission of TSEs. Development of reliable methods to remove or inactivate the agents of TSE agents during the processing of blood and plasma remain of paramount importance and are to be encouraged, as are efforts to develop and validate sensitive and specific antemortem TSE diagnostic tests suitable for donor screening and tests for qualifying donor units as free of TSE agents.

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Web addresses:

Working Group on International Reference Materials for Diagnosis and Study of TSEs:

<http://www.who.int/biologicals>

Joint WHO/FAO/OIE Technical Consultation on BSE:

<http://www.who.int/csr/resources/publications/bse>

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Annex I: Major Categories of Infectivity: Table IA, IB, IC

The information in these Tables is based exclusively upon observations of naturally occurring disease, or primary experimental infection by the oral route (in ruminants), and does not include data on models using strains of TSE adapted to experimental animals, because passaged strain phenotypes can differ significantly and unpredictably from those of naturally occurring disease. Also, because the detection of misfolded host prion protein (PrP^{TSE}) has proven to be a reliable indicator of infectivity, PrP^{TSE} testing results have been presented in parallel with bioassay data. Tissues are grouped into three major infectivity categories, irrespective of the stage of disease:

IA: High-infectivity tissues:

CNS tissues that attain a high titre of infectivity in the later stages of all TSEs, and certain tissues that are anatomically associated with the CNS.

IB: Lower-infectivity tissues:

Peripheral tissues that have tested positive for infectivity and/or PrP^{TSE} in at least one form of TSE.

IC: Tissues with no detectable infectivity:

Tissues that have been examined for infectivity and/or PrP^{TSE} with negative results.

Data entries are shown as follows:

- + Presence of infectivity or PrP^{TSE}
- Absence of detectable infectivity or PrP^{TSE}
- NT Not tested
- NA Not applicable
- ? Controversial results
- () Limited or preliminary data

The placement of a given tissue in one or another category can be disease-specific and subject to revision as new data accumulate from increasingly sensitive tests. In fact, it is conceivable that the detection of infectivity using transgenic mice that over-express genes encoding various prion proteins, or the detection of PrPTSE using some newly developed amplification methods, might prove to be more sensitive than transmission studies in wild-type bioassay animals, and thus may not correlate with disease transmission in nature.

It is also important to understand that categories of infectivity are not the same as categories of risk, which require consideration not only of the level of infectivity in tissue, but also of the amount of tissue to which a person or animal is exposed, and the route by which infection is transmitted. For example, although the level of tissue infectivity (concentration of infectivity in tissue as reflected by titre) is the most important factor in estimating the risk of transmission by instrument cross contamination during surgical procedures (e.g., neurosurgery versus general surgery), it is only one determinant of the risk of transmission by blood transfusions, in which a large amount of low infectivity material is administered directly into the circulation, or the risk of transmission by food that, irrespective of high or low infectivity, involves the comparatively inefficient oral route of infection.

Table IA: High-infectivity tissues

CNS tissues that attain a high titre of infectivity in the later stages of TSE and certain tissues anatomically associated with the CNS								
Tissues	Human TSEs				Cattle		Sheep & goats	
	vCJD		Other TSEs		BSE		Scrapie	
	Infectivity ¹	PrP ^{TSE}	Infectivity ¹	PrP ^{TSE}	Infectivity ¹	PrP ^{TSE}	Infectivity ¹	PrP ^{TSE}
Brain	+	+	+	+	+	+	+	+
Spinal cord	+	+	+	+	+	+	+	+
Retina	NT	+	+	+	+	NT	NT	+
Optic nerve ²	NT	+	NT	+	+	NT	NT	+
Spinal ganglia	+	+	NT	+	+	NT	NT	+
Trigeminal ganglia	+	+	NT	+	+	NT	NT	+
Pituitary gland ³	NT	+	+	+	-	NT	+	NT
Dura mater ³	NT	-	+	-	NT	NT	NT	NT

Table IB: Lower-infectivity tissues

Peripheral tissues that have tested positive for infectivity and/or PrP ^{TSE} in at least one form of TSE								
Tissues	Human TSEs				Cattle		Sheep & goats	
	vCJD		Other TSEs		BSE		Scrapie	
	Infectivity	PrP ^{TSE}	Infectivity	PrP ^{TSE}	Infectivity	PrP ^{TSE}	Infectivity	PrP ^{TSE}
<i>Peripheral Nervous system</i>								
Peripheral nerves	+	+	(-)	+	+	+	+	+
Enteric plexuses ⁴	NT	+	NT	(-)	NT	+	NT	+
<i>Lymphoreticular tissues</i>								
Spleen	+	+	+	+	-	-	+	+
Lymph nodes	+	+	+	-	-	-	+	+
Tonsil	+	+	NT	-	+	-	+	+
Nictitating membrane	NA	NA	NA	NA	+	-	NT	+
Thymus	NT	+	NT	-	-	NT	+	NT
<i>Alimentary tract</i>								
Esophagus	NT	-	NT	-	-	NT	NT	+
Fore-stomach ⁵ (ruminants only)	NA	NA	NA	NA	-	NT	NT	+
Stomach/abomasum ⁶	NT	-	NT	NT	-	NT	NT	+
Duodenum	NT	-	NT	NT	-	NT	NT	+
Jejunum ⁶	NT	+	NT	-	-	NT	NT	+
Ileum ^{6,7}	NT	+	NT	-	+	+	+	+
Appendix	-	+	NT	-	NA	NA	NA	NA
Large intestine ⁶	+	+	NT	-	-	NT	+	+
<i>Reproductive tissues</i>								
Placenta ⁸	NT	-	(+)	-	-	NT	+	+
<i>Other tissues</i>								
Lung	NT	-	+	-	-	NT	-	-
Liver	NT	-	+	-	-	NT	+	NT
Kidney	NT	-	+	-	-	-	-	-
Adrenal	NT	+	-	-	NT	NT	+	NT
Pancreas	NT	-	NT	-	-	NT	+	NT
Bone marrow	-	-	(-)	-	(+)	NT	+	NT
Skeletal muscle ⁹	NT	+	(-)	+	(+)	NT	-	+
Tongue ¹⁰	NT	-	NT	-	-	NT	NT	+
Blood vessels	NT	+	NT	+	-	NT	NT	+
Nasal mucosa ¹¹	NT	NT	NT	+	-	NT	+	+
Salivary gland	NT	-	NT	NT	-	NT	+	NT
Cornea ¹²	NT	-	+	-	NT	NT	NT	NT
<i>Body fluids</i>								
CSF	-	-	+	-	-	NT	+	NT
Blood ¹³	+	?	-	?	-	?	+	?

Table IC: Tissues with no detected infectivity or PrP^{TSE}

Tissues	Human TSEs				Cattle		Sheep & goats	
	vCJD		Other TSEs		BSE		Scrapie	
	Infectivity	PrP ^{TSE}	Infectivity	PrP ^{TSE}	Infectivity	PrP ^{TSE}	Infectivity	PrP ^{TSE}
<i>Reproductive tissues</i>								
Testis	NT	-	(-)	-	-	NT	-	NT
Prostate/Epididymis/ Seminal vesicle	NT	-	(-)	-	-	NT	-	NT
Semen	NT	-	(-)	-	-	NT	NT	NT
Ovary	NT	-	NT	-	-	NT	-	NT
Uterus (non-gravid)	NT	-	NT	-	-	NT	-	NT
Placenta fluids	NT	NT	(-)	NT	-	NT	NT	NT
Fetus ¹⁴	NT	NT	NT	NT	-	NT	-	-
Embryos ¹⁴	NT	NT	NT	NT	-	NT	?	NT
<i>Musculo-skeletal tissues</i>								
Bone	NT	NT	NT	NT	-	NT	NT	NT
Heart/pericardium	NT	-	-	-	-	NT	-	NT
Tendon	NT	NT	NT	NT	-	NT	NT	NT
<i>Other tissues</i>								
Gingival tissue	NT	-	-	-	NT	NT	NT	NT
Dental pulp	NT	-	NT	-	NT	NT	NT	NT
Trachea	NT	-	NT	-	-	NT	NT	NT
Skin	NT	-	NT	-	-	NT	-	NT
Adipose tissue	NT	-	(-)	-	-	NT	NT	NT
Thyroid gland	NT	-	(-)	-	NT	NT	-	NT
Mammary gland/udder	NT	NT	NT	NT	-	NT	-	NT
<i>Body fluids, secretions and excretions</i>								
Milk ¹⁵	NT	NT	(-)	NT	-	-	-	NT
Colostrum ¹⁶	NT	NT	(-)	NT	(-)	-	-	NT
Cord blood ¹⁷	NT	NT	(-)	NT	-	NT	NT	NT
Saliva	NT	-	-	NT	NT	NT	-	NT
Sweat	NT	NT	-	NT	NT	NT	NT	NT
Tears	NT	NT	-	NT	NT	NT	NT	NT
Nasal mucus	NT	-	-	NT	NT	NT	NT	NT
Bile	NT	NT	NT	NT	NT	NT	NT	NT
Urine ^{16,17}	NT	NT	-	-	-	NT	NT	NT
Feces	NT	NT	-	NT	-	NT	-	NT

Annex 1 Footnotes:

1. Infectivity bioassays of human tissues have been conducted in either primates or mice (or both); bioassays of cattle tissues have been conducted in either cattle or mice (or both); and most bioassays of sheep and/or goat tissues have been conducted only in mice. In regard to sheep and goats, not all results are consistent for both species.
2. In experimental models of TSE, the optic nerve has been shown to be a route of neuroinvasion and contains high titres of infectivity.
3. No experimental data about infectivity in human pituitary gland or dura mater have been reported, but cadaveric dura mater allograft patches, and growth hormone derived from cadaveric pituitaries have transmitted disease to hundreds of people and therefore must be included in the category of high-risk tissues.
4. In cattle, PrPTSE was limited to enteric plexus in the distal ileum.
5. Ruminant fore-stomachs (reticulum, rumen, and omasum) are widely consumed, as is the true stomach (abomasum). The abomasum of cattle (and sometimes sheep) is also a source of rennet.
6. In vCJD, transmission to mice has so far been limited to rectal tissue, and PrPTSE was detected only in gut-associated lymphoid and nervous tissue (mucosa, muscle, and serosa were negative). In goats, PrPTSE was also limited to gut-associated lymphoid and nervous tissue [Andreoletti, unpublished data].
7. In cattle and sheep, only the distal ileum has been bioassayed for infectivity.
8. A single report of transmission of CJD infectivity from human placenta has never been confirmed and is considered improbable.
9. Muscle homogenates have not transmitted disease to primates from humans with sCJD, or to cattle from cattle with BSE. However, intracerebral inoculation of a semitendinosus muscle homogenate (including nervous and lymphatic elements) from a single cow with BSE has transmitted disease to PrP over-expressing transgenic mice at a rate indicative of only trace levels of infectivity. Also, recent published and unpublished studies have reported the presence of PrPTSE in skeletal muscle in experimental rodent models of scrapie and vCJD, in

experimental and natural infections of sheep and goats, in sheep orally dosed with BSE [Andreoletti, unpublished data], and in humans with sCJD, iCJD and vCJD. Bioassays to determine whether PrP^{TSE} is associated with transmissibility in these experimental or natural infections are in progress.

10. In cattle, infectivity bioassay was negative, but the presence of PrP^{TSE} in palatine tonsil has raised concern about possible infectivity in lingual tonsillar tissue at the base of the tongue that may not be removed at slaughter.
11. In sCJD, PrP^{TSE} is limited to olfactory mucosa.
12. Because only one or two cases of CJD have been plausibly attributed to corneal transplants among hundreds of thousands of recipients, cornea is categorised as a lower-risk tissue; other anterior chamber tissues (lens, aqueous humor, iris, conjunctiva) have been tested with a negative result both in vCJD and other human TSEs, and there is no epidemiological evidence that they have been associated with iatrogenic disease transmission.
13. A wealth of data from studies of blood infectivity in experimental animal models of TSE has been extended by recent studies documenting infectivity in the blood of sheep with naturally occurring scrapie, and (from epidemiological observations) three blood-associated vCJD transmissions in humans. Blood has not been shown to transmit disease from patients with any other form of TSE, or from cattle with BSE (including fetal calf blood). However, several laboratories using new, highly sensitive methods to detect PrP^{TSE} claim success in studies of plasma and/or buffy coat in a variety of animal and human TSEs. Because the tests are all in a preliminary stage of development (and do not yet include results on blinded testing of specimens from naturally infected humans or animals), the Consultation felt that it was still too early to evaluate the validity of these tests with sufficient confidence to permit either a negative or positive conclusion.
14. Embryos from BSE-affected cattle have not transmitted disease to mice, but no infectivity measurements have been made with fetal calf tissues other than blood (negative mouse bioassay). Calves born of dams that received embryos from BSE-affected cattle have survived for observations periods of up to seven years, and examination of the brains of both the unaffected dams and their offspring revealed no spongiform encephalopathy or PrP^{TSE}.

15. Evidence that infectivity is not present in milk includes temporo-spatial epidemiologic observations failing to detect maternal transmission; clinical observations of over a hundred calves nursed by infected cows that have not developed BSE; and experimental observations that milk from infected cows has not transmitted disease when administered intracerebrally or orally to mice. Also, PrP^{TSE} has not been detected in milk from cattle incubating BSE following experimental oral challenge.
16. Early reports of transmission of CJD infectivity from human cord blood, colostrum, and urine have never been confirmed and are considered improbable. A recent bioassay in PrP over-expressing transgenic mice of colostrum from a cow with BSE gave a negative result; and PrP^{TSE} has not been detected in colostrum from cattle incubating BSE following experimental oral challenge.
17. IgG short chains mimicking the Western blot behavior of PrP^{TSE} have been identified in the urine of sporadic, variant, and familial CJD patients.

7. Table References

Tables IA, IB and IC are an update from tables created in an earlier consultation (WHO Guidelines on Transmissible Spongiform Encephalopathies in relation to Biological and Pharmaceutical Products, 2003, <http://www.who.int/bloodproducts/tse/en>) by an *ad hoc* expert interim group composed of Dr O. Andreoletti, Mr R. Bradley, Dr P. Brown, Prof Dr H. Budka, Prof Dr M.H. Groschup, Prof J.W. Ironside, Prof M. Pocchiari, and Mr G.A.H. Wells. Dr P. Brown coordinated the group and consolidated the information for review by all Consultation participants. Most of the observations that form the basis for the Tables have been published in original reports (or cited in reviews) that follow. Most studies published since the previous Consultation have been listed, but no attempt has been made to list the many earlier reports in which only one or two tissues were examined unless they concerned tissues of exceptional current interest. Also, a number of observations made by or known to members of the expert group that assembled the Tables have not yet been published.

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Viral Safety of Human Blood Plasma Medicinal Products

Part I: Guidelines on viral inactivation and removal procedures intended to assure the viral safety of human blood plasma products

Part II: Nucleic acid testing (NAT) for human immunodeficiency virus type 1 (HIV – 1) and hepatitis c virus (HCV): testing, product disposition, and donor deferral and reentry

Part I: Guidelines on viral inactivation and removal procedures
intended to assure the viral safety of human blood plasma products

List of abbreviations and definitions used in this Annex

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

AHF: Antihaemophilic factor. Blood coagulation factor VIII, missing in patients with classic haemophilia.

Blood components: These typically refer to red blood cell concentrates, platelet concentrates and plasma.

BEV: Bovine enterovirus. A non-enveloped, single stranded RNA virus used as a model for hepatitis A virus.

BVDV: Bovine viral diarrhea virus. An enveloped, single-stranded RNA virus used as a model for hepatitis C virus.

CMV: Cytomegalovirus. An enveloped, double stranded DNA virus, typically cell-associated.

Coxsackie virus: A non-enveloped, single-stranded RNA virus.

CPV: Canine parvovirus. A non-enveloped, single stranded DNA virus.

Donor retested plasma: A process for reducing window period transmissions whereby fresh frozen plasma is held in the inventory for a designated period of time until the donor returns and tests negative for virus exposure. The initial unit is then released for use. Also called quarantine plasma.

Dry heat: A process of heating protein following lyophilization, typically at 80°C or higher.

EBV: Epstein–Barr virus. An enveloped, double stranded DNA virus, typically cell-associated.

EMCV: Encephalomyocarditis virus. A non-enveloped, single-stranded, RNA virus.

Factor IX: Blood coagulation factor IX, missing in patients with haemophilia B.

Factor VIII: Blood coagulation factor VIII, missing in patients with haemophilia A. Also called antihaemophilic factor.

FFP: Fresh frozen plasma.

Fluence: The total quantity of light delivered. Expressed in J/cm².

Gamma-irradiation: A process of virus inactivation or bacterial sterilization using gamma-irradiation of liquid, frozen or lyophilized product.

GE: Genome equivalents. The amount of nucleic acid of a particular virus assessed using nucleic acid testing.

GMPs: Good manufacturing practices. Sometimes referred to as current good manufacturing practices.

HAV: Hepatitis A virus. A non-enveloped, single stranded RNA virus.

HBsAg: Hepatitis B surface antigen. The antigen on the periphery of hepatitis B virus.

HBV: Hepatitis B virus. An enveloped, double stranded DNA virus.

HCV: Hepatitis C virus. An enveloped, single stranded, RNA virus.

HDV: Hepatitis delta virus. A defective virus which requires co-infection by hepatitis B virus.

High purity factor VIII: Factor VIII concentrate with a specific activity typically greater than 100 IU/mg.

HIV: Human immunodeficiency virus. An enveloped, single-stranded RNA virus.

HSV: Herpes simplex virus. An enveloped, double stranded DNA virus, typically cell-associated.

HTLV 1 and 2: Human T-cell lymphotropic virus, types 1 and 2. Enveloped, single-stranded RNA viruses, typically cell-associated.

ID₅₀: The quantity of virus or other infectious agent that will infect 50% of subjects or tissue cultures. Frequently expressed on a log scale; thus, 6 log₁₀ ID₅₀ represents 1 million infectious units.

Immunogenic: Causing the formation of antibody. Harsh processing conditions may modify the structure of a protein so as to make it immunogenic.

Intermediate purity factor VIII: Factor VIII concentrate with a specific activity between 1 and 50 IU/mg.

IVIG: Intravenous immunoglobulin.

Limiting dilution: A way of determining titre by diluting the sample continually until the positive signal is lost.

LRF: Log reduction factor. The quantity of virus, expressed on a log 10 scale, inactivated or removed.

MB-plasma: Methylene blue-treated plasma intended as a substitute for fresh frozen plasma.

Nanofilters: Filters that usually have effective pore sizes of 50 nm or less, designed to remove viruses from protein solutions.

NAT: Nucleic acid testing, using amplification techniques such as polymerase chain reaction.

Pasteurization: A process of heating protein in solution, typically at 60°C.

Polio virus: A non-enveloped, single-stranded, RNA virus.

PPRV: Porcine pseudorabies virus. An enveloped, double-stranded DNA virus.

PPV: Porcine parvovirus. A non-enveloped, single stranded DNA virus.

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

PRV: Pseudo rabies virus. An enveloped, double stranded DNA virus.

Psoralen: A furocoumarin ring structure, which when exposed to light, cross-links nucleic acid.

Quarantine plasma: See donor retested plasma.

RT3: Reovirus type 3. A non-enveloped, double stranded RNA virus.

Rutin: A flavonoid used as an antioxidant that reduces the action of reactive oxygen species.

Solvent/detergent treatment: A process of treating protein in solution, usually with the organic solvent, tri(n-butyl) phosphate, and a detergent such as Tween 80 or Triton X-100.

SD-Plasma: Solvent/detergent-treated plasma intended as a substitute for FFP.

Sindbis virus: An enveloped, single-stranded RNA virus.

SLFV: Semliki forest virus. An enveloped, single stranded, RNA virus.

Titre: The quantity of virus, typically expressed on a log₁₀ scale. Six logs of virus are equal to 1 million infectious units.

TNBP: Tri(n-butyl)phosphate. The organic solvent used with solvent/detergent treatment.

Triton X-100: A non-ionic detergent frequently used as part of solvent/detergent treatment.

Tween 80: A non-ionic detergent frequently used as part of solvent/detergent treatment.

UVC: Ultraviolet irradiation, usually at a wavelength of 254 nm.

Vaccinia virus: An enveloped, double-stranded DNA virus.

Vapour heating: A process of heating protein following lyophilization and then reintroducing moisture normally at 60°C and in some cases at 80°C.

Viral inactivation: A process of enhancing viral safety in which virus is intentionally “killed”.

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

VSV: Vesicular stomatitis virus. An enveloped, single-stranded RNA virus.

West Nile virus: An enveloped, single-stranded RNA virus.

1. Introduction and Scope

Human blood is the source of a wide range of medicinal products used for the prevention and treatment of a variety of often life-threatening injuries and diseases. Despite measures such as donor selection, testing of donations and of plasma pools, the transmission of blood-borne viruses by plasma and purified plasma products is still considered to constitute a risk to patients. Over the past 15–20 years, the transmission of the principal viral threats historically associated with these products — hepatitis B virus (HBV), hepatitis C virus (HCV) and human immunodeficiency virus (HIV) — has been greatly reduced or eliminated in many areas of the world. This is a consequence of the more sensitive methods being used to screen donated blood and plasma pools, and of the establishment of manufacturing practices that lead to significant virus inactivation and removal. Several procedures for virus inactivation and removal have proven to be robust and to contribute substantially to blood product safety. Viral inactivation methods should be applied to all blood plasma-derived protein solutions.

Continuing concerns about the quality and safety of plasma-derived medicinal products have resulted in a number of urgent requests from countries for support and advice from WHO. Moreover, the World Health Assembly Resolution No 50.20, of 13 May 1997 on the “Quality of biological products moving in international commerce”, requested WHO to extend the assistance offered to countries to develop and to strengthen their national regulatory authorities and control laboratories to increase competence in the area, and to extend efforts to upgrade the quality and safety of all biological products worldwide.

The present Guidelines on viral inactivation and removal procedures intended to assure the viral safety of human blood plasma products were developed to complement the Requirements for the collection, processing and quality control of blood, blood components and plasma derivatives”(1), in response to the above requests. These Guidelines pertain to the validation and assessment of the steps for viral inactivation and removal employed in the manufacture of human blood plasma derivatives and virally inactivated plasma for transfusion, prepared either from plasma pools or from individual donations. It is hoped that this document, by summarizing current experience with well-recognized methods, will help set expectations, serve as a guide to speed implementation, and ensure that implementation is appropriate.

The document does not address products of animal origin or those manufactured by recombinant techniques.

2. General Considerations

Viral safety derives from three complementary approaches during manufacture, i.e. donor selection, testing of donations and plasma pools, and the introduction of viral inactivation and removal procedures in the course of manufacture, each of which requires strict adherence to good manufacturing practices (GMPs). Although these Guidelines address only viral inactivation and removal, no individual approach provides a sufficient level of assurance, and safety will only be achieved by using a combination of the three.

Some of the principles that relate to viral inactivation and removal procedures as applied to purified blood plasma products and to plasma intended for transfusion are listed below.

Viral contamination can arise from the donor, or, less commonly, from other sources introduced during manufacture (e.g. from the reagents employed).

Viral validation studies are intended to assess the degree to which virus infectivity is eliminated during manufacture. These studies can only approximate the inactivation and removal that occur during routine manufacture because the model viruses employed in the studies may differ from those present in blood, and it may be difficult or impossible to truly model the conditions employed during manufacture. Thus, the appropriateness of the studies needs to be reviewed on a case-by-case basis, and the manufacturer should justify the choice of viruses and the validation conditions employed.

Viruses to be studied, where required, include: HIV; a model for hepatitis C such as Sindbis virus or bovine viral diarrhoea virus (BVDV); one or more non-enveloped viruses such as hepatitis A virus, encephalomyocarditis virus (EMCV), or porcine parvovirus; and an enveloped DNA virus such as pseudo rabies virus or duck hepatitis B virus. The ability of a process to inactivate or remove viruses should take into account:

- The reduction in virus titre achieved;
- For inactivation processes, the rate of inactivation and the shape of the inactivation curves; for removal, mass balance;
- How robust the step is in response to changes in process conditions; and
- The selectivity of the process for viruses of different classes.

Data should be analyzed using appropriate statistical procedures.

Virus removal should be distinguished from virus inactivation. This is important in ensuring the accurate modeling of a process step and identifying the parameters that are most effective

in reducing infectivity in that process. For example, if a chromatography step removes viruses, flow rates and column dimensions are important process variables, whereas if the buffer used inactivates viruses, temperature and pH are likely to be more significant.

Purification procedures such as precipitation or chromatography can contribute to virus removal; however, removal depends critically on the protein composition and the separation conditions used, and it is difficult to scale down partition processes for validation purposes. Therefore, all appropriate specifications and accepted tolerances should be stated, and control data provided. For chromatographic columns and media, the conditions of storage, preservation and regeneration should be described.

Validation studies need to be well documented to ensure proper execution of the procedure. The highest titre of virus that can reasonably be employed should be added (spiked) into the solution to be tested at a ratio not exceeding one part virus to nine parts sample. Virus infectivity starting titre should be measured, ideally after addition to the sample, and then with time during the virus inactivation and removal procedure. Worst-case conditions must be studied. Appropriate controls should be run to demonstrate the validity and sensitivity of the assay.

All viral infectivity tests suffer from the limitation that the ability to detect low viral concentrations depends for statistical reasons on the size of the sample. Consequently, the largest sample size that can be practically assayed should be chosen if the study indicates that all viruses are inactivated or removed.

Appropriate procedures should be employed throughout the manufacturing process to prevent recontamination following use of a virus inactivation or removal method.

Priority for validating the viral inactivation steps used in the manufacture of plasma protein solutions should be given to those products with the highest risk potential, such as coagulation factors, proteolytic inhibitors and intravenous immunoglobulins.

3. Infectious Agents

3.1 Viruses, viral burden and screening methods

Medicinal products made from human blood include clotting factors, immunoglobulins and albumin among others, have all at some time transmitted serious virus infections to recipients. The object of viral inactivation and removal procedures is to improve viral safety so that such transmissions no longer occur. The viruses of particular concern, HBV, HCV and HIV, have all been transmitted by some plasma products, and all cause life-threatening diseases. Other viruses of concern include hepatitis A virus and parvovirus B19, both of which have been transmitted by clotting factor concentrates. Some of the properties of these viruses are listed in Table 1.

Table 1

Selected properties of some plasma-borne viruses

Virus	Genome	Envelope	Size (nm)
Hepatitis B virus	dsDNA	yes	40–45
Hepatitis C virus	ssRNA	yes	40–50
Human immunodeficiency virus	ssRNA	yes	80–130
Hepatitis A virus	ssRNA	no	28–30
Parvovirus B19	ssDNA	no	18–26

ds, double-strand; ss, single-strand

The pathogenicity of a virus may depend on the patient group and on the product being administered. For example parvovirus B19 infects the red blood cell precursors and effectively eliminates them for a period. Parvovirus infections are usually relatively mild in the general population because most people have a substantial buffer of mature red cells. However, in patients with haemolytic anaemias (such as sickle-cell anaemia), parvovirus infections can be fatal because the lifespan of mature red cells is shorter. Parvovirus B19 may be of greater concern in Africa where sickle-cell anaemia is relatively more common than in Europe, and it is possible that other agents (e.g. hepatitis E virus) would be significant in other geographical settings depending on their prevalence in the donor population. Other examples include cytomegalovirus and human T lymphotropic virus I and II (HTLV I + II) which are strongly cell-associated and are therefore not considered to pose a significant risk in therapeutic proteins derived from human plasma, although they have been transmitted by cellular components in blood transfusions, and HAV, which can be transmitted by purified coagulation factor concentrates, but is not usually a problem with products such as intravenous immunoglobulin (IVIG) that contain anti-HAV antibodies.

For the product to be safe, the production process must inactivate and/or remove all the virus present. The quantity of virus depends on the number of infected donors contributing to the pooled starting material and the titre (concentration) of infectious virus in those donations. Estimates of the frequency of occurrence of hepatitis viruses, HIV and parvovirus and their titres prior to the implementation of screening tests, in European and US donor populations are given in Table 2.

Table 2
Viruses in plasma from unscreened donor blood

Virus	Prevalence in donor blood	Viral titre (GE/ml)	Calculated titre in plasma pool (GE/ml) ^a
Hepatitis B virus	1/10 000	10^3 – 10^8	0– 10^4
Hepatitis C virus	1/50–1/100	10^4 – 10^6	10^2 – 10^4
Human immunodeficiency virus	1/1000–1/10 000	10^3 – 10^7	0– 10^4
Hepatitis A virus	1/500 000	10^3 – 10^5	0– 10^1
Parvovirus B19	1/1000–1/7000	10^2 – 10^{12}	0– 10^9

^a Assumes the pooling of 10 000 units.

Table 3
Frequency of HCV RNA-positive plasma pools following testing of single donations for anti-HCV antibody

Screening test on individual unit	Number of pools (positive/total)	Percentage hepatitis C virus PCR positive
None	8/8	100
First-generation antibody test	65/85	76
Second-generation antibody test	49/123	39

Source: Nübling, Willkommen & Löwer (5).
PCR, polymerase chain reaction.

For example, before tests for HCV antibody were developed, approximately 1–2% of donors were unknowingly infected with HCV. Parvovirus is now known to be present in 1/1000–1/7000 blood donors, largely because it is a common infection in the general population, and tests for it are not routinely employed. Most pools of 10 000 or more unscreened donor units would therefore be expected to be contaminated with HCV and parvovirus. When this information is combined with the titre of virus in contaminated units and the number of donors contributing to the plasma pool, the titre in the plasma pool can be calculated (Table

2). Because the titres of HCV RNA in an infected individual may range from 10^4 to 10^6 genome equivalents (GE)/ml and those of parvovirus B19 DNA from 10^2 to 10^{12} GE/ml, plasma pools would be expected to contain 10^2 – 10^4 GE/ml of HCV and 0– 10^9 GE/ml of parvovirus. Put more simply, most pools of 10,000 or more unscreened donor units would be expected to be contaminated with HCV and parvovirus, whereas contamination with HBV, HIV and HAV would occur at a lower frequency. Viral titres of HBV, HCV and HIV in the plasma pool can reach 10^4 GE/ml. It should be noted that the incidence of virally infected units depends on several factors including the population from which the donors are drawn and, for parvovirus, on seasonal variations.

A study conducted at the Paul Ehrlich Institute, Germany, determined the frequency of HCV RNA-positive pools before and after screening of donors was introduced, using first- or second-generation tests for HCV antibody (Table 3) (5). Although screening reduced the number of antibody-positive pools, it is important to note that the viral titre in those pools that were contaminated was not reduced. This is a consequence of using a test for the antibody rather than for the virus and because in the case of HCV, and many other viruses, peak titres occur before the appearance of the antibodies in the circulation (i.e. the so-called window period). Nonetheless, because the screening of donors for markers of infection such as hepatitis B surface antigen or antibodies to HIV or HCV can reduce the number of positive pools and, in certain circumstances, the virus load in the starting material, screening is an important element in assuring viral safety.

Nucleic acid amplification technology (NAT) has been introduced in some instances to detect viral nucleic acid. As nucleic acid is associated with the virus itself rather than the host response to infection, NAT minimizes the window period and reduces the total quantity of virus in the plasma pool (6,7). Window period estimates are given in Table 4. As an additional measurement of the effectiveness of donor screening, the quantity of viral genomic nucleic acid present in the plasma pool can be assessed by NAT. Even if only carried out intermittently, performing NAT on plasma pools provides a basis for assessing product safety when coupled with the data quantifying virus removal or inactivation.

Table 4

Average window period estimates for HIV, HCV and HBV

Virus	Window period without nucleic acid amplification technology (days)	Window period with minipool nucleic acid amplification technology (days)
Human immunodeficiency virus	22	10
Hepatitis C virus	82	9
Hepatitis B virus	59	49

Sources: Schreiber et al. (8); Kleinman et al. (9).

Finally, it should be recognized that all screening methods are subject to the criticisms that they are unable to detect virus infection below a certain level, and that errors in the screening process may occur, particularly where large numbers of donations are used. Additionally, screening is limited to the viruses being tested for. Thus, while screening helps to ensure that the virus load is kept to a minimum, it is not sufficient to ensure safety in itself, and the ability of the production process to remove or inactivate viruses is a crucial second element. The proportion of potential donors who are infected with viruses will depend on the particular geographical region. In donors from certain areas, HBV or HIV infections may be far more common than in those from countries where the strategies for ensuring viral safety have evolved. Where this is the case, the ability of the production process to inactivate or remove viruses will be even more important.

3.2 Other infectious agents

Bacteria and parasitic infections including malaria and trypanosomes do not pose a risk in plasma products that have been sterile filtered with a 0.2µm filter.

Prions, the putative causative agent of the transmissible spongiform encephalopathies including Creutzfeld Jakob Disease (CJD) of humans, are a matter of concern, especially as a result of the occurrence of variant CJD (vCJD) in the United Kingdom following the epidemic of bovine spongiform encephalopathy. The continuing concern stems, in part, from experimental evidence in animal models that infectivity could be present in blood, albeit late in infection and at low levels. However, there has been no increase in the incidence of classic CJD (currently one death per million head of population per year wherever it has been measured), despite the increased transfusion of blood and the extremely hardy nature of the

agent. As with CJD, there is no evidence that vCJD has been transmitted by blood, blood components or plasma-derived products in clinical practice. However, since vCJD is a newly emerging disease, it is too early to conclude that there is no risk. Measures to minimize the risks to humans from human- and bovine-derived materials are summarized in the GCC guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products (10).

3.3 Validation of viral inactivation and removal procedures

3.3.1 Selection of relevant and model viruses

The viruses that may contaminate blood and blood products encompass all of the viral types, including viruses with a DNA or RNA genome, with and without a lipid membrane, and ranging in size from the smallest, such as parvovirus, to the middle range, such as HBV. The processes employed should therefore be shown to be able to remove or inactivate a wide range of viruses if they are to be considered satisfactory; typically, validation studies have involved at least three viruses, chosen to represent different kinds of agent.

Viruses have been selected to resemble those that may be present in the starting material (Table 5). All are laboratory strains that may be grown to high titre and assayed readily. The models for hepatitis C virus include BVDV, Sindbis virus, Semliki forest virus and yellow fever virus as they share many properties, including a lipid membrane, an RNA genome and a particle size of 40–50 nm. Laboratory strains of HIV or hepatitis A virus are used, and canine and porcine parvovirus have been used as models for parvovirus B19. Suitable models for hepatitis B virus have been more difficult to identify, because few viruses of this family can be grown in culture. Duck hepatitis virus has been used, but the pseudo rabies virus has also been employed as a large DNA virus. This list is not exhaustive and other appropriate viruses are acceptable. The main viruses of concern are HIV, HBV and HCV, and laboratory viruses are almost always used to represent them. During the developmental phase, viruses that are particularly resistant to the approach taken often serve as useful surrogates. As an example, the use of vesicular stomatitis virus (VSV) has proven useful when first qualifying a viral inactivation step based on low pH or solvent/detergent treatments. Nonetheless, for product registration, the use of viruses that better resemble those present in the starting material should be used. Precautions needed for the safe handling of the viruses for both human and animal contacts should be taken into account in the design and execution of the studies. Readers are directed to existing guidance documents for additional details on the selection and assay of model viruses (2, 3).

Table 5

Plasma-borne viruses and their models

Virus	Examples of viruses used to model inactivation/removal studies
Hepatitis B virus	Duck hepatitis B virus, pseudorabies virus ^a
Hepatitis C virus	Bovine viral diarrhoea virus, Sindbis virus, Semliki forest virus, Yellow fever virus
Human immunodeficiency virus	Human immunodeficiency virus
Hepatitis A virus	Hepatitis A virus, encephalomyocarditis virus
Parvovirus B19	Canine parvovirus, porcine parvovirus

^a Because there are no convenient models for hepatitis B virus, pseudorabies virus is frequently used: both pseudorabies virus and hepatitis B virus are enveloped, double-stranded DNA viruses.

3.3.2 Modeling (downscaling) the production process

The production process can be viewed as a series of steps, and it is the obligation of the manufacturer to identify those steps likely to remove or inactivate virus and to demonstrate the degree of virus reduction achieved by following them. Not every step needs to be evaluated. The ability of the steps in a process to remove or inactivate viruses is measured on a laboratory scale and not in the production facility where it would be inappropriate to introduce infectious virus deliberately. The accuracy of the model is crucial, and should be assessed by comparing the characteristics of the starting material and the product for that step for both laboratory and full-scale processes. In the model of the process, physical factors (e.g. temperature, stirring, column heights and linear flow rates, and sedimentation or filtration conditions) and chemical factors (e.g. pH, ionic strength, moisture and the concentration of inactivating agents) should be equivalent to the real process) where possible. It should be noted that whereas many process steps can be modeled readily, models of ethanol fractionation processes have proved particularly variable, in part because of difficulties in scaling down centrifugational processes and in controlling subzero temperatures on a small scale.

Once the step is accurately modeled, virus is introduced into material derived from the fractionation process just prior to the step being evaluated, and the amount remaining after the modeled process step is measured. The results are conventionally expressed in terms of the logarithm of the reduction in infectivity reported. Total infectivity or viral load is calculated as the infectious titre (infectious units per ml) multiplied by the volume. Viral clearance

compares the viral load at the beginning with that at the conclusion of the step being evaluated.

For viral inactivation procedures, both the kinetics and extent of virus inactivation need to be demonstrated. The kinetics of inactivation are important since the rapid kill of large amounts of virus is a further indication of the virucidal potential of the step and, for well characterized procedures for viral inactivation, enables comparison of a process with similar processes executed by others. For viral removal systems, an attempt should be made to show mass balance, i.e. to account for all of the virus added. If the buffers used are virucidal, it is important to distinguish virus inactivation from virus removal.

It is necessary to evaluate the effect of possible variations in the process conditions on the virus clearance observed, for example the effects of changes in temperature or composition of the starting material for the particular step. A robust, effective and reliable process step will be able to remove or inactivate substantial amounts of virus, typically 4 logs or more, be easy to model convincingly and be relatively insensitive to changes in process conditions. For example, if at the start of a step the viral titre is 10^5 /ml and the volume is 20ml and at the conclusion of the step the viral titre is 10^1 /ml and the volume is 60ml, then the viral load at the start is 6.3 logs and at the end is 2.8 logs, and the viral clearance is 3.5 logs. Steps removing 1 log of virus or less cannot be regarded as significant. A production process that includes two robust steps able to remove or inactivate enveloped viruses is likely to give a safe product, particularly if the steps act by different mechanisms (e.g. inactivation by a chemical treatment followed by a robust physical removal step). Non-enveloped viruses are more difficult to remove or inactivate. A process that includes one robust step effective against non-enveloped viruses may give a safe product; failing this, other approaches including implementing screening procedures, e.g. NAT, may prove helpful in excluding infectious material.

Virus validation studies are subject to a number of limitations. The subdivision of the process into individual steps which are separately assessed assumes that the effects of different procedures can be added up in some way. This is true only if the fraction of virus surviving one step is not resistant to another, which is not always the case. If virus is resistant to a chemical treatment because it is present as an aggregate that the chemical cannot penetrate, it may also be resistant to a second, different, chemical treatment. Care must be taken to not count the same treatment twice, for example if ethanol has a direct inactivating effect on a virus, steps in fractionation involving increasing concentrations of ethanol may all inactivate

the virus in the same way, and will therefore have no additive effects. In contrast, if the reduction in viral infectivity results from the removal of virus particles at one ethanol concentration, followed by the inactivation of virus at a higher concentration, the effects may be summed. Care must therefore be taken to provide justification for summing the effects of different steps which, ultimately, is dependent on the steps removing or inactivating viruses by different mechanisms. Other limitations are that the properties of the virus used in the laboratory studies may differ in from that occurring in nature, the plasma may contain antibodies to the virus of interest that may affect virus inactivation or removal in unpredictable ways, there may be fractions of virus that are resistant to a number of steps, and the modeling of the process may be imperfect. The clearance figures obtained are therefore approximate.

The difficulties of establishing an adequate laboratory model for virus inactivation and removal mean that the figures produced are unlikely to fully reflect manufacturing operations. In general, for a product to be safe, the process must remove or inactivate virus infectivity to a much greater extent than the level of virus in the starting materials. The use of two complementary steps for virus inactivation and removal may be especially important if the population of donors contributing to the plasma pool has a high incidence of blood borne viruses, leading to a high viral load in the material being processed. A second advantage in employing two complementary methods of virus inactivation and removal is the potential to increase the spectrum of viruses covered.

3.3.3 Other considerations

In practice many inactivation and removal processes result in a product that is safe. For bacteria, a sterile product is conventionally defined as one having fewer than one infectious organism in one million doses. No comparable figure has been agreed upon for viral sterility because viruses are more difficult to assay in the final product, the titre of virus in the stocks used to spike product is limited, and assessing the ability of a process to remove or inactivate viruses is subject to significant sources of error.

The testing of a final product for viral markers, as part of the routine batch release, has generally been found to contribute little to safety. Still, The regulatory authority requires that serology and NAT testing are done prior to batch release. Commercially available serological tests are generally not designed or validated for use with purified fractions. For most products, the purification process is likely to remove viral antibody or antigen to levels below the limit of

sensitivity of the test, and for immune globulin preparations testing by ELISA typically yields a very high rate of false-positive results because of their high immune globulin content. With respect to genomic tests, NAT testing of plasma pools has proven useful; however, NAT cannot distinguish virus that has survived an inactivation step from inactivated virus, and if infectious virus is present, it is likely to be at very low concentration. Therefore NAT testing of final product is not recommended. Should final product testing be performed, the tests used must be shown to be suitable for their intended purpose.

3.3.4 Measurement of infectivity

The provision of details on the methods used to measure viral infectivity is beyond the scope of this document, and readers are referred to other available guidelines (see references 2–4). A sample final report of a viral inactivation study is given in Appendix 1. A few points to consider are given below.

Care should be taken when preparing virus stocks with high titres to avoid aggregation which may enhance physical removal and decrease inactivation thus distorting the correlation with actual production.

The virus spike should be added to the product in a small volume so as to not dilute or alter the characteristics of the product. Typically, a spike of 5–10% of the total volume is employed.

Buffers and product should be evaluated independently for toxicity or interference in viral infectivity assays used to determine viral titres, as these components may adversely affect the indicator cells. If the solutions are toxic to the indicator cells, dilution, adjustment of the pH, dialysis of the buffer, or other steps to eliminate toxicity or interference will be necessary. Sufficient controls to demonstrate the effect of procedures used solely to prepare the sample for assay (e.g. dialysis and storage) on the removal or inactivation of the spiking virus should be included.

If samples are frozen prior to assay, sufficient controls need to be run to show that the freeze/thaw cycle does not affect virus infectivity. Inactivating agents should be removed prior to freezing.

The reliability of the viral assays employed needs to be demonstrated. This may necessitate repeat runs of the experiment with or without slight changes in conditions to evaluate the robustness of the procedure, and use of viral assay systems of appropriate statistical

reliability. A well-controlled in vitro virus assay should have a within-assay 95% confidence interval of plus or minus 0.5 log 10.

4. Review of Well-recognized Methods for Viral Inactivation and Removal

The methods described in this section are generally recognized as contributing substantially to viral safety based on the following factors:

- Their application to a variety of products;
- Use by several manufacturers;
- The availability of a substantial body of preclinical and clinical information; and
- Their robust nature.

Well-recognized methods of inactivation (pasteurization, dry heat, vapour heat, solvent/detergent and low pH) are described in section 4.1, and well recognized methods of removal (precipitation, chromatography and nanofiltration) are described in section 4.2. The selection of the methods to be employed for viral inactivation and removal depends on the size and liability of the protein being prepared, the method(s) of purification the manufacturer wishes to use, and the nature and titre of the viruses of concern. Each method of inactivation and removal has special characteristics that need to be taken into account. For example, solvent/detergent is very effective against enveloped viruses, but does not inactivate non-enveloped viruses. If HBV is a principal concern, solvent/detergent may have an advantage over methods that employ heating because HBV is known to be relatively heat stable. On the other hand, several methods of heating have been shown to inactivate 4 logs or more of HAV; therefore if HAV is the virus of concern, heat has an advantage over solvent/ detergent. As mentioned above, from a virus safety perspective, the best procedures will use a combination of complementary methods because combinations have the advantage of increasing the spectrum of viruses covered as well as of increasing the total quantity of virus that is eliminated. Whether one or more methods of inactivation and removal are used, the maintenance of protein structure and function is equally as important as viral safety and must be evaluated thoroughly. The general characteristics of well-recognized methods of inactivation and removal are listed in Tables 6a and 6b, and examples of the successful application of individual, dedicated viral inactivation and removal procedures to commercialized products are provided in Table 6c. Subsequent sections provide representative data applicable to a variety of products; nonetheless, manufacturers are obligated to evaluate virus inactivation and removal in each of their products.

4.1 Methods of inactivation

4.1.1 Pasteurization of albumin

Albumin solutions are heated as a liquid at $60\pm0.5^{\circ}\text{C}$ for 10–11 hours continuously, usually following sterile filtration and dispensing into final containers (glass vials). If pasteurization is conducted before filling, care must be taken to prevent post-treatment contamination, and bacterial sterility may be compromised. To prevent denaturation of albumin, low concentrations of sodium caprylate alone or with N-acetyl tryptophan are added prior to sterile filtration. Safety with respect to hepatitis viruses and HIV has been demonstrated for decades, with few exceptions (11). Much of this history derives from albumin manufactured using cold ethanol fractionation, which also contributes to safety. The inactivation of model viruses added to 5% albumin solution on heating at 60°C is shown in Figure 1. Infectious virus can no longer be detected after 10 minutes of treatment. Because the conditions of treatment are well established and, in some countries, specified by regulation, manufacturers are not required to validate the effectiveness of the treatment itself; however, they need to demonstrate that the process parameters of temperature and time are met. Homogeneity of temperature is typically achieved by total immersion of the vials in a water-bath or by placing them in a forced-air oven. In both cases, temperature-mapping studies are required to demonstrate homogeneity, including measurements of both the temperature of the water or air and of the product itself. These studies must be performed with representative loads. Once validated, temperature probes are placed at strategic points in the water-bath or oven during each pasteurization run. Albumin used to stabilize other parenteral drugs should conform to the same requirements as albumin for therapeutic use.

Table 6a
Characteristics of well recognized virus inactivation procedures

Treatment	Advantages	Points to consider	Most relevant properties to be recorded
Pasteurization	- Inactivates both enveloped and some non-enveloped viruses including HAV - Relatively simple equipment	- Protein stabilizers may also protect viruses - HBV is relatively heat stable - Does not inactivate parvovirus 819 - Process validation required	- Temperature - Temperature homogeneity - Duration - Stabilizer concentration - Freeze cycle - Lyophilization cycle - Temperature homogeneity - Residual moisture
Terminal dry heat	- Inactivates both enveloped and some non-enveloped viruses including HAV - Treatment applied on the final container	- At least 120°C usually required for elimination of hepatitis viruses - Does not inactivate parvovirus 819 - Requires strict control of moisture content - Freezing and lyophilization conditions require extensive validation	- Freeze cycle - Initial lyophilization cycle - Temperature homogeneity - Moisture before and after heating - Temperature - Duration - Reagent concentration
Vapour heat	Inactivates both enveloped and some non-enveloped viruses including HAV	- Does not inactivate parvovirus 819 - Freezing and lyophilization conditions require extensive validation - Relatively complex to implement	- Freeze cycle - Initial lyophilization cycle - Temperature homogeneity - Moisture before and after heating - Temperature - Duration - Reagent concentration
Solvent/detergent	- Very efficient against enveloped viruses - Does not denature proteins - High process recovery - Relatively simple equipment	- Non-enveloped viruses unaffected - Not generally affected by buffers used - Solvent/detergent reagents must be removed	- Temperature - Duration - Reagent concentration
Acid pH	- Effective against enveloped viruses - Relatively simple equipment	- Limited efficacy against non-enveloped viruses - Use largely restricted to IgG - At pH 4, effective virus kill requires elevated temperatures - Process validation required	- pH - Temperature - Duration

I-IAV, hepatitis A virus; HBV, hepatitis B virus.

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Table 6b

Characteristics of well recogized virus removal procedures			
Treatment	Advantages	Points to consider	tvbst relevant properties to be recorded
Precipitation	• Purifies protein • Can be effective against both enveloped and non-enveloped viruses including HAV and parvovirus 819	• Virus removal usually modest • Difficuk to model	• Concentration of precipitation agent(s) • A-otein concentration. pH. and possibly onic strength . • Temperature • Timing for the addition of p-ecipitation agent and for precipitate ageing • Degree of contamination of precipitate w•h supernatant (or vice versa)
Chromatography	• Purifies protein • Can be effective against both enveloped and non-enveloped viruses including HAV and parvovirus 819	• Virus removal highly dependent on choice of resin, p-otein solution and buffers • May be highly variable from one virus to another or virus removal may • Degree change as resin ages • Resin must be sanitized between lots	• Resin packing by e.g. HETP • Protein elution profile • Flow rate and buffer volumes • Number of cycles of resin use
Nanofiltration	• Effective against enveloped viruses • Can be effective against non-enveloped viruses including HAV and parvovirus 819 • Does not denature proteins • High recovery of "smaller" proteins such as coagulation factor IX • Risk of downstream contamination limited when performed just prior to aseptic filling	• Degree of virus removal depends on the pore size of filter used • Elimination of small viruses may be incomplete • Fiker defects may not be detected by integrity testing	• Pressure • Flow-rate • Filter integrity • Protein concentration • Ratio of product volume to filter surface area

HAV, Hepatitis A virus: HETP, light-equivalent the o etia l plates.



Table 6c

Established applications of dedicated viral inactivation and removal procedures to marketed plasma products

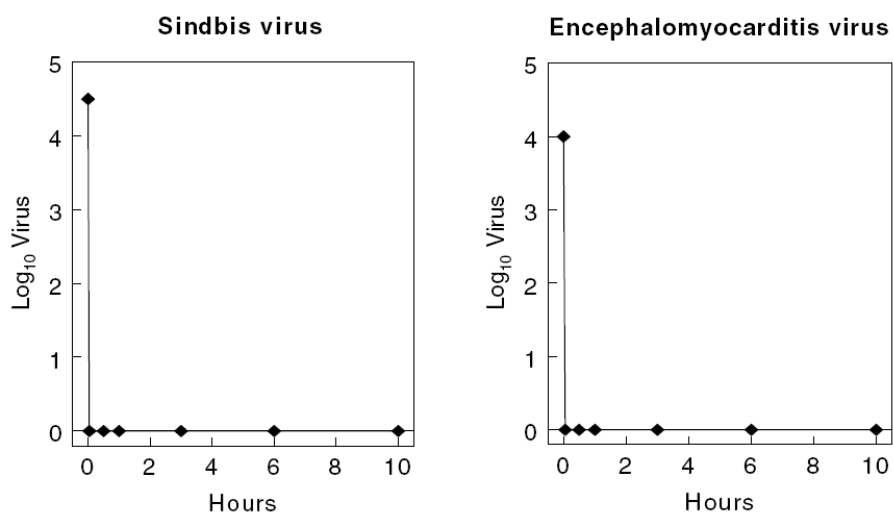
Treatment	Product type
In-process	
Solvent/detergent treatment	• IgG • Coagulation factors (e.g. factor VIII, factor IX, prothrombin complex, fibrin sealant) • Protease inhibitors (e.g. antithrombin III) • Plasma
Pasteurization	• IgG • Coagulation factors (e.g. factor VIII, factor IX, von Willebrand factor, prothrombin complex, fibrin sealant) • Protease inhibitors (e.g. antithrombin III and alpha-1-proteinase inhibitor)
Steam-treatment	• Coagulation factors (e.g. factor VIII, factor IX, fibrin sealant) • Protease inhibitors (e.g. C1-inhibitor)
Incubation at pH 4	• IgG
Nanofiltration (35 nm or less)	• IgG • Coagulation factors (e.g. factor VIII, factor IX, von Willebrand factor, prothrombin complex) • Protease inhibitors (e.g. antithrombin III)
Terminal (final container)	
Terminal pasteurization	• Albumin
Terminal dry-heat treatment	• Coagulation factors (e.g. factor VIII, factor IX and factor XI)

4.1.2 Pasteurization of other protein solutions

Most proteins denature when heated in solution at 60°C. To maintain the biological function of the more labile proteins, general stabilizers such as amino acids, sugars or citrate are added. Because these may also stabilize viruses, virus inactivation procedures need to be validated in model studies for each product under the conditions of treatment specified by the manufacturer. Following pasteurization, the stabilizers usually need to be removed. This is typically accomplished by diafiltration, size exclusion chromatography, or positive adsorption chromatography where the protein of interest binds to a chromatographic resin. Pasteurization has been used successfully with a variety of plasma protein products including coagulation factors and immune globulin solutions, although in rare instances transmission of HBV has been reported (13). A common method of preparing factor VIII is to heat it at 60°C for 10 hours in the presence of high concentrations of glycine and sucrose or selected salts. Published results showing the extent and rate of virus inactivation of blood coagulation factor VIII are illustrated in Figures 2 and 3. Prior to heating, the solution is typically filtered through a 1µm or finer filter to eliminate particles that might entrap and further stabilize viruses. Heating is conducted in a jacketed tank and the solution is usually stirred throughout the heating cycle. Temperature-mapping studies are conducted to ensure that the temperatures at all points in the tank are within the range specified by the process record. Care must be taken to ensure that all parts of the tank, including the lid, where solution might splash, are heated. Viral inactivation studies, conducted under worst-case conditions, are performed at the lowest temperature that might be encountered in an acceptable production run. Protein recovery should be monitored during virus inactivation studies and should be comparable to that achieved at scale.

Figure 1

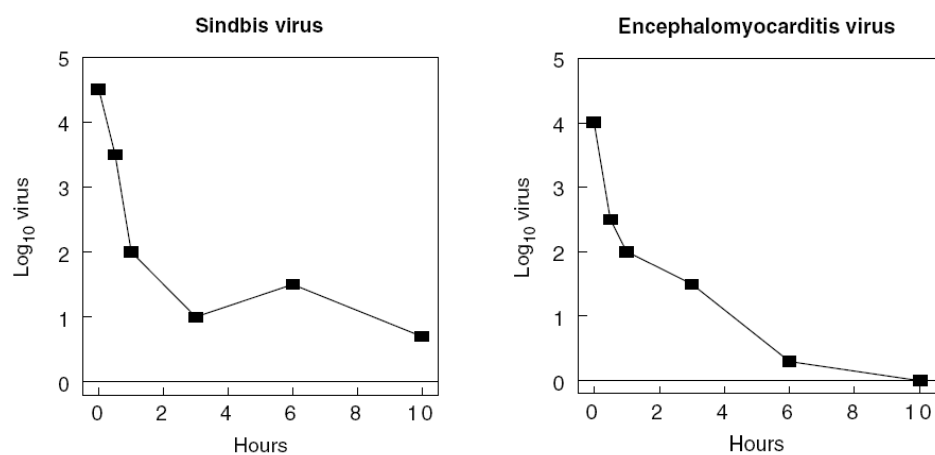
Rate of virus inactivation on pasteurization of 5% albumin at 60°C¹



¹ On this and other similar graphs, "0" indicates that no infectious virus was detected.
Source: Horowitz et al. (12).

Figure 2

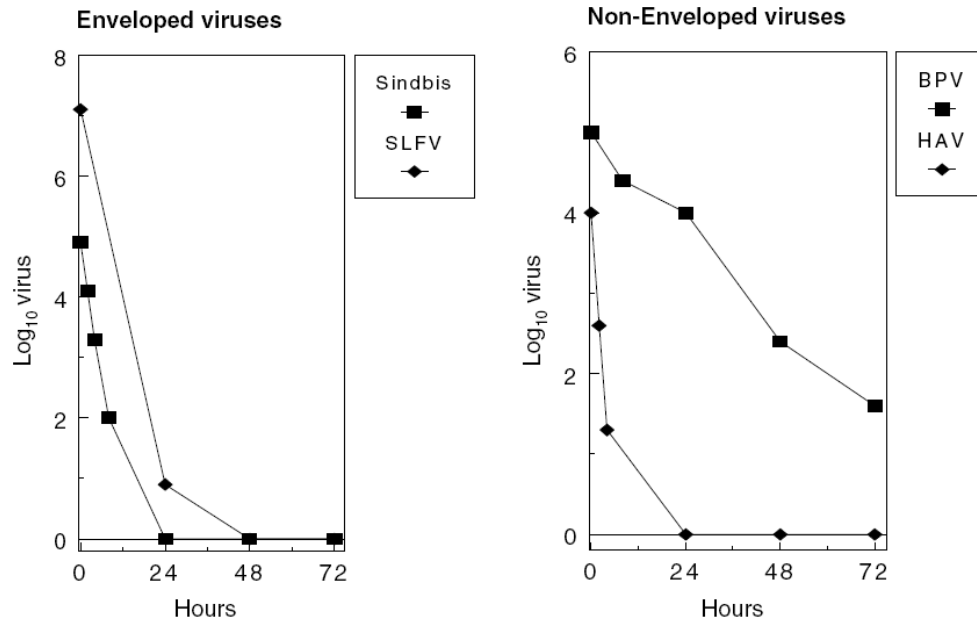
Rate of virus inactivation on pasteurization of blood coagulation factor VIII at 60°C in the presence of 50% sucrose and 2.75M glycine



Source: Horowitz et al. (12).

Figure 3

Rate of inactivation of enveloped and non-enveloped viruses during dry-heat treatment of blood coagulation factor VIII at 80 °C



BPV, bovine parvovirus; HAV, hepatitis A virus; SLFV, Semliki forest virus.
Results generously provided by the Scottish National Blood Transfusion Service.

Table 7

Treatment of a solution of blood coagulation factor VIII by pasteurization

Virus	Extent of inactivation (log ID ₅₀)	Inactivation time (hours required)
Human immunodeficiency virus	≥5.0	1.0
Cytomegalovirus	≥6.0	8
Epstein–Barr virus	≥3.3	0.5
Herpes simplex virus	≥5.9	4
Poliovirus	≥7.1	10
Vaccinia virus	6.2	10

Source: Hilfenhaus, et al. (14).

4.1.3 Heating of dry (lyophilized) products

Proteins can withstand being heated at temperatures of 60–80°C or higher when they are first lyophilized to remove water. Heating at 60– 68°C for up to 72 hours has generally not been found to eliminate hepatitis transmission (15), whereas heating at 80°C has produced favourable results with respect to transmission of HBV, HCV, HIV and HAV. (16) Recently, at least one manufacturer has been treating its coagulation factor VIII with solvent/detergent and also heats final product for 30 minutes at 100°C. All HAV (≥ 5 logs) was inactivated within 4 minutes (17). Since viruses may be more stable following lyophilization, virus inactivation needs to be validated for each product under the conditions of treatment specified by the manufacturer. Viral inactivation is influenced by residual moisture, the formulation (e.g. content of protein, sugars, salts and amino acids), and by the freezing and lyophilization cycles. Residual moisture is influenced by the lyophilization cycle and may be introduced inadvertently by the rubber stoppers.

Since virus inactivation is very sensitive to residual moisture content, the setting of upper and lower limits for moisture should be based on viral validation studies, and the variation of moisture content between vials should be within the limits set. To ensure reproducibility, one manufacturer has stipulated that, during the freeze-drying process, the temperature in three or more product vials, the shelf coolant temperature and the chamber pressure must remain within defined limits for each timed phase of the lyophilization cycle for every batch manufactured. Following freeze-drying, vials are stoppered under sterile, dry nitrogen at atmospheric pressure to ensure a constant atmosphere from vial-to-vial during dry-heat treatment. In addition, from every lyophilization run, the residual moisture content of five vials out of a lot of 1500 is measured following heat treatment. The moisture contents of these vials are used to calculate the 95% confidence interval for the batch, and this interval must be within the upper and lower limits of moisture defined for the product. Again using the specifications of one manufacturer, the dry-heat treatment, itself, is performed at $80.25 \pm 0.75^\circ\text{C}$ for 72 hours. Process monitoring during heat treatment is carried out by means of temperature sensors located in 10 vials distributed throughout the load and two “air” probes located at the previously determined warmest and coldest points in the oven. All temperature sensors (both those in the vials and those measuring air temperature) must reach 79.5°C before the cycle timer starts. Temperatures recorded by all sensors should remain stable between 79.5°C and 81°C for a continuous period of 72 hours. In addition, the dry-heat ovens are validated every 6 months, when a further 12 independent probes (10 in vials and two “air”

probes) linked to a separate chart recorder are included to increase the temperature coverage to 24 points. In this way the temperature control is tested and the temperature spread within the cabinets established. The cycle time on the automatic control is also checked for accuracy. Typical results achieved by heating factor VIII at 80°C are given in Table 8 and Figure 3.

Table 8

Treatment of lyophilized blood coagulation factor VIII at 80°C for 72 hours

Virus	Extent of inactivation (log ID ₅₀)	Inactivation time (hours required)
Sindbis virus	8	72
Human immunodeficiency virus	≥6.4	72
Vaccinia virus	2.6–3.3	72
Herpes simplex virus	2.2	48
Semliki forest virus	≥6.9	24
Hepatitis A virus	≥4.3	24
Canine parvovirus	≥2.1	48

Sources: Knevelman et al. (18) Winkelman et al. (19) and Hart et al. (20).

Table 9

Virus inactivation by Vapour heating at 60°C for 10 hours

Product	Virus	Extent of inactivation (log ID ₅₀)	Inactivation time (hours required)
Factor VIII: intermediate purity	HAV	>3.3	8
	HIV	>6.8	10
	PRV	5.9	10
Factor VIII: high purity	HAV	3.9	10
	HIV	6.7	10
	PRV	5.6	10
Factor IX: intermediate purity	HAV	>5.7	6
	HIV	>6.5	6
	PRV	>7.1	8
Factor IX: high purity	HAV	>6.7	3
	HIV	>7.9	8
	PRV	>6.8	8

Data and process information provided courtesy of Baxter/Immuno. See also Barrett et al. (24) and Dorner and Barrett (25)
HAV, Hepatitis virus A; HIV, Human immunodeficiency virus; PRV, pseudorabies virus.

4.1.4 Heating of lyophilized products under humidified conditions (vapour heating)

At equivalent temperatures, a higher level of virus inactivation can be achieved by the addition of water vapour before initiating the heat cycle. To assure proper application of this approach, the material to be heated, the addition of moisture and the heat cycle need to be tightly controlled. In one case, freeze-dried intermediate bulk product is homogenized by a combination of sieving and milling. After determination of the residual water content, the freeze-dried intermediate is transferred into a stainless steel tank where an amount of water vapour, that has been predetermined based on the weight and the residual water content of the lyophilized product, is slowly added to adjust the water content to 7–8% (w/w). After an equilibration period, the water content is measured again before the product is ready for vapour heating. The intermediate product is transferred to a stainless steel cylinder. The cylinder is flushed with dry nitrogen to remove oxygen, and a pressure test is performed to ensure that the cylinder is airtight. This cylinder is then transferred to a heating cabinet equipped with an electric heater and a fan to ensure even temperature distribution. The intermediate product within the cylinder is heated according to the temperature regimen specified for the particular product. The cylinder is subjected to an oscillating rotation, changing direction every half-turn, until the end of vapour heating. During the heating process the pressure inside the vessel rises due to heating of the enclosed nitrogen, which cannot expand in the closed cylinder, and also due to evaporating water vapour from the moist intermediate product. After vapour heating, the heating cabinet is opened from the other side, and the product is further processed in a different and isolated manufacturing zone to prevent cross-contamination from non-inactivated product.

To assure consistency from lot-to-lot, the ranges for protein, salt and water content are set on the basis of the results of preliminary viral infectivity and protein functional studies. Additionally, the ratio of product weight to cylinder volume is specified for each product. A pressure test is performed before the start of vapour heating to ensure that the cylinder is airtight. During heating, product temperature and air temperature (one temperature sensor each) and pressure within the cylinder are measured continuously and must conform to the specifications set for each. Following vapour heating, the water content of the intermediate is measured again.

Although historical reports indicate some cases of transmission of enveloped virus (21, 22), the preponderance of clinical data indicate safety with respect to transmission hepatitis viruses and HIV. (23) It should be noted that some products are heated at 60°C for 10 hours and

others are additionally heated at 80°C for 1 hour; however, this cannot be considered as the use of two independent steps and the viral kill observed cannot be summed. Typical results achieved by vapour heating are given for several products in Table 9.

4.1.5 Solvent/detergent treatment

Organic solvent/detergent mixtures disrupt the lipid membrane of enveloped viruses. Once disrupted, the virus can no longer bind to and infect cells. Non-enveloped viruses are not inactivated. The conditions typically used are 0.3% tri(n-butyl) phosphate (TNBP) and 1% nonionic detergent, either Tween 80 or Triton X-100, at 24°C for a minimum of 4 hours with Triton X-100, or 6 hours with Tween 80. When using TNBP and Triton X-100, some preparations can be treated successfully at 4°C. Since high lipid content can adversely affect virus inactivation, the final selection of treatment conditions must be based on studies demonstrating virus inactivation under worst-case conditions; i.e. lowest permitted temperature and reagent concentration and the highest permitted product concentration. Prior to treatment, solutions are filtered through a 1 µm filter to eliminate virus entrapped in particles. Alternatively, if filtration is performed after addition of the reagents, the process should be demonstrated to not alter the levels of solvent and detergent added. The solution is stirred gently throughout the incubation period. When implementing the process in a manufacturing environment, physical validation should be used to confirm that mixing achieves a homogeneous solution and that the target temperature is maintained throughout the designated incubation period. Mixture homogeneity is best verified by measuring the concentrations of TNBP or detergent at different locations within the tank, although measuring dye distribution might be an acceptable substitute. To ensure that every droplet-containing virus comes into contact with the reagents, an initial incubation for 30–60 minutes is typically conducted in one tank after which the solution is transferred into a second tank where the remainder of the incubation takes place.

In this manner, any droplet on the lid or a surface of the first tank that might not have come into contact with the solvent/detergent reagents is excluded. The use of a static mixer where reagents and plasma product are mixed before being added to the tank is an acceptable alternative. The tank in which viral inactivation is completed is located in a separate room in order to limit the opportunity for post-treatment contamination. This room typically has its own dedicated equipment and may have its own air supply. When the treatment is complete, the solvent/detergent reagents must be removed. This is usually accomplished by extraction

with 5% vegetable oil, positive adsorption chromatography (where the protein of interest binds to a chromatographic resin), or adsorption of the reagents on a C-18 hydrophobic resin. Depending on the volume of product infused and the frequency of infusion, the permitted residual levels of TNBP, Tween 80 and Triton X-100 are generally, 3–25, 10–100 and 3–25 ppm, respectively.

When performing viral validation studies, the reaction is stopped either by dilution or, in some cases, adsorption of the TNBP and Triton X-100 by a C18 hydrophobic resin. An appropriate control needs to be run to establish that virus inactivation does not continue following the use of the stop procedure. Safety with respect to HBV, HCV and HIV has been demonstrated in numerous clinical studies that reflect the high level of virus inactivation demonstrated in both laboratory and chimpanzee studies. Typical results achieved on treating a coagulation factor VIII concentrate and fibrinogen at 24°C are given in Table 10 and Figure 4.

Table 10

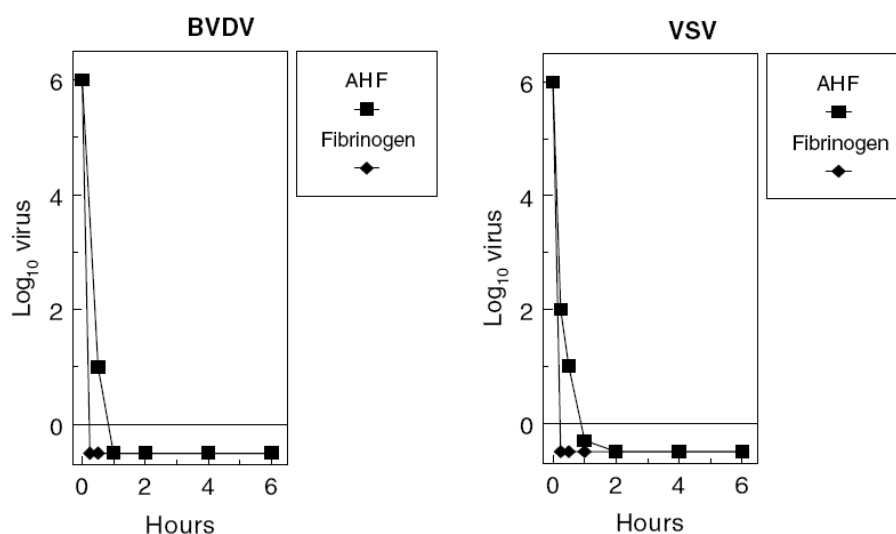
Treatment of blood coagulation factor VIII solution with 0.3% TNBP and Tween 80

Virus	Extent of inactivation (log ID ₅₀)	Inactivation time (hours required)
Vesicular stomatitis virus	≥4.5	2
Sindbis virus	≥5.5	1
Sendai virus	≥6.0	1
Hepatitis B virus	≥6.0	6 ^a
Hepatitis C virus	≥5.0	6 ^a
Hepatitis D virus	≥4.0	6 ^a
Human immunodeficiency virus-1	≥6.0	0.25

Sources: Horowitz (26) and Horowitz et al. (27).

^a These studies were conducted in the chimpanzee model; 6 hours was the only time-point tested.

Figure 4

Treatment of AHF and fibrinogen by solvent/detergent^a

AHF, blood coagulation factor VIII; BVDV, bovine viral diarrhoea virus; TNBP, tri(*n*-butyl) phosphate; VSV, vesicular stomatitis virus.

^a AHF was treated with 0.3% TNBP and 1% Tween 80 at 24 °C and fibrinogen was treated with 0.3% TNBP and 1% Triton X-100 at 24 °C. At the time-points indicated, BVDV and VSV infectivity were measured.

Data provided courtesy of V.I. Technologies.

Table 11

Log reduction factors for four different viruses and for three precipitation steps used during the manufacture of albumin

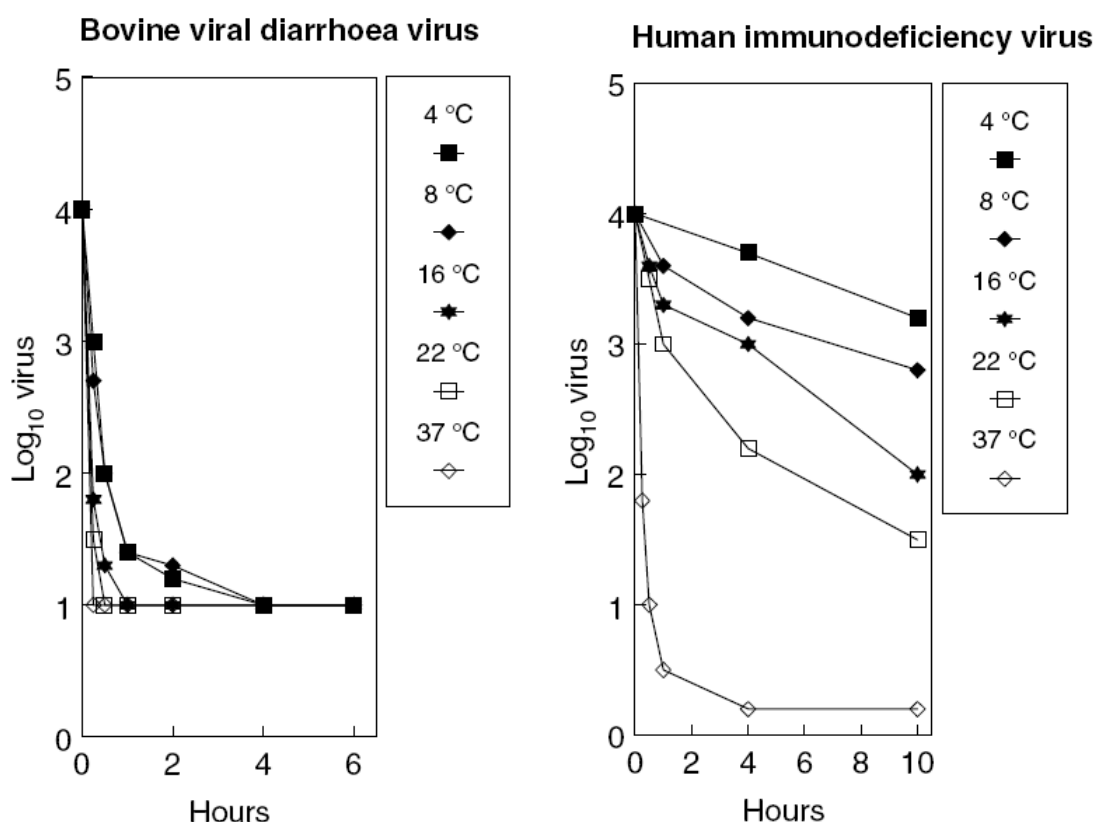
Step	Ethanol %	pH	Log reduction factor			
			HIV	PRV	Sindbis	BEV
Step A	19	5.85	3.3	3.7	4.2	4.2
Step IV	40	5.85	4.4	5.7	5.4	3.6
Step D	10	4.60	0.9	1.7	3.1	1.2

BEV, bovine enterovirus; HIV, human immunodeficiency virus; PRV, pseudorabies virus.

Source: reference 29.

Figure 5

Inactivation of viruses in IgG at pH 4 in the presence of pepsin



Source: Omar et al. (28).

4.1.6 Low pH

Most proteins are damaged by exposure to the acidic conditions needed to kill viruses. For example, few viruses are killed at pH 5.0–5.5, a condition known to inactivate factor VIII. Immune globulin solutions are an exception. Various studies have shown that low pH, such as in the pH 4-treatment used in the preparation of immunoglobulins, inactivates several enveloped viruses. (28) The presence of trace concentrations of pepsin added to reduce anticomplementary activity during this procedure has been shown to contribute little to virus inactivation. Since acid treatment was originally designed to reduce IgG aggregation and anticomplementary activity, a number of variants of this procedure have been developed; hence, the conditions being used may or may not inactivate virus efficiently. Each manufacturer's process needs to be validated separately because virus inactivation is influenced by pH, time, temperature, pepsin content, protein content and solute content. As an example, the effects of time and temperature on the inactivation of BVDV and HIV in one

preparation are given in Figure 5. On the basis of these and other results, one manufacturer incubates its immunoglobulin preparation at pH 4.0 for at least 6 hours at 37°C whereas another follows solvent/detergent treatment by incubating in the container at pH 4.25 for a minimum of 21 days at 20°C.

4.2 Methods of virus removal

Before the 1980s, conditions for the fractionation of plasma were selected largely on the basis of considerations of protein purification and less on the capacity of the process to remove virus. Modern purification procedures frequently consider both protein purification and virus removal. For example, an ion-exchange or monoclonal antibody column may be selected for the degree of protein purification provided, but also be characterized fully with respect to virus removal. Based on this characterization, additional wash buffers or greater volumes of wash buffer may be used to increase the degree of virus removal. Additionally, in the last few years, specific removal methods such as nanofiltration have been developed, and others, such as viral affinity adsorbents, are under development. Such methods are intended to remove viruses. Where virus removal is believed or claimed to be an important consideration for a particular purification step, whether intended or not, the same discipline in validating and implementing that step should be used as is applied to a virus inactivation step.

4.2.1 Precipitation

Precipitation with ethanol is the single most widely used plasma fractionation tool worldwide, although other reagents have been used. In addition to its use as a precipitant, ethanol is also a disinfectant. Unfortunately, it acts as a disinfectant mostly at room temperature or above, whereas plasma fractionation is carried out at a low temperature to avoid protein denaturation. The contribution of ethanol to viral safety through inactivation is, therefore, marginal at best. Nonetheless, ethanol can also partially separate virus from protein. Viruses, as large structures, tend to precipitate at the beginning of the fractionation process when the ethanol concentration is still relatively low. As with any other precipitation reaction, the distribution of viruses between precipitate and supernatant is never absolute.

The following log reduction factors (LRFs) were reported for three distinct steps in albumin production by cold-ethanol precipitation (Table 11; the designations of the steps correspond to the Kistler/ Nitschmann fractionation scheme) and for the production of immunoglobulin (Table 12). (Note that LRFs should not be summed across steps unless the mechanism of action has been shown to be independent, or other data demonstrate that the summing is legitimate).

Because the result of any precipitation step is a partitioning of components between a solid and a liquid phase it should be borne in mind that, in the absence of inactivation, fractionation results in distribution of viruses between these phases. Therefore, if viruses are indeed removed from one fraction, the bulk of virus will be found in another fraction, which may or may not be that used for making the final product. Many manufacturers separate the precipitated proteins by centrifugation whereas others have introduced filtration as an alternative. To prevent clogging of the filters, filtration is carried out using filter aids. Because these substances (diatomaceous earth or similar products) may also adsorb virus, it is often possible to remove more of the viral infectivity from the supernatant than would be expected based on precipitation alone. This may also explain some of the discrepancies found in the literature. Some authors concluded that BVDV, as a model for HCV, was not removed to any significant extent by Cohn–Oncley fractionation, (31) whereas others found substantial partitioning in several steps of cold-ethanol fractionation when separation was carried out in the presence of filter aids, as shown for one step in Table 13.

When virus inactivation steps are implemented, it is usually relatively easy to ensure that every drop of a large batch is treated in exactly the same way, e.g. by thorough mixing or by transfer of the whole volume from one tank to another (see above). This is much more difficult to achieve for precipitation; the first volumes that come into contact with a filter press encounter an environment that is quite different from that encountered by the last volumes of the same batch. Although it is probable that these changes occur in a reproducible way in each batch, this could be difficult to prove. Similarly, model experiments are relatively easy to perform in a homogeneous system, as may be the case during chemical or physical inactivation. However, large scale centrifugation is usually done in continuous-flow machines and although this could be reduced in size to laboratory scale, parameters such as path lengths and residence times are unlikely to be the same. Filtration is not any easier to model on a small scale. In either case, manufacturers need to show with carefully selected parameters (e.g. protein composition and enzyme activity) that both large-scale and small-scale processes achieve the same level of phase separation. Demonstration that the downscaled method provides a similar product to that achieved at full scale is at least as important as the demonstration of virus removal.

In spite of all the problems associated with precipitation as a means of removing viruses, ethanol precipitation has proven its value over the years. There can be little doubt that partitioning through precipitation has contributed substantially to the safety of some plasma-

based products, e.g. intravenous and intramuscular immunoglobulins, which have very rarely transmitted viral diseases although until very recently the manufacturing processes for these products did not include a dedicated virus inactivation step. Nonetheless, reliance on virus removal alone is not recommended because small changes in process conditions can affect virus partitioning and safety. As an example, HCV partitioning was modified on introduction of anti-HCV screening, with the result that an IVIG became infectious (33).

Table 12

Log reduction factors for five different viruses and for four precipitation steps used during the manufacture of intravenous immunoglobulin

Step	Ethanol %	pH	Log reduction factor				
			HIV	PRV	Sindbis	SFV	BEV
Step A	19	5.85	4.0	3.6	3.2	3.6	3.4
Step B	12	5.10	5.3	4.7	4.6	2.2	4.1
Step C	25	7.00	4.0	4.7	2.9	3.5	3.8
Step D	—	—	2.2	3.0	1.7	—	2.8

Source: reference 30.

BEV, bovine enterovirus; HIV, human immunodeficiency virus; PRV, pseudorabies virus; SFV, semliki forest virus.

Table 13

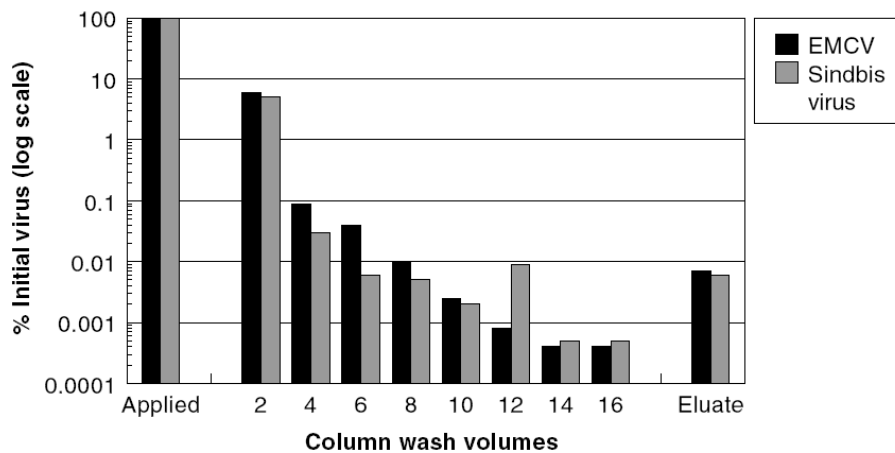
Removal of various viruses from an immunoglobulin solution by filtration in the presence of Celite

Virus	Log reduction factor
Semliki forest virus	3.4
Vesicular stomatitis virus	2.5
Bovine viral diarrhoea virus	3.1
Pseudorabies virus	3.4
Sindbis virus	4.1
Human immunodeficiency virus	5.4
Coxsackie virus	>6
Bovine parvovirus	3.4
Bovine enterovirus	4.1

Source: Omar & Morgenthaler, (32).

Figure 6

The reduction of model viruses during method M immunoaffinity purification of factor VIII^a



EMCV, encephalomyocarditis virus; TNBP, tri (*n*-butyl) phosphate.

^a For EMCV, TNBP and Triton X-100 were present; for Sindbis virus, they were omitted due to the rapid inactivation that would otherwise occur.

Source: Griffith (37).

4.2.2 Chromatography:

Chromatography has been designed to separate closely related molecules; some variants of chromatography, e.g. affinity chromatography, are specific for a single molecular species. The logical expectation would therefore be the chromatography is a good way to physically separate viruses from therapeutic proteins. Both enveloped and non-enveloped viruses can be removed. The log reduction factors are usually of the order of 2–3 for ion exchange chromatography and may reach 5 for very specific steps, e.g. affinity chromatography. However, because viruses can bind to protein or to the resin backbone, success in removing viruses by chromatography is influenced by a number of factors, including column geometry, the composition and flow rate of the buffers used, intermediate wash steps, the protein composition of the preparation and the ageing of the chromatographic resin. All of these factors need to be defined and controlled.

Relatively modest reduction factors were reported for three consecutive chromatographic purification steps used in an albumin isolation scheme. LRFs of <0.3, 0.3 and 1.5 were reported for removal of HBsAg during chromatography on DEAE-Sepharose FF, CM-Sepharose FF and Sephacryl S200 HR, respectively (34). The same group recorded LRFs of 5.3, 1.5 and 4.2 for HAV for the same three steps (35). In another study, the first two

chromatographic steps of the same process were investigated for their potential to remove poliovirus and canine parvovirus from albumin. When the two steps were conducted in sequence, overall LRFs of 5.3 and 1.8 were obtained for poliovirus type 1 and canine parvovirus, respectively (36).

A second and more commonly applied approach is the use of affinity chromatography, frequently antibody-mediated, of the protein of interest. In the preparation of monoclonal antibody-purified factor VIII, approximately $4 \log_{10}$ of EMCV and Sindbis virus were removed. Extensive washing of the column prior to eluting factor VIII contributed to the overall removal factor (Figure 6).

Sanitization of resins and associated chromatography equipment between runs is essential because viruses tend to stick to resins and a complete wash-out is often impossible. Discarding used resin is, for financial reasons, normally not a practical option. Many resins withstand chemical or physical treatments that inactivate viruses. Typical treatments include overnight incubation with 0.1–1N sodium hydroxide or hydrochloric acid, oxidizing conditions such as provided by sodium hypochlorite; very high temperatures, or autoclaving. The selection of a sanitization procedure depends on the column matrix in use. For example, silica backbones are degraded on exposure to alkali, and immobilized antibody used in affinity chromatography can be degraded by harsh chemical treatments (and by enzymes present in the material being purified).

Since sanitization is an essential part of the production process, it must be validated to the same extent as virus inactivation or elimination steps. The aim of the validation is to prove that there is no cross contamination from one batch to the next. If it can be shown convincingly that at least one of the solutions used during the regeneration cycle completely inactivates all relevant viruses under the conditions used during cleaning, validation will be relatively simple and can be limited to demonstrating that the column material and all associated equipment has been exposed to the cleaning solution. However, in most cases, inactivation of certain viruses will be incomplete. In such cases, wash-out of viruses during the sanitization cycle needs to be monitored. If necessary, washing may be prolonged until no more virus is removed from the column. Finally, an attempt should be made to demonstrate that no infectious virus remains on the resin, usually by subjecting it to the next purification cycle. These validation experiments need to be done with fresh resin as well as with resin that has been used for the specified maximum number of cycles.

4.2.3 Nanofiltration

Nanofiltration is a technique that is specifically designed to remove viruses. Simplistically, nanofiltration removes viruses according to their size while permitting flow-through of the desired protein. However, large proteins — particularly those that tend to form aggregates — are as large as or larger than small viruses so nanofiltration cannot be used with all products. Effective removal requires that the pore size of the filter be smaller than the effective diameter of the virus. Filters with a pore size that exceeds the virus diameter may still remove some virus if it is aggregated such as by inclusion in antibody/ antigen or lipid complexes. In reality, nanofiltration is a more complex process. Apart from sieving effects, adsorption of the virus to the filter surface may also contribute to virus removal, though this will be strongly influenced by the intrinsic characteristics of the solution being filtered. Only a careful validation of the down-scaled process with several virus species will reveal the potential of the method for specific applications.

Nanofilters are usually available in many different sizes (surface areas), which makes it easy to increase to production scale and to decrease to laboratory scale for validation experiments. Careful monitoring of the performance of the nanofilters in every run is mandatory. Filter integrity should be ascertained before and after use, and every filter manufacturer offers test methods that have been developed specifically for this purpose. If a filter fails the integrity test after use, the filtration step has to be repeated. So far, nanofilters may be used only once.

Although nanofiltration is a gentle method, proteins are subjected to shear forces that may damage their integrity and functionality. Appropriate tests should be conducted during the development phase to rule out this possibility, keeping in mind that several filters may be used in series.

Membranes with 15 and 35 nm pore size were reported to remove 6–7 log₁₀ of murine xenotropic retrovirus, SV40 and pseudo rabies virus from IgG and IgM solutions (38). Troccoli and coworkers found that all viruses larger than 35 nm spiked into an IVIG-solution were completely removed by cascade filtration through one 75 nm pre-filter, followed by two 35 nm virus removal filters; the pre-filter was used to increase the capacity of the small-pore filters. Even smaller viruses like EMCV, HAV and PPV were removed to a significant extent (LRFs were 4.3, >4.7 and 2.6, respectively). The removal of some small viruses (BPV, Sindbis and SV40) could not be evaluated due to neutralization by cross-reacting antibodies (39). A single dead-end filtration was able to remove HIV, BVDV, PPRV, RT3 and SV40

with LRFs of >5.7 to >7.8, when these viruses were added to high purity factor IX and factor XI concentrates (40). Numerous other studies have also demonstrated the efficiency of virus removal with appropriate membranes, either with model solutions or in the presence of purified plasma proteins. Protein recovery has almost always been reported to be excellent. It should however be borne in mind that the virus stocks used in validation studies may be artifactually aggregated as a consequence of achieving high titres in culture systems or of the concentration methods used.

4.3 Protein issues

When considering processes that inactivate or remove viruses, just as with other manufacturing procedures, manufacturing consistency and the integrity of the final product with respect to protein function and structure must be demonstrated. Several analytical tests are typically applied to in-process samples and to the final product. These almost always include total protein, one or more functional assays for the protein of interest, and an assessment of its aggregation/fragmentation. Additional final product protein assays are occasionally employed depending on the product being manufactured. For example, anticomplementary activity in IVIG and activated coagulation factor activity in prothrombin complex concentrate are usually measured in every production lot, while the *in vivo* measurement of the thrombogenic potential of prothrombin complex concentrates is usually assessed during process development.

4.3.1 Measurements of protein structure

Depending on the experience with the process methods being employed, laboratory and animal studies such as those described below can be of value for characterizing products under development.

Electrophoresis is a fast and easy method for evaluating the overall integrity of a protein. Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS–PAGE) is particularly useful for analyzing protein composition, aggregation and fragmentation. The procedure separates proteins approximately according to their relative molecular mass, although protein shape and glycosylation can affect migration. Under nonreducing conditions, disulfide-linked protein chains usually remain together. For instance, under nonreducing conditions, immunoglobulins migrate as a single molecule with a relative molecular mass of approximately 160 kD while under reducing conditions, the chains that were linked by disulfide bridges fall apart,

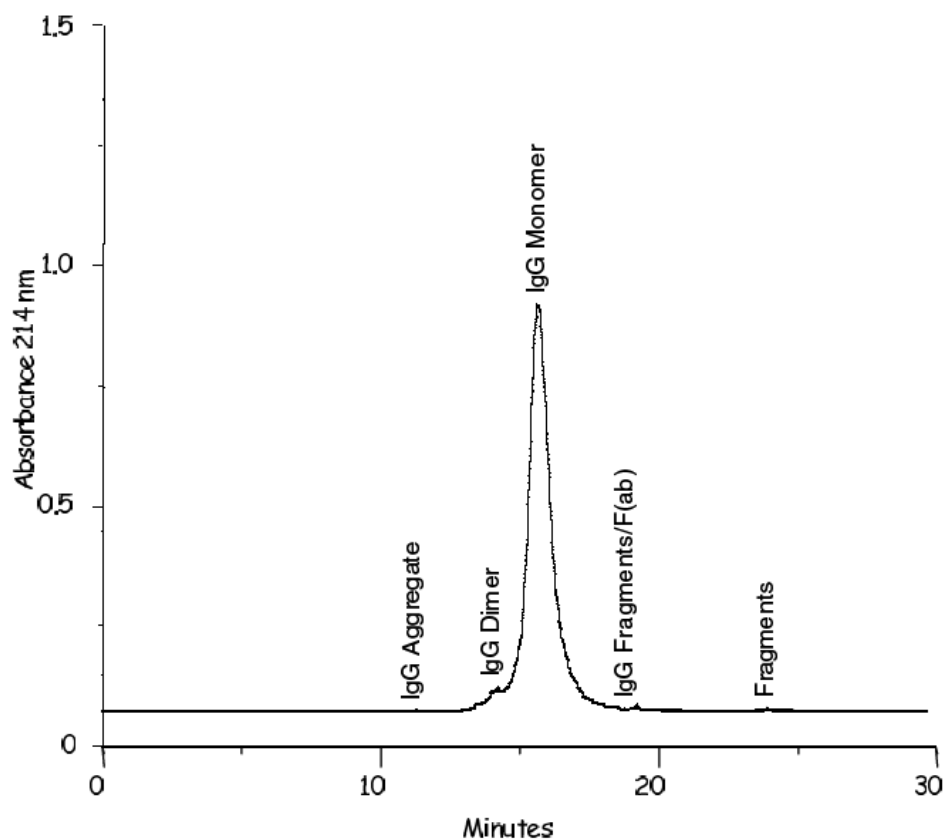
producing two bands with approximate relative molecular masses of 50kD (heavy chain) and 25 kD (light chain). Cleavage in the primary sequence of protein is usually easily detected. SDS-PAGE will not normally reveal changes in higher-order structures or covalent modifications of amino acids.

Capillary electrophoresis has recently been introduced as an adjunct method to PAGE. Although it is more amenable to automation, and therefore useful for high-throughput analyses, it does not yield substantially more information than PAGE and requires sophisticated equipment.

Size-exclusion gel chromatography separates proteins according to their overall size and shape. The use of size-exclusion high performance liquid chromatography (SE-HPLC) allows rapid analysis and high resolution of protein components and also better reproducibility than that obtained using conventional gel permeation chromatography. Fragmentation and/or aggregation of plasma proteins are usually easily demonstrated and quantifiable and gross modifications to the molecular shape of the protein may also be detectable. More subtle changes may not be detected, and the method is insensitive to chemical modifications of amino acids. An example of SE-HPLC analysis of IVIG is given in Figure 8.

Isoelectric focusing separates proteins according to their isoelectric point. Separation is normally performed in the presence of a supporting matrix (e.g. polyacrylamide), and the proteins may be subjected to this method either in a native or denatured state. As covalent modification of amino acids usually changes their electric charge, it also affects their isoelectric point and therefore the protein's position in an isoelectric focusing gel. Isoelectric focusing combined with PAGE is a very powerful tool for the detection of even small differences in the product consists primarily of IgG monomers with small amounts of dimer and trace amounts of fragments and aggregates. Figure reproduced with the kind permission of R. Thorpe, National Institute of Biological Standards and Control, England. Protein structures and properties, although the gel patterns can be very complex depending on the purity of the sample.

Figure 8
SE-HPLC of an intravenous immunoglobulin product showing the expected distribution of molecular species^a



^a The product consists primarily of IgG monomers with small amounts of dimer and trace amounts of fragments and aggregates.
 Figure reproduced with the kind permission of R. Thorpe, National Institute of Biological Standards and Control, England.

Antigen/activity ratio. During process qualification, it may be useful to measure protein functional activity and antigen concentration simultaneously in an immunoassay. A constant ratio of activity to antigen during the isolation process and before and after virus inactivation provides evidence that protein structure was not affected while a decline in this ratio is indicative of detrimental effects.

Neoimmunogenicity may be regarded as a special case of changes to the higher-order structure of proteins, which do not necessarily impair the protein's functionality, but result in an immune response in the recipient. Products made with current methods of viral inactivation and removal do not generally stimulate an antibody response in humans. There are, however, two documented instances (one involving a pasteurized product and the other a product treated with solvent/detergent combined with pasteurization) where treated products had unexpected immunogenicity and were therefore withdrawn from the market (41–43).

The detection of neoimmunogenicity preclinically is very difficult, and several animal models have been developed. One approach is to immunize one group of laboratory animals (e.g. rabbits) with the preparation to be tested and another group with the same preparation in which the viral inactivation step has been omitted, or a similar preparation with proven lack of neoimmunogenicity. The resulting antisera are compared with one another in a cross-over experiment; if the antibodies raised against the new preparation are completely adsorbed by the old preparation, the preparation under test is not likely to contain neoantigens. However, these experiments have to be conducted in a heterologous system and there is no guarantee that the human immune system recognizes the same epitopes as those recognized by the immune systems of laboratory animals.

Because the models are not believed to adequately predict human response, animal neoimmunogenicity studies are not generally required for products manufactured using well recognized techniques for viral inactivation and removal. If the manufacturing conditions differ substantially from well recognized treatment conditions, such as the use of a higher temperature than that normally employed during solvent/detergent or heat treatments, new combinations of treatments, or the use of a new method of virus inactivation and removal, then animal neoimmunogenicity studies using one of the available models should precede first use of the product in humans.

The best proof of absence of neoantigens is derived from careful clinical studies involving a number of patients determined on a case-by-case basis. The determination of circulatory recovery and half-life in repeatedly infused subjects can be very useful and such measurements are typically made prior to licensure. A full assessment of immunogenicity is best monitored over the long term and is, therefore, typically monitored in humans following licensure of the product. This has proven to be especially important in the care of patients with haemophilia, and recommendations for the conduct of such studies have been published (44). If there is no increase in the appearance of clinically relevant antibodies or of other adverse immunological reactions in patients (as compared with the incidence expected from earlier studies, when available), it is reasonable to assume that the newly developed product does not exhibit neoantigens.

The following are not usually applied to well-established procedures, products and processes, but may prove valuable with new viral inactivation and removal procedures.

Amino acid analysis determines the overall quantitative composition of a protein. It may help to detect changes that were inadvertently introduced, e.g. covalent modifications of amino acids.

Amino acid terminus analysis may identify changes in the covalent structure of proteins because cleavage of protein chains may produce new terminal sequences that can be identified and located by alignment with the native sequence (if it is known) or when comparing pretreatment to post-treatment samples.

Cleavage with proteolytic enzymes can be used to assess protein integrity because denatured proteins or proteins with altered conformation often contain new sites that are now recognized by sequence-specific proteases. Comparing the fragmentation patterns produced by addition of proteolytic enzyme(s) before and after virus inactivation and removal may give clues to subtle changes that have occurred during treatment. The degradation patterns may be analysed by several of the methods already mentioned such as SDS-PAGE and size-exclusion gel chromatography (45).

Circulatory survival may be considered as an *in vivo* variant of using proteolytic enzymes, albeit a difficult, time-consuming and expensive variant. It is carried out by injecting the protein intravenously into a suitable animal (e.g. rat or rabbit) and comparing the half-life with that of a reference preparation of the same protein, possibly with the protein in its native state, i.e. in plasma. The Kinetics of removal of a foreign protein from circulation are quite sensitive to minor changes in protein structure. As a demonstration of the utility of this method, the circulatory half-life of human albumin prepared by standard procedures was shown to be unaltered whereas that of chemically modified albumin was halved (46). Analogous experiments performed with virally inactivated (by solvent/detergent) immunoglobulins and coagulation factors demonstrated no change from historical controls (47).

Other methods that could be indicative of overall changes in shape include measurements of sedimentation and diffusion coefficients, viscosity, circular dichroism and optical rotatory dispersion. Most of these methods are difficult and slow to perform. They are of limited value because their results are hard to interpret and can only be evaluated by comparison with a difficult to establish standard preparation of the same protein.

4.3.2 Final product characterization

The specifications for many plasma products are provided in several pharmacopoeias, national regulations of the GCC requirements for the collection, processing and quality control of blood, blood components and plasma derivatives (48). Common tests that are generally considered in the characterization of plasma derivatives in final product are listed in Figure 9. Tests to be conducted on each final bulk solution or filling lot should comply with methods and specifications approved by the national regulatory authority and international pharmacopial monographs.

Figure 9
Tests commonly applied to final product characterization

Albumin

- Protein composition (albumin content)
- Molecular size distribution (polymers, aggregates)

Normal and specific immunoglobulins

Intramuscular administration

- Protein composition (IgG content)
- Molecular size distribution (aggregates, dimers, monomers, fragments)
- Potency tests of antibody reactivity against selected antigens

Intravenous administration

- Protein composition (IgG content)
- Molecular size distribution (aggregates, dimers, monomers, fragments)
- Anti-complementary activity
- Potency tests of antibody reactivity against selected antigens

Coagulation factor concentrates

Anti-haemophilic factor

- Factor VIII coagulant activity
- von Willebrand factor activity (if required)

Prothrombin complex / factor IX

- Factor IX coagulant activity
- Factor II, VII, X coagulant activities
- Measurement of activated clotting factors

Tests common to all products

- Total protein
- Moisture and solubility (if lyophilized)
- pH

4.3.3 Stability assessment

The purpose of stability studies is to prove that a product remains stable, safe and efficacious during the shelf-life that is claimed for it by the manufacturer. A set of relevant parameters has to be chosen and measured at regular, predefined intervals. These parameters will include measures of potency as well as indicators of protein integrity. Limits for these parameters that may not be exceeded are generally predefined.

True stability tests can only be conducted in real time. Since most plasma-protein products have a shelf-life of 2–3 years, stability tests are usually incomplete at the time of licensure. Real-time stability studies need to be done under worst-case conditions. For example, if the storage conditions for a particular product are specified to be within a temperature range of 2–8°C, the minimum testing would be carried out within the specified temperature range and at some other higher temperature.

To obtain an indication of product stability before the data from real time stability studies are available, it is possible to conduct accelerated stability studies (49, 50). For these, the product is exposed to more severe conditions than are normally expected to be encountered during routine storage and shipping, e.g. higher temperatures, and stability is assessed over a shorter period than that used for the real time study. The data can be used to predict stability under the prescribed storage conditions, but cannot replace real-time studies because predictions from accelerated studies do not always correlate with what occurs during real time. Other stress factors that are often incorporated into an accelerated stability test include humidity, light, mechanical stress (shaking) and combinations of these. Parameters that are identified as critical during accelerated testing will receive particular attention during real-time testing.

In both accelerated and real-time studies, time points need to be chosen such that both transient and permanent deviations from the initial value will be recognized. Should the predefined limits for any parameter be exceeded, a reconsideration of the storage conditions will be unavoidable.

4.4 Clinical trials to assess safety

Historically, the role of clinical trials has been to assess efficacy, both general and viral safety, and immunogenicity. Trial design for established products is a subject of considerable discussion with an overall trend towards simplifying design and reducing the number of patients required. Viral safety is assessed principally by the review of donor demographics, test procedures and process validation. Within the EU, there is a trend towards assessment of

viral safety in humans after, rather than before, receipt of marketing authorization (post-marketing surveillance). This trend takes into account the safety exhibited by current products, recent reductions in viral loads, the universal use of well validated methods of virus removal and inactivation, and the relative insensitivity of small clinical trials.

Special circumstances in individual countries and the diverse medical uses of the established products make the setting of generally applicable guidelines a daunting task. Prior to licensure, all products typically undergo safety evaluation in a minimum of 5–10 volunteers, and in many cases, 25 or more. More patients are included in testing of products made by new processes.

4.5 Implementation in a manufacturing setting

A set of measures should be implemented to ensure that virus inactivation and removal procedures are correctly carried out in a manufacturing process and that cross-contamination following these procedures is avoided. The examples of viral reduction treatment practices given below should not be understood as requirements, but rather as general points for consideration. They are not the only acceptable way of conducting viral reduction treatments but provide examples of the solutions employed by some manufacturers when addressing this issue.

4.5.1 Overall process design

When planning to implement a new viral inactivation and removal treatment, the following conditions should be set ahead of time to facilitate their implementation:

batch-size or volume at the stage of the viral reduction step, and potential up-scaling in the future;

floor area in the manufacturing facility required for the viral reduction step itself, and for downstream processing (e.g. for removal of stabilizers or chemicals);

the possibility of creating a “safety area” where successive production steps are arranged in a clear and logical way so as to avoid cross contamination from a consideration of how the various flows (operators, product, equipment, wastes) will be organized during and after the viral reduction step.

Whether cleaning or sanitation procedures will be in that place or executed in another location.

4.5.2 Equipment specifications

Because viral reduction treatments are critical to product safety, the specifications of the equipment employed in these steps are of particular importance. The following examples are illustrative:

In-process/bulk virus inactivation (e.g. solvent/detergent, low pH, pasteurization)

Ideally, incubation vessels should be fully enclosed fitted with an appropriate mixing device. Usually, these are temperature-controlled vessels in which the source of heat is a jacket or a heating coil. They often have hygienic, polished internal finishes, flush-fitting valves, hygienic entry ports for the addition of reagents and sampling (e.g. to control pH and osmolality), and probe ports for relevant in-process monitoring (such as measurement of temperature).

There should be no “dead points” i.e. areas where the temperature defined in the specification or where homogeneity of the mixture cannot be ensured.

For heat inactivation processes, temperature-monitoring equipment should provide a continuous, accurate and permanent record of temperature during the treatment cycle.

Terminal virus inactivation (e.g. pasteurization or dry heat in the final container)

The heating device (such as a water-bath, steam autoclave, or forced-air oven) should provide even temperature distribution across the range of batch sizes encountered. This should be demonstrated as part of the equipment qualification.

The temperature-monitoring equipment should be capable of providing a continuous, accurate and permanent record of the heat treatment cycle.

4.5.3 Pre-qualification and validation

Once the equipment for the virus inactivation or removal step has been received, the following steps are usually followed prior to routine use.

The installation qualification verifies that the viral inactivation and removal equipment conforms to the predefined technical specifications and relevant good manufacturing practices regulations applicable at the time of installation in the manufacturing environment. This includes confirmation that the required services (e.g. voltage, cooling/heating fluid and steam) are available and appropriate.

The operational qualification demonstrates experimentally, typically without product, that the equipment for the inactivation and removal of viruses functions within the specified limits

and under the requirements for good manufacturing practices in the manufacturing environment.

The performance qualification establishes that the equipment for the inactivation and removal of viruses operates to the predetermined performance requirements in the presence of product under routine manufacturing conditions.

Product validation provides evidence that intermediate and/or final product prepared with the newly installed equipment reproducibly meets its specifications.

4.5.4 Process design and layout

The benefit of viral inactivation and removal will be negated if the plasma fractions from preceding steps are permitted to recontaminate the intermediates or products that follow; thus, the manufacturer must describe how the operating procedures reduce the likelihood of cross-contamination. Usually, decisions are made after a multidisciplinary team consisting of responsible staff from manufacturing, engineering, quality assurance and microbiology has made its recommendations.

The simplest and best solution to the problem of cross-contamination, from a facility management perspective, is to transfer product from one room to the next in the course of the specific inactivation and removal procedure. This serves to create different safety zones, which, when arranged in a clear and logical way, help avoid cross contamination. In the best implementations, every zone has its own dedicated staff, equipment, entrance, air-handling and other services. When this arrangement is not practical, the same effect can be achieved through appropriate management practices. For example, some facilities utilize the same staff in both downstream and upstream areas, and personnel moving into a safer zone must change their outer overalls, shoes or shoe covers, gloves, etc before entering. Equipment must also be decontaminated when moving it into a safer zone. Preferably, the equipment in one safety zone should not be shared with a second zone. Strict segregation has generally been adopted for continuous flow centrifuges, column chromatography matrices and ultra- filtration membranes which are notoriously difficult to decontaminate with the methods that are currently available.

The following points illustrate how some manufacturers have addressed these cross-contamination issues.

In-process/bulk virus inactivation (e.g. solvent/detergent, low pH, pasteurization)

Inactivation procedures are usually carried out in two stages. For example, the first stage may be a treatment at acid pH 4 which takes place in a normal production room, followed by a second incubation in another tank located in a segregated, contained area.

For solvent/detergent treatment, most of the inactivation is usually during the first 30–60 minutes of the 4–6 hour total treatment time.

If bacterial growth during virus inactivation is a consideration, the solution is sterile-filtered (pore size 0.45µm or less) before treatment.

Samples are usually taken to confirm that the process conditions for inactivation meet the specified limits (e.g. for pH, stabilizer concentrations and concentration of virus inactivating agent).

On completion of the first stage of inactivation, the product is aseptically transferred (sterile coupling) into the second vessel, which is located in a safety zone, for completion of the second stage of viral inactivation.

Ideally, the “safety area” has an independent air-handling system, designated controlled clothing for personnel, and defined routes of entry for all equipment, reagents (including process buffers) and consumables.

The process water and the reagents supplied to the safety area are of water for injection (WFI) standard or demonstrated to be free of infectious agents.

All processing after virus inactivation and prior to sterile filtration and dispensing (e.g. removal of solvent/detergent or stabilizers and further purification steps) are carried out in the safety area.

All the equipment used in the safety area that is in contact with the product is dedicated, or decontaminated in a manner that can be shown to inactivate any remaining virus.

In some cases, a dedicated aseptic filling area is used for virus inactivated products while a separate dispensing area is used for products that have not been virally inactivated during the purification process and are treated at the end of the process. Alternatively, products that will be inactivated in the final container can also undergo a preliminary virus inactivation in bulk, or the filling line is cleaned by procedures that can be shown to inactivate virus.

In-process virus removal (e.g. nanofiltration, specified purification steps)

The principles relating to product segregation described above also apply to procedures for virus removal.

Terminal virus inactivation (e.g. pasteurization or dry heat in the final container) Inherently, terminal virus inactivation procedures greatly reduce the likelihood of recontamination.

Temperature is monitored at several locations throughout the load including the previously determined locations at which the highest and lowest temperatures occur.

The temperature control probe is independent from the probes used to monitor product temperature during the heat treatment.

A maximum time is specified for the temperature to reach its set point.

The specified temperature is maintained by all probes for the required period.

4.5.5 Process control

Quality assurance is a critical part of the manufacturing process because completeness of virus inactivation and removal cannot be guaranteed by testing the final product. It is the responsibility of quality assurance to ensure that the execution of virus inactivation and removal methods in a production setting conforms to the conditions that were validated in the virus spiking studies. Additionally, it is their responsibility to ensure that the procedures that are designed to avoid cross-contamination are strictly followed. In the case of any departure from the standard, specified manufacturing processes or in environmental conditions, the independent quality assurance team, typically with the assistance of a select committee, will conduct a deviation investigation to determine whether or not the product can be released. Generally, the quality assurance team has final authority to release or reject product.

The following points should be taken into consideration.

As with all other procedures, viral inactivation and removal procedures should be described in approved standard operating procedures.

The standard operating procedures should contain critical process limits for the viral inactivation and removal methods.

Because of the critical importance of the viral inactivation and removal step, quality assurance personnel may review and approve the recorded conditions for viral inactivation and removal while the batch is being processed; i.e. not just as part of the final overall review of the batch file.

5. Virally Inactivated Plasma for Transfusion

In the past, plasma has been used to treat a variety of haemostatic disturbances and immune deficiencies and even to provide a source of nutrients. This has led to a significant increase in the often inappropriate use of fresh frozen plasma (FFP). For many of those applications, alternative, safer and more economical treatments are now a better choice than FFP. According to the recommendations of consensus development conferences in various countries, there are a limited number of indications for the use of FFP (51–53). These include patients who require massive transfusions, patients with multiple coagulation factor deficits who are bleeding or who need an invasive procedure, patients with thrombotic thrombocytopenic purpura and patients with protein-losing enteropathy. In addition, FFP is indicated where there are no concentrates or purified preparations available such as for congenital coagulation factor deficiencies and immune deficiencies.

Regulatory approvals have been granted to three approaches designed to enhance the viral safety of transfusion plasma, namely:

- quarantine or donor-retested plasma;
- solvent/detergent-treated plasma; and
- methylene blue-treated plasma.

Each of the three approaches is described below, and all have been recently reviewed (54). All transfusion plasma options, including the continued use of FFP from well-screened donors, have advantages and disadvantages, and it is up to the local medical community and relevant regulatory bodies to determine which option is preferable and most suitable for the particular setting. Implementation should adhere to the applicable measures described in section 4.5.

5.1 Quarantine or donor-retested plasma

One approach to reducing window-period transmissions is to hold donor units in quarantine for a suitable period of time until the donor returns and can be retested. This method is useful only for the viruses being tested for, although interviewing the donor at the time of the second test may help to identify any transient illnesses that occurred between the two donations. The length of the quarantine period is related to estimates of the window period, which differs for each virus. To reduce transmission of HIV, HBV and HCV, a sufficient hold period should be chosen to give a 95% confidence level of not releasing a product during a window period. Periods of 3–4 months have typically been considered to prevent almost all window-period

transmissions. The option to quarantine is made possible by the relatively long outdating (shelf-life) period of FFP, typically 1 year.

Although the transmission of HBV, HCV and HIV will be greatly reduced by use of quarantine plasma, it will not have been eliminated. For example, HCV has been reported to have been transmitted by quarantine plasma (55), blood donations that are not screened by genomic techniques continue to harbour HIV and other viruses of concern (56), and quarantine has little or no impact on viruses that are not tested for. However, the advantages of this method are that the plasma itself is unchanged and thus has the same properties and indications as FFP, and no sophisticated equipment, other than that used for donor tracking, is required. On the other hand, supply logistics may prove difficult in some circumstances where a large number of donors need to re-donate well before the expiry date of the initial FFP unit. This is of special concern in an environment based on blood donations volunteers, where many donors give blood infrequently, with consequent losses of plasma units.

Implementation requires systems that correctly match donated units with the returning donors and that prevent premature release of units labeled as being either “quarantined” or “donor retested”. Although manual systems may be used, computerization greatly facilitates this process and provides improved security.

Table 14

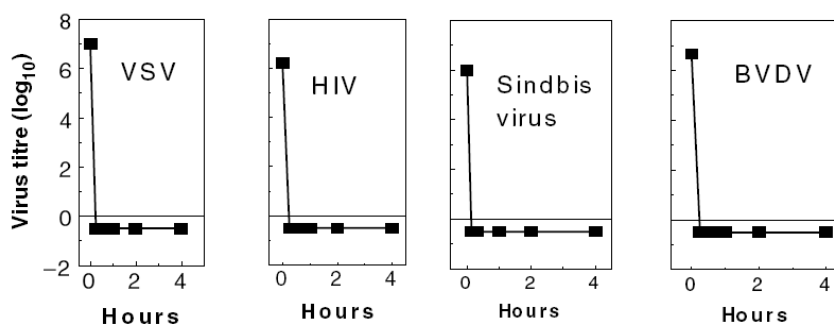
Inactivation of viruses on treatment of plasma with 1% tri(*n*-butyl) phosphate and 1% Triton X-100 at 30 °C for 4 hours

Virus	Inactivation (log ₁₀)	Inactivation time (hours required)
Vesicular stomatitis virus	≥7.5	0.25
Sindbis virus	≥6.9	0.25
Duck hepatitis B virus	≥7.3	2.5
Bovine viral diarrhoea virus	≥6.1	0.25
Human immunodeficiency virus	≥7.2	0.25
Hepatitis B virus	≥6.0	4 ^a
Hepatitis C virus	≥5.0	4 ^a

^a Only one time point tested.

Figure 10

Rate of virus inactivation on solvent/detergent treatment of plasma



BVDV, bovine viral diarrhoeavirus; HIV, human immunodeficiency virus; VSV, vesicular stomatitis virus. Data generously provided by V.I. Technologies, Inc.

5.2 Solvent/detergent-treated plasma

Routinely collected source, recovered, or FFP is pooled and treated with 1% TNBP and 1% Triton X-100 at 30°C for a minimum of 4 hours to inactivate enveloped viruses. The reagents are removed by hydrophobic chromatography to near undetectable levels (57). The compounds used are non-mutagenic and have an overall benign toxicology profile. Leukocytes, bacteria and parasites are removed by sterile filtration. The final product is available frozen and, in some countries, also in a lyophilized form. Inactivation of HIV, HBV and HCV and of many other enveloped viruses has been demonstrated (Table 14, Figure 10). To reduce the risk from non-enveloped viruses, the application of NAT can eliminate positive pools. Little change is observed in the level of most procoagulant factors, and bag-to-bag consistency is ensured through the pooling process.

Clinical trials conducted in both Europe and the USA have shown that solvent/detergent (SD)-treated-plasma can replace FFP in all of its indications, including the replacement of coagulation factors and the treatment of thrombotic thrombocytopenic purpura (58–61). More recently, several deaths were reported in liver transplant patients who received a product provided by one manufacturer in the USA. Although the link with this product or with reduced levels of some anticoagulant proteins in SD-plasma is uncertain, the US manufacturer's product label has been amended to indicate that this product should not be

used in patients undergoing liver transplant or in patients with severe liver disease and known coagulopathies.

Additionally, the coagulation status of patients receiving large volumes of SD-plasma should be monitored for evidence of thrombosis, excessive bleeding or exacerbation of disseminated vascular coagulation.

The same parameters need to be defined and controlled as for other solvent/detergent-treated products. In addition, some regulatory bodies have instituted a maximum for the number of donors that can contribute to an individual lot; the maximum number permitted ranges from 100 to 2500.

5.3 Methylene blue and visible light

Methylene blue is a photosensitizer, and in conjunction with light and in the presence of oxygen it can inactivate biological systems. The virucidal action of methylene blue is well known (62) but the mechanism of action is not entirely clear. Nucleic acid damage usually results from photosensitization with methylene blue. This was ruled out as the cause of virus kill in one case (63), but not in others (64). In the current procedure, individual plasma units are treated with 1mM methylene blue and white fluorescent light for 1h at 45000lux (65) or with low-pressure sodium lamps at 200 Joules/cm² for 20 minutes. The individual units are re-frozen and stored for later use. Added methylene blue is not usually removed although special filters for its removal are being developed (66). Model enveloped viruses and cell-free HIV are inactivated effectively, but non-enveloped viruses, (Table 15 and Figure 11) (67–68) cell-associated HIV and other cell-associated viruses are less affected. The latter must be removed completely by filtration or other means. A recent study has suggested that parvovirus may be inactivated (69). The in vitro coagulation capacity of plasma treated with methylene blue is well maintained, but the activities of fibrinogen and factor VIII are reduced (70). Photodynamic methylene blue treatment of plasma resulted in no adverse reactions in a controlled clinical study (71) and there is no evidence of neoantigen formation (72). The advantage of this approach compared with solvent/detergent-treatment (see above) is the absence of pooling, i.e. recipients would receive plasma from individual donations, rather than from a plasma pool made from hundreds or thousands of donations. Because it is well known that methylene blue and its reaction products are mutagenic (genotoxic) in bacteria, some regulatory authorities in Europe have requested additional data on the mutagenic potential of these substances in mammals and/or on the validated use of filters to efficiently remove them from treated plasma units.

On the basis of the above considerations, the following factors are likely to affect outcome and therefore need to be defined and controlled:

- the volume of plasma being treated;
- the geometry of the sample;
- the light intensity and duration of exposure;
- the effect of residual cells;
- the transparency of the bag;
- mixing efficiency; and
- residual levels of reagent and its photoproducts.

Some of this information may be available from the manufacturer of the specialty equipment employed during this procedure.

6. Review of Newer Viral Inactivation Methods Under Development

Several new viral inactivation procedures are being investigated, with the principal objectives of providing broader viral coverage, complementing existing methods, reducing cost and/or improved applicability to FFP. Several of these newer approaches are reviewed here, but it should be noted that in many cases, there is little or no clinical experience with these methods.

6.1 Psoralen-treated fresh frozen plasma

The use of the psoralen, S-59, together with ultraviolet (UVA) irradiation is being investigated with both FFP and platelet concentrates. Published data on viral kill are provided in Table 16. The amount of virus killed by S-59 treatment of platelet concentrates is somewhat greater than that in plasma because of its lower protein content. In phase 1 studies involving six healthy volunteers, infusion of up to 1 l of plasma resulted in no adverse events and no significant clinical changes in blood chemistries or haematological measurements (73). Three phase 3 trials are under way. In an open-label trial in patients (to date, n = 34) with congenital deficiencies in blood clotting factors, infusion of S-59-treated plasma resulted in a similar increase in coagulation factor levels to those reported with untreated plasma (74) in historical data. Based on the above considerations, the following factors are likely to affect outcome and therefore need to be defined and controlled:

- The volume of plasma being treated;
- The geometry of the sample;
- The light intensity and duration of exposure;

- The effect of residual cells;
- The transparency of the bag;
- Mixing efficiency; and
- Residual levels of the reagent and its photoproducts.

Some of this information may be available from the manufacturer of the specialty equipment employed during this procedure.

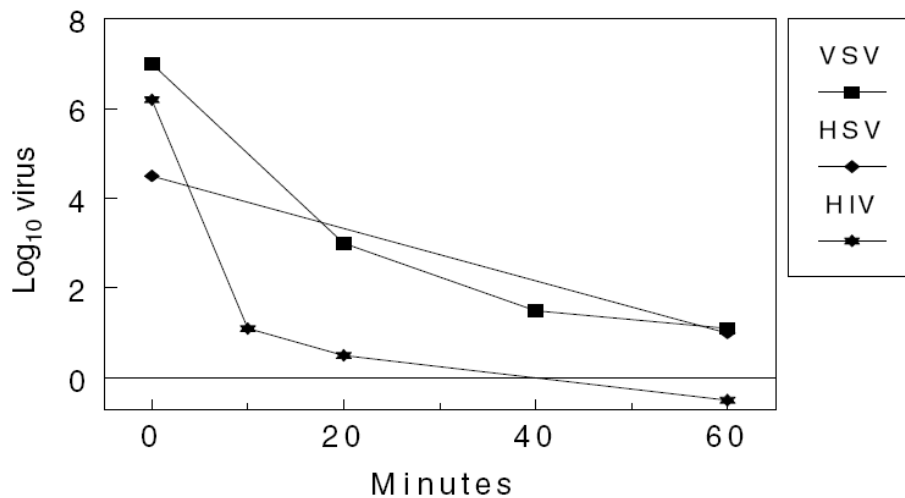
Table 15

Inactivation of viruses on treatment of plasma with 1 μ M methylene blue and white light for 1 hour

Virus	Inactivation (log ₁₀)	Inactivation time (minutes required)
Vesicular stomatitis virus	5.0	60
Simian immunodeficiency virus	≥ 6.3	—
Semliki forest virus	≥ 7.0	10
Herpes simplex virus	≥ 5.5	60
West Nile virus	≥ 6.5	—
Sindbis virus	≥ 9.7	—
Bovine viral diarrhoea virus	≥ 5.9	2
Human immunodeficiency virus (extracellular)	≥ 6.3	10–30
Human immunodeficiency virus (cellular)	0	—
Duck Hepatitis B virus	3.9	60
Hepatitis A virus	0	60
Porcine parvovirus	0	60

Figure 11

Rate of virus inactivation on methylene blue treatment of plasma



HIV, human immunodeficiency virus; HSV, herpes simplex virus; VSV, vesicular stomatitis virus.

Results generously provided by H Mohr, DRK Blutspenddienst, Springe.

6.2 Irradiation with ultraviolet light (UVC)

Ultraviolet irradiation, typically at a wavelength of 254nm (UVC) targets nucleic acid, thus a wide variety of viruses are inactivated irrespective of the nature of their envelope. Viruses containing single stranded nucleic acids are more sensitive, because they are unable to repair damage in the absence of a complementary strand, and sensitivity increases with genome size (75), because a larger target is hit more often. Attempts to use UVC in the 1950s failed to prevent hepatitis transmission by whole plasma, but this probably reflects the relatively high titre of HBV present in donor plasma at that time and the fact that HBV is a double-stranded DNA virus. Based on these principles, HAV and parvovirus should be relatively sensitive to UVC. Following the early efforts, considerable thought was given to the factors that affect UVC efficacy, particularly to the various ways in which a uniform thin film can be formed in continuous flow. For most protein solutions thin films are necessary to ensure complete penetration of the UVC light because the protein solutions at least partially absorb UVC energy. The difficulty in assuring maintenance of an appropriate thin film may be the reason that a prothrombin complex concentrate treated with UVC was reported to transmit HIV (76). UVC has also been shown to damage protein. For example, albumin prepared from whole plasma irradiated with UVC was reported to be appreciably less stable during storage than albumin prepared from unirradiated plasma (77, 78).

The most practical applications use a light source that emits at 254nm. With such a source, Hart et al. (79) have shown that both albumin and IVIG solutions could be treated with 5000 Joules/m² UVC before an unacceptable level of IgG aggregates was observed. Non-enveloped and heat and/or acid-resistant viruses (e.g. polio 2, T4 phage and vaccinia) were effectively inactivated. The results of validation studies performed with albumin appear encouraging (80). Horowitz et al. have shown that the addition of quenchers of reactive oxygen species enhances the specificity of virus inactivation by UVC in protein solutions. By adding the plant flavonoid rutin to the protein solution prior to treatment with UVC, these investigators found that the inactivation of several viruses was largely unaffected (Figure 12), but that several coagulation factors were protected against UVC induced damage (81). The beneficial effect of including rutin during UVC treatment was also observed with fibrinogen incorporated into a fibrin sealant, albumin and IVIG (82).

The above mechanistic considerations and experimental findings indicate that the following factors are likely to affect outcome and therefore need to be defined and controlled:

- UVC dose;
- Uniformity of dose over time;
- Flow rate; and
- Optical density of the material being treated.

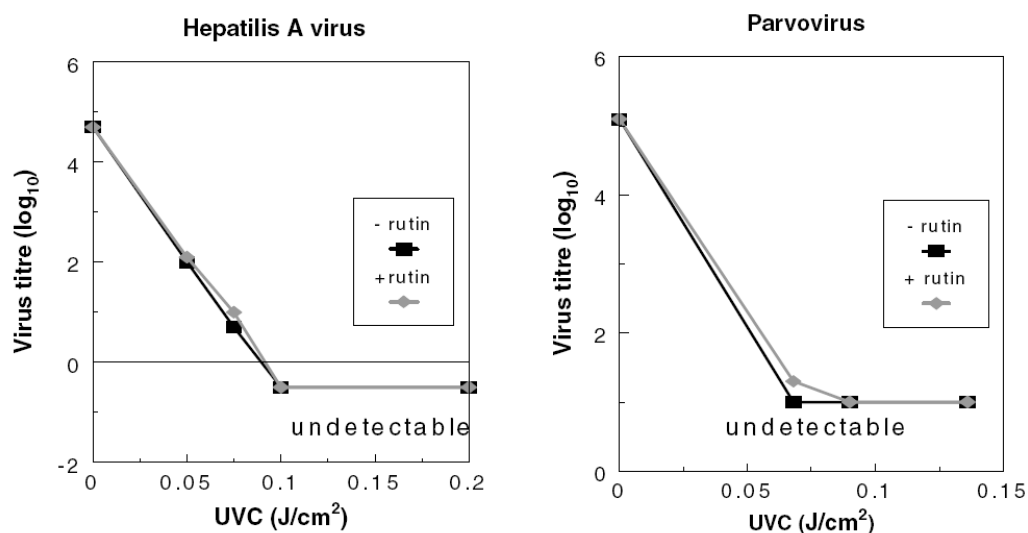
Table 16

Inactivation of viruses on treatment of plasma with 150µM psoralen S-59 and 3 J/cm² UVA

Virus	Inactivation (log ₁₀)
Duck hepatitis B virus	5.4
Hepatitis B virus	≥4.5
Hepatitis C virus	≥4.5
Bovine viral diarrhoea virus	≥6.7
Human immunodeficiency virus	≥5.9
Human immunodeficiency virus (cellular)	6.4

Figure 12

Inactivation of non-enveloped viruses added to a concentrate of coagulation factor VIII with UVC



0.5 mM rutin was either present or absent
Source: Chin et al. (81).

6.3 Gamma-irradiation

The use of gamma irradiation has been studied extensively for a range of applications from sterilizing hospital supplies to reducing bacterial and viral contamination of meats, other foods and sewage sludge. In most installations, ⁶⁰cobalt serves as the source. Gamma irradiation can act by two different mechanisms. The first is the direct rupture of covalent bonds in target molecules including both proteins and nucleic acids. The second is an indirect, mechanism, such as with water, producing reactive free radicals and other active, radiolytic products, which in turn can react with a variety of macromolecules including both proteins and nucleic acids. Indirect reactions can be reduced by adding radical scavengers, removing water by lyophilization, and/or working at cold temperatures. More recently, for the same total dose of radiation, reducing the dose rate has been reported to improve the balance between protein recovery and virus inactivation. The kinetics of viral kill are typically linear in a semi-logarithmic plot of virus titre versus radiation dose, suggesting that inactivation occurs with a single hit of radiation that is absorbed or directly by the nucleic acid is the likely basis of the inactivation.

The principal challenge in using gamma irradiation is the inactivation of the desired quantity of virus while maintaining the structural and functional integrity of protein. For example, Hiemstra et al. showed that on treating plasma, the inactivation of 5–6 logs of HIV required

5–10 mRad, whereas recovery of at least 85% of factor VIII demanded that the dose not exceed 1.5 mRad (Figure 13). Coagulation activity present in a lyophilized blood coagulation factor VIII, concentrate was even more sensitive whether the treatment was at -80°C or +15°C. Moreover, following irradiation of either lyophilized antihæmophilic factor or lyophilized prothrombin concentrates; high-pressure size-exclusion chromatography revealed protein changes at doses as low as 0.5–1mRad.

These results contrast with those of Kitchen et al. (84), who reported a recovery of 85% for factor VIII and of 77% for factor IX on treatment of frozen plasma with 4mRad gamma irradiation. This dose of radiation resulted in the inactivation of 4.3 logs of HIV and more than 4 logs of several other viruses including polio and measles. It has not yet been possible to explain the different findings in these two studies.

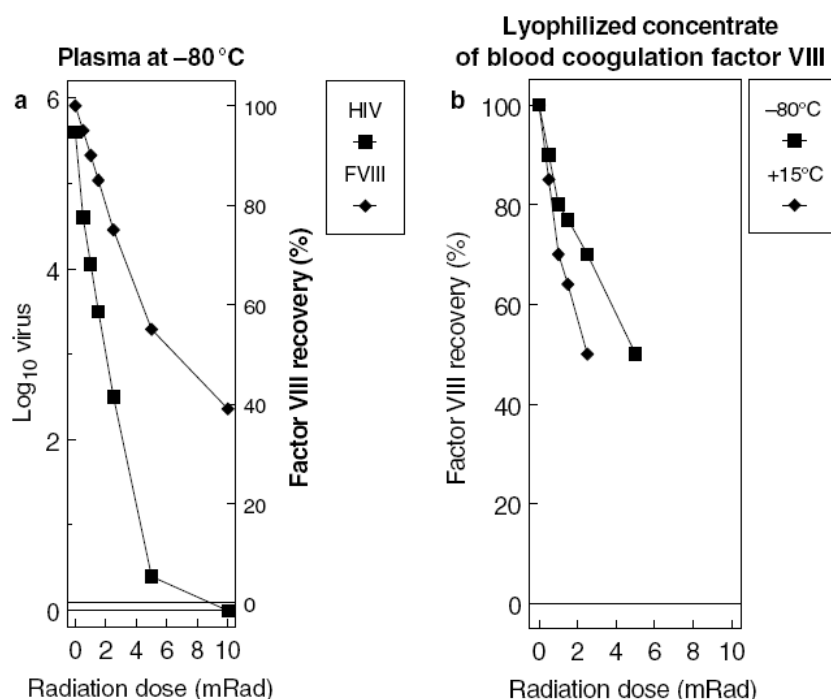
More recently, Miekka et al. (85) reported that treatment of lyophilized preparations of blood coagulation factor VIII with 2–3mRad of gamma irradiation resulted in the inactivation of 4 logs of porcine parvovirus while retaining 93% of fibrinogen solubility, 67% of factor VIII activity and over 80% of α -1-proteinase inhibitor activity. The dose rate may have been an important variable in these studies. Since then, Drohan et al. have reported that treatment of a monoclonal antibody preparation in the presence of an antioxidant protein protection cocktail resulted in the inactivation of $\geq 4.8 \log_{10}$ of PPV. The retention of antigen-binding activity was improved 3- to 4-fold by the presence of the protectant cocktail.

On the basis of the above mechanistic considerations and experimental findings, the following factors are likely to affect outcome and therefore need to be defined and controlled:

- Total dose;
- Dose rate and dose uniformity;
- Composition;
- Oxygen content;
- Temperature; and
- (for lyophilized products) residual moisture.

Figure 13

Gamma irradiation of plasma and of blood coagulation factor VIII



FVIII, factor VIII; HIV, human immunodeficiency virus.

a Effect on HIV (squares) and factor VIII activity (diamonds) on treatment of frozen plasma. **b** Effect on factor VIII activity in a lyophilized concentrate of blood coagulation factor VIII.

Source: Hiemstra et al. (83).

6.4 Iodine

Iodine is a strong oxidizing agent and, as a result, is a powerful microbicidal agent. However, in its free form iodine is not sufficiently selective. When bound to polymers, such as polyvinylpyrrolidone (86), cross-linked starch (87), or dextran chromatographic medium such as Sephadex, the virucidal action of iodine is more controlled. The iodine in these bound forms is slowly released into the protein solution, and virus inactivation occurs over the course of hours. For example, starch-bound iodine at a concentration of 1.05mg/ml resulted in more than 7 log₁₀ inactivation of model lipid enveloped and non-enveloped viruses while more than 70% of the activity of the clotting factors in plasma was retained. In another implementation, protein was passed through a bed of iodine–Sephadex followed immediately by a bed of Sephadex used to trap and remove free iodine.

Based on the above mechanistic considerations and experimental findings, the following factors are likely to affect outcome and therefore need to be defined and controlled:

- iodine concentration;
- age of iodine–Sephadex;
- temperature;
- contact and incubation times; and
- composition of protein solution being treated.

In addition, careful studies evaluating the covalent incorporation of iodine into macromolecules are required.

6.5 Pasteurized fresh frozen plasma

A system for pasteurizing pooled plasma in bulk at 60°C for 10 hours with 80–90% retention of coagulation factor activity has been described (88). Added stabilizers are removed by diafiltration. Data on viral kill are provided in Table 17. No changes in blood pressure or heart rate were observed when the treated plasma was infused in rat at 4 ml/kg body weight, and there was no sign of toxicity on infusion of a single dose of 25 ml/kg body weight of treated plasma into mice. Clinical studies have not been initiated. One alternative that does not require a manufacturing plant, described in a preliminary report, is to heat plasma from a single donor at 50°C for 3 hours in the presence of lower concentrations of stabilizers, thus avoiding the need for diafiltration (89). Although this approach results in lower levels of virus inactivation with some viruses, complete inactivation of HIV (≥ 6.6 logs) was achieved.

The same factors need to be defined and controlled as for other pasteurized products. In addition, if single units of plasma are treated, the effect of varying the ratio of plasma volume to stabilizer mixture needs to be evaluated.

7. Summary

A number of procedures for the inactivation and removal of viruses are now in common use and are well recognized as contributing substantially to the virus safety of plasma products and plasma for transfusion. Adoption of these or equivalent methods is encouraged. For the virus inactivation and removal procedures commonly employed, the information above should help define criteria for acceptance often based on a decade or more of experience. For new products or products from new manufacturers, the rate of virus kill and the extent of virus kill or removal should match those shown for products with good safety records. Assuming this requirement is met for selected viruses, the details of how a process is installed in the production facility, including staff training, equipment selection, steps taken to monitor the process and process controls, and measures taken to prevent recontamination, probably deserve more emphasis than increasing the number of different viruses studied or the number of slight variations explored.

Which method is most appropriate depends on a variety of factors such as the type of virus, the nature of the product and the characteristics of the production process. The method selected needs to guarantee both viral and general safety without affecting clinical effectiveness, and full safety may require the use of more than one method. The use of more than one robust virus inactivation and removal procedure may be especially important if the viral load present in plasma is substantially higher than that encountered in the countries where the strategies for ensuring viral safety have evolved.

National regulatory authorities frequently need to address the question of how much viral and protein data should be required prior to initiation of clinical trials or routine clinical use. No definitive answer to this question is yet available. Decisions of this nature need to take local circumstances into consideration. For example, to initiate clinical trials, the US Food and Drug Administration usually limits its virus requirements to studies demonstrating the adequate inactivation and removal of HIV, a model for HCV such as BVDV, and a single non-enveloped virus such as parvovirus or HAV.

This guidance document is intended to define the scientific principles that should be taken into consideration as a common basis in the evaluation of the safety of a plasma-derived product, both by the regulatory authorities and the manufacturer.

The following principles should be applied.

Viral inactivation and removal are part of an integrated process designed to guarantee product safety; they cannot replace other safety measures such as donor selection, donation screening or overall compliance with current good manufacturing practices.

The preparation of all purified plasma products should incorporate two independent and complementary methods able to eliminate enveloped viruses, at least one of which is a viral inactivation step.

The inactivation and removal of non enveloped viruses with current methods is frequently incomplete. Manufacturers are therefore encouraged to develop procedures to deal with such viruses.

Studies that assess viral clearance are required for all products. An exception can be made for albumin produced by the established methods using ethanol fractionation followed by pasteurization. This means that even if the manufacturing process, including virus inactivation and removal, has been validated by other manufacturers and has a history of use, additional viral validation by the new manufacturer is still required.

When validating virus inactivation and removal, viruses should not be brought into the production facility.

When applying established methods of viral inactivation to a particular product, the kinetics and extent of viral inactivation should be assessed with reference to existing data derived from products with a history of safety in which viral inactivation has been carried out by the same or similar procedures.

When applying established methods of virus removal to a particular product, the extent of removal should be assessed with reference to existing data derived from products with a history of safety that have been manufactured by the same or similar procedures. Studies should include an attempt to show mass balance, i.e. to account for the entire quantity of virus added.

A robust, effective, reliable process step will be able to remove or inactivate substantial amounts of virus, typically 4 logs or more, be easy to model convincingly and be relatively insensitive to changes in process conditions.

The manufacturer should demonstrate, using appropriate methodologies, that the viral inactivation step(s) has (have) not adversely affected the required characteristics of the product.

Manufacturing aspects such as facility layout, equipment, product flow, staff training and standard operating procedures need to comply with current good manufacturing practices, including measures to prevent the recontamination of product or intermediates.

Regulations of inactivation and removal of viruses can be established only by the regulatory authority. Products imported into a country should comply with both the requirements in the country of origin and in the GCC state. Batches of plasma derivatives recalled or withdrawn in one country should under no circumstances be exported to another country.

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9. Appendix

Example of a study on the inactivation of human immunodeficiency virus-1 by treating a therapeutic plasma protein preparation with tri (n-butyl) phosphate and Tween 80

A solvent/detergent procedure was evaluated for its ability to inactivate human immunodeficiency virus type 1 (HIV-1) added to a therapeutic plasma protein preparation. The study evaluated the rate and extent of HIV-1 inactivation under “worst-case” conditions in that the concentrations of tri(n-butyl)phosphate (TNBP) and Tween 80 were 85% of that specified and the temperature was at the minimum specified under routine manufacturing conditions. Samples were titrated by 50% tissue culture infectious dose (TCID₅₀) end-point assay using C8166 cells.

The calculated log reduction factor for the solvent/detergent procedure was:

$$\geq 6.00 \pm 0.31 \log_{10} \text{TCID}_{50}.$$

Validation study report

Objective

The objective of this viral validation study is to provide information concerning the inactivation of HIV-1 on treatment of a therapeutic plasma protein (hereinafter “Test Article”) with a solvent/detergent procedure.

Testing facility

Responsibilities for preparing the spiking virus, performing the scale down process, performing the virus titration, writing the final report and maintaining an archive with the raw data were defined. The validation studies were reviewed by the quality assurance unit.

The following records were stored in the archives: virus spiking records, sample records, cell culture records, culture treatment records, virus titration records, dilution records, inoculation records and records of examination of cells.

Selection criteria for viruses

Validation of virus removal and inactivation should include relevant viruses that are known to, or likely to, contaminate the source material. The virus proposed for this study is HIV-1, a potential contaminant of human blood products. The characteristics of HIV-1 are given in Table 1.

Table 1

Virus used in this viral clearance study

Virus	Genome	Envelope	Family	Size (nm)	Resistance to physicochemical reagents
HIV-1	RNA	Yes	Retro	80–110	Low

Equipment and supplies

All equipment and supplies required for this study, including pipettes, pH meters, water-bath, biohazard hoods and incubators were provided. All had been calibrated and certified within the past 6 months.

Test article

Responsibilities for the preparation, stability, purity and concentration of the Test Article were defined. The Test article was sampled from the point in manufacture just prior to virus inactivation, frozen at -70°C or below, and shipped to the testing facility on dry ice. Once received, the test Article was stored at -70°C or below.

Virus preparation

Stock virus was prepared at the testing facility. Its titre was determined with three independent assays of its TCID₅₀ using 5-fold dilutions and eight replicates per dilution. The certified titre was the average of these three determinations.

Cytotoxicity and viral interference

A previous study had been conducted to determine whether the test article, in the presence or absence of the solvent/detergent reagents, was cytotoxic to the indicator cells used in assessing infectivity of the virus, or interfered with its detection. The results indicated that cytotoxicity could be overcome by diluting the Test Article 81-fold (3^4) with RPMI-1640 + 10% FBS (culture medium) and that, at this dilution, the Test Article did not interfere with the detection of 100 TCID₅₀ of HIV-1.

Protocol

1. On the day of testing, the Test Article was thawed in a water bath at 37°C for approximately 1 hour and clarified by centrifugation at 5000xg for 10 minutes and the precipitate discarded. The pH following centrifugation was 7.2, and the A_{280} was 25.6.
2. (a) HIV-1 stock (1 ml) was added to Test Article (19ml) at a dilution of 1 : 20 and mixed thoroughly. This was divided into two aliquots, one of 15 ml (to receive solvent/detergent) and one of 5 ml (to receive water). Both were brought to $21 \pm 1^\circ\text{C}$ in a shaking water-bath.

(b) An additional aliquot (50 μl) of HIV-1 stock was diluted 1000- fold into culture medium containing 10% FBS to serve as the positive control. This was placed on ice during the remainder of the experiment.
3. (a) To the 15 ml aliquot was added 667 μl of 20% Tween 80 followed immediately (after mixing) by 40 μl of TNBP.

(b) To the 5 ml aliquot was added 222 μl water for injection.

(c) Both were returned to the shaking water-bath set at $21 \pm 1^\circ\text{C}$.
4. (a) From the vessel to which solvent/detergent had been added (the +SD vessel), 0.5 ml was removed after 0, 15, 30, 60, 120 and 240 minutes and diluted immediately 81-fold with culture medium containing 10% FBS. Following dilution, the samples were placed on ice.

(b) From the -SD vessel, 0.5ml was removed after 0 and 240 minutes and diluted immediately 81-fold with culture medium containing 10% FBS. Following dilution, the samples were placed on ice.

Assay of infectivity

1. Samples were assayed for HIV infectivity on the day of sampling using C8166 as indicator cells, 3-fold serial dilutions with eight replicates per dilution and 50 μl /well. In addition, to increase the sensitivity of the assay, the +SD, 240-minute time point was also assayed in “large volume” using 800 replicates without further dilution and 50 μl /well. Excess samples of the original dilution (approximately 10 ml) were placed on ice and stored at -70°C or below until completion of the study in case additional assays were required.
2. For the test to be valid, the titre of the positive control must be within ± 1 log of the certified titre.

3. Calculation of titre

The following formula for the calculation of TCID₅₀ is based on the Karber method:

$$LT = LT_{\min} + (\log SDF)/2 + \log SDF \sum P_i$$

where:

LT = log titre for the sample volume tested

LT_{min} = log of smallest dosage causing infection in all cultures

SDF = serial dilution factor (usually 3, 5 or 10)

$\sum P_i$ = the sum of the proportion of positive results observed at all dilutions greater than that causing infection in all cultures.

4. Calculation of 95% confidence interval

The 95% confidence interval was calculated using the following formula:

$$SE^2 = (\log SDF)^2 \times \sum \{ (P_i (1 - P_i)) / (n_i - 1) \};$$

and the 95% confidence interval is: $\pm 1.96 \times SE$

where:

SE = the standard error

SDF = serial dilution factor (usually 3, 5 or 10)

P_i = proportion of positive results at dilution i

n_i = the number of replicates at dilution i

$\sum$ = the summation over all dilutions

5. Calculation of viral reduction factor (RF)

$$RF = \log_{10} (\text{input virus titre (per unit volume)} \times \text{input volume}) / (\text{output virus titre (per unit volume)} \times \text{output volume})$$

For example:

$$RF = \log (108 \text{ IU/ml} \times 10 \text{ ml}) / (102 \text{ IU/ml} \times 20 \text{ ml})$$

Results

The controls met the criteria for a valid test. The positive control was within ± 1 log of the certified titre of the stock virus, and the negative control did not elicit any cytopathology during the test period. The raw data recorded are given in Table 2.

The TCID₅₀ titres of the samples tested were as shown in Tables 3 and 4.

Table 3
Calculation of TCID₅₀ values

Sample	Titre $\pm$ 95% CI (log ₁₀ TCID ₅₀ /ml)	Volume (ml)	Volume correction (dilution prior to titration)	Viral load (log ₁₀ TCID ₅₀)
Certified titre of spiking virus	8.45 $\pm$ 0.24			
Positive control	4.94 $\pm$ 0.20		1000	7.94 $\pm$ 0.20
Negative control	no virus detected			
Solvent/detergent treatment				
+SD; T = 0min	3.69 $\pm$ 0.21	15.67	81	6.79 $\pm$ 0.21
+SD; T = 15min	1.96 $\pm$ 0.25	15.67	81	5.06 $\pm$ 0.25
+SD; T = 30min	$\leq 0.70^a$	15.67	81	≤ 3.80
+SD; T = 60min	$\leq 0.70^a$	15.67	81	≤ 3.80
+SD; T = 120min	$\leq 0.70^a$	15.67	81	≤ 3.80
+SD; T = 240min	$\leq 0.70^a$	15.67	81	≤ 3.80
+SD; T = 240min (large volume)	$\leq -1.12^a$	15.67	81	≤ 1.98
-SD; T = 0min	4.88 $\pm$ 0.31	15.67	81	7.98 $\pm$ 0.31
-SD; T = 240min	5.24 $\pm$ 0.29	15.67	81	8.34 $\pm$ 0.29

+SD, solvent/detergent present; -SD, solvent/detergent absent; TCID₅₀, 50% tissue culture infectious dose.

^a No virus detected. The theoretical titre was based on the Poisson distribution.

Table 4
Reduction factors (viral clearance)

Process step	Initial load (log ₁₀ TCID ₅₀)	Output load (log ₁₀ TCID ₅₀)	Log ₁₀ reduction
Solvent/detergent treatment (240 min)	7.98 $\pm$ 0.31	≤ 1.98	$\geq 6.00 \pm 0.31$

TCID₅₀, 50% tissue culture infectious dose.

Table 2
Raw data

Sample	No. of wells positive for HIV-1/total ^a																	
Serial dilution factor (3 ^x)	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Positive control	8/8	8/8	8/8	8/8	8/8	8/8	8/8	7/8	1/8	1/8	0/8	0/8	0/8	0/8	0/8	0/8	0/8	0/8
Negative control	0/8	0/8	0/8	0/8	0/8	0/8												
+SD T = 0 min	8/8	8/8	8/8	8/8	8/8	3/8	1/8	0/8	0/8	0/8	0/8							
+SD T = 15 min	8/8	4/8	3/8	0/8	0/8	0/8	0/8	0/8	0/8	0/8	0/8							
+SD T = 30 min	0/8	0/8	0/8	0/8	0/8	0/8	0/8	0/8	0/8									
+SD T = 60 min	0/8	0/8	0/8	0/8	0/8	0/8	0/8	0/8	0/8									
+SD T = 120 min	0/8	0/8	0/8	0/8	0/8	0/8	0/8	0/8	0/8									
+SD T = 240 min	0/8	0/8	0/8	0/8	0/8	0/8	0/8	0/8	0/8									
+SD T = 240 min	0/800																	
(large volume)																		
-SD T = 0 min				8/8	8/8	8/8	7/8	5/8	2/8	0/8	1/8	1/8	0/8	0/8	0/8	0/8	0/8	0/8
-SD T = 240 min				8/8	8/8	8/8	8/8	7/8	3/8	3/8	1/8	0/8	0/8	0/8	0/8	0/8	0/8	0/8

+SD, solvent/detergent present; -SD, solvent/detergent absent.

^a 50 μ l were tested per well.

Part II: Nucleic acid testing (NAT) for human immunodeficiency virus type 1 (HIV-1) and hepatitis c virus (HCV): testing, product disposition, and donor deferral and reentry

Nucleic acid testing (NAT) for human immunodeficiency virus type 1 (HIV –1), hepatitis C virus (HCV) and hepatitis B virus (HBV): testing, product disposition, and donor deferral and reentry

1. Introduction

During the past decade there has been a dramatic reduction in the transmission of Human Immunodeficiency Virus Type 1 (HIV-1) and Hepatitis C Virus (HCV) by human blood and blood components. Primarily, this is due to the implementation of sensitive tests for viral antibody, antigen (for HIV-1), and nucleic acids, and in the case of plasma derivatives, the use of effective virus removal and inactivation methods. The sources of remaining risk of HIV-1 and HCV transmission are marker-negative “window period” donations (made during the period that the donor is infected with a virus, but neither the virus nor antibodies to the virus are detectable by current tests), donors infected with immunovariant viral strains, persistent antibody-negative (immunosilent) carriers, and laboratory test procedure errors. According to a recent report, donations during the window period constitute most of the risk of HIV-1 and HCV transmission (Ref. 1). Therefore, measures to reduce the window period could further reduce significantly the low residual risk of HIV-1 and HCV transmission by human blood and blood components.

Studies performed using seroconversion panels indicate the value of Nucleic Acid Testing (NAT) in reducing the window period for HIV-1 and HCV. The estimated mean window-period reduction of HIV-1 ribonucleic acid (RNA) by pooled sample NAT is approximately 11 to 15 days relative to antibody and 5 to 9 days relative to HIV-1 P24 antigen testing (Refs. 2-4). NAT for detection of HCV has been estimated to reduce the window period by 50-60 days relative to that for HCV antibody. In large-scale studies performed in the US, NAT for HIV-1 detected four cases that were antigen-negative/antibody-negative. Window period donations and in the case of NAT for HCV detected 42 additional antibody-negative window period donations. As a result, subsequent to implementation of NAT, the residual risk of HIV-1 and HCV in screened human blood and blood component donations is currently estimated to be approximately 1 in 2,135,000 donations for HIV-1 and in 1,935,000 donations for HCV (Ref. 3).

Currently, for HBV screening, Whole Blood and components for transfusion are routinely tested for both hepatitis B surface antigen (HBsAg) and antibody to hepatitis B core antigen (anti-HBc) consistent with current regulations and guidance documents (see References). Source Plasma for further manufacture into injectable plasma derivatives is routinely screened for HBsAg, but not for anti-HBc. Therefore, for HBV screening, centers that implement HBV NAT will be using a total of three assays to test Whole Blood and blood components (HBV NAT, HBsAg and anti-HBc), and two assays to test Source Plasma (HBV NAT and HBsAg). Thus, these blood establishments will encounter a number of possible test result combinations, and they will need to make suitable decisions regarding the management of donors and units based on these test result combinations.

2. Definitions

Master Pool: A pool of donor samples on which NAT is performed as screening test. A Master Pool is formed by pooling of samples from subpools or by directly pooling samples from individual donors.

Subpool: A pool of donor samples that was used with other (sub) pools to form the Master Pool or that was formed during “deconstruction” of the Master Pool.

Deconstruction: Resolution of the reactivity of a Master Pool by testing subpools (original or freshly made) or samples from individual donors that formed the Master Pool. Deconstruction of a Reactive Master Pool to individual units is a required step for all approved tests.

Multiplex NAT: A NAT that simultaneously detects HIV-1 RNA and HCV RNA.

Discriminatory NAT: A NAT that uses specific primers for HIV-1 or HCV to identify the RNA in the Reactive multiplex NAT sample as HIV-1 RNA or HCV RNA. Performing a discriminatory NAT is a required step for those establishments using a multiplex test such as the Procleix HIV-1/HCV NAT.

Additional NAT: A NAT that uses an amplification technology and/or primers that are different from those that were used for the original NAT screening test, and that has been validated for use with samples from individual donors. This test is not used to make the initial determination of donor suitability, but is used for donor counseling and to determine whether lookback should include notification of transfusion recipients.

Lookback: A series of actions taken by a blood establishment based on donor test results indicating infection with HIV-1 or HCV. These actions relate to prior donations from that donor that possibly were donated during the window period when HIV-1 or HCV RNA and antibody were not detectable by screening tests by the infectious agent might be present in the donor’s blood. These actions include: quarantining of prior collections from that donor that remain in inventory, notifying consignees to quarantine prior collections, further testing of the donor, destroying or relabeling potentially infectious prior collections, and notifying transfusion recipients who received human blood or blood components from that donor, when appropriate.

In the proposed HCV lookback rule published in November 2000 (Ref. 8) the FDA proposed changes that would require lookback to be performed on the basis of a reactive NAT result, even when serological testing is non-reactive. When that rule becomes final, lookback for

HIV-1 and for HCV will be required. In the meantime, we recommend that you perform lookback for HIV-1 and for HCV when donor samples test Reactive using HIV-1 NAT or HCV NAT.

Donor Reentry: A procedure that qualifies a donor who was deferred as eligible to donate again. Donor reentry procedures may be used following a false positive test result and typically require the passage of time to allow for possible seroconversion prior to the performance of additional serologic testing and NAT (See section 4.7. and 4.8.).

3. Background and Discussion

In September 1994 the FDA held a workshop to discuss the potential application of nucleic acid based methods to donor screening for HIV-1. They concluded at the time that these methods clearly were sensitive, but they were not ready for implementation on a large scale.

The industry actively pursued the development of NAT for screening donors of human blood and blood components. Because of the cost and labor intensiveness of NAT, there was much interest in testing pools of plasma donor samples (minipools) by NAT, and by 1997, some manufacturers in Europe had voluntarily instituted NAT on minipools. At about that time, the European Union issued a directive that, by July 1, 1999, HCV RNA testing would be required in Europe for all plasma for fractionation, and that the requirement for HIV-1 RNA testing would follow at a later date.

Large-scale clinical studies were needed to demonstrate the efficacy of NAT because of the low frequency of window period donations. Small-scale studies would not identify adequate numbers of window period donations. Test kit manufacturers and testing laboratories submitted Investigational New Drug (IND) applications describing their test method and in-house validation of that method. Blood organizations and establishments intending to use the assay for donor screening also filed INDs to describe their clinical trial protocol for validation of pooled-donor sample NAT and individual donor sample NAT.

In December 1999, the FDA issued guidance for industry on the validation of NAT methods to screen plasma donors (Ref. 9). This document provided guidance on test standards, manufacturing requirements, and clinical trial requirements for licensure of the test method for use in donor screening for transfusion-transmitted viruses.

In September 2001, the FDA licensed the first NAT system, the National Genetics Institute (NGI) UltraQual™ HIV-1 and HCV Reverse Transcription Polymerase Chain Reaction (RT-PCR) assays. Under that license, NGI performs these assays on pooled samples from donors of source plasma.

In February 2002, the FDA licensed the Procleix™ HIV-1/HCV Assay, a qualitative NAT for detection of HIV-1 RNA and/or HCV RNA in plasma from donors of human blood and blood components for transfusion. This assay was approved for use with individual donor samples or pooled donors samples.

In December 2002, the FDA licensed the COBAS AmpliScreen™ HCV Test, v 2.0 and the COBAS AmpliScreen™ HIV-1 Test, v 1.5. These tests are qualitative in vitro tests for the direct detection of HCV RNA and HIV-1 RNA in plasma samples from individual human donors, including donors of whole blood and blood components, Source Plasma, and other living donors. They are also intended for use in screening organ donors when specimens are obtained while the donor's heart is still beating. These assays were approved for use with individual donor samples or pooled donor samples.

In 2003, the FDA issued a final guidance, "Use of Nucleic Acid Tests on Pooled and individual Samples from Donors of Whole Blood and Blood Components (including Source Plasma and Source Leukocytes) to Adequately and Appropriately Reduce the Risk of Transmission of HIV-1 and HCV". That guidance combined and finalized the draft guidance "Use of Nucleic Acid Tests on Pooled Samples from Source Plasma Donors to Adequately and Appropriately Reduce the Risk of Transmission of HIV-1 and HCV" dated December 2001 (January 31, 2002, 67 FR 4719) and the draft guidance "Use of Nucleic Acid Tests on Pooled and Individual Samples from Donors of Whole Blood and Blood Components for Transfusion to Adequately and Appropriately Reduce the Risk of Transmission of HIV-1 and HCV" dated march 2002 (April 9, 2002, 67 FR 17077).

The FDA has recently approved the COBAS Ampliscreen™ HBV Test. This is the first licensed NAT to screen blood donors for infection with HBV. This NAT can be used to test minipools (MP) of up to 24 plasma samples and can also be used to test individual donations (ID). FDA has decided that the use of the test is optional and that its use cannot replace HBV serologic testing. Therefore, at the present time, blood centers may screen blood donations by using COBAS HBV NAT voluntarily as an additional test to currently recommended serologic testing.

That guidance informed establishments that collect blood and blood components that FDA has licensed NAT as tests to screen blood donors for HIV-1 RNA, HCV RNA and HBV NAT, that these licensed tests can detect evidence of infection at a significantly earlier stage than is possible under previously approved tests using antibody or antigen detection technology, including the HIV-1 p24 antigen test, and that we believe that these newly licensed tests are now widely available and meet the criteria for screening tests that are necessary to reduce adequately and appropriately the risk of transmission of communicable disease through blood products.

In that guidance the FDA recommend the use of HIV-1 NAT, HCV NAT and HBV NAT on units that are not reactive on a donor-screening test for the detection of antibodies to HIV, HCV and HBV antigen, respectively. However, for donations that are reactive on a test for the detection of antibodies to HIV-1 and are to be discarded or used in the manufacture of non-injectable products, we do not believe that HIV-1 NAT, HCV NAT and HBV NAT are necessary as part of the adequate and appropriate testing requirements. Nevertheless, you may decide to perform HIV-1 and HCV NAT for these donations in order to obtain useful information regarding the donor's infection status. This information may be useful as part of donor notification.

This guidance is intended to assist you with testing, product disposition, donor deferral, donor notification, donor reentry, and lookback. We have written this document in general form because additional NAT may be approved in the future. However, where appropriate, we will identify sections that apply to NAT that are already approved. You must follow manufacturers' instructions regarding testing. Note that screening of donors of human blood and blood components for HIV-1 024 antigen may be replaced by a NAT that has been validated by the manufacturer as a replacement for the HIV-1 p24 antigen EIA.

NAT Algorithms

If you perform NAT on pooled samples and obtain a Reactive NAT result on a Master Pool, the manufacturer's instructions instruct you to perform subsequent testing to identify the individual unit(s) that contains the RNA identified in the Master Pool test. Once you have identified a positive unit, either by subsequent testing of a Master Pool, or by initial individual test, you must not use the donation for transfusion or for manufacturing into injectable products unless an exception applies. You must defer the donor and you must inform the donor of the deferral and the basis for the deferral including test results. A reactive NAT result may indicate ongoing infection of the donor, and thus prior donations from that donor, although NAT-Non-Reactive, may pose a risk to transfusion recipients. We recommend that you perform lookback when donor samples test Reactive for HIV-1 NAT or HCV NAT.

At the meeting of the Blood Products Advisory Committee (BPAC) in March 2001, the FDA requested advice on appropriate algorithms for management of donations of human blood and blood components tested by pooled donor sample NAT for both HIV-1 RNA and HCV RNA. In particular, FDA sought comment on actions to be taken in the even of discrepant testing results, such as when the Master Pool is Reactive but individual donor samples test Non-

Reactive. Data generated using NAT under IND that was presented in the BPAC meeting showed that in each discrepant case it was the Master Pool that was falsely Reactive, due to contamination either during specimen handling or during the assay run. In response to FDA questions, the BPAC voted to consider the NAT result on samples from individual donors as the definitive test result, and recommended release from quarantine for donations from those donors, on the basis of Non-Reactive test results.

This guidance document contains six recommended algorithms for use when NAT-Reactive results are obtained on individual samples or pooled samples from donors of human blood and blood components. This guidance also contains recommendations on product disposition, donor deferral criteria, follow-up testing of the donor, donor notification and donor reentry criteria that combine NAT and serologic test results, and lookback. This guidance is not intended to replace manufacturers' instructions for testing using approved tests.

The first and second algorithms (See Recommendations 4.1. and 4.2, Figures 1 and 2, and Tables 1 and 2) recommend actions to be taken when a NAT-Reactive result is obtained on an individual sample from a donor of human blood or blood components. The third and fourth algorithms (See Recommendations 4.3 and 4.4, Figure 3 and 4, and Table 3 and 4) recommend actions to deconstruct a comparatively small Reactive Master Pool by testing individual donors. The fifth and sixth algorithms (See Recommendations 4.5 and 4.6, Figures 5 and 6, and Tables 5 and 6) recommend actions to deconstruct a larger Reactive Master Pool by testing archived or freshly pooled subpools to identify the Reactive individual donor(s).

B. Donor Reentry

Each year, thousands of donors are deferred from donating blood for an indefinite period, because of a false positive test result on a serological test, followed by a Negative or Indeterminate supplemental test for antibodies to HIV-1 or HCV. In addition to these deferrals, the implementation of individual donor sample and pooled donor sample NAT for HIV-1 RNA and HCV RNA has resulted in deferrals of several hundred donors each year due to potentially false Reactive NAT results.

These deferred donors are eligible to be considered for reentry to donate blood or blood components. A deferred donor subsequently may be found to be suitable as a donor by a re-qualification method or process found acceptable for such purposes by FDA. However, some establishments are not attempting to reenter donors because of the complexity of the current reentry algorithms and concerns about inappropriately reentering a donor. Although we do not

require reentry of donors deferred because of false positive test results, FDA issued guidance in April of 1992 and August of 1993 on reentry of donors deferred because of HIV or HCV test results (Refs. 5, 7).

For those establishments that choose to perform donor reentry, this guidance recommends criteria for reentry of donors deferred because of Reactive HIV-1 or HCV or certain other test results.

These recommendations include two new reentry algorithms based on the combined use of NAT and serologic testing: one for donors deferred because of HIV-1 test results, and a second for donors deferred because of HCV test results. In this guidance we recommend that you consider for reentry three classes of donors deferred because of HIV-1 test results (See Recommendation 4.7, Figure 7, and Table 7):

Donors who had HIV NAT-Reactive results but were seronegative. This includes donors previously deferred because of Reactive test results on an investigation HIV-1 NAT.

Donors with Non-Reactive NAT who have a Repeatedly Reactive screening test for HIV-1 antibody, and Negative or Indeterminate HIV-1 Western Blot or immunofluorescence assay (IFA) results or an HIV-1 Western Blot or IFA was not performed. This includes donors previously deferred because of Repeatedly Reactive HIV serologic test results prior to the initiation of testing by NAT. this class actually includes three subsets of donors, those with a Western Blot that was: (1) Indeterminate with viral bands present, (2) Indeterminate with non-viral bands only, and (3) Negative or not performed.

Donors with a Repeatedly Reactive result on an HIV-1 P24 antigen test and with an Indeterminate (an invalid or a non-neutralized) result on the HIV-1 p24 Neutralization test (Ref. 6). In addition, donors with a Positive result on the HIV-1 p24 antigen Neutralization test also may be eligible for reentry because there are many donors who had (false) positive Neutralization test results who are currently Non-Reactive by HIV-1 NAT and Negative by anti- HIV-1/2 EIA. FDA has advised that it no longer recommends that blood and plasma establishments using certain approved NAT methods perform screening for HIV-1 p24 antigen. If antigen testing continues to be performed concurrent with NAT and antibody testing, donors deferred because of HIV-1 p24 test results would continue to be eligible for reentry.

Data presented at the June 2001 BPAC meeting demonstrated that an 8-week waiting period encompasses the pre-seroconversion window period for HIV-1 with sufficient confidence that

Negative tests after at least 8 weeks have passed rule out HIV-1 infection (Ref. 10). Absent evidence for seroconversion, the Negative NAT on follow-up testing would be evidence that any prior Reactive (but unconfirmed) NAT result was an error.

Accordingly, for all three classes of donors, after a minimum time period of 8 weeks, we recommend that you take a follow-up sample from the donor for testing by both HIV-1 NAT and HIV-1 antibody enzyme immunoassay (EIA). Performing follow-up testing first on a sample from the donor before they donate again may prevent a potentially contaminated unit from being collected and placed in inventory at the blood establishment. If the NAT is Non-Reactive and the EIA is Negative on the follow-up sample, the donor may be reentered. The donor would then be tested again at the time of his/her next donation using the battery of screening tests. Thus, two HIV-1 NAT tests would be performed and must be Non-Reactive and two HIV-1 EIA tests would be performed and must be Negative before a unit from that donor could be used. For purpose of donor counseling, you may choose to test the deferred donor with an HIV-1 NAT and an anti- HIV-1/2 EIA test at any time prior to the end of this 8-week waiting period after the original donation. However, if an HIV-1 NAT is Reactive or an anti- HIV-1/2 EIA is Repeatedly Reactive prior to the end of this 8-week waiting period, the donor would not be eligible for reentry and we recommend that you defer the donor permanently.

In this guidance we recommend that you consider for reentry two classes of donors deferred because of HCV test results. (See Recommendation 4.8., Figure 8, and Table 8):

Donors who had HCV NAT-Reactive results but were seronegative on the HCV antibody test. This includes donors previously deferred because of Reactive test results on an investigational HCV NAT.

Donors with Non-Reactive NAT who have a Repeatedly Reactive screening test for HCV antibody, and radioimmunoblot assay (RIBA) results that were Indeterminate or Negative or a RIBA was not performed. This includes donors previously deferred because of Repeatedly Reactive HCV serologic test results prior to the initiation of testing by NAT.

Data presented at the June 2001 BPAC meeting demonstrated that a 6-month follow-up period encompasses the pre-seroconversion window period with sufficient confidence that Negative serologic tests after at least 6 months have passed rule out HCV infection (Ref. 10).

For purpose of reentering both of these classes of deferred donors, we recommend that a sample be taken from the donor, after a minimum time period of 6 months, for follow-up

testing by both HCV NAT and anti-HCV EIA. Current research indicates that detectable viremia may be intermittent or may also be resolved in about 15-25% of cases of HCV infection (Refs. 11, 12). If the NAT is Non-Reactive and the EIA is Negative on the follow-up sample, the donor may be reentered. For purposes of donor counseling and to detect possible HCV viremia, you may also choose to test the deferred donor with an HCV NAT and an anti-HCV EIA test at any time prior to the completion of this 6-month period after the original donation. However, if an HCV NAT is Reactive or an anti-HCV EIA is Repeatedly Reactive prior to the end of this 6-month period, the donor would not be eligible for reentry and we recommend that you defer the donor permanently.

4. Recommendations

Currently approved tests on individual donor samples for HIV-1 RNA and HCV RNA may be either Multiplex NAT for the simultaneous detection of HIV-1 RNA and/or HCV RNA or separate tests for the RNA of the two viruses.

1. **Testing, product disposition, and donor management for an individual donor sample that is Reactive on a Multiplex NAT after a Negative Antibody Screening Test:**

If you obtain a Reactive Multiplex HIV-1 RNA/HCV RNA NAT result on an individual donor sample, you must do the following (See Figure 1 and Table 1):

Follow the manufacturer's instructions, which instruct you to test the Reactive donation using Discriminatory NAT(s) (Ref. 13).

If the Discriminatory NAT is Reactive for HIV-1 RNA and/or HCV RNA, you must quarantine the unit. You must not ship or use the unit. If you choose not to destroy the unit, you may release it for research or further manufacture with written approval from regulatory authority. If released for one of these uses, you must re-label the unit consistent with the labeling requirements. The unit must be labeled as "Biohazard" and with one of the following cautionary statements as applicable:

"Reactive for HIV-1 RNA

OR

"Reactive for HCV RNA"

OR

"Reactive for HIV-1 RNA and HCV RNA"

AND EITHER

"Caution: For Further Manufacturing Into In Vitro Diagnostic Reagents For Which There Are
No Alternative Sources"

OR

"Caution: For Laboratory Research Use Only".

Further, we recommend that you include on the label after the appropriate Reactive test results one of the following statements:

"Increased risk of transmission of HIV"

OR

"Increased risk of transmission of HCV"

OR

“Increased risk of transmission of HIV and HCV”.

You must defer the donor. The donor may be eligible for reentry (See sections 4.7 and 4.8).

You must notify the donor of his/her deferral, providing information about the test results.

We recommend that you perform lookback (product quarantine/retrieval and notification of recipients of prior collections for HIV-1 and/or HCV), as appropriate.

If the Discriminatory NAT is Non-Reactive for both HIV-1 RNA and HCV RNA, you must quarantine the unit and destroy or relabel the unit as described in section 4.1.a.i. above. You must defer the donor. The donor may be eligible for reentry (See sections 4.7 and 4.8). You must notify the donor of his/her deferral, providing information about the test results. We recommend that you perform lookback for HIV-1 and HCV.

Alternatively, for purposes of donor notification, you may choose to perform another NAT (the original NAT, or Discriminatory NAT(s), or an Additional NAT)) on a new sample or the same sample from the original donation. If an Additional NAT is performed, we recommend that the test be one that has been validated for use with individual donor samples.

If you perform another test on a sample from the original donation and it is Reactive, you must quarantine the unit and destroy or relabel the unit as described in section 4.1.a.i above. You must defer the donor. The donor may be eligible for reentry (See sections 4.7 and 4.8). You must notify the donor of his/her deferral, providing information about the test results. We recommend that you perform lookback for HIV-1 and/or HCV, as appropriate.

If you perform another test on a sample from the donation and it is Non-Reactive, you must quarantine the unit and destroy or relabel the unit as described in section 4.1.a.i above. You must defer the donor. The donor may be eligible for reentry (See sections 4.7 and 4.8). You must notify the donor of his/her deferral, providing information about the test results. In this case you may explain to the donor that the test result, while initially Reactive, is not conclusive. There is a slight risk that the initial test result was a Positive result that cannot be excluded without follow-up testing of the donor. We recommend that you quarantine/retrieve prior collections; however, due to the low probability that any of the prior collections was infectious, we do not recommend that you notify transfusion recipients.

2. Testing, Product Disposition, and Donor Management for an Individual Donor Sample that is Reactive on an Individual NAT after a Negative Antibody Screening Test:

If you obtain a Reactive HIV-1 RNA/HCV RNA NAT result for an individual donor sample (not by Multiplex NAT), you must do the following (See Figure 2 and Table 2):

Quarantine the unit.

You must not ship or use the unit unless one of the exceptions described in 610.40(h)(2) applies. If you choose not to destroy the unit, you may release it for research or further manufacture with written approval from FDA. If released for one of these uses, you must re-label the unit as described in section 4.1.a.i.

Defer the donor. The donor may be eligible for reentry if serologic test results are negative. (See sections 4.7. and 4.8).

Notify the donor of his/her deferral including information about the test results.

We recommend that you perform lookback (product quarantine/retrieval and notification of recipients of prior collections for HIV-1 and/or HCV), as appropriate.

Currently approved tests on Master Pools of donor samples for HIV-1 RNA and HCV RNA may be either Multiplex NAT for the simultaneous detection of HIV-1 RNA and/or HCV RNA or separate tests for the RNA of the two viruses.

In general, there are two approaches to resolving a Master Pool that is Reactive on a Multiplex NAT or a Master Pool that is Reactive using separate tests for the RNA of the two viruses. If you would like to directly test all individual donor samples in sections IV.3 and IV.4. These test algorithms are illustrated in Figures 3 and 4 and described in Tables 3 and 4. If you would like to test subpools that are used to construct a NAT-Reactive Master Pool, we recommend that you follow the test algorithms described in sections IV.5 and IV.6. These test algorithms are illustrated in Figures 5 and 6 and described in Tables 5 and 6.

3. Testing, Product Disposition, and Donor Management for a Master Pool that is Reactive on a Multiplex NAT: Resolution by Testing Individual Donor Samples:

If you obtain a Reactive Multiplex HIV-1 RNA/HCV RNA NAT result for a Master Pool, the test instructions for use instruct you to perform subsequent testing to identify the donor sample(s) that is (are) NAT-Reactive as the basis for the NAT-Reactive result on the pool. For comparatively small Master Pools, you may wish to directly test individual donor samples (See Figure 3 and Table 3). You must follow the instructions in the package insert for a licensed NAT test that provides a specific testing algorithm.

If you directly test the samples from individual donors that constituted the Multiplex NAT-Reactive Master Pool, consistent with the manufacturer's instructions you must test the individual donor samples using the same Multiplex NAT method that was used in the original NAT on the Master Pool (Ref. 13).

Note: In some cases the manufacturer's instructions provide for a different sample preparation procedure. However, the primers and probes would be the same as those used in the original NAT on the Master Pool.

If all individual donor samples are Non-Reactive, you may release from quarantine all individual donations (if serologic tests on those donor samples are Negative and the donations are otherwise suitable for release). However, you must investigate the unexplained discrepancy in testing. Laboratory control procedures must make adequate provisions for monitoring the reliability, accuracy, precision, and performance of laboratory test procedures and instruments, and must include adequate identification and handling of all test samples. Use of supplies and reagents must be in a manner consistent with the instructions provided by the manufacturer. In addition, as part of an overall Quality Assurance Program, we recommend that you conduct additional investigation to determine the cause of the initial reactivity of the Master Pool.

If one (or more) individual donor sample(s) is (are) Reactive, perform the step in section IV.1.a. above.

You may release from quarantine all Non-Reactive individual donations (if serologic tests on those donor samples are Negative and the donations are otherwise suitable for release).

Testing, Product Disposition, and Donor Management for a Master Pool that is Reactive on an Individual NAT: Resolution by Testing Individual Donor Samples.

If you obtain a Reactive Multiplex HIV-1 RNA/HCV RNA NAT result for a Master Pool, the test instructions for use instruct you to perform subsequent testing to identify the donor sample(s) that is (are) NAT-Reactive as the basis for the NAT-Reactive result on the pool. For comparatively small Master Pools, you may wish to directly test individual donor samples (See Figure 3 and Table 3). You must follow the instructions in the package insert for a licensed NAT test that provides a specific testing algorithm.

If you directly test the samples from individual donors that constituted the Multiplex NAT-Reactive Master Pool, consistent with the manufacturer's instructions you must test the individual donor samples using the same Multiplex NAT method that was used in the original NAT on the Master Pool (Ref. 13).

Note: In some cases the manufacturer's instructions provide for a different sample preparation procedure. However, the primers and probes would be the same as those used in the original NAT on the Master Pool.

If all individual donor samples are Non-Reactive, you may release from quarantine all individual donations (if serologic tests on those donor samples are Negative and the donations are otherwise suitable for release). However, you must investigate the unexplained discrepancy in testing. Laboratory control procedures must make adequate provisions for monitoring the reliability, accuracy, precision, and performance of laboratory test procedures and instruments, and must include adequate identification and handling of all test samples. Use of supplies and reagents must be in a manner consistent with the instructions provided by the manufacturer. In addition, as part of an overall Quality Assurance Program, we recommend that you conduct additional investigation to determine the cause of the initial reactivity of the Master Pool.

If one (or more) individual donor sample(s) is (are) Reactive, perform the step in section 4.1.a. above.

You may release from quarantine all Non-Reactive individual donations (if serologic tests on those donor samples are Negative and the donations are otherwise suitable for release).

Testing, Product Disposition, and Donor Management for a Master Pool that is Reactive on a Multiplex NAT: Resolution by Testing Individual Donor Samples.

If you obtain a Reactive Multiplex HIV-1 RNA/HCV RNA NAT result for a Master Pool, the test instructions for use instruct you to perform subsequent testing to identify the donor sample(s) that is (are) NAT-Reactive as the basis for the NAT-Reactive result on the pool.

Deconstruction of the NAT Reactive Master Pool may be performed by testing the subpools (original or freshly made), that formed the Master Pool. This deconstruction of the Master Pool to determine the basis for the reactivity may involve several layer of testing using original or freshly pooled subpools, followed by testing of individual donor samples in the reactive subpool(s). (See Figure 5 and Table 5). You must follow the instruction in the package insert for a licensed NAT that provides a specific testing algorithm.

If you test subpools that were used to construct a Multiplex NAT Reactive Master Pool, consistent with the manufacturer's instructions you must test the original subpools of freshly pooled subpools using the same Multiplex NAT method that was used in the original NAT on the Master Pool (Ref. 13).

Note: In some cases the manufacturer's instructions provide for a different sample preparation procedure. However, the primers and probes would be the same as those used in the original NAT on the Master Pool.

If all subpools are Non-Reactive, you may release from quarantine all individual donations that comprise the Non-Reactive subpools (if serologic tests on those donor samples are Negative and the donations are otherwise suitable for release). However, you must investigate the unexplained discrepancy in testing. Laboratory control procedures must make adequate provisions for monitoring the reliability, accuracy, precision, and performance of laboratory test procedures and instruments, and must include adequate identification and handling of all test samples. Use of supplies and reagents must be in a manner consistent with the instructions provided by the manufacturer. In addition, as part of an overall Quality Assurance Program, we recommend that you conduct additional investigation to determine the cause of the initial reactivity of the Master Pool.

If one (or more) of the subpools is (are) Reactive, You may release from quarantine the individual donations that comprise the Non-Reactive subpools (if serologic tests on those donor samples are Negative and the donations are otherwise suitable for release). Consistent with the manufacturer's instructions, you must test the individual donor samples that comprise the Reactive Subpool using the same Multiplex NAT method that was used in the original NAT on the Master Pool (Ref. 13).

If all individual donor samples are Non-Reactive, you may release from quarantine all individual donations (if serologic tests on those donor samples are Negative and the donations

are otherwise suitable for release). However, you must investigate the unexplained discrepancy in testing. Laboratory control procedures must make adequate provisions for monitoring the reliability, accuracy, precision, and performance of laboratory test procedures and instruments, and must include adequate identification and handling of all test samples. Use of supplies and reagents must be in a manner consistent with the instructions provided by the manufacturer. In addition, as part of an overall Quality Assurance Program, we recommend that you conduct additional investigation to determine the cause of the initial reactivity of the Master Pool.

If one (or more) individual donor sample(s) is (are) Reactive, perform the steps in section 4.1.a above. You may release from quarantine all Non-Reactive individual donations (if serologic tests on those donor samples are Negative and the donations are otherwise suitable for release).

Testing, Product Disposition, and Donor Management for a Master Pool that is Reactive on an Individual NAT: Resolution by Testing Individual subpools.

If you obtain a Reactive result for a NAT for HIV-1 RNA and / or HCV RNA performed separately on a Master pool Pool, the test instructions for use instruct you to perform subsequent testing to identify the donor sample(s) that is (are) NAT-Reactive as the basis for the NAT-Reactive result on the pool. Deconstruction of the NAT Reactive Master Pool may be performed by testing the subpools (original or freshly made), that formed the Master Pool. This deconstruction of the Master Pool to determine the basis for the reactivity may involve several layer of testing using original or freshly pooled subpools, followed by testing of individual donor samples in the reactive subpool(s). (See Figure 6 and Table 6). You must follow the instruction in the package insert for a licensed NAT that provides a specific testing algorithm.

If you test subpools that were used to construct a NAT Reactive Master Pool, consistent with the manufacturer's instructions you must test the original subpools of freshly pooled subpools using the same NAT method that was used in the original NAT on the Master Pool (Ref. 13).

Note: In some cases the manufacturer's instructions provide for a different sample preparation procedure. However, the primers and probes would be the same as those used in the original NAT on the Master Pool.

If all subpools are Non-Reactive, you may release from quarantine all individual donations that comprise the Non-Reactive subpools (if serologic tests on those donor samples are

Negative and the donations are otherwise suitable for release). However, you must investigate the unexplained discrepancy in testing. Laboratory control procedures must make adequate provisions for monitoring the reliability, accuracy, precision, and performance of laboratory test procedures and instruments, and must include adequate identification and handling of all test samples. Use of supplies and reagents must be in a manner consistent with the instructions provided by the manufacturer. In addition, as part of an overall Quality Assurance Program, we recommend that you conduct additional investigation to determine the cause of the initial reactivity of the Master Pool.

If one (or more) of the subpools is (are) Reactive, You may release from quarantine the individual donations that comprise the Non-Reactive subpools (if serologic tests on those donor samples are Negative and the donations are otherwise suitable for release). Consistent with the manufacturer's instructions, you must test the individual donor samples that comprise the Reactive Subpool using the same NAT method that was used in the original NAT on the Master Pool (Ref. 13).

If all individual donor samples are Non-Reactive, you may release from quarantine all individual donations (if serologic tests on those donor samples are Negative and the donations are otherwise suitable for release). However, you must investigate the unexplained discrepancy in testing. Laboratory control procedures must make adequate provisions for monitoring the reliability, accuracy, precision, and performance of laboratory test procedures and instruments, and must include adequate identification and handling of all test samples. Use of supplies and reagents must be in a manner consistent with the instructions provided by the manufacturer. In addition, as part of an overall Quality Assurance Program, we recommend that you conduct additional investigation to determine the cause of the initial reactivity of the Master Pool.

If one (or more) individual donor sample(s) is (are) Reactive, perform the steps a-d in section 4.2. above. You may release from quarantine all Non-Reactive individual donations (if serologic tests on those donor samples are Negative and the donations are otherwise suitable for release).

Reentry for Donors Deferred Because of HIV-1 Test Results

Currently, FDA has not approved a process for reentry of donors with the following HIV-1 test results:

NAT-Reactive for HIV-1 (either by a Discriminatory NAT after a Reactive Multiplex NAT or by a separate NAT for HIV-1 RNA) and anti-HIV-1/2 EIA Repeatedly Reactive (regardless of HIV-1 Western Blot or IFA or HIV-1 p24 EIA test result);

OR

NAT-Reactive for HIV-1 (either by a Discriminatory NAT after a Reactive Multiplex NAT or by a separate NAT for HIV-1 RNA) and anti-HIV-1 p24 EIA Repeatedly Reactive (regardless of anti HIV-1/2 EIA test result);

OR

NAT Non-Reactive for HIV-1 (or HIV-1 NAT not performed) and anti-HIV-1/2 EIA Repeatedly Reactive, HIV-1 Western Blot Positive (regardless of HIV-1 p24 EIA test result);

FDA has approved a method or process for reentry of deferred donors in the following classes:

Donors who were NAT-Reactive and seronegative. This includes donors previously deferred because of Reactive test results on an investigation HIV-1 NAT. The HIV-1 p24 antigen EIA may not have been performed if it was replaced by an approved NAT that was validated to replace the HIV-1 P24 antigen test. The HIV-1 Discriminatory NAT may have been either Positive or Negative. If an Additional NAT for HIV-1 (validated for use with individual donor samples) was performed, it must have been Non-Reactive.

Note: If the original donation that was NAT-Reactive was Negative on the Discriminatory NAT for HIV-1 but was Positive on the Discriminatory NAT for HCV, you may attempt to reenter the donor according to the recommendations in section 4.8. (See Figure 8 and Table 8). If the original donor sample that was NAT-Reactive was Positive or Negative on both the Discriminatory NAT for HIV-1 and on the Discriminatory NAT for HCV, you may attempt to reenter the donor according to the recommendations in both sections 4.7 and 4.8 (See Figures 7 and 8, Tables 7 and 8).

Donors who were NAT-Non-Reactive (or NAT was not performed) and who were Repeatedly Reactive on a screening test for HIV-1 antibody, with an HIV-1 Western Blot or IFA that was Negative (or was not performed), or an HIV-1 Western blot result that was Indeterminate

(viral bands may be present). This includes donors previously deferred because of Repeatedly Reactive HIV serologic test results prior to the initiation of testing by NAT.

These donors may be eligible for reentry only if the HIV-1 p24 antigen EIA (if done) was Negative and if a second, different, licensed HIV-2 EIA was Negative, or, if the second HIV-2 EIA was Repeatedly Reactive, an investigational HIV-2 supplemental test was not Positive. Currently, we have not approved a supplemental (additional, more specific) test for HIV-2.

Donors who were NAT Non-Reactive and who were Negative on a screening test for HIV-1 antibody, but who were Repeatedly Reactive on an HIV-1 p24 antigen EIA with a Positive or an Indeterminate (that is, an invalid or a Non-Neutralized) result on the Neutralization test.

To reenter a donor who meets FDA eligibility criteria (i.e., the donor is otherwise eligible to donate again), we recommend that you do the following (See Figure 7 and Table 7):

At least 8 weeks after the original donation obtain a new sample from the donor (no donation is made at this time) and perform follow-up testing using:

A licensed HIV-1 NAT that is the same as the NAT (i.e., the Discriminatory NAT for HIV-1) that was run on the original donor sample or a licensed HIV-1 NAT that is labeled as sensitive for HIV-1 group O and HIV-1 group M variants;

AND

A licensed anti-HIV-1/2 EIA. If the original donor sample was repeatedly Reactive on the anti-HIV-1/2 EIA, we recommend that you use that same EIA to test this follow-up sample. If the original donor sample was Negative on the anti-HIV-1/2 EIA, we recommend that you use an Alternative EIA that is labeled as sensitive for HIV-1 Group O.

Note: If you wish to perform follow-up testing on a donor who is deferred because of HIV-1 test results, you may do so prior to the end of this 8-week waiting period for donor notification purposes or for medical reasons. Negative results on a follow-up HIV-1 test conducted before the 8-week period ends may be useful in donor counseling. However, only a Negative screening test result obtained at least 8 weeks after the NAT-Reactive or Repeatedly Reactive anti-HIV-1/2 or HIV-1 p24 EIA test result would qualify the donor for reentry. If you again obtain a Reactive NAT or a Repeatedly Reactive anti-HIV-1/2 EIA result during this 8-week waiting period, the donor would not be eligible for reentry and we recommend that you defer the donor permanently.

Evaluate the results of the follow-up testing on the donor's new sample as follows:

If the NAT is Reactive and the anti-HIV-1/2 EIA is Repeatedly Reactive, we recommend that you defer the donor permanently.

if the NAT is Reactive and the anti-HIV-1/2 EIA is Negative, we recommend that you defer the donor permanently.

If the NAT is Non-Reactive and the anti-HIV-1/2 EIA is Repeatedly Reactive, you may reconsider the donor for reentry by additional follow-up testing after a second waiting period of 8 weeks.

When there is a persistent anti-HIV-1/2 EIA Repeatedly Reactive result, you may wish to further test the donor's new sample using an HIV-1 Western Blot. If the Western Blot test result is Negative, or an Indeterminate blot pattern has not progressed, you may reconsider the donor for reentry by additional follow-up testing after a second waiting period of 8 weeks. If the Western blot result is Positive, we recommend that you defer the donor permanently.

If the NAT is Non-Reactive and the anti-HIV-1/2 EIA is Negative, you may reenter the donor (i.e., The donor is eligible to donate in the future, provided the donor meets all donor eligibility criteria).

Reentry for Donors Deferred Because of HCV Test Results

Currently, FDA has not approved a process for reentry of donors with the following HCV test results:

NAT-Reactive for HCV (either by a Discriminatory NAT after a Reactive Multiplex NAT or by a separate NAT for HCV RNA) and anti-HCV EIA Repeatedly Reactive (regardless of HCV RIBA test result);

OR

NAT Non-Reactive for HCV (or HCV NAT not performed) and anti-HCV EIA repeatedly reactive, HCV RIBA Positive.

FDA has approved a method or process for reentry of deferred donors in the following classes:

Donors who were NAT-Reactive and seronegative. This includes donors previously deferred because of Reactive test results on an investigation HCV NAT. The HCV Discriminatory NAT may have been either Positive or Negative. If an Additional NAT for HCV (validated for use with individual donor samples) was performed, it must have been Non-Reactive.

Note: If the original donor sample that was NAT-Reactive was Negative on the Discriminatory NAT for HCV but was Positive on the Discriminatory NAT for HCV, you may attempt to reenter the donor according to the recommendations in section 4.7. (See Figure 7 and Table 7). If the original donor sample that was NAT-Reactive was Positive or Negative on both the Discriminatory NAT for HCV and on the Discriminatory NAT for HIV-1, you may attempt to reenter the donor according to the recommendations in both sections 4.7 and 4.8 (See Figures 7 and 8, Tables 7 and 8).

Donors who were NAT-Non-Reactive (or NAT was not performed) and who were Repeatedly Reactive on a screening test for HCV antibody, with an HCV RIBA that was Indeterminate or Negative (or was not performed). This includes donors previously deferred because of Repeatedly Reactive HCV serologic test results prior to the initiation of testing by NAT.

To reenter a donor who meets FDA eligibility criteria (i.e., the donor is otherwise eligible to donate again), we recommend that you do the following (See Figure 8 and Table 8):

At least 6 Months after the original donation obtain a new sample from the donor (no donation is made at this time) and perform follow-up testing using:

A licensed HCV NAT.

AND

A licensed anti- HCV EIA.

Note: If you wish to perform follow-up testing on a donor who is deferred because of HCV test results, you may do so prior to the end of this 6 months waiting period for donor notification purposes or for medical reasons. Negative results on a follow-up HCV test conducted before the 6 months period ends may be useful in donor counseling. However, only a Negative screening test result obtained at least 6 months after the NAT-Reactive or Repeatedly Reactive anti- HCV test result would qualify the donor for reentry. If you again obtain a Reactive NAT or a Repeatedly Reactive anti- HCV EIA result during this 6 months waiting period, the donor would not be eligible for reentry and we recommend that you defer the donor permanently.

Evaluate the results of the follow-up testing on the donor's new sample as follows:

If the NAT is Reactive and the anti- HCV EIA is Repeatedly Reactive, we recommend that you defer the donor permanently.

if the NAT is Reactive and the anti- HCV EIA is Negative, we recommend that you defer the donor permanently.

If the NAT is Non-Reactive and the anti- HCV -1/2 EIA is Repeatedly Reactive, you may reconsider the donor for reentry by additional follow-up testing after a second waiting period of 6 months.

When there is a persistent anti-HCV EIA Repeatedly Reactive result, you may wish to further test the donor's new sample using an HCV RIBA. If the test result is Negative, you may reconsider the donor for reentry by additional follow-up testing after a second waiting period of 6 months. If the RIBA result is Positive or Indeterminate, we recommend that you defer the donor permanently.

If the NAT is Non-Reactive and the anti- HCV EIA is Negative, you may reenter the donor (i.e., The donor is eligible to donate in the future, provided the donor meets all donor eligibility criteria).

Recommendations for HBV NAT:

A. In regard to donor and unit management when the HBV DNA NAT result is negative:

- If a unit tests negative or is part of a minipool that tests negative for HBV DNA, donor and unit management should be consistent with recommendations in regard to testing donors for HBsAg and anti-HBc (see References and the following two paragraphs).
- Whole Blood units and units of blood components that test HBV DNA negative using the COBAS AmpliScreen HBV Test in ID format or in MP format and that test negative for HBsAg and anti-HBc can be used for transfusion, provided that all other blood screening tests were negative and the units were otherwise suitable for release.
- Source Plasma units that test HBV DNA negative using the COBAS AmpliScreen HBV Test in ID or MP format and that test negative for HBsAg can be used for further manufacture, provided that all other screening tests were negative and the units were otherwise suitable for release.

B. In regard to donor and unit management when the HBV DNA NAT result is positive:

- Units that test HBV DNA positive using the COBAS AmpliScreen HBV Test in MP or in ID format should be discarded and not used for blood transfusion or further manufacture.
- Donors whose units test positive for HBV DNA should be indefinitely deferred until reentry algorithms are formulated, validated, and approved.

The algorithm that the GCC is considering for management of donors who test HBV DNA positive is as follows:

1. Whole Blood and Blood Components for Transfusion (See Table 1):

Whenever a donor tests HBV NAT-positive and HBsAg repeatedly reactive (RR), and the latter result is confirmed by neutralization (irrespective of the anti-HBc test results), or tests HBV NAT-positive and anti-HBc RR (irrespective of the HBsAg test results), that donor should be permanently deferred (Categories 1, 2, 3 and 4, below). Whenever a donor tests positive for HBV DNA and tests non-reactive (NR) for anti-HBc, and either HBsAg NR, or HBsAg RR /not confirmed by neutralization (Category 5 and 6, below), that donor should be indefinitely deferred, but may be reevaluated for possible reentry, as described below. (A unit that tests positive by HBV NAT, or that is RR for HBsAg or anti-HBc should not be used.)

Table 1

Category	HBV NAT Result	HBsAg Result	Anti-HBc Result	Donor and Unit
1	Positive	Repeat Reactive / Neutralized	Non-Reactive	Unit Not Used, Donor Permanently Deferred
2	Positive	Repeat Reactive / Neutralized	Repeat Reactive	
3	Positive	Repeat Reactive / Non Neutralized	Repeat Reactive	
4	Positive	Non-Reactive	Repeat Reactive	
5	Positive	Non-Reactive	Non-Reactive	Unit Not Used, Donor Indefinitely Deferred Donor possibly may be re-entered (see below)
6	Positive	Repeat Reactive / Non Neutralized	Non-Reactive	

2. Source Plasma for Further Manufacture into Plasma Derivatives (See Table 2):

Whenever a donor tests HBV NAT-positive and HBsAg repeatedly reactive, which is confirmed by neutralization, that donor should be permanently deferred (Category 1, below). Whenever a donor tests positive for HBV DNA and tests NR for HBsAg or HBsAg RR /not confirmed by neutralization (Category 2 and 3, below), that donor should be indefinitely deferred, but may be reevaluated for possible reentry, as described below. (A unit that tests positive by HBV NAT, or that is RR for HBsAg should not be used.)

Table 2

Category	HBV NAT Result	HBsAg Result	Donor and Unit
1	Positive	Repeat Reactive / Neutralized	Unit Not Used, Donor Permanently Deferred
2	Positive	Non-Reactive	Unit Not Used, Donor Indefinitely Deferred
3	Positive	Repeat Reactive / Not Neutralized	Donor possibly may be re-entered (see below)

3. Donor Reevaluation (See Table 3):

- i. In regard to reevaluation for possible reentry of a donor of Whole Blood or blood components for transfusion, at least 6 months after the original donation, a new sample from the donor should be obtained (no donation is made at this time), and follow-up testing using individual sample HBV NAT, HBsAg, and anti-HBc licensed assays should be performed.
 - a) If a positive individual sample HBV NAT is obtained, then, irrespective of HBsAg and anti-HBc test results, the donor is permanently deferred.
 - b) If a negative individual sample HBV NAT result and a non-reactive HBsAg result and a non-reactive anti-HBc result are obtained on the sample, the donor may be reentered (that is, the donor is eligible to donate in the future, provided the donor meets all donor eligibility criteria).
 - c) If a negative individual sample HBV NAT result is obtained, together with a repeatedly reactive HBsAg result and/or a repeatedly reactive anti-HBc result, the donor should be further evaluated as described in the FDA recommendations.^{2, 3} (Note: FDA's guidance documents do not yet address reentry of donors of blood and blood components intended for transfusion, who test anti-HBc repeatedly reactive on more than one occasion.)

Follow-up testing on a donor of Whole Blood or blood components for transfusion, who is deferred because of HBV NAT results, may be performed prior to the end of this 6-month waiting period for donor notification purposes or for medical reasons. However, if a positive HBV NAT is obtained, during this 6-month waiting period, irrespective of HBsAg and anti-HBc results, the donor should be permanently deferred. Negative and non-reactive results on the follow-up HBV tests may be used in donor counseling. However, only a negative

individual sample HBV NAT result and a non-reactive HBsAg result and a non-reactive anti-HBc obtained from a sample collected at least 6 months after the original donation qualify the donor in category 5 or 6, Table 1, for reentry.

- ii. In regard to reevaluation for possible reentry of a donor of Source Plasma, at least 6 months after the original donation, a new sample from the donor should be obtained (no donation is made at this time), and follow-up testing using individual sample HBV NAT and HBsAg licensed assays should be performed.
 - a) If a positive individual sample HBV NAT is obtained, then, irrespective of the HBsAg test result, the donor is permanently deferred.
 - b) If a negative individual sample HBV NAT result and a non-reactive HBsAg result are obtained on the sample, the donor may be reentered (that is, the donor is eligible to donate in the future, provided the donor meets all donor eligibility criteria).
 - c) If a negative individual sample HBV NAT result is obtained, together with a repeatedly reactive HBsAg result, the donor should be further evaluated as described in the FDA recommendations.

Follow-up testing on a donor of Source Plasma, who is deferred because of HBV NAT results, may be performed prior to the end of this 6-month waiting period for donor notification purposes or for medical reasons. However, if a positive HBV NAT is obtained during this 6-month waiting period, irrespective of the HBsAg result, the donor should be permanently deferred. Negative and non-reactive results on the follow-up HBV tests may be used in donor counseling. However, only a negative individual sample HBV NAT result and a non-reactive HBsAg result obtained from a sample collected at least 6 months after the original donation qualify the donor in category 5 or 6 for reentry.

Table 3

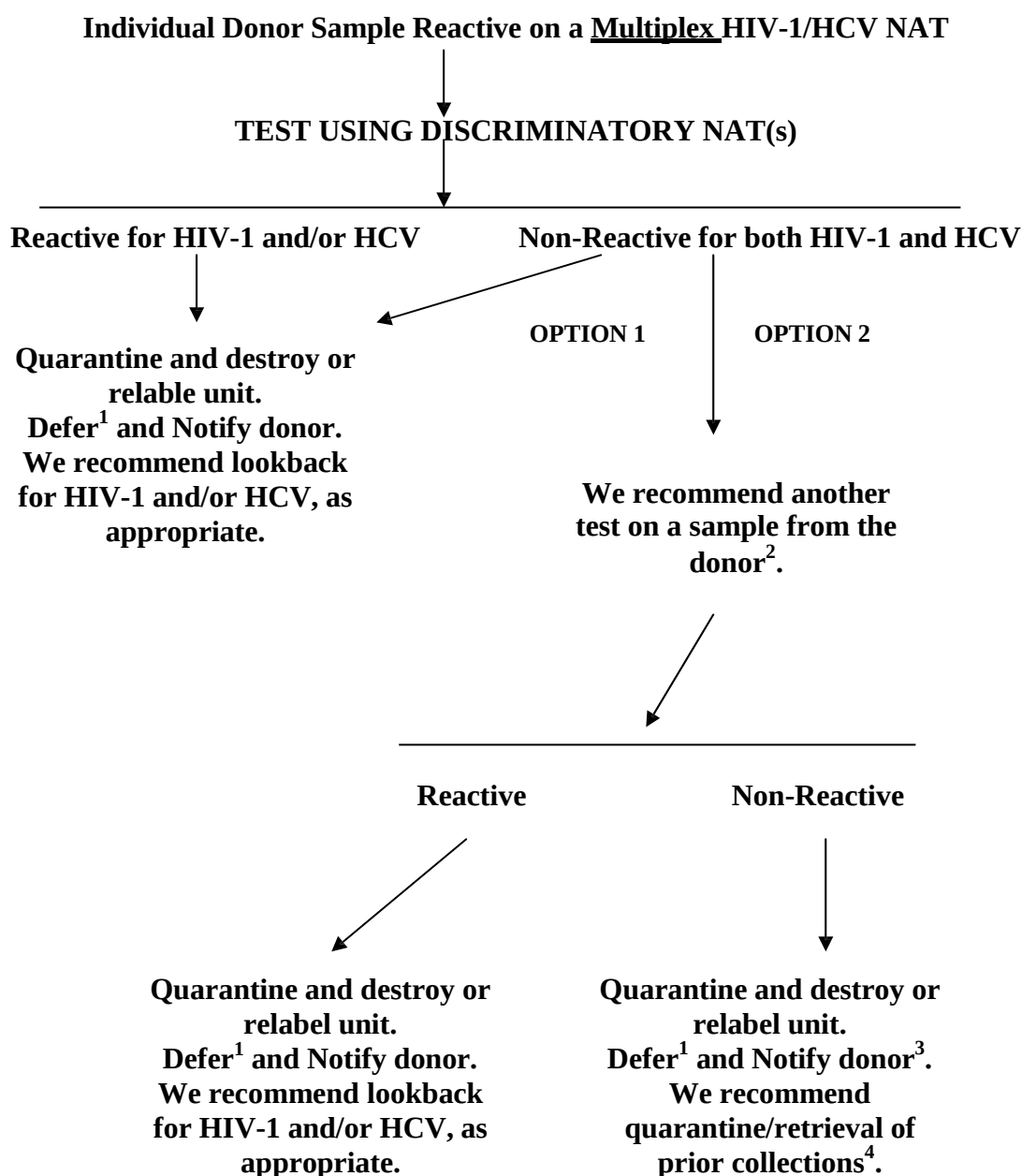
HBV NAT Result	HBsAg and/or Anti-HBc Result		Donor
a	Positive	Any test result	Permanently Deferred
b	Negative	Negative	Reenter
c	Negative	Repeat Reactive	Further evaluation

6. References

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7. FDA Memorandum to All Registered Blood Establishments: “Revised Recommendations for Testing Whole Blood, Blood Components, Source Plasma and Source Leukocytes for Antibody to Hepatitis C Virus Encoded Antigen (Anti-HCV),” August 5, 1993.
8. Federal Register, 11/16/00 (65 FR 69378), Proposed Rule: Curent Good Manufacturing Practice for Blood and Blood Components; Notification of Consignees and Transfusion Recipients Receiving Blood and Blood Components at Increased Risk of Transmitting HCV Infection (Lookback).
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<http://www.fda.gov/cber/memo.htm>
17. Recommendations for the Quarantine and Disposition of Units from Prior Collections from Donors with Repeatedly Reactive Screening Tests for Hepatitis B Virus, Hepatitis C Virus (HCV) and Human T-Lymphotropic Virus Type I (HTLV-I) - **7/19/96** <http://www.fda.gov/cber/memo.htm>
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Figure 1. Testing, product disposition, and donor management for an individual donor sample that is reactive on a multiplex NAT after a negative antibody screening test



¹The donor may be eligible for reentry (See Figures 7 and 8).

²If you test a new sample from the original donation, you may use the original NAT or Discriminatory NAT(s) or an Additional NAT. Alternatively, you may test the same sample as in the previous NAT tests (e.g., using an Additional NAT).

³You may explain to the donor that the test result, while initially Reactive, is not conclusive. There is a slight risk that the initial test result was a Positive result that cannot be excluded without follow-up testing of the donor.

⁴We do not recommend that you notify transfusion recipients.

Figure 2. Testing, product disposition, and donor management for an individual donor sample that is reactive on an individual NAT after a negative antibody screening test

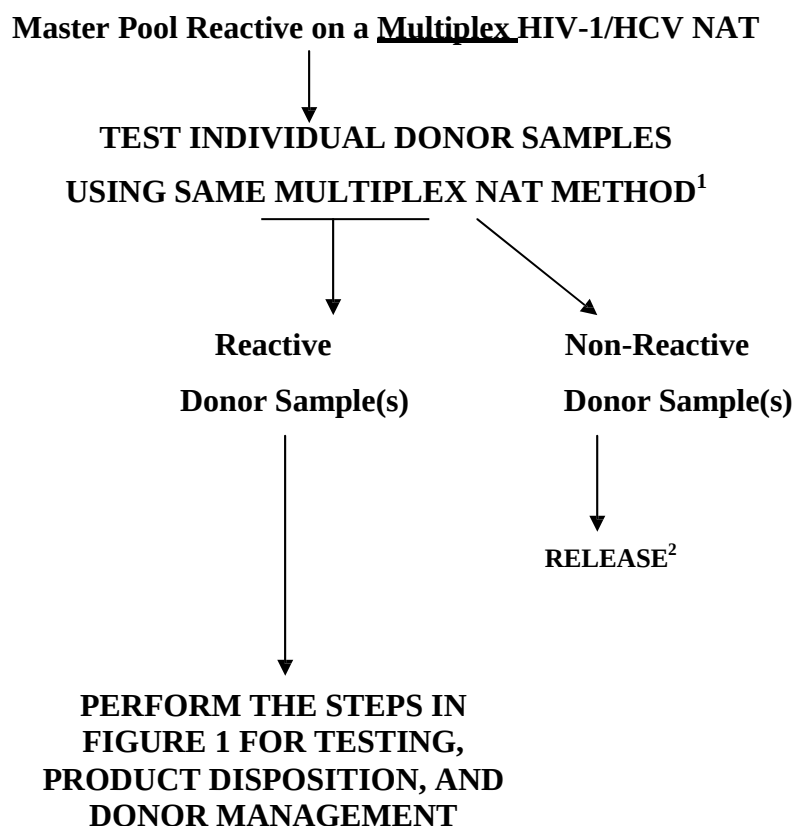
Individual Donor Sample Reactive on a HIV-1 NAT and/or /HCV NAT

↓
**Quarantine and destroy or relabel unit.
Defer donor¹
Notify donor.**

We recommend lookback for HIV-1 and/or HCV, as appropriate.

¹The donor may be eligible for reentry (See Figures 7 and 8)

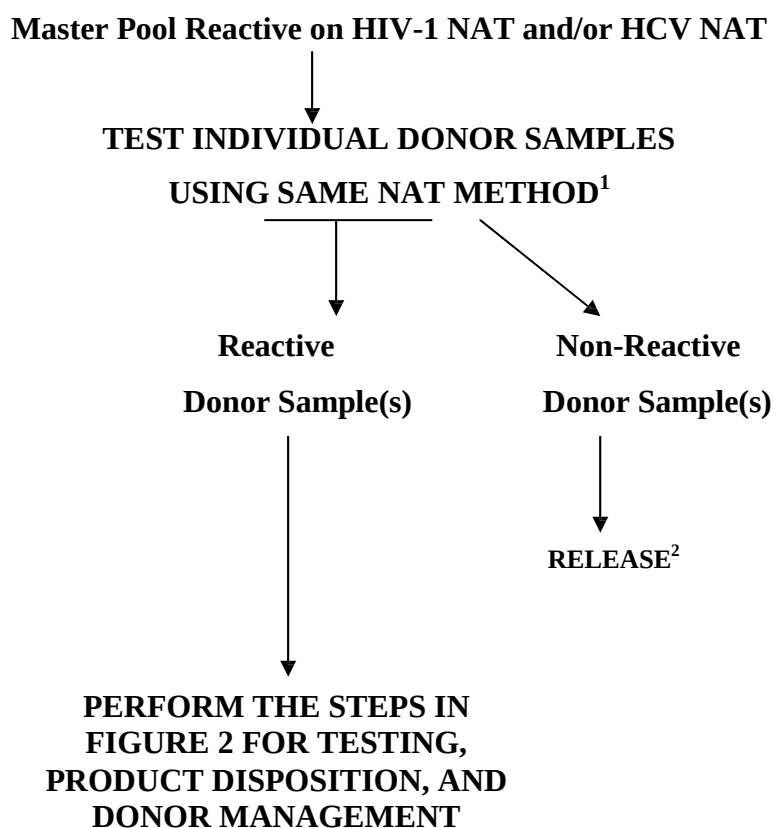
Figure 3. Testing, product disposition, and donor management for a master pool that is reactive on a multiplex NAT: resolution by testing individual donor samples



¹In some cases a different sample preparation procedure may be used per manufacturer's instruction. However, primers and probes should be same as those used in the NAT on Master Pool

²Units may be released only if serologic tests for HIV-1 and HCV are Negative and the units are otherwise suitable for release.

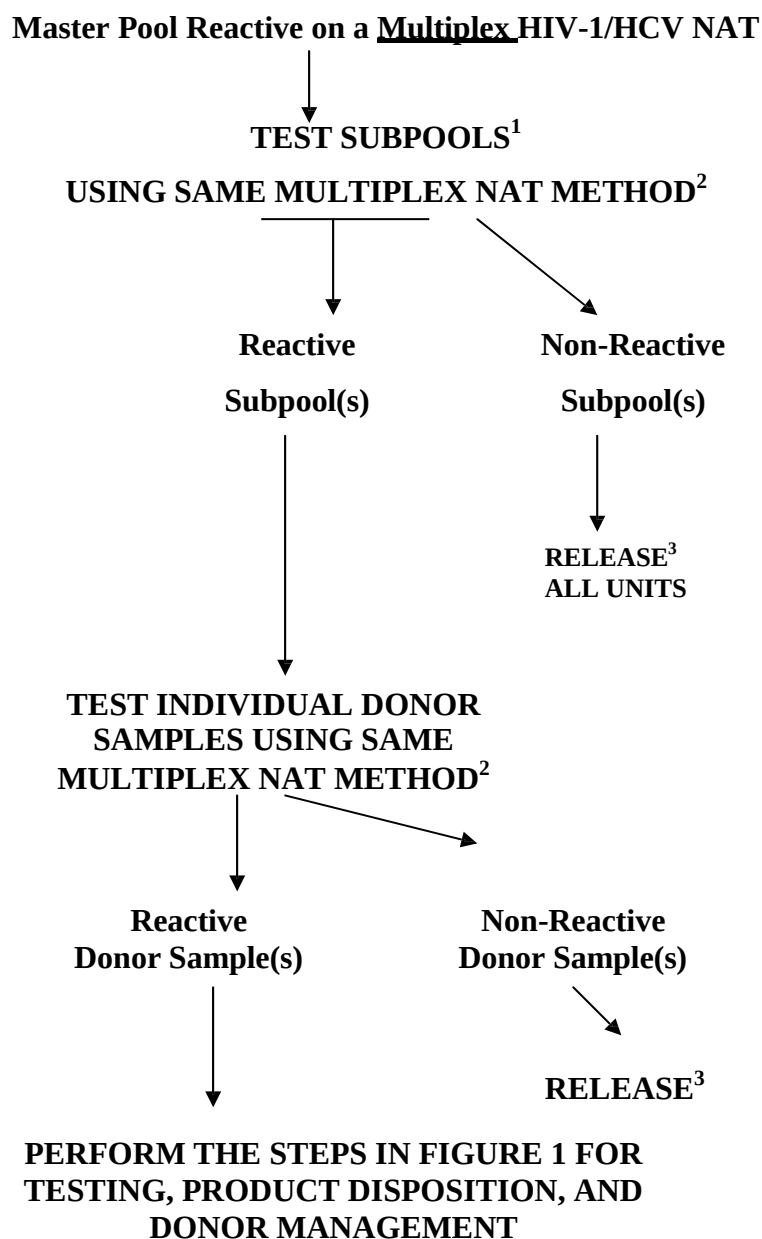
Figure 4. Testing, product disposition, and donor management for a master pool that is reactive on a individual NAT resolution by testing individual donor samples



¹In some cases a different sample preparation procedure may be used per manufacturer's instruction. However, primers and probes should be same as those used in the NAT on Master Pool

²Units may be released only if serologic tests for HIV-1 and HCV are Negative and the units are otherwise suitable for release.

Figure 5. Testing, product disposition, and donor management for a master pool that is reactive on a multiplex NAT: resolution by testing subpools.

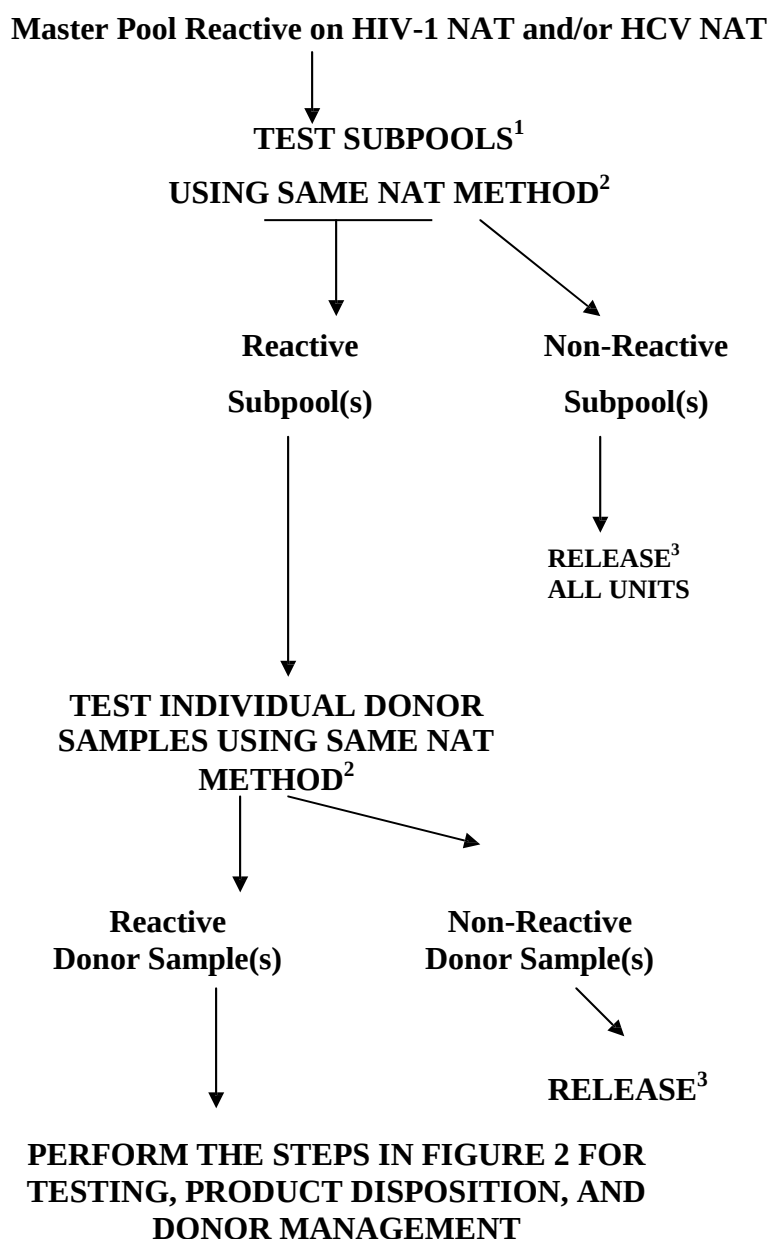


¹can be several layers of deconstruction using original or freshly pooled subpools.

²In some cases a different sample preparation procedure may be used per manufacturer's instruction. However, primers and probes should be same as those used in the NAT on Master Pool.

³Units may be released only if serologic tests for HIV-1 and HCV are Negative and the units are otherwise suitable for release.

Figure 6. Testing, product disposition, and donor management for a master pool that is reactive on an individual NAT: resolution by testing subpools

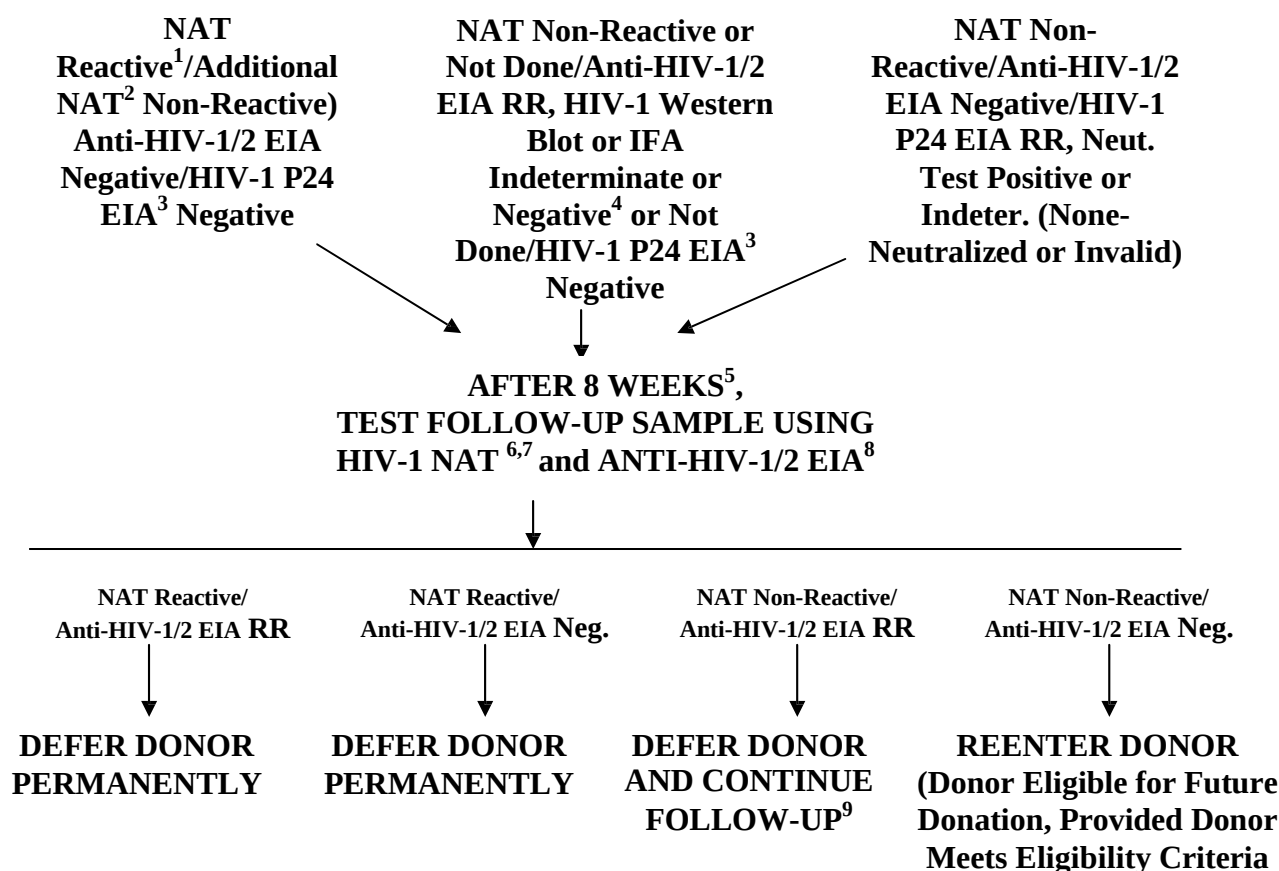


¹can be several layers of deconstruction using original or freshly pooled subpools.

²In some cases a different sample preparation procedure may be used per manufacturer's instruction. However, primers and probes should be same as those used in the NAT on Master Pool.

³Units may be released only if serologic tests for HIV-1 and HCV are Negative and the units are otherwise suitable for release.

Figure 7. Reentry for donors deferred because of HIV-1 test results



¹HIV-1 Discriminatory NAT may be Positive or Negative; however, if Negative and if HCV Discriminatory NAT is Positive, use HCV Reentry Algorithm only (See Figure 8).

²An additional NAT that has been validated for use with individual donor samples.

³May not have been performed, depending upon conditions of specific NAT approval.

⁴If a second, different, licensed HIV-2 EIA was Negative or, if Repeatedly Reactive, an investigational HIV-2 Supplemental Test was not Positive.

⁵HIV-1 NAT and/or anti-HIV1/2 EIA, if performed prior to 8 weeks, must be negative.

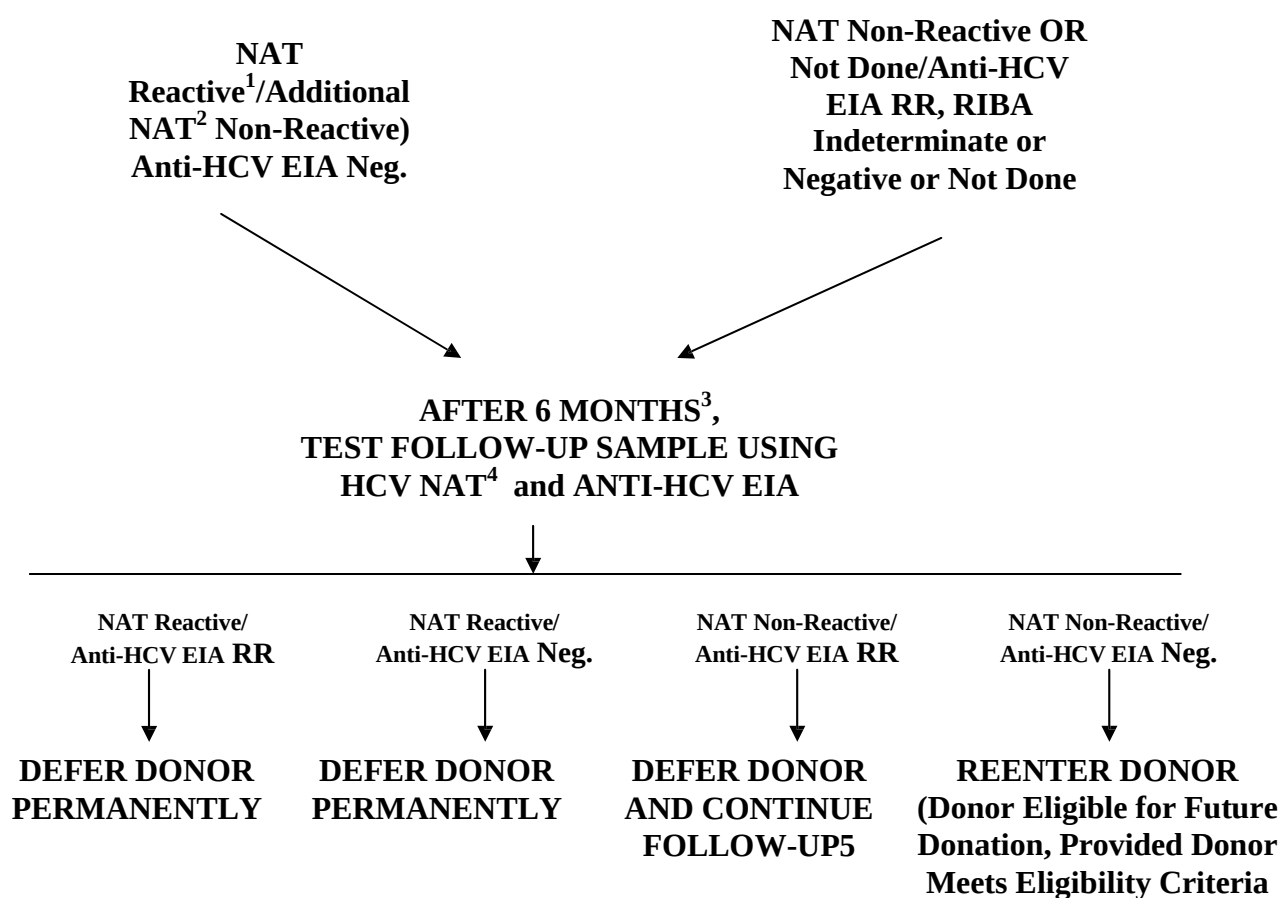
⁶If the original donor sample was Non-Discriminated using Discriminatory NAT for HIV-1 and HCV or was Positive on both of the Discriminatory NAT tests, test a follow-up sample using HCV NAT and Anti-HCV EIA also, as in HCV Reentry Algorithm (See Figure 8).

⁷Using the same NAT (i.e., the Discriminatory NAT for HIV-1) or a NAT labeled as sensitive for HIV-1 Group O and HIV-1 Group M variants.

⁸If the original donor sample was Repeatedly Reactive on the anti-HIV-1/2 EIA, we recommend that you use that same EIA to test this follow-up sample. If the original donor sample was Negative on the anti-HIV-1/2 EIA, we recommend that you use an Alternate EIA that is labeled as sensitive for HIV-1 Group O.

⁹At your option you may further test the donor's sample using HIV-1 Western Blot. If Western Blot is Negative, or if an Indeterminate blot pattern has not progressed, you may reconsider the donor for reentry by additional follow-up testing after a second waiting period of 8 weeks. If Western Blot is Positive, defer the donor permanently.

Figure 8. Reentry for donors deferred because of HCV test results



¹HCV Discriminatory NAT may be Positive or Negative; however, if Negative and if HIV-1 Discriminatory NAT is Positive, use HIV-1 Reentry Algorithm only (See Figure 7).

²An additional NAT that has been validated for use with individual donor samples.

³HCV NAT and/or anti-HCV EIA, if performed prior to 6 months, must be negative.

⁴If the original donor sample was Non-Discriminated using Discriminatory NAT for HIV-1 and HCV or was Positive on both of the Discriminatory NAT tests, test a follow-up sample using HIV-1 NAT and Anti-HIV-1/2 EIA also, as in HIV-1 Reentry Algorithm (See Figure 7).

⁵At your option you may further test the donor's sample using HCV RIBA. If RIBA is Negative, you may reconsider the donor for reentry by additional follow-up testing after a second waiting period of 6 months. If RIBA is Positive or indeterminate, defer the donor permanently.

Table 1. Testing, product disposition, and donor management for an individual donor sample that is reactive on a multiplex NAT after a negative antibody screening test

If:	Then:	After that if:	Then:	After that if:	Then:
Individual donor sample reactive on a Multiplex HIV-1/HCV NAT	Test the sample using Discriminatory NAT(s)	Reactive for HIV-1 and/or HCV	Quarantine and destroy or relabel unit; defer ¹ and notify donor; we recommend lookback for HIV-1 and/or HCV appropriate		
		Non-Reactive for both HIV-1 and HCV	Quarantine and destroy or relabel unit; defer ¹ and notify donor; we recommend lookback for HIV-1 and/or HCV as appropriate		
		OR: We recommend another test ² on a sample from the donor		Another test is Reactive	Quarantine and destroy or relabel unit; defer ¹ and notify donor; we recommend lookback for HIV-1 and/or HCV as appropriate
				Another test is Non-Reactive	Quarantine and destroy or relabel unit; defer ¹ and notify donor ³ ; we recommend quarantine/retrieval of prior collections ⁴

1. The donor may be eligible for reentry (See Figures 7 and 8).
2. If you test a new sample from the original donation, you may use the original NAT or Discriminatory NAT(s) or an Additional NAT. Alternatively, you may test the same sample as in the previous NAT tests (e.g., using an Additional NAT).
3. You may explain to the donor that the test result, while initially Reactive, is not conclusive. There is a slight risk that the initial test result was a Positive result that cannot be excluded without follow-up testing of the donor.
4. We do not recommend that you notify transfusion recipients.

Table 2. Testing, product disposition, and donor management for an individual donor sample that is reactive on an individual NAT after a negative antibody screening test

If:	Then:
Individual Donor Sample Reactive on HIV-1 NAT and/or HCV NAT	Quarantine the unit
	Destroy or relabel the unit
	Defer the donor ¹
	Notify the donor
	We recommend lookback for HIV-1 and/or HCV, as appropriate

1. The donor may be eligible for reentry (See Figures 7 and 8).

Table 3. Testing, product disposition, and donor management for master pool that is reactive on a multiplex NAT: resolution by testing individual donor samples

If:	Then:	After that if:	Then:
Master Pool Reactive on a Multiplex HIV- 1/HCV NAT	Test the individual donor samples using same Multiplex NAT methd ¹	Reactive donor sample(s)	Perform the steps in Table 1 for Testing, Product Disposition, and Donor Management
		Non-Reactive donor samples	Release ²

1. In some cases a different sample preparation procedure may be used per manufacturer's instructions. However, primers and probes should be same as those used in NAT on Master Pool.
2. Units may be released only if serologic tests for HIV-1 and HCV are Negative and the units are otherwise suitable for release.

Table 4. Testing, product disposition, and donor management for master pool that is reactive on an individual NAT: resolution by testing individual donor samples

If:	Then:	After that if:	Then:
Master Pool Reactive on HIV-1 NAT and/or HCV NAT	Test the individual donor samples using same NAT methd ¹	Reactive donor sample(s)	Perform the steps in Table 2 for Testing, Product Disposition, and Donor Management
		Non-Reactive donor samples	Release ²

1. In some cases a different sample preparation procedure may be used per manufacturer's instructions. However, primers and probes should be same as those used in NAT on Master Pool.
2. Units may be released only if serologic tests for HIV-1 and HCV are Negative and the units are otherwise suitable for release.

Table 5. Testing, product disposition, and donor management for a master pool that is reactive on a multiplex NAT: resolution by testing subpools

If:	Then:	After that if:	Then:	After that if:	Then:
Master Pool reactive on a Multiplex HIV-1/HCV NAT	Test subpools ¹ using same Multiplex NAT method ²	Reactive subpools	Test the individual donor samples using same Multiplex NAT method ²	Reactive donor sample(s)	Perform the steps in Table 1 for Testing, product disposition, and donor management
				Non-Reactive Donor Samples	Release ³
		Non-Reactive subpools(s)	Release all units ³		

1. Can be several layers of deconstruction using original or freshly pooled subpools.
2. In some cases a different sample preparation procedure may be used per manufacturer's instruction. However, primer and probes should be same as those used in the NAT on Master Pool.
3. Unit may be released only if serologic tests for HIV-1 and HCV are Negative and the units are otherwise suitable for release.

Table 6. Testing, product disposition, and donor management for a master pool that is reactive on an individual NAT: resolution by testing subpools

If:	Then:	After that if:	Then:	After that if:	Then:
Master Pool reactive on HIV-1 NAT and/or HCV NAT	Test subpools ¹ using same NAT method ²	Reactive subpools	Test the individual donor samples using same NAT method ²	Reactive donor sample(s)	Perform the steps in Table 2 for Testing, product disposition, and donor management
				Non-Reactive Donor Samples	Release ³
		Non-Reactive subpools(s)	Release all units ³		

1. Can be several layers of deconstruction using original or freshly pooled subpools.
2. In some cases a different sample preparation procedure may be used per manufacturer's instruction. However, primer and probes should be same as those used in the NAT on Master Pool.
3. Units may be released only if serologic tests for HIV-1 and HCV are Negative and the units are otherwise suitable for release.

Table 7. Reentry for donors deferred because of HIV-1 test results

If:	Then:	After that if:	Then:
NAT Reactive ¹ /(Additional NAT ² non-Reactive)/Anti-HIV-1/2 EIA Negative/HIV-1 p24 EIA ³ Negative OR NAT Non-Reactive or Not Done/Anti-HIV-1/2 EIA RR, HIV-1 WB or IFA Indeterminate or Negative ⁴ or Not Done/HIV-1 p24 EIA ³ Negative OR NAT Non-Reactive/Anti-HIV-1/2 EIA Negative/HIV-1 p24 EIA RR, Neut. Test Positive or Indeterminate (Non-Neutralized or Invalid)	After 8 weeks ⁵ test follow-up sample using HIV-1 NAT ^{6,7} and Anti-HIV-1/2 EIA ⁸	NAT Reactive/Anti-HIV-1/2 EIA RR	Defer donor permanently
		NAT Reactive/Anti-HIV-1/2 EIA Negative	Defer donor permanently
		NAT Non-Reactive/Anti-HIV1/2 EIA RR	Defer donor and continue follow-up ⁹
		NAT Non-Reactive/Anti-HIV-1/2 Negative	Reenter donor (donor eligible for future donation, provided donor meets eligibility criteria)

1. HIV-1 Discriminatory NAT may be Positive or Negative; however, if Negative and if HCV Discriminatory NAT is Positive, use HCV Reentry Algorithm only (See Table 8).
2. An additional NAT that has been validated for use with individual donations.
3. May not have been performed, depending upon conditions of specific NAT approval.
4. If a second, different, licensed HIV-2 EIA was Negative or, if repeatedly Reactive, an investigational HIV-2 Supplemental Test was not Positive.
5. HIV-1 NAT and/or anti-HIV1/2 EIA, if performed prior to 8 weeks, must be negative.
6. If the original donor sample was Non-Discriminated using Discriminatory NAT for HIV-1 and HCV or was Positive on both of the Discriminatory NAT tests, test a follow-up sample using HCV NAT and Anti-HCV EIA also, as in HCV Reentry Algorithm (See Table 8).
7. Using the same NAT (i.e., the Discriminatory NAT for HIV-1) or a NAT labeled as sensitive for HIV-1 Group O and HIV-1 Group M variants.
8. If the original donor sample was Repeatedly Reactive on the anti-HIV-1/2 EIA, we recommend that you use that same EIA to test this follow-up sample. If the original donor sample was Negative on the anti-HIV-1/2 EIA, we recommend that you use an Alternate EIA that is labeled as sensitive for HIV-1 Group O.

9. At your option you may further test the donor's sample using HIV-1 Western Blot. If Western Blot is Negative, or if an Indeterminate blot pattern has not progressed, you may reconsider the donor for reentry by additional follow-up testing after a second waiting period of 8 weeks. If Western Blot is Positive, defer the donor permanently.

Table 8. Reentry for donors deferred because of HCV test results

If:	Then:	After that if:	Then:
NAT Reactive ¹ /(Additional NAT ² non-Reactive)/Anti-HCV EIA Negative OR NAT Non-Reactive or Not Done/Anti-HCV EIA RR, RIBA Indeterminate or Negative or Not done	After 6 months ³ test follow-up sample using HCV NAT ⁴ and Anti-HCV EIA	NAT Reactive/Anti-HCV EIA RR	Defer donor permanently
		NAT Reactive/Anti-HCV EIA Negative	Defer donor permanently
		NAT Non-Reactive/Anti-HCV EIA RR	Defer donor and continue follow-up ⁵
		NAT Non-Reactive/Anti-HCV Negative	Reenter donor (donor eligible for future donation, provided donor meets eligibility criteria)

1. HCV Discriminatory NAT may be Positive or Negative; however, if Negative and if HIV-1 Discriminatory NAT is Positive, use HIV-1 Reentry Algorithm only (See Table 7).
2. An additional NAT that has been validated for use with individual donations.
3. HCV NAT and/or anti-HCV EIA, if performed prior to 6 months, must be negative.
4. If the original donor sample was Non-Discriminated using Discriminatory NAT for HIV-1 and HCV or was Positive on both of the Discriminatory NAT tests, test a follow-up sample using HIV-1 NAT and Anti-HIV-1/2 EIA also, as in HIV-1 Reentry Algorithm (See Table 7).
5. At your option you may further test the donor's sample using HCV RIBA. If RIBA is Negative, you may reconsider the donor for reentry by additional follow-up testing after a second waiting period of 6 months. If RIBA is Positive or Indeterminate, defer the donor permanently.



Executive Board of the Health Ministers' Council for GCC States

Guideline on the Scientific Data Requirements for Plasma Master File (PMF)

Version 1.0

Date issued	01/08/2016
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Document Control

Version	Date	Author(s)	Comments
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This document provides guidance on the structure and requirements for presentation of data on starting material in a Plasma Master File (PMF). This guidance shall also apply when the PMF certification scheme is not followed.

Introduction and Principle of a PMF

The PMF contains common information on plasma, from collection to plasma pool, relevant to the manufacture of all human plasma-derived medicinal products and medical devices for which this PMF is applicable.

Cryoprecipitates and any other intermediates are not part of the PMF dossier; these should be described in the relevant sections of the dossier for each individual medicinal product, medical device or investigational medicinal product. This Guideline describes the structure and scientific data required to be submitted in a PMF.

Applicants using the PMF certification system need to clearly identify' and make reference to the PMF in the dossier of each medicinal product. Reference to more than one PMF is possible but this must be clearly indicated. Where information is specific to a particular product (e.g. immunization scheme used for specific immunoglobulin) this should be included in the dossier for the relevant product and not in the PMF, unless otherwise stated in this guideline.

Annual updates:

The PMF shall be updated and re-submitted for approval on an annual basis. The scientific documentation for the annual update should include the following:

- Update as described in annex 1 “check list on annual update”.
- A list of all changes applied for with the annual update
- List of commitments or follow up measures and accompanying requested data.
- A compiled updated integrated PMF including:
 - All changes submitted during the year and with the annual update, including updated lists with highlighted changes, and specifying historic information where this is still relevant for batches that may be on the market e.g. information on the period when an organization was actively supplying plasma.
 - Update in sections 1.2, 2.1.3 and 2.3. In addition, when relevant, update on deletions of country(ies) and/or organization(s)/establishment(s) used for blood/plasma collection, or in which testing of donation and plasma pools is carried out, and deletion of blood bag(s) may be submitted at the annual update. The reason for the deletion should be specified.

- The newly available epidemiological data of the blood/plasma collection establishments together with its scientific evaluation.
- Update of inspection/audit status of blood establishments (see annexes).
- Cases for which it was retrospectively found that a donation should have been excluded from processing or has been excluded and a viral marker found retrospectively to be positive.
- The results of NAT (Nucleic Acid Amplification Technique) testing of plasma pools indicating how many positive pools/mini-pools were detected and how many positive donations were identified.
- Participation in proficiency studies (viral marker testing and NAT testing).

1. General Information (Summary)

1.1. Plasma-derived products' list

The Plasma Master File shall provide a list of the medicinal products for which the Plasma Master File is valid, whether the medicinal products have been granted a marketing authorization or are in the process of being granted such an authorization, with the relevant Competent Authority(ies) for each plasma-derived product. This list should also include medical devices incorporating stable derivatives of human blood or human plasma.

1.2 Overall safety strategy

Critical evaluation of the contribution of each of the significant steps in the processing of plasma from collection to preparation of the plasma pool to the overall safety strategy should be provided. It should demonstrate how different aspects of the PMF interrelate to contribute to the overall safety of the plasma. This critical evaluation should incorporate all aspects of the PMF and draw together the following information; the epidemiological data on blood transmissible infections known for the donor population, criteria for the use of donations from first time donors (when applicable), the system of donor selection criteria including measures that reduce the risk of (v)CJD, screening of donations, minipool strategy if relevant, the testing of the plasma pools, viral load limits for plasma pools and normal size of manufacturing pool, inventory hold and “look-back” procedures, The critical evaluation should be supported by diagrams, e.g. to describe the plasma donation test system and strategy of (mini/plasma) pool testing. The aim should be to demonstrate how the company's strategy integrates to robustly ensure that all measures taken throughout the collection, processing, testing, storage and transport of the plasma work together to provide a safe plasma pool.

The estimated residual risk of missing viraemic donations that may enter the production pool should be described.

1.3 General logistics

A flow-chart, describing the complete supply-chain for plasma from collection to the manufacture of the plasma pool, should be provided. This should/include all relevant organizations involved in the collection, testing, storage and transport of blood or plasma. The arrangements in place between collection establishments and testing laboratories should be clearly indicated in the flow chart.

2. Technical Information on Starting Materials

The quality and safety of products derived from human plasma rely both on the source plasma material and the further manufacturing processes. Therefore, the collection, testing, storage and transportation of human plasma are major factors in the quality assurance of the manufacture of plasma-derived products. These operations should be subject to periodic inspections in order to ensure the expected product quality.

An exhaustive list of names and addresses of blood establishments and dedicated collection centers excluding mobile or temporary equipped sites, in which collection and/or testing, storage and transport of donations and testing of plasma pools is carried out, including any sub contractors should be provided using the tabular format given in the annexes II, III, IV and V together with the relevant supporting documents.

2.1 Plasma Origin:

2.1.1 Information on centers or establishments in which blood/plasma collection is carried out, including inspection and approval, and epidemiological data on blood transmissible infections.

A.Information on centers or establishments in which blood/plasma collection is carried out
See Annex II.

An exhaustive list of names and addresses of blood establishments and dedicated collection centers, excluding mobile or temporary equipped sites, from which plasma is still available, should be provided as an appendix to this section. Suppliers of plasma should also indicate the address, duties and approval status by the regulatory authority of their look back department. The suppliers of plasma, for which special criteria have been defined (such as anti-D), should be identified.

Blood establishments and dedicated collection centers excluding mobile or temporary equipped sites, should be inspected and approved by a GCC regulatory authority in accordance with the (GCC guidelines for collection and testing blood and blood products). If blood/plasma centers are closed and plasma is still available, they should be kept in the list clearly indicating the date of closure and the reason. For those centers that are temporarily closed and or have stopped delivering plasma, explanation of their status should be provided. Traceability should be guaranteed in all cases when the center is closed.

If mobile or temporary equipped sites are used, the PMF holder should indicate the total number of them and how they relate to the organization.

B.Characteristics of donations

For any organization responsible for collection it should be specified whether the donors are non-remunerated or remunerated. The nature of any compensation for donation should be described if applicable. “A donation is considered voluntary and non-remunerated if the person gives blood, plasma or cellular components of his/her own free will and receives no payment of it, either in the form of cash or in kind which could be considered a substitute for money. This would include time off work other than that reasonably needed for the donation and travel. Small tokens, refreshments and reimbursements of direct travel costs are compatible with voluntary, non-remunerated donations.

C. Epidemiological data on blood transmissible infections should be submitted in accordance with Guideline on Epidemiological Data on Blood Transmissible Infections.

2.1.2 Information on centers or establishments in which testing of donations and plasma pools is carried out, including inspection and approval status.

See Annex III.

The test laboratory used for each center should be specified.

If confirmatory tests are performed at separate laboratories full details should be supplied.

If test laboratories are no longer used they should be listed in a stand-alone table indicating the date when use of the laboratory ceased and the reason.

2.1.3. Selection / exclusion criteria for blood /plasma donors.

Confirm for each organization the compliance with the Selection / exclusion criteria for blood /plasma donors according to the requirements of the European pharmacopeia's Monographs. Where appropriate, indicate compliance with GCC regulations and international recommendations. In addition, specify any requirements versus emerging infectious agents in a specific country and confirm that Selection / exclusion criteria for blood /plasma donors are in compliance with such requirements.

2.1.4. System in place which enables the path taken by each donation to be traced from the blood / plasma collection establishment through to finished products and vice versa.

Describe the system in place, which enables the path taken by each donation to be traced from the blood / plasma collection establishment through to finished products, including testing facility and vice versa. Confirm compliance with GCC guidelines especially concerning traceability including labeling and record keeping. If several organizations / countries are involved, the information is given for each system. Include information on how traceability is maintained for closed collection centers.

Given information on steps that would be taken if it was found retrospectively that donation (S) should have been excluded from processing (look-back procedure , any system in place to retain samples) and justify the system . Specify the length of look-back period in accordance with GCC guidelines.

The information on system for traceability and post-donation information measures should also be provided in the case of intermediates and plasma- derived products supplied to third parties (e.g. albumin supplied for use as excipient).

2.2 Plasma quality and safety

2.2.1 Compliance with European Pharmacopoeia Monographs.

Confirm compliance with the Ph. Eur, Monograph for Human Plasma for Fractionation. Confirm compliance with any requirements for particular products for which Ph. Eur. Monographs exist.

2.2.2 Testing of blood/plasma donations and pools for infectious agents, including information on test methods and, in the case of plasma pools, validation data on the tests used.

Information should be provided:

- On screening tests for markers of infection required according to the GCC guidelines for collection and testing blood and blood products and the European Pharmacopoeia.
- On any other screening tests carried out

Test	Tests Performed on:		
	Individual Donation	Minipool (size) (if few, nil)	Plasma Pool
HBsAg			
HIV 1 and 2 Antibody			
HCV Antibody			
HCV RNA			
B19 DNA			
Other tests			

When minipools of donations are tested, the rationale and full details of this testing should be provided, including the size of the minipools. It should be clarified whether all donations are tested in the same way. Criteria for acceptance or rejection of donation/pool and re-testing policy should be described.

Validation of testing methods:

a. Testing of donations

Confirm that tests are carried out in accordance with the manufacturers' directions for use.

Copies of instructions for use of commercial kits are not needed.

For CE-marked test kits submission of complete validation data are not required. In the absence of a CE mark, the applicant should demonstrate that the test kit can be considered "state of the art" according to Common Technical Specifications (CTS), with particular attention to evidence for seroconversion sensitivity and sub-type sensitivity in comparison with a CE marked test kit .

In case of mini pool testing by NAT as part of the screening of individual donations, a brief description of the analytical procedures for all the NAT methods should be provided, whether non CE-marked in-house methods or commercial kits are used. A summary of the validation reports should also be provided and should include specificity, detection limit and robustness. The description of the analytical procedures and validation are not required for the testing of small pools by NAT if the test is CE marked for this purpose. In the latter case, though, information on the detection limit as related to the single donation, should be provided.

b. Viral marker testing of the plasma pool(s)

For every testing laboratory, provide a description of the test method and a report on the validation of the test methods used on each plasma pool & according to the guidelines:

- Guideline on Validation of Immunoassay for the Detection of Antibody to human immunodeficiency Virus (Anti-HIV) in Plasma Pools, (EMEA/CHMP/BWP/298388/2005) .
- Guideline on Validation of Immunoassay for the Detection of Hepatitis B Virus Surface Antigen (HBsAg) in Plasma Pools, (EMEA/CHMP/BWP/298390/2005).

Information on the sensitivity of the test for each marker as a function of pool size should also be included.

c. NAT testing of the Plasma pool(s)

For every testing laboratory, provide a description of the test method and a validation report for each of the NAT tests performed.

NAT for HCV RNA is required by the Ph. Eur. Monograph “Human Plasma for Fractionation”. Validation is carried out according to the Ph. Eur. Guidelines for validation of NAT for the detection of HCV RNA in plasma pools. As recommended in this guideline, the ability of the analytical procedure to detect all HCV genotypes is demonstrated.

If the list of plasma-derived products for which the PMF is valid includes anti-immunoglobulin for intravenous and/or intramuscularly administration and/or human plasma (pooled and treated for virus inactivation), NAT for B 19 DNA is also carried out as required by the respective Ph. Eur. Monographs. The maximum B19 virus burden should be in accordance to the current version of the Ph. Eur. monograph. Validation is performed according to the guideline for validation of NAT for quantification of B 19 virus DNA in plasma pools . Included in this guideline is the recommendation that for the design of primers and probes the existence of the B19 variants A6 and V9 is taken into account, The International Committee on the Taxonomy of Viruses (ICTV) classifies these variants under B19 virus (Eighth report of the ICTV, Eds.; C.M. Fauquet, M.A. Mayo et al., Elsevier, page 361).

In case that the applicant performs NAT testing for viruses other than HCV and B 19 the validation studies are carried out according to the following guidelines:

- ICH Topic Q2A Note for guidance on validation of analytical methods: definitions and terminology (CPMP/ICH/38 1/95).
- ICH Topic Q2B Note for guidance on validation of analytical procedures: methodology (CPMP/ICH/28 1/95).
- Ph. Eur. General method 2.6.21 “Nucleic acid amplification techniques” (NAT).

For practical purposes, in the case of NAT qualitative methods, validation is carried out taking into consideration the above mentioned guideline Validation of NAT for the Detection of 1-WV RNA in Plasma Pools.

Provide information on specificity, including the ability of the assays to detect different genotypes, sensitivity and robustness.

Proficiency Studies: Results arising from participation in proficiency studies should be reported.

2.2.3 Technical characteristics of bags for blood and plasma collection, including information on anticoagulant solutions used.

Justification should be given when the bag is not CE marked under Council Directive 93/42/EEC concerning Medical Devices, as amended. In addition, for non-CE marked bags the following information should be provided in an Appendix to this section:

Describe the material of the bag, the composition of the bag and its specification; confirm that materials comply with Ph. Eur., describe the sterilization procedure including its validation. Where the bag contains an anticoagulant solution, give information on production and quality control as for a medicinal product and confirm compliance with Ph. Eur. requirements.

2.2.4 Conditions of storage and transport of plasma.

See Annex IV and V.

Describe the conditions for freezing and storage of plasma for every establishment responsible for collecting blood/plasma (i.e. time to freezing, time to reach the target core temperature, at which temperature, to which temperature,) including the following:

- Compliance with Ph. Eur. with respect to freezing and storage.

- Sites/organizations which are involved in the storage and indicate whether they have been inspected by a Competent Authority.
- Conditions of storage (temperature and maximum time).
- Data on the validation of the freezing conditions.

Describe the conditions of transport of plasma including the following:

- Confirm compliance with requirements in the Ph. Eur. Monograph for Human Plasma for Fractionation and if applicable, with any Ph. Eur. requirements for particular products.
- Transport flows from centers of collection to interim storage, if relevant, and further to fractionation sites.
- Organizations which are involved in the transport (own and contractors) and indicate whether they have been inspected by a Competent Authority.
- Provide a summary of the system in place to ensure the transport is performed under appropriate conditions. (Time, temperature and GMP compliance). Provide validation data to support the storage and transport conditions.

2.2.5 Procedures for any inventory hold period.

Provide details of any inventory hold procedure and provide the justification for the chosen period. Specify whether the procedure applies to all plasma or specify for which plasma it is applicable.

2.2.6 Characterization of the plasma pool.

Plasma pool preparation:

Provide details of all sites at which the pooling of plasma is performed.

(Give a short description of all the relevant procedures in preparation of the plasma pool: thawing process, inspection of individual bags or bottles before pooling, opening and pooling.

Indicate the size of the plasma pool, in number of donations and in liters.

Clarify whether or not the plasma pool is the same for all products.

Sampling of plasma pool:

Define and justify the plasma pool (e.g. cryosupernatant or complete plasma pool) from which the samples for the viral marker testing are obtained.

Describe the sampling procedure, any manipulation of samples and the storage conditions of the pool samples.

Testing of the plasma pools for all sites should be performed in accordance with the details provided in this plasma master file.

2.3 System in place between the plasma-derived medicinal product manufacturer and/or plasma fractionators/processor on the one hand, and blood establishments on the other hand, which defines the conditions of their interaction and their agreed specifications.

Confirm that a contract exists between the blood establishments on one hand and the manufacturer on the other hand to ensure the interaction between them. In addition, confirm that adequate criteria have been agreed between these organizations in order to allow action to be taken when appropriate.

Concerning systems for notification, confirm compliance with Directive 2002/98/EC of the European Parliament and of the Council of 27 January 2003 setting standards of quality and safety for the collection, testing, processing, storage and distribution of human blood and blood components and amending Directive 2001/83/EC, and Commission Directive 2005/61/EC of 30 September 2005 implementing Directive 2002/98/EC of the European Parliament and of the Council as regards tractability requirements and notification of serious adverse reactions and events.

It should be declared that all blood establishments have signed the contracts mentioned above.

ANNEX I: CHECK LIST ON THE ANNUAL UPDATE

Checklist to be used with the Annual Update of the PMF

Change/Update	Submitted with annual update (AU) or Notified/Approved during the year (N or A)	Scope and reason for change	Type ¹¹	Variation Number ¹²	PMF procedural number	Date of Notification /Approval	Comment/ Implementation date	Implemented current PMF ¹³
Item: 1.1 Plasma-derived products' list								
Change								
Item: 2.1.1 Information on centres or establishments in which blood/plasma collection is carried out, including inspection and approval, and epidemiological data on blood transmissible infections								
Addition of country of blood/plasma collection centres or establishments								
Deletion of country of blood/plasma collection centres or establishments								
Change in the organisation								
Change in the name of organisation								

Change/Update	Submitted with annual update (AU) or Notified/Approved during the year (N or A)	Scope and reason for change	Type ¹¹	Variation Number ¹²	PMF procedural number	Date of Notification /Approval	Comment/ Implementation date	Implemented current PMF ¹³
Addition of organisation								
Deletion of organisation								
Addition of a new blood/plasma collection establishment for an organisation already included in the PMF.								
Addition of a blood/plasma collection establishment for an organisation not yet included in the PMF								
Deletion of a blood/plasma collection establishment								
Change of characteristics of donations								
Item: 2.1.2 Information on centres or establishments in which testing of donations and plasma pools is carried out, including inspection and approval status								
Addition or change of a site testing the donations within an organisation already included in the PMF								

Change/Update	Submitted with annual update (AU) or Notified/Approved during the year (N or A)	Scope and reason for change	Type ¹¹	Variation Number ¹²	PMF procedural number	Date of Notification /Approval	Comment/ Implementation date	Implemented current PMF ¹³
Addition or change of a site testing the donations within an organisation not yet included in the PMF								
Deletion of a site testing the donations within an organisation included in the PMF								
Addition or change of a site testing mini-pools/plasma pools within an organisation already included in the PMF								
Addition or change of a site testing mini-pools/plasma pools within an organisation not yet included in the PMF								
Deletion of a site testing mini-pools/plasma pools within an organisation included in the PMF								
Item 2.1.4. System in place which enables the path taken by each donation to be traced from the blood/plasma collection establishment through to finished products and vice versa								
Change in the system in place, which enables the path taken by each donation to be traced from the blood/plasma collection establishment through to finished products and vice versa								

Change/Update	Submitted with annual update (AU) or Notified/Approved during the year (N or A)	Scope and reason for change	Type ¹¹	Variation Number ¹²	PMF procedural number	Date of Notification /Approval	Comment/ Implementation date	Implemented current PMF ¹³
Change in 'look-back' procedure								
Item: 2.2.2 Testing of blood/plasma donations and pools for infectious agents, including information on test methods and, in the case of plasma pools, validation data on the tests used								
Change of test performed on donations/mini-pools/plasma pools (specification)								
Change in kits/methods to test donations/mini-pools/plasma pools								
Item: 2.2.3 Technical characteristics of bags for blood and plasma collection, including information on anticoagulant solutions used								
Addition of or replacement with a CE marked blood bag								
Addition of or replacement with a non -CE marked blood bag								
Changes of the composition, production, shelf life and control of non-CE marked blood bags								
Deletion of CE marked blood bag								

Change/Update	Submitted with annual update (AU) or Notified/Approved during the year (N or A)	Scope and reason for change	Type ¹¹	Variation Number ¹²	PMF procedural number	Date of Notification /Approval	Comment/ Implementation date	Implemented current PMF ¹³
Item: 2.2.4 Conditions of storage and transport of plasma								
Change in the sites/organisations involved in the storage and/or transport								
Change in storage and/or transport conditions								
Item: 2.2.5 Procedures for any inventory hold period								
Introduction/extension/ a more stringent procedure e.g. release only after retesting of donors								
Removal or reduction in length of period								
Item: 2.2.6 Characterisation of the plasma pool								
Change in plasma pool preparation (e.g. manufacturing method, pool size, storage, control procedures, sampling)								

**ANNEX II: INFORMATION ON CENTRES OR ESTABLISHMENTS
IN WHICH BLOOD/PLASMA COLLECTION IS CARRIED OUT**

Address	Sequential Number ¹⁴	Activity		Inspection by an EEA competent authority			Inspection by a non-EEA competent authority			Audit/Certification			
		Plasma- pheresis	Whole Blood collection	Member State	Date of last inspection	Final outcome (observations ¹⁵)	Country	Date of last inspection	Final outcome (observations ¹⁶)	Description ¹⁷	Name of organi- sation ¹⁸	Outco- me	Date
Organisation 1 responsible for collection													
Country 1													
Full address site 1 Organisation 1 Country 1		☐	☐										
Full address site 2 Organisation 1 Country 1		☐	☐										
Full address site 3 Organisation 1 Country 1		☐	☐										
Organisation 2 responsible for collection													
Country 1													
Full address site 1 Organisation 2 Country 1		☐	☐										
Full address site 2 Organisation 2 Country 1		☐	☐										
Country 2													
Full address site 3 Organisation 2 Country 2		☐	☐										

**ANNEX III: INFORMATION ON CENTRES OR ESTABLISHMENTS
IN WHICH TESTING OF DONATIONS AND PLASMA POOLS IS CARRIED OUT**

Address	Specify the sequential number(s) of the collection centre(s) for which the testing is performed	Testing				Inspection by an EU competent authority			Inspection by a non-EU competent authority			Audit			
		Viral Marker		NAT testing		Member State	Date of last inspection	Final outcome (observations ¹⁹)	Country	Date of last inspection	Final outcome observations	Description ²¹	Name of organisation ²²	Outcome	Date
		Donations	Plasma Pools	Donations	Plasma Pools										
Organisation 1 responsible for collection															
Country 1															
Full address site 1 Organisation 1 Country 1		☐	☐	☐	☐										
Full address site 2 Organisation 1 Country 1		☐	☐	☐	☐										
Country 2															
Full address site 3 Organisation 1 Country 2		☐	☐	☐	☐										
Organisation 2 responsible for collection															
Country 1															
Full address site 1 Organisation 2 Country 1		☐	☐	☐	☐										
Full address site 2 Organisation 2 Country 1		☐	☐	☐	☐										

Documented evidence of satisfactory outcome should be submitted (e.g. certificate, close-out letter, inspection report). If the final outcome is not satisfactory, a summary objections found during the last inspection, the follow-up and the appropriate corrective actions should be included.

Describe nature of and frequency of audit/certification

Specify name of organization carrying out the audit/certification

**ANNEX IV: INFORMATION ON CENTRES AND ESTABLISHMENTS
IN WHICH STORAGE OF DONATIONS AND PLASMA POOLS IS CARRIED OUT**

Address	Specify the sequential number(s) of the collection centre(s) for which the storage is performed	Storage		Inspection by an EU competent authority			Inspection by a non-EU competent authority			Audit (Frequency and date of last audit)				Conditions of storage (temperature and maximum time) Compliance with Ph Eur with respect to freezing and storage
		Freezer	Freezing Room	Member State	Date of last inspection	Final outcome (observations ²⁵)	Country	Date of last inspection	Final outcome observations	Description ²⁵	Name of organisation ²⁶	Out come	Date	
Organisation 1 responsible for collection														
Country 1														
Full address site 1 Organisation 1 Country 1	☐	☐												
Full address site 2 Organisation 1 Country 1	☐	☐												
Country 2														
Full address site 3 Organisation 1 Country 2	☐	☐												
Organisation 2 responsible for collection														
Country 1														
Full address site 1 Organisation 2 Country 1	☐	☐												

Documented evidence of satisfactory outcome should be submitted (e.g. certificate, close-out letter, inspection report). If the final outcome is not satisfactory, a summary of the objections found during the last inspection, the follow-up and the appropriate corrective actions should be included.

Describe nature of and frequency of audit/certification
Specify name of organization carrying out the audit/certification.

**ANNEX V: INFORMATION ON CENTRES AND ESTABLISHMENTS
IN WHICH TRANSPORT OF DONATIONS AND PLASMA POOLS IS CARRIED OUT**

Address	Specify the sequential number(s) of the collection centre(s) for which the storage is performed	Inspection by an EU competent authority			Inspection by a non-EU competent authority			Audit			Conditions of transport (temperature and maximum time) Compliance with Ph Eur with respect to freezing and storage
		Member State	Date of last inspection	Final outcome (observations ²⁷)	Country	Date of last inspection	Final outcome observations	Description ³⁰	Name of organisation ³⁰	Out come	
Organisation 1 responsible for collection											
Country 1											
Organisation site 1											
Organisation 1											
Country 1											
Organisation site 2											
Organisation 1											
Country 1											
Country 2											
Organisation site 3											
Organisation 1											
Country 2											
Organisation 2 responsible for collection											
Country 1											
Organisation site 1											
Organisation 2											
Country 1											
Organisation site 2											
Organisation 2											
Country 1											

Documented evidence of satisfactory outcome should be submitted (e.g. certificate, close-out letter, inspection report). If the final outcome is not satisfactory, a summary of the objections found during the last inspection, the follow-up and the appropriate corrective actions should be included.

Describe nature of and frequency of audit/certification
Specify name of organization carrying out the audit/certification.



Executive Board of the Health Ministers' Council for GCC States

Labeling of Blood Products

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Definition of labeling

The important information which must be stuck on the drug or the pharmaceutical product container, or put on the outer package or the leaflet accompanying the package or a certificate of analysis accompanying the article, as decided by the competent authority.

Importance of labeling

Information will make it easier for health care practitioners to access, read and use such information in prescription drug labeling. Also it will enhance the safe and effective use of prescription drug products and reduce the number of adverse reactions.

A. Package labeling Requirements

1. Brand name of the product:

The label must contain the brand name (i.e. the commercial name) which must be implemented by the manufacturing company. This name is considered as a trade mark for the producing company, which enables it legally to have the rights of producing such a product.

2. Name of the active ingredient:

The name(s) of the active ingredient(s) must be written in the label. The scientific name must be used to the drug substances.

3. Name, concentration and the function of other excipients and additives:

This is very important in terms of drug pharmacological action and the mutual interactions which may happen among such excipients and the drug during storage. Thus, the name and the concentration and the specific function of each additive in the formulae or the biological product must be written.

4. Concentration of the active ingredient(S):

The concentration of the active ingredient(s) must be written on the label. The concentration terms may be percent or moles per liter or equivalents per liter or the international units in certain biological products.

5. Total volume of the product:

If the final product is a liquid dosage form like solution or suspension or emulsion, the total volume of the product must be mentioned and written on the label, also the single dose which must be administered must be mentioned. In case of injectable solutions, it must be mentioned on the label whether the whole bottle will be transfused intravenously or many doses will be injected separately. The volume of the dose and the way of injection must be mentioned in the label.

6. Name of the pharmaceutical dosage form and the method of administration:

The name of the pharmaceutical dosage form must be included in the label; also the method of administration of such a dosage form must be mentioned as some methods need experience and skill as in the case of all types of injections. Obviously, mentioning the name of the dose sometimes specify the method of drug using.

7. Name and address of the manufacturer:

The name, address and the country of the pharmaceutical manufacturer must be written in the label. That is because the consumer of the product or the patient may need to contact or return to this manufacturer in case of manufacturing or packaging mistakes or ambiguous information.

8. Batch and lot number:

A group of numbers, letters or combination must identify the batch and the lot number of each unit of the product. This is very important in case of product withdrawal from the market or when the producing company needs to give certain instructions concerning certain batch or lot especially in risk conditions.

9. Manufacturing date:

The manufacturing date must be included in the label as it is very important and it may specify a lot of things as the expiration dating and the priority of the product distribution over the market.

10. Expiry date:

The expiry date must be mentioned in the label which basically depends on the stability testing of the product by calculating the T 90, which is the time for the product to retain 90 %

of its potency in accelerated stability testing. This date is very important since it specifies the period at which the product or the drug is legible for use.

11. Route of the administration:

Sometimes just mentioning the type of the dosage form of the product on the label specifies the route of this product administration, despite, the route of the product administration must be clearly mentioned. Some routes of drug administration needs certain skill and experiences as all types of injections. Some injectable drugs need some sensitivity tests before injections as in some blood products, some allergic materials and penicillins.

12. Storage conditions:

The storage conditions must be specified in the label and it must stick to such conditions as many improper storage conditions may deteriorate the active ingredients physically, chemically and/or biologically. It worth mentioning that lower temperature storage not always of benefit for all products as it may cause separation of some pharmaceutical systems as emulsions, or it may cause difficulty or no reconstitution of some other pharmaceutical biological products. Thus some storage conditions like keep frozen, means keep below zero degree centigrade. “Keep cool” means from 2-8 degree centigrade. Store at “ambient conditions” means 25-35 degree centigrade .The storage conditions also specify the percentage of relative humidity which must not be exceeded since, certain products deteriorates by hydrolysis.

13. Keep out of the reach of children:

This warning must be written over the outer carton as well as the internal container as well as the leaflet to warn against the hazards which may be encountered when the children wrongly ingest or get in contact with some drugs. It must be written in red and with big clear letters.

14. For indications and dosage see enclosed leaflet:

This statement is written over the outer carton to guide the user of the medication to read more knowledge about the drug in the accompanying leaflet specially the contraindications of the drug, the side effects and the precautions during administration and how to treat adverse drug reactions if happened.

15. Specific requirements for human blood product:

Because blood products are highly sensitive biological materials which need special precautions during manufacturing, processing, packing, storage and administration, special information and warnings must be written on the label. If blood products are stored in polyethylene bags, the name and compositions of such bags, also the storage temperature and humidity must be mentioned as these bags may affect such components on storage and may lead to deteriorations. Some other additive information must be written on the label:

- The content of protein expressed in gm per liter.
- The content of sodium expressed in milli-mole per liter.
- The product is not to be used if it is cloudy or if a deposit formed.
- The name and concentration of any added substances such as anticoagulant factor and stabilizer.
- The name and volume of solvent to be used for reconstitution.
- The amount of albumin used as a stabilizer.
- The transitions of infectious agents cannot be totally excluded when medicinal products prepared from human blood or plasma is administered.
- Where applicable, the name and concentration of the antimicrobial preservative in the preparation.
- The distribution of subclasses of immunoglobulin G present in the preparation (Immunoglobulin injection).
- If a whole blood sample be considered, it must mention over the label the date of this blood withdrawal, the person from which this blood withdrawn and the place or hospital at which this operation takes place.

B. Requirements of Leaflet contents

1. Brand name of the product

The label must contain the brand name” the commercial name “which must be implemented by the manufacturing company. This name is considered as a trade mark for the producing company, which enables it legally to have the rights of producing such a product.

2. Name and chemical structure of the active ingredient

The name(s) and chemical structure(s) of the active ingredient(s) must be written in the label. The scientific name must be used to the drug substances.

3. Composition and concentration of the additives

The used additives must be mentioned in the leaflet, some manufacturers mentioned just the names, and others mentioned the corresponding concentration. These additives are divided into formulating and bioavailability improving additives.

4. The clinical and pharmacological effect of the drug

The clinical and pharmacological effects of the formulated drug(s) must be mentioned in full detail in the accompanying leaflet.

5. Pharmacokinetics and pharmacodynamics

The absorption, distribution, action, metabolism and excretion of the drug must be mentioned in the leaflet beside the characteristic pharmacodynamics of the drug.

6. Indications

All the modes of drug use as a treating or diagnostic agent must be mentioned in full detail, as many specialists and non-specialists are very eager to know this topic. Some drugs have more than one indication.

7. Contraindications

The cases or the conditions in which this drug must not be administered must be emphasized for the fear of the complications if the contrary happened. These cases must be clearly stated.

8. Side Effects

The drug side effects and how can these side effects will be symptomatically treated if happened must be mentioned in the leaflet. This warns the drug user of such effects and makes him ready to treat them.

9. Special Precaution

These precautions may include the way of administration, the accompanying drugs and to avoid some activities after drug administration as in case of antihistamines. Sometimes the type of diet may affect the drug action, as in tetracyclines therapy. Thus such precautions are numerous and depend upon drug type and the patient administering the drug. In case of blood products, some products make hypersensitivity reactions and may cause anaphylactic shock through antigen-antibody reactions.

10. Drug Interactions

Adverse drug-drug reactions must be specified and written clearly in the accompanying leaflet. The drug-drug interactions are classified into 3 categories, synergistic, antagonistic and no effect. Sometimes drug-drug interactions are very dangerous leading to toxic levels or products, which must identified and warned against.

11. Dosage and Administration

The dose of the drug must be specified and the schedule of drug administration must be specified according to the age, gender and weight of the patient. So other many factors may interfere in the quantity of the dose of the drug calculation, which must be put in consideration.

12. Overdosage

As mentioned, it is very important to specify the drug dosing according to the case of the patient, the weight, age, and gender. The maximum permissible dose must be calculated and must not be exceeded at any condition.

13. Batch number

As mentioned before under packaging labelling requirements.

14. Expiry date

As mentioned before under packaging labelling requirements.

15. Rout of the administration

As mentioned before under packaging labelling requirements.

16. Storage conditions

As mentioned before under packaging labelling requirements.

17. Special notice

As a highly sensitive biological material, blood products must be treated with caution regarding storage temperature and the % of relative humidity, also in terms of handling and administration and the tests of allergy to the administering patient

18. Name and address of the pharmaceutical manufacturer

As mentioned before under packaging labelling requirements.

19. Human blood product

As mentioned before under packaging labelling requirements.



Executive Board of the Health Ministers' Council for GCC States

Changes to an Approved Application for Blood Products Intended for Further Manufacture

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I. Introduction

Frequently, a licensed blood product manufacturer determines that it is appropriate to make a change in the product, labeling, production process, quality controls, equipment, or facilities as documented in its approved license application(s). This guideline describes the requirements for you, the licensed blood product manufacturer, to report such changes for your licensed blood products to the concerned regulatory authority. This regulation only applies to the manufacture and distribution of licensed products. It does not apply to unlicensed products manufactured at unlicensed, registered-only facilities.

Under these requirements, you must report a change in the approved product, labeling, production process, quality controls, equipment, or facilities to the regulatory authority. You may report the change in: 1) a supplement requiring approval prior to distribution; 2) a supplement submitted at least 30 days prior to distribution of the product made using the change; or 3) an annual report, depending on its potential to have an adverse effect on the “identity, strength, quality, purity, or potency of the blood product as they may relate to the safety or effectiveness of the product” (referred to as “The safety or effectiveness of the product”). Before distributing a licensed product manufactured using a change, you are required to demonstrate, through appropriate validation and/or clinical or non-clinical laboratory studies, the lack of adverse effect of the change on the safety or effectiveness of the product.

The three reporting categories for changes to an approved application are defined:

- 1) Changes that have a substantial potential to have an adverse effect on the safety or effectiveness of the product, that require submission of a supplement and approval by FDA prior to distribution of the product made using the change (major changes);
- 2) Changes that have a moderate potential to have an adverse effect on the safety or effectiveness of the product, that require submission of a supplement to the regulatory authority at least 30 days prior to distribution of the product made using the change or, for some changes, the 30 days may be waived (moderate changes); and
- 3) Changes that have minimal potential to have an adverse effect on the safety or effectiveness of the product, those are to be described by the applicant in an annual report (minor changes).

If the company makes a change to an approved license application, it should conform to other applicable laws and regulations, including the current good manufacturing practice (cGMP) requirements of the GCC

II. Definitions

Acquisition - the purchase of a facility previously operated by a new applicant. The acquired facility will no longer be connected to the original US. License Number. We will either revoke or modify the original license to delete the facility. The license application for the legal entity acquiring the facility will be supplemented to include the manufacture of product at the acquired facility. If a new applicant has acquired a facility, we will grant a new license. We previously referred to acquisitions as ‘rollovers’ (Ref 7).

Applicant - any person or legal entity that has submitted an application to manufacture a product. The applicant assumes responsibility for compliance with the applicable product and establishment standards and for Quality Assurance (QA) oversight of all manufacturing steps (Ref 2). Also see **manufacturer**.

Application - request submitted by the applicant for produce license, including supportive documentation, in order to manufacture and distribute.

AR- Annual Report.

Authorized Official - person(s) designated by the applicant to communicate with the regulatory authority on behalf of the applicant. Authorized officials can initiate applications or supplements to a license application, discuss submissions with our representatives, provide additional information in support of the submissions, and withdraw applications or supplements (Ref 3). The applicant or manufacturer should immediately notify us in writing if there is a change in the authorized official(s).

Circular of Information - instruction circular that provides adequate directions for the use of blood products intended for transfusion. The circular contains descriptions of the blood products, information on the tests performed on the components, indications for use, contraindications, cautions and administration recommendations .

CBE — Changes being effected (30 days waived).

CBE30 — Changes being effected in 30 days .

Contractor - any person or entity, other than the applicant, that performs part or all of the manufacturing of the licensed product as a service to the applicant. The applicant assumes responsibility for the contractor's compliance with the applicable product and establishment standards. Both the applicant and the contractor are legally responsible for the work performed by the contractor (Ref 4).

Contractual Agreement — agreement between a manufacturer and a contractor, that describes the manufacturing steps performed by the contractor. Although you do not need to include the specific legal contract in your submission, you should include a description of the services requested from all contractors performing a manufacturing step for you (e.g., outside testing laboratories performing routine donor/product testing and confirmatory testing & indication facilities, and storage facilities). This should also be available for review during inspection.

CP — Comparability Protocol.

Disease-Associated Antibody Donors - donors who meet all the required/recommended normal Source Plasma donor suitability criteria, except that their plasma contains pre-existing IgG antibodies as a result of previous exposure to certain diseases or cellular antigens (Ref. 5).

Disease-State/High-Risk Donors - a donor whose plasma contains or lacks a specific property (e.g., protein, antibody, inherited trait) as a result of their disease. These donors may not meet all the required or recommended normal Source Plasma donor suitability criteria.

Establishment/Facility - includes any and all establishments used by the manufacturer for collection, processing, product testing, compatibility testing, and storage. Any facility in which a manufacturing step is performed must meet the specifications and procedures established in the license application designed to insure the continued safety, purity and potency of the product. Establishment and facility have the same meaning (Ref. 4). For the purposes of this document, the facilities are separated into three categories determined by the manufacturing steps they perform: Major Facilities, Auxiliary Facilities and Transfusion Services.

Major Facilities:

Collection Facility — facility that collects Whole Blood and/or apheresis products, and/or performs infrequent plasmapheresis, but that does not perform FDA required or recommended blood and plasma donor testing or prepare components from Whole Blood. Collection facilities may also label, store, and distribute blood products.

Community Blood Bank – government blood collection/processing facility, not part of a hospital system, that may perform manual and/or automated blood collection.

Product Testing Laboratory - facility that performs routine GCC required or recommended blood and plasma donor testing.

Auxiliary Facilities:

Distribution Center - facility that stores blood or blood products under specific controlled conditions prior to shipment to the final user, including suppliers of source material for further manufacture.

Donor Center - facility that only performs manual collection of Whole Blood and does not collect blood product by automated methods.

Transfusion Services:

Transfusion Service - facility that performs compatibility testing for blood and blood components, but does not routinely collect blood.

Fractionated Blood Derivatives - sterile solutions of a specific protein(s) derived from human blood, e.g., albumin, plasma protein fraction and immune globulin.

Inspection - an on-site evaluation conducted by the regulatory authority personnel of operations at a regulated establishment to assess whether it is in compliance with applicable laws.

- **Pre-Approval Inspection** — an announced or unannounced inspection conducted by regulatory authority personnel. This inspection is conducted as part of the review of a supplement to an approved biologics license application. Examples of supplements that would require pre- approval inspections include submissions for irradiated blood products, or implementation of a red blood cell immunization program under a Source Plasma license.

- **Pre-License Inspection** — an announced inspection conducted by a team from regulatory authority.

This comprehensive inspection is conducted as part of the review of an application for a national license, or a supplement to an approved biologics license application for a new facility or product. Examples of applications and supplements that would require pre-license inspections include a new blood establishment; or an additional major facility operating under an existing national license.

- **Post-Approval Inspection** — a periodic unannounced inspection conducted by the regulatory authority investigators. This inspection is a surveillance activity, conducted to assess whether the operations of licensed and unlicensed blood establishments are in compliance with applicable laws and regulations and with commitments made in the approved license application (for licensed establishments).

Manufacturer - any person or legal entity engaged in the manufacture of a product. Manufacturer also includes any person or legal entity that is an applicant for a license where the applicant assumes responsibility for compliance with the applicable product and establishment standards.

Manufacturer's Instructions — instructions for use of equipment, test kits, reagents, supplies, etc., used in the manufacture of blood components that are prepared by the manufacturer of the equipment, test kits, reagents, supplies, etc. Manufacturer's instructions may be described in the equipment operator's manuals and reagents or supplies package inserts.

Manufacturing - all steps involved in the preparation of a product intended for transfusion or for further manufacture into injectable or non-injectable products. Manufacturing includes determining donor suitability, the informed consent and collection procedure, component preparation, product/donor testing (including quality control), labeling, storage of the product, compatibility testing, and the quarantine and destruction of unsuitable blood products. The steps may be performed by the manufacturer holding the biologics license or by a contractor who performs one or more of the manufacturing steps.

PAS — Prior approval supplement.

Source Material - blood component derived from human blood that is collected by either manual or automated apheresis techniques and is intended for further manufacturing into injectable or non-injectable products.

Supplement - written request submitted to the regulatory authority.

Transfusion Blood Components - blood components (plasma) derived from human blood collected by either manual whole blood collection or automated apheresis techniques and intended to be transfused to human recipients.

III. Changes requiring supplement submission and approval prior to distribution of the product made using the change (major changes). [PAS]

If the company makes any change to a product, production process, quality controls, facilities, manufacturing plant or equipment, that has a substantial potential to have an adverse effect on the safety or effectiveness of the product, they must submit a supplement and receive our approval before distributing the product made using the change. For a change under this category, the company must submit a supplement to the regulatory authority that includes the following:

- A detailed description of the proposed change;
- The products involved;
- The manufacturing site(s) or area(s) affected;
- A description of the methods used and studies performed to evaluate the effect of the change on the product's safety or effectiveness;
- The data derived from those studies;
- Relevant validation protocols and data;
- Appropriate labels; and
- Relevant standard operating procedure(s) (SOP) or a list referencing previously approved relevant SOP.

We consider the following types of changes to be major changes, for which submission and approval of a supplement prior to distribution of product made using the change must occur:

A. Product Manufacturing/Procedural Changes

1. Addition or revision of SOP for the following categories if the change is less restrictive than previously approved:
 - a. Donor suitability, including donor deferral;
 - b. Blood collection, including arm preparation;
 - c. High risk behavior questions, including AIDS information;
 - d. Donor history forms, including informed consent,

- e. Product manufacturing for licensed products; and
- f. Quarantine and disposition of unsuitable product.

NOTES:

- Report changes in the content of the procedure or form. You do not need to report changes in format only and minor editorial changes to SOP and forms.
 - You may reference previously approved SOP and forms. You should include the registration number referencing a previously approved SOP or form.
 - You should submit to us for review SOP revisions in the above areas prepared in response to post-approval inspectional observations.
2. Implementation of procedures and/or donor history forms that differ from and are less restrictive than the ones previously approved by the regulatory authority. This includes a change in quality control procedures. If the modifications are more restrictive or if the procedure is performed following the manufacturer's directions, you may report the change in the annual report. You may report the addition of procedures or tests that are not required or recommended by FDA in the annual report.
 3. Use of an abbreviated donor history questionnaire for repeat or frequent donors.
 4. Implementation of a computer-assisted donor history questionnaire where the computer system can be accessed from remote locations is able to make decisions about donor suitability or is interfaced with other computer systems, either at the same collection center or at other facilities.
 5. Change from manufacturing a sole product by automated apheresis to manufacturing additional products as by-products.
 6. Addition of an immunization program for unlicensed vaccines.
 7. Implementation of a physician substitute program in a Source Plasma facility (Ref.).
 8. Collection of Source Plasma from disease-state or high-risk donors.
 9. Request for approval of a comparability protocol.
 10. Request for approval of an alternative procedure under for which there is no published guidance.

B. Equipment Changes

1. Conversion from manual to automated collection of blood components; e.g. Plasma (both Fresh Frozen and Source), Red Blood Cells, Source Leukocytes.
2. Changes or upgrades in automated apheresis equipment that affect the purity, potency or quality of the product(s). These changes include but are not limited to: increase in product yield; change in storage conditions; change in anticoagulant.
3. Change in manufacturer of automated apheresis equipment used in the collection of Red Blood Cells.

C. Contractor Changes

1. Use of or change to, a new facility or any facility not previously engaged in blood product testing as a contract testing laboratory to perform the routine serologic and infectious disease screening testing, and supplemental and/or confirmatory testing for blood and blood products. These laboratories perform the tests of record (tests used to determine donor/product suitability).
2. Use of or change in, a contractor that was not previously engaged in performing a manufacturing step on blood products, to perform the manufacturing step. This includes, but is not limited to, contractors who irradiate blood products or supply blood cells for immunization.

D. Facility Changes

1. Expanding operations by adding a major facility where licensed products are manufactured. This includes the addition of a contractor to perform the manufacturing step.
 - a. Major facilities where Fresh Frozen Plasma, Pheresis are collected using automated collection systems, Source Plasma and Source Leukocytes are collected using either manual or automated collection methods, or routine FDA required or recommended blood and plasma testing is performed.
2. Relocation of a major facility where product manufacturing is performed that results in a change in core center personnel, and/or a change in SOP or equipment. You should also report the relocation of any contractor that results in a change in personnel and/or a change in SOP or equipment

IV. Changes requiring supplement submission at least 30 days prior to distribution of the product made using the change (moderate changes). [CBE3O]

If the company makes any change in your product, production process, quality controls, equipment, or facilities that has a moderate potential to have an adverse effect on the safety or effectiveness of the product, the Company should submit a supplement to the regulatory authority at least 30 days prior to distribution of a licensed product made using the change. The requirements for the content of these supplements are the same as for PAS.

The company must specify that the changes are being reported in this category by labeling the submission: “Supplement - Changes Being Effected in 30 Days.” Within 30 days of the date the regulatory authority receive the submission, then determine if the change or changes have been reported in the proper category and will notify you if they have not. If we have not notified you otherwise within 30 days after we receive the supplement, you may distribute your product under licensure, using the change described in your supplement. You do not have to wait for our written approval before distributing a product made using a change reported in this category. If we do not notify you, it does not mean that we have approved the changes reported in your supplement, merely that you have reported the changes in the proper category. Our review of your submission will proceed after we have determined that the changes are reported in the proper category.

We consider the following types of changes to be moderate changes, for which submission of a supplement at least 30 days prior to the distribution of the product made using the change should occur:

A. Product Manufacturing/ Procedural Changes

1. Addition of the collection of plasma as a by-product in an approved plasmapheresis program provided the applicant is otherwise approved to manufacture the plasma product.
2. Request to manufacture the following products:
 - a. Plasma Cryoprecipitate-Reduced, provided the applicant is approved to manufacture Cryoprecipitate AHF and Fresh Frozen Plasma.
 - b. Fresh Frozen Plasma Donor Retested, provided the applicant is approved to manufacture Fresh Frozen Plasma.

3. Implementation of an immunization program for licensed vaccines where the program is consistent with the vaccine insert instructions.
4. Implementation of a self-administered donor history questionnaire where the information is presented to the donor on a printed form that the donor must read or that is presented using audio/visual tools.
5. Implementation of a computer-assisted donor history questionnaire where the computer system operates as a stand-alone system, does not make decisions about donor suitability, and is not interfaced with other computer systems, either at the same collection center or at other facilities.
6. Request for an alternative procedure for which published guidance is available and implementation conforms with the guidance.
7. Implementation of recommendations described in FDA guidance documents, if followed without modifications and directed to be reported in this manner by the guidance document.

B. Equipment Changes

1. Change in manufacturer of automated plasma apheresis equipment.

C. Contractor Changes

1. Use of, or change to, an international registered contract testing laboratory currently engaged in blood product testing, to perform the routine serologic and infectious disease screening testing, and supplemental and/or confirmatory testing for blood and blood products. These laboratories perform the tests of record (tests used to determine donor/product suitability).
2. Use of, or change to, an international registered contractor, currently engaged in performing manufacturing steps on blood products, to perform a specific manufacturing step, e.g., irradiation of blood products.
3. Use of an off-site contract storage facility to store unlicensed product collected under a pending license application or for the storage of excess licensed product that meets all product release criteria. The storage facility may also distribute licensed product to the final user.

D. Facility Changes

1. Change in legal name of the applicant. This will cause the issuance of a new license number.
2. Relocation of a major facility where product manufacturing is performed and there is no change in SOP, equipment; and core center personnel, especially center management and medical personnel. Report relocations of facilities that result in a change in core center personnel as a prior approval supplement.
 - a. Include relocation of all contractors where there is no change in SOP, equipment; and core personnel.
 - b. Do not include move of auxiliary facilities. You must report this submitting at the time of the move and by updating your organizational report in the annual report.

V. Changes requiring supplement submission prior to distribution of the product made using the change (30 days is waived). [CBE]

A. Product Manufacturing/ Procedural Changes

- a. Implementation of another manufacturer's previously approved SOP, with written permission from the manufacturer.
- b. Implementation of recommendations described in final GCC guidance documents, if followed without modifications and directed to be reported in this manner by the guidance document.

B. Facility Changes

- a. Voluntary revocation or permanent closure of a major facility. You may report closure of auxiliary facilities in the annual report.
- a. Temporary move or closure and reopening of a major facility provided there are no changes in SOP, equipment, and core center personnel. You should indicate the estimated time for the change and describe the plans for restarting operations at the original site in your submission.

VI. - Changes To Be Described In An Annual Report (Minor Changes). [AR]

The company document changes to the product, production process, quality controls, equipment; or facilities, that have minimal potential to have an adverse effect on the safety or effectiveness of the product in an annual report submitted within 60 days of the anniversary date of approval of your first product application in each year you have changes to report in this category. You must include a list of all licensed products involved, and a full description of the manufacturing and controls changes including: the manufacturing site(s) or area(s) involved, the date each change was made, and a cross-reference to relevant validation protocol(s) and/or approved SOP in your annual report.

Annual reports should contain information about minor changes to any and all licensed products implemented since the prior annual report submit one original annual report and two copies to us for review. If there are no minor changes, you need not submit an annual report.

We will review the annual report to determine if the changes were reported in the proper category. If the annual report contains changes that should have been reported as supplements, we will notify you in writing of those changes that should be submitted as supplements.

We consider the following types of changes to be minor changes, to be reported in an annual report:

A. Product Manufacturing/Procedural Changes

1. Revision of SOP for the following categories if the change is more restrictive than previously approved or is not described in published GCC guidance documents.
 - a. Donor suitability, including donor deferral
 - b. Blood collection, including arm preparation (if changing to an approved method and following manufacturer's instructions)
 - c. High risk behavior questions, including AIDS information
 - d. Donor history forms, including informed consent
 - e. Product manufacturing for licensed products
 - f. Quarantine and disposition of unsuitable product

2. Implementation of a program to collect Source Plasma from normal donors with pre-existing disease-associated, red blood cell and/or HLA antibodies in Source Plasma (Ref).
3. Implementation of approved Uniform Donor History Questionnaire, if used without modifications or if modifications are more restrictive.
4. Implementation of recommendations described in final GCC guidance documents, if followed without modifications and directed to be reported in this manner by the guidance document.
5. Change in the quality control method if the procedure is consistent with the manufacturer's directions. This includes the methods used to quality control the systems involved in product manufacturing, e.g., blood products, equipment; reagents, supplies.
6. Implementation of additional procedures or tests which are not required or recommended by the regulatory authority.
7. Change in collection sets or leukocyte reduction filters for products prepared from Whole Blood, if used according to manufacturer's instructions.

B. Equipment Changes

1. Changes or upgrades by the device manufacturer of automated apheresis equipment that does not affect the purity, potency or quality of the product(s), if the facility is already approved for the original procedure.
2. Change in indication equipment used by you or your contractor.
3. Implementation of a blood establishment computer system that maintains data used by blood establishment personnel to make decisions regarding the suitability of donors and the release of blood and blood components for further manufacture. Do not include the use of a computer-assisted donor history questionnaire. Report the name of the software manufacturer, name and version number of the software. You should include the following in the annual report:
 - a. Installation of both commercially developed software and software developed and used in-house.

- b. Implementation of data entry and retrieval or library database systems.
 - c. Change in blood establishment computer software manufacturers or software versions, provided there are no major changes in the processes performed by the computer (e.g., adding an electronic cross match function) or no modifications made by the user that changes the intended functionality of the new system.
 - d. Implementation of a computer/electronic cross match.
4. Implementation of automated equipment to perform ABO/Rh, syphilis and infectious disease screening testing on donor blood samples.
 5. Change in infectious disease screening testing methodology if the procedure is consistent with manufacturer's directions.
 6. Change in equipment that performs total protein and serum/plasma protein electrophoresis on donor specimens.
 7. Change in equipment that performs vital sign testing (e.g., pulse, blood pressure, temperature) and hemoglobin/hematocrit testing on blood donors or donor specimens.
 8. Use of sterile connecting (docking) device to manipulate product in a sterile manner (e.g., take samples; attach transfer bag, needle, saline, anticoagulant or other processing solutions; prepare aliquots; pool products) if approved to manufacture the product and use of the device is consistent with manufacturer's directions.
 9. Changes or upgrades in automated apheresis equipment that result in a decrease in donation time.

C. Contractor Changes

1. Use of, or change in, a contract testing laboratory that performs reference or quality control testing or tests that are not required or recommended by the regulatory authority. This does not include a change in a contract testing laboratory that performs the infectious disease tests of record. Such a change must be reported as a PAS or CBE3O. (See sections III. C. 1. and IV. C. 1.)

2. Temporary use of a previously approved alternate or back-up contractor to perform a manufacturing step. Include the dates the alternate contractor was used. A permanent change in a contractor should be reported as a PAS or CBE3O. (See sections III. C. 1. and IV. C. 1.)
3. Use of, or change in, a contractor to provide personnel responsible for collecting blood products or performing quality assurance activities.

D. Facility Changes

1. Addition or deletion of a self contained motorized vehicle used for blood and blood product collection.
2. Change in “doing business as” name that does not affect the legal entity name on the license.
3. Openings, moves and closures of auxiliary facilities operating.

The following information should not be included in the annual report:

- Major or moderate changes that have received prior approval as supplements during the reporting period, unless they are included in the organizational changes.
- Major or moderate changes submitted as supplements and currently under our review.
- Shipment of source blood, plasma or serum that is repeatedly reactive for an infectious disease marker and is to be used in the manufacture of vaccines and licensed or unlicensed *in-vitro* diagnostic biological products.
- Notification of the development of unexpected antibodies in donors participating in red blood cell immunization programs.
- Biological product deviation (error and accident) or incident reports, fatalities and recalls.
- Validation data compiled during the installation and qualification of new or upgraded equipment computer systems or software.
- You should report the following corporate changes to us at the time they occur:
 - Change in corporate mailing address of the legal entity.
 - Change in, or addition of: an authorized official.

Reporting Format for the Annual Report

We recommend that you use the following reporting format for the annual report. However, you may choose to use a different format. In either case, we request that you include the information listed below in your report. See appendices for examples of annual reports.

- We ask that you submit a cover letter describing the contents of your annual report.
- Application to Market a new blood products.
- Description of the current organizational systems involved in the manufacture of Whole Blood and blood components, to include any quality assurance activities.
 1. If organizational changes have occurred since the last report; submit a current organization chart with descriptive job titles.
 2. List the licensed products you are currently approved to distribute in interstate commerce.
- Full description of minor changes reported to approved applications, as described in section VI.
 1. List products affected by each change.
 2. List the address of the facility or facilities where the change was implemented. Include the registration number of the facility.
 3. Include the date the change became effective.
 4. Describe the SOP or process affected by the change.

VII. Labeling Changes

You must report changes to labeling in one of the following ways:

1. As a supplement requiring approval prior to distribution of a product with the labeling change.
2. As a supplement requiring approval but permitting distribution of a product bearing such change prior to approval.
3. In an annual report

We have listed below examples of changes to labeling (product labels, circular of information, package inserts) that we currently consider being appropriate for submission in each of the categories.* This list is not intended to be all-inclusive.

"Transmittal of Labels and Circulars Form" should accompany each submission.

NOTE: Report changes in the content of the label. Product labels must be consistent with requirements stated in GCC guidelines.

A. Labeling changes requiring approval prior to product distribution.

Where applicable, circular of information must also be submitted as part of the labeling submission.

1. Labels submitted as part of a pending application or Prior Approval Supplement.
2. Labeling which contains an additional claim. You may need to provide documentation to support these claims.
3. Labels representing a change in the volume of Whole Blood collected, e.g., 450 mL to 500 mL., with an approved SOP stating donor must weigh at least 110 lbs.
4. If you are already approved to manufacture Source Plasma for injectable products and now want to include the manufacture of Source Plasma for non-injectable products, you should submit your non-injectable product label(s) in this category.
5. If you are already approved to manufacture Source Plasma for non-injectable products and now want to include the manufacture of Source Plasma for injectable products, you should submit your injectable product label(s) in this category.

6. Green base labels for units intended for autologous use only and labels printed using black ink for all text.

B. Labeling changes requiring regulatory authority approval but product may be distributed prior to FDA approval.

1. Labels submitted as part of a Changes Being Effected (CBE3O or CBE) supplement.
2. Labels consistent with a GCC-approved uniform Codebar labeling guideline.
3. Labels representing a change in an approved additive/anticoagulant solutions used in blood product collection.

C. Labeling changes requiring submission in an annual report:

Labels for Source Plasma collected from normal donors with pre-existing disease-associated, red blood cell and/or HLA antibodies.

Labels representing a change in “doing business as” name that does not affect the legal entity name on the license. Your legal entity name should appear on the label.

VIII. References

1. Federal Register, 7/24/97 (62 FR 39890), Final Rule: Changes to an Approved Application. (<http://www.fda.gov/cber/mles/chng072497btm>)
2. Federal Register, 10/20/99 (64 FR 56441), Final Rule: Biological Products Regulated Under Section 351 of the Public Health Service Act Implementation of Biological License; Elimination of Establishment license and Product License. (<http://www.fda.gov/cber/rules/elaplatxt>) (or substitute PDF for txt in the URL)
3. Federal Register, 5/14/96 (61 FR 24227), Final Rule: Elimination of Establishment License Application for Specified Biotechnology and Specified Synthetic Biological Products. (http://www.fda.gov/cber/niles/elao5_1496.pdf)
4. Federal Register, 10/15/97 (62 FR 53536), Final Rule: Revision of the Requirements for a Responsible Head for Biological Establishments. (<http://www.fda.gov/cber/genadmin/resphed.him>)
5. Federal Register, 11/25/92 (57 FR 55544), Notice: FDA's Policy Statement Concerning Cooperative Manufacturing Arrangements for Licensed Biologics.
6. Draft Reviewer's Guide: 'Disease Associated Antibody Collection Program,' October 1, 1995. (by fax at 888-CBER-FAX)
7. Memorandum to All Licensed Manufacturers of Source Plasma: 'Physician Substitutes,' August 15, 1988. (http://www.fdagov/cber/bldmem/08_1588.txt) (or substitute PDF for txt in the URL)