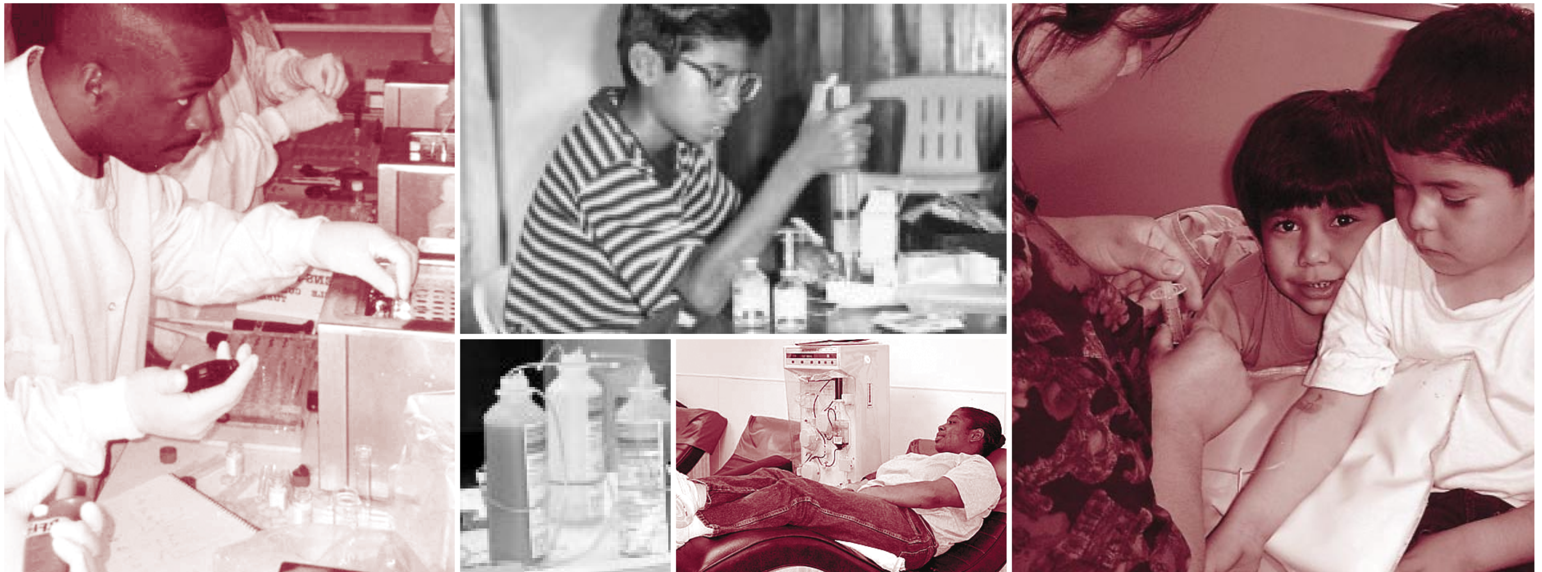
GUIDE FOR THE ASSESSMENT OF CLOTTING FACTOR CONCENTRATES



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Treatment for All

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INTRODUCTION

Selecting therapeutic products for the treatment of hemophilia is a difficult task. In well-resourced countries, key decisions on whether a product is sufficiently safe and of high quality are made by regulatory agencies, such as the U.S. Food and Drug Administration (FDA) and the European Medicines Evaluation Agency (EMEA), which are dedicated to assessing products and granting marketing licences. Many countries do not have the resources to set up such an agency, however, even in the absence of an established regulatory agency, good decisions regarding the purchase of products for the treatment of hemophilia can be made. For this to happen, authorities need to understand and use a number of well-established principles when evaluating the different features of products offered. The aim of this guide is to provide these principles to help government officials and others responsible for selecting therapeutic products for the treatment of hemophilia for their national health system.

Hemophilia treatment products are of two main types, those made from plasma donated by human blood donors and those made using recombinant technology. This guide focuses on plasma-derived products. Current processes for manufacturing hemophilia treatment products, when well managed, can produce products with risks as low as most other pharmaceuticals in use today. However, because hemophilia treatment products sourced from human blood have a well-established history of transmission of blood-borne infectious agents, such as HIV and hepatitis, it is very important to ensure that products being considered for purchase are safe and free from viral infection. Section 1 of this guide describes the factors contributing to the quality, safety, and efficacy of hemophilia treatment products and, in particular, the provisions made for ensuring that they are free of viruses. The impact of blood plasma quality on product safety is explored in some depth. Viral reduction steps at the manufacturing stage are also covered in detail.

Regulatory systems in the U.S. and the European Union (EU) for regulation and control of pharmaceutical medicinal products are well established, and the approaches used may be helpful for countries that want to develop their own framework for assessing and choosing products. North American and European practices are summarized with comment in Section 2. However, it is important to note that these arrangements are very complex, and may not be appropriate in a country that is setting up new regulatory arrangements.

Section 3 provides guidance for regulatory authorities in countries that have no established system for regulating plasma products and that want to develop procedures to ensure the safety and quality of plasma products. It also explores aspects of finished product testing, and the potential contribution (and limitations) of such testing to evaluate the safety of individual batches of product with regard to infectious risks.

Drawing on the principles outlined in previous sections, Section 4 offers a model for evaluating products for decision makers in countries without established regulatory agencies. It includes minimum requirements that must be met for a product to be considered, and example scenarios outlining how products are evaluated.

Many developing countries still use locally produced cryoprecipitate (cryo) to treat hemophilia A. Section 5 looks at measures to assure the safety and quality of locally produced cryo, which are somewhat different than for concentrates because of the nature of the product.

The appendices include various useful materials to help authorities assess products. Appendix 1 includes the "Registry of Clotting Factor Concentrates", which is a listing of currently available hemophilia treatment products, and includes information on plasma source, serological tests done on donor plasma, and viral reduction procedures. It also provides information on the manufacturers and the regulatory agencies that have reviewed submissions and granted licences for their distribution.

Appendix 2 is a model questionnaire for assessing hemophilia products, which includes the necessary information to assess the safety and quality of a product. It should be completed by the manufacturer before any assessment of products begins.

Often the language and acronyms used by regulators and government officials can be difficult to understand. Appendix 3 is a glossary defining the processes used in the manufacture and control of hemophilia treatment products. Appendix 4 is a list of other WFH resources.

This guide was written with hemophilia treatment products specifically in mind but many of the same principles apply to all plasma-derived medicinal products. The term "fractionated plasma products" is used throughout this guide to include all products derived from large plasma pools (more than 1000 donations) by a process which incorporates subsequent purification steps.

SECTION 1

FACTORS AFFECTING THE QUALITY AND SAFETY OF CLOTTING FACTOR CONCENTRATES

Introduction – determinants of the viral safety of plasma products

Because hemophilia treatment products sourced from human blood have been responsible for transmitting blood-borne infectious agents (such as HIV and hepatitis) in the past, it is very important to ensure that products being considered for use are safe and free from viral infection. Since the 1980s, manufacturers and the agencies regulating the manufacture of fractionated plasma products have responded to concerns about transmission of blood-borne viruses by developing a comprehensive set of measures designed to reduce, if not eliminate, infectious risk. These measures are based on the following principles:

1) Selection of appropriate blood and plasma donors
2) Screening of the plasma raw material with laboratory tests
3) Elimination of any contaminating viruses through the manufacturing process

Of these three principles, the elimination of viruses through the manufacturing process has enhanced the safety of hemophilia treatment products the most.

Measures to enhance the viral safety of plasma products include:

- Selection procedures which ensure that donors with high-risk behaviour are excluded
- Mandatory serological testing on all plasma donations for HIV, hepatitis B, and hepatitis C
- Plasma inventory hold and exclusion based on post-donation information
- Nucleic acid testing (NAT) of minipools for HCV-RNA (and increasingly for other viruses including HIV, HBV, B19, and HAV) and exclusion of reactive donations
- Testing start-manufacturing plasma pool samples for viral markers and viral genomic material
- Inclusion of one or more validated specific viral inactivation and/or removal steps in the manufacturing process
- Full traceability of plasma from donors to end products

In addition, some agencies and manufacturers also test finished products for viral markers and genomic material. The merit of this as a measure of the safety of hemophilia products is discussed in detail in the section on end-product testing in Section 3, page 19.

The combination of appropriate donor selection procedures, screening with the current generation of standard serological tests, and, in particular, the inclusion of measures to inactivate or remove viruses has made fractionated plasma products free from serious known blood-borne viruses such as HIV, HBV and HCV. Fractionated plasma products manufactured by today's processes, and manufactured with attention to good manufacturing practices (GMPs), are among the lowest risk therapeutic products in use today.

Plasma quality

Factors which have an impact on plasma quality and safety include:

1) Plasma handling factors such as separation, storage, and transport, which also depend on the methods used for collecting plasma (*recovered* from whole blood or obtained by *plasmapheresis*)
2) Donor epidemiology (viral infection, prion disease)
3) Donor selection and testing procedures (including NAT) to reduce the window period for infection with different viruses

All these factors affect the safety of fractionated plasma products with respect to transmissible infectious agents. They also affect the yield and specific activity of products.

Donor selection

Donor selection procedures are designed to identify and exclude donors at risk of being infected with viruses that can be transmitted by blood transfusion. In developed countries, donor selection procedures have reached a high level of sophistication and complexity, and regulators have included these procedures in their assessment of overall safety of material used to manufacture plasma products.

Exclusion criteria for donors used in different regulatory climates include:

- History of blood-borne infections
- Intravenous drug use
- High risk sexual behaviour (male-to-male sex, prostitution)
- Having received blood, tissues, etc.
- Risky behaviour (tattoos, piercing, etc.)
- Medical procedures, such as certain illnesses, surgery etc.

Like all the measures described in this guide, the ability of different countries to implement these measures may vary. Each regulatory authority must assess a country's local needs before mandating specific measures.

Plasma types

Plasma types may be distinguished based on donor remuneration status (paid or unpaid) and method of collection (recovered or source plasma). Recovered plasma is a by-product of donated whole blood and is generally procured from unpaid donors. Source plasma is collected from donors, most of whom are paid, through a process known as plasmapheresis, which removes only the donor's plasma. When collected and processed with steps that exclude and inactivate or eliminate enveloped viruses (HIV, HCV, and HBV), both recovered and source plasma have the same level of viral safety in the derived products.

In the past, before the introduction of regulation in the blood sector, plasma for fractionation from paid donors was considered to be of higher risk of viral infection than plasma from voluntary donors drawn from the same population. However, nowadays, in the developed blood systems of North America and Europe, this is no longer the case. This is the result of the strict regulatory regimens found in these areas and the introduction of similarly strict industry standards. The inclusion of nucleic acid testing (NAT) for plasma for fractionation in these systems has greatly reduced the viral load for HIV and HCV for all donor types. This equivalence in safety is not necessarily the case in other donor populations,

and authorities need to assess each plasma source for the safety factors described in this guide, whether it is from paid or unpaid donors.

The incorporation of viral reduction steps inactivates or removes this low viral load with equal efficacy for both recovered and source plasma. Furthermore, the introduction of measures by the source plasma industry (which is mostly drawn from paid donors), such as inventory hold and donor qualification, has made this plasma, in terms of its safety as a raw material, potentially safer than plasma recovered from whole blood for which many of these measures are not possible.

Paid and unpaid plasma sources have to be assessed individually, and evaluated in relation to the whole range of safety measures outlined in this guide. It is known that in certain source plasma donor populations in developing countries, the risk from paid donors is high and may be higher than from unpaid donors, although good data to assess this is lacking. Essentially, authorities need to assess each plasma source on all its merits.

Donor screening

Individual donations of blood are screened to ensure that blood-borne viruses do not enter the plasma pool. Screening is currently available for hepatitis B (HBV), hepatitis C (HCV), and HIV. All plasma donations should be tested for these three viruses.

Tests for detecting viral infection through the immune response of the donor are limited as there is a "window" period before the body's immune response generates sufficient levels of the immunological marker. During this period the donor is infectious but the infection is undetectable. In HBV infection, the serological marker detected in traditional blood screening is an antigen (HbsAg) associated with the virus, rather than an indicator of the immune response, but the window period still exists. With NAT, this period is shortened by the detection of the viral genome, which appears in the blood before the immunological markers. The recent introduction of NAT has decreased the viral load of plasma pools and therefore increases the margin of safety should viral reduction procedures break down. However, it is very expensive and, given the effectiveness of viral reduction procedures, it is not an essential requirement.

TABLE 1: Donor screening tests for blood-borne viruses

TEST	RECOMMENDED	MANDATORY
Anti-HIV	Yes	Yes
Anti-HCV	Yes	Yes
HbsAg (hepatitis B)	Yes	Yes
HIV RNA[1] (NAT)	Yes	No
HCV RNA (NAT)	Yes	No
HBV DNA (available soon)	Yes	No
B19[2] DNA (available soon)	Yes	No
HAV[3] RNA (available soon)	Yes	No

[1]DNA = Deoxyribonucleic Acid; RNA = Ribonucleic Acid. These constitute the essential elements of the genetic code.
[2]B19 = Human Parvovirus B19
[3]HAV = Hepatitis A Virus

Inventory hold

Inventory hold is the holding of plasma in (frozen) storage before it is processed into concentrates. A plasma donation is held until testing of the donor ensures that the donation was not collected while the donor was in the window period of disease. The use of inventory hold pending qualification of plasma donors further enhances safety and is an attractive, although not mandatory, feature. This measure is generally only possible for source plasma, as apheresis donors can donate more frequently which may result in more donations during the infectious window period. The particular features of an inventory hold vary across the organizations which practice it. It is most effective when donations which are not re-tested are not used, whether the donor returns or not. This is not always the case, and the particular features of a plasma supplier's inventory hold should be kept in mind in assessing the relative safety merits of paid and unpaid plasma sources.

Ensuring the safety of raw materials

Donor selection procedures that exclude high-risk donors, combined with serological screening of plasma donations are the mainstay of ensuring safe raw material for the fractionation process. The safety of the raw material can only be ensured by the fractionator through the use of suppliers that exclude high-risk donors and use good quality viral screening tests. Further guidance on how regulatory authorities can make certain of the safety of raw material used for blood products is provided in Sections 2, 3, and 4. Some fractionators may purchase plasma from the open or so-called "spot" plasma market, rather than obtain it from their own centres or from centres subscribing to their own standards. The use of such "spot" plasma will not be subject to the same level of safety and regulatory control as the use plasma from well-accredited centres, and authorities should not consider using products manufactured from this type of plasma.

Viral reduction processes

There are two types of viral reduction processes: inactivation (viral kill) and removal of virus through purification of protein. Viral elimination procedures in the manufacturing process have had the greatest impact on enhancing the safety of hemophilia treatment products. While all the components of the blood safety chain described in this guide are required for product safety, manufacturing processes can have an especially significant role. For example, solvent-detergent treatment made pooled hemophilia treatment products safe from hepatitis C before the introduction of testing for the virus increased the safety of normal blood transfusion and single-donor cryoprecipitate from HCV transmission. Authorities tasked with assessing which measures are essential for ensuring safe products – as opposed to those which, while enhancing safety, are not essential – need to keep in mind the features of plasma derivatives, such as hemophilia products, relative to the hospital products of mainstream blood banking.

While donor selection and screening of donations, combined with appropriate NAT testing (and inventory hold where it can be achieved), have significantly reduced the risk of blood-borne viruses entering the fractionation pool, we must presume that any plasma pool for fractionation may contain levels of virus capable of transmitting infection. The inclusion, in the fractionation process, of one or more steps with validated capability to inactivate and/or remove relevant viruses, primarily enveloped viruses (HIV, HBV, and HCV), results in plasma products that are essentially free from risk of these viruses. However, current inactivation and removal processes are less effective for non-enveloped viruses (mainly HAV and parvovirus B19, and the concern can be extended to "unknown" viruses and infectious agents also).

There are a number of different viral reduction methods available, including solvent-detergent, heat treatment (pasteurization, dry-heat, or steam heat), and nanofiltration. The advantages and limitations of these are outlined in Table 2, page 8.

Failures in testing, processing, or critical quality systems are more likely to result in the release of a batch of product with increased risk of infection than any fundamental deficiency in process design or competence. Because of the importance of viral elimination in the ultimate safety of plasma products, there is no room for failure in the process steps upon which viral elimination depends. Process validation, and those systems at the heart of good manufacturing practices — traceability, segregation of product manufacturing steps to avoid cross-contamination, training, documentation, change control, deviation reporting — are the keys to the reliable manufacture of safe, effective plasma products.

Non-enveloped viruses

Current viral inactivation and/or removal steps are effective for enveloped viruses but less effective for non-enveloped viruses. While some viral elimination steps, notably nanofiltration, have been shown to offer at least a partial reduction of the viral burden from non-enveloped viruses during product manufacture, other strategies, particularly vaccination when possible (for HAV, for example) of people receiving plasma concentrates on a lifelong basis, should be used. For known non-enveloped viruses, several manufacturers have established schemes involving limit testing of the plasma pool using NAT in which a maximum level of viral contamination, rather than an absolute elimination, is the aim. In the absence of validated viral reduction in-process steps, this offers probably the current best general approach for reducing the viral burden of the plasma pool and, therefore, for reducing the transmission potential for viruses tested using this methodology.

Variant Creutzfeldt-Jakob disease (vCJD)

Experiments with different animal species indicated that the diseases known as transmissible spongiform encephalopathies (TSEs) are transmissible through blood, plasma, and plasma fractions. These experiments indicated that a considerable portion of the infectivity in blood is actually found in the plasma, and is carried into plasma fractions when the plasma is processed into therapeutic products. However, depending on the fraction studied and the manufacturing technique used, much of the infectivity is removed or cleared from the therapeutic product as a result of the manufacturing process.

At least some of the animal studies have been confirmed in humans as four recipients of blood or red cells from donors who developed a human TSE — variant Creutzfeldt-Jakob disease (vCJD)[1] — have also been infected with the disease. Therefore, this illness is a blood safety risk and is addressed through the same combination of measures which are used to minimize the risk of viral transmission. The only donor selection measure which is currently possible for minimizing the risk of donors incubating the disease entering the plasma pool is the deferral of individuals who have been exposed to BSE through travel or residence in a country where the disease has entered the food chain. These deferral measures have been introduced by a number of countries. The main target of these policies has been travel or residence in the United Kingdom during the period when BSE was entering the human food chain in that county, but some countries have also included deferral of donors with a history of travel and residence in other countries with a minor BSE epidemic.

[1]CJD is the human form of bovine spongiform encephalopathy (BSE), a disease in cattle which has affected animals in the U.K. and the European continent and which is thought to have entered the human food chain through the consumption of contaminated meat products. For current information on the prevalence of BSE in different countries go to www.oie.int

Table 2: Advantages and other points to consider when selecting viral reduction treatments of factor concentrates

Treatment	Advantages	Points to consider
Solvent-detergent (SD) Treatment with a mixture of chemicals – solvents and detergents – which inactivate viruses through removal of the lipid envelope which coats some types of viruses. Hence it is not effective against agents which lack this envelope.	• Extremely efficient against enveloped viruses • Relatively simple equipment • Non denaturing effect on proteins • High recovery of protein functional activity	• Requires a subsequent manufacturing step to eliminate the SD agents • Not effective against non-enveloped viruses, e.g. B19 or HAV
Pasteurisation This is a generic term for heat treatment of a protein in solution at 60°C for 10 hours. Its efficacy in inactivating viruses is dependent on the exact conditions under which it is performed. When it is used to treat proteins which are fragile, such as clotting factors, the solution has to include protective chemicals so as to preserve the protein; these may also preserve the virus.	• Potential to inactivate enveloped and non-lipid enveloped viruses, including HAV. Each process needs to be evaluated on the basis of the data submitted by the manufacturer. • Relatively simple equipment	• Dependent on conditions • Protein stabilizers may protect viruses • Does not inactivate B19 • Low recovery of fragile coagulation factors • Potential generation of neoantigens
Vapor-heat Currently restricted to one manufacturer.	• May inactivate enveloped and non-enveloped viruses, including HAV	• Possible risk of transmission of HCV and HBV • Does not inactivate B19
Terminal dry-heat • This involves heating the final product in the lyophilized state in the container used to issue and reconstitute the concentrate. The efficacy of viral kill is strongly dependent on the exact combination of time and temperature which the product is exposed to. These conditions have been described by manufacturers: • 60°C for 72 hours • 80°C for 72 hours • 100°C for 30 minutes • 100°C for 120 minutes • 65°C for 96 hours For example, 60°C is known to be less effective than 80°C for similar lengths of time. Each process needs to be evaluated on the basis of the data submitted by the manufacturer.	• May inactivate enveloped and non enveloped viruses, including HAV • Treatment applied on the final container	• Does not inactivate B19 • 10 to 20% loss of coagulation factor activity • Requires strict control of residual moisture content

Treatment	Advantages	Points to consider
Nanofiltration on 15 nm membranes	• Elimination of viruses based on size-exclusion effect. • Eliminate all major viruses including HAV and B19. • May possibly eliminate prions • Filter's integrity and removal capacity is validated after use • High recovery of protein activity • Non denaturing for proteins • Risks of downstream contamination are limited when filtration is performed prior to aseptic filling • Filters are commercially available; no royalties	• Non applicable to high molecular weight protein concentrate (without significant protein loss)
Nanofiltration on 35 nm membranes	• Similar to 15 nm membranes • Applicable to some factor VIII and von Willebrand factor concentrates	• Elimination of small viruses not total

Adapted from: Ala F, Burnouf T, and El Nagueh M, *Plasma Fractionation Programmes for Developing Countries*,WHO Regional Publications, Eastern Mediterranean Series, No. 22, 1999, and Burnouf T and Radosevich M,"Reducing the risk of infection from plasma products; specific preventative strategies," *Blood Reviews*, 2000, 14: 94-110.

Authorities faced with making a decision on excluding donors at risk of vCJD need to assess carefully the effect of such deferral measures on the overall blood supply. In many developing countries, blood is in short supply and these countries cannot afford to lose donors because of possible vCJD risk. Also, in areas of high prevalence for other more established risks, such as HIV and HCV infection, the deferral of well-accredited repeat donors because of possible vCJD risk may mean that new donors with a higher prevalence of these established infections are used instead. New donors always have higher viral marker rates than repeat donors, and authorities in developing countries, where selection and screening procedures may not be optimized, need to ensure that unproven risks are not replaced by real ones.

A test for vCJD is currently unavailable, and is unlikely to be available for some years, despite considerable effort to develop such a test. The exclusion of infective donations to the extent that pooled plasma products are rendered safe is probably not achievable through selection and testing measures, if the experience with viral infections is any guide[2].

This leaves the clearance of infectivity through the manufacturing process as the main route for minimizing the risk of vCJD from plasma products.

The level of clearance attained by different processes is considerable and has probably contributed to the absence of vCJD infection in recipients of plasma products, including people with hemophilia, observed so far. It is known that plasma from people who subsequently developed vCJD has been

[2] Infections such as HIV and HCV continued to be transmitted through hemophilia products after the introduction of selection and testing measures, which are generally of insufficient sensitivity to exclude infected donations from being included in plasma pools containing thousands of units.

used to manufacture these products, including clotting factor concentrates. Assessments for estimating the risk posed by these products have been developed by regulatory authorities including the U.S. FDA[3], using principles also described by the WFH. We have several relevant publications at www.wfh.org.

All the estimates agree that the risk is strongly dependent on the level of clearance contributed by the process, and that higher purity products pose lower risk levels as the infective agent is removed more effectively. This should be considered when assessing the safety of concentrates, while keeping in mind that even the recipients of low purity products manufactured from plasma pools collected during the period of BSE penetration into the U.K. food chain have not developed vCJD, despite the U.K. authorities' conclusion that these patients have an enhanced risk of developing vCJD compared to the general U.K. population[4].

In summary, it is important to reiterate that there have been no cases of vCJD transmitted by plasma products, including clotting factor concentrates. Bleeding continues to be the major cause of mortality and morbidity for people with hemophilia, and it is important to retain access to products which prevent this event. Regulatory authorities and manufacturers are now fully aware of the risk of vCJD from plasma derivates and have instituted measures to ensure that manufacturing processes are optimized to clear potential infectivity. The inclusion of such measures should form part of the evaluation process of clotting factor concentrates.

Purity versus safety

Purity refers to the percentage of the desired ingredient (e.g., factor VIII) in concentrates, relative to other ingredients present. Concentrates on the market vary widely in their purity (see "Registry of Clotting Factor Concentrates" in Appendix 1, page 33). Generally, products which are produced at higher purity tend to be associated with low manufacturing yields, due to less von Willebrand factor (the natural carrier protein of factor VIII), and are therefore costlier.

In some products, higher purity leads to clinical benefit. For example, high purity factor IX concentrates lacking factors II, VII, and X are preferable for the treatment of hemophilia B to the so-called prothrombin complex concentrates made of a mixture of these factors, as the risk of thromboembolic complications is decreased. The purity of factor VIII concentrates has not been convincingly demonstrated to enhance the safety of these products, as long as adequate viral elimination measures are in place. However, concerns regarding the currently unknown risk of vCJD have stimulated manufacturers to validate their processes for the potential to eliminate vCJD-like agents from the final product. These studies have shown that several processes for manufacturing factor VIII and factor IX products are capable of eliminating significant levels of contaminating vCJD-like agents. Generally, the more purified the product, the higher the level of such elimination.

Conclusions

Since the 1980s, various measures have been introduced to reduce the risk of viral transmission by fractionated plasma products. Not all practices are considered as mandatory standards by regulatory agencies, and their use by different fractionators must be assessed in the overall context of safety, availability, and cost. For example, donor source can be significant but other practices, such as NAT to narrow the window period and inventory hold, reduce the risk of infectious units being pooled. Some

[3] http://www.fda.gov/cber/blood/vcjdrisk.htm

[4] http://www.hpa.org.uk/webw/HPAweb&HPAwebStandard/HPAweb_C/1195733818681?p=1191942152861

measures may have only limited benefits for users of hemophilia products, and may possibly affect the yield and financial viability of fractionation processes. For example, limiting donor pool size can reduce the risk of viral transmission, but probably only for infrequent users of plasma products. These possibilities must be kept in mind when making decisions about purchasing products.

Donor selection procedures that exclude high-risk donors and serological screening of plasma donations are the mainstay of ensuring safe raw material for the fractionation process. However, it is in-process inactivation that has had the most profound impact on the safety of fractionated plasma products. Even allowing for limited effectiveness against non-lipid enveloped viruses (for which NAT may be used to limit plasma pool viral burden), in-process viral inactivation or removal has made the risks of receiving an infected product extremely low – assuming adherence to validated process conditions. Establishment and maintenance of good manufacturing practices (GMPs) and licence-compliant (i.e., validated) conditions are critical to eliminating these areas of risk.

SUMMARY

- Fractionated plasma products have a history of transmitting blood-borne viruses (HBV, HCV, and HIV).
- Plasma products manufactured by today's processes, and manufactured with attention to good manufacturing practices (GMPs), rank among the lowest risk therapeutic products in use today.
- Product safety is the result of efforts in several areas:
  - Improved donor selection (exclusion of at-risk donors)
  - Improved screening tests of donations (including NAT)
  - Type and number of in-process viral inactivation and/or removal steps

  Of these, in-process viral inactivation is the single largest contributor to product safety.
- Plasma types are distinguished based on:
  - Donor remuneration status (paid or unpaid), which, when regulated to current standards, are similar in the safety of manufactured products
  - Method of collection. In practice, all will yield safe, effective products if processes are properly optimized and GMPs are observed.
- The inclusion in the fractionation process of one or more steps with validated capability to inactivate or remove relevant viruses, primarily enveloped viruses (HIV, HBV, and HCV), results in plasma products that are essentially free from risk of these viruses. Inactivation and removal processes are less effective for non-enveloped viruses (mainly HAV and B19).
- Currently, there is no screening test for vCJD, and no established manufacturing steps to inactivate the agent. vCJD in the U.K. donor population made it necessary to exclude plasma for fractionation from U.K. donors and has led to exclusion of perceived at-risk donors from other donor populations. There is no established risk of transmission of vCJD by plasma products.

SECTION 2

LICENSING, REGULATION, AND CONTROL OF CLOTTING FACTOR CONCENTRATES IN EUROPE AND NORTH AMERICA

Introduction

Arrangements for the licensing, regulation, and control of medicinal products have been developed and formalized to ensure that the risk-to-benefit relationship, which is involved in any medical intervention, may be optimized to assure patient safety. The responsibilities of national regulatory authorities (NRAs) under such arrangements include:

- Establishing and maintaining a system of licensing and control, including
    - Dossier review and pre-approval inspection
    - Facility and product registration
    - Facility and product inspection and enforcement
- Providing standards and guidelines
- Requiring that licence holders adopt and maintain appropriate quality systems
- Providing arrangements for post-marketing surveillance of products

Regulatory systems in Europe and North America are highly evolved and very complex, and are beyond the capacity of most healthcare systems in developing countries with limited resources. However, it is beneficial if authorities in developing countries are aware of the approaches used by the main regulatory agencies, which may help them to develop their own framework for assessing and choosing hemophilia treatment products. The approaches of the Food and Drug Administration and the European Medicines Evaluation Agency (EMEA) are outlined in this section, along with other approaches aimed at harmonization.

FDA regulations and guidelines

The U.S. Food and Drug Administration (FDA) is the largest regulatory body, with wide responsibilities for assuring the quality of foodstuffs, medicines, and medical devices manufactured for sale and supply in the U.S. Regulations to be observed in the manufacture and supply of pharmaceuticals are defined in Title 21 of the Code of Federal Regulations (21CFR) and in sections 1-999 of the United States Pharmacopoeia (USP). The parts of 21CFR with specific relevance to plasma products are[5]:

- Parts 210 and 211, which describe current good manufacturing practices (cGMPs)
- Parts 600 to 680, which set out the requirements for biological products

Additional guidance (distinct from regulations) is provided to manufacturers (and inspectors) in a range of paper and web-based publications, including:

[5]Available from http://www.access.gpo.gov

- FDA draft guidelines
- FDA inspection guides
- USP sections 1000 et seq.

Biologics, including plasma products, are presently overseen by the Center for Biologics Evaluation and Research (CBER), with the following broad areas of oversight:

- Regulatory oversight, which addresses all aspects of licensing and enforcement
- Product evaluation and research, including standardization
- Acquisition and evaluation of new information, including surveillance

EMEA regulations and guidelines

Regulatory provisions in Europe are defined through a comprehensive set of "directives", published by the European Union (EU) and through the European Pharmacopoeia (EP), published by the Council of Europe (CoE) – an entity with wider membership than the EU. Individual member states of the EU are required to incorporate EU directives into national legislation for implementation.

The key directive[6] relevant to plasma products manufacture is 2001/83/EEC, which summarizes and supplants the previous directives: 65/65/EEC, which provides the basis for regulation of proprietary medicinal products; 75/318/EEC, which provides standards for products regulated under 65/65/EEC; 75/319/EEC, which establishes administrative procedure for use with 65/65/EEC; and 89/381/EEC, which extends the above to cover blood products.

The specification of medicinal products in Europe is achieved through the European Pharmacopoeia Monograph 853 "Human Plasma for Fractionation", the only EP monograph specifying a source material. Other monographs cover all plasma products supplied to the EU by two or more manufacturers. These monographs represent only the minimum specification for the product described. Products must comply with the relevant pharmacopoeial specification throughout their in-date period.

Standards for the manufacture and supply of pharmaceutical products for the EU are described in a nine-volume compendium, "The Rules Governing Medicinal Products in the European Union". Volume 4, entitled "Good Manufacturing Practices", sets out the minimum GMP requirements for compliance. Annex 1 (Sterile Products) and Annex 14 (Blood Products) have special relevance for hemophilia treatment products.

The EMEA's Biotechnology Working Party (BWP) also publishes, through its Committee for Human Medicinal Products (CHMP), guidelines on several aspects of plasma derivatives which reflecte best practices in the field (available on www.emea.eu.int)

In addition, since 2003, the European Commission has introduced a number of directives[7] which have established a system of centralized oversight for blood and plasma collection establishments in the EU. Of immediate relevance for hemophilia products are the implications for plasma for fractionation, which is included in the scope of these measures. For example, plasma imported into the EU for the purpose of contact fractionation and re-export to the country of origin is captured in this framework, which therefore requires that such plasma, even when imported into the EU, needs to meet certain standards. While these standards may be viewed as being difficult to attain by emerging countries wishing to use established European manufacturers for contract fractionation, the benefits on all parts

[6]Search for the relevant documents by entering the directive number in the search field at http://europa.eu.int/geninfo/query_en.htm. The first two digits of a directive's designation corresponds to the year the directive was drafted.

[7]Helpfully collated at http://www.mhra.gov.au/home/idcplg?IdcService=SS_GET_PAGE&nodeId=209

of the blood product system for these countries, including but not restricted to hemophilia products, are clear. Amongst other measures, the use of the plasma master file in Europe provides a high level of assurance of the quality of the raw material.

Plasma master file concept

The concept of the plasma master file (PMF) was developed by the EMEA.[8] The purpose of the PMF is to specify plasma for different plasma products and establish a rapid and simplified way to assure adequate levels of quality and safety in the plasma raw material. Key elements in the PMF are:

- Requirement for a formal contract governing purchase and supply of plasma
- Description of the quality assurance system applying to plasma supply and use
- Arrangements for donor selection (including population epidemiology)
- Requirements for testing of samples of donations and pools
- Arrangements for communication and review of post-donation information

The PMF is mandatory in Europe and is supported by guidelines on the submission of the relevant data, in particular the data for describing the epidemiology of the plasma and blood donor populations which, in conjunction with the data for the manufacturing process, allows manufacturers and authorities alike to estimate the residual risk posed by the final product for different infectious agents. Since the different manufacturers obtain their plasma from a dynamic environment where companies change the source of their raw material according to convenience and market pressure, the safety profile of the raw material can change from one year to the next. Through the mandatory updating of the PMF, authorities are able to monitor these changes and, if necessary, intervene to exclude plasma of an unacceptable safety profile.

The key tenets of the plasma master file include:

- Exclusion of at-risk donors
- Mandatory serology on all plasma donations
- Exclusion of donations on the basis of post-donation information
- Traceability from donor to product

While the PMF has been developed and proposed for the European environment, it is an excellent model for assessing the safety of plasma, and can be adapted for other countries to be a stand-alone document tailored to the needs of particular countries. It includes all the information national authorities need to have on plasma as a raw material to ensure its quality and safety. The model product assessment questionnaire in Appendix 2 includes elements drawn from the PMF guideline. Authorities should insist on the submission of a full PMF from manufacturers, and should ensure that raw material providers whose safety profile does not conform to the PMF's requirements are excluded from the pool for hemophilia concentrates. This is especially important in today's globalized plasma industry, where some of the big companies deploy manufacturing plants both in countries under the EMEA's oversight and others outside the EMEA's mandate. Plasma not acceptable to the EMEA might be incorporated, through manufacture in non-EMEA regulated plants, in products destined for outside the EMEA regulated markets, a situation which is not desirable in terms of patient safety. Despite the pivotal role which viral inactivation plays in the manufacturing process, a manufacturer who is unable to secure reliable supplies of safe raw material as specified by the PMF is unlikely to be able to assure an authority of its capacity to manufacture safe and effective plasma products.

[8] http://www.emea.europa.eu/htms/human/humanguidelines/quality.htm

Common strengths of U.S. & EU regulatory provisions

- Review of data in marketing authorization application:
  - Commitments on plasma source – "plasma master file"
  - Process/batch consistency including effectiveness of viral inactivation/viral removal steps
  - Review data on safety and efficacy and of pharmacokinetics
- Inspection and enforcement of:
  - Plasma donor base, collection facilities, and quality systems
  - Manufacturing facility, process, and quality systems
- Control agency batch review and release
  - Batch-specific review of protocols and testing of samples
  - Availability of trend information on batch performance over time
- Post-marketing surveillance – mandatory follow-up

Source: Snape T, "Assessment of Products Not Licensed by the FDA and the EMEA," WFH Global Forum, January 21-22, 2002.

Harmonizing established regulatory requirements

A program is in place to facilitate harmonization of the requirements for manufacture and supply of pharmaceutical medicinal products in the U.S., EU, and Japan, the three trade areas where requirements are most formally established. This program, under the auspices of the International Conference on Harmonisation (ICH), has made some progress with respect to definitions, but much remains to be done in terms of implementation. Guidances[9] presently established include:

- ICH Common Technical Document (format for registration submissions)
- ICH quality guidelines (testing and validation of test methods)
- ICH efficacy guidelines (good clinical practice)

SUMMARY

- Arrangements for regulation, licensing, and control of plasma products are well established under U.S. and EU legislative procedures.
- The plasma master file concept allows safety assessments and facilitates the movement of plasma, intermediates, and products across national boundaries.
- Attempts are being made to harmonize the requirements for the manufacture and supply of pharmaceutical medicinal products in the U.S., EU, and Japan.

[9] Guidances are available from www.ifpma.org/ich5.html

SECTION 3

ESTABLISHING LICENSING, REGULATION, AND CONTROL PROCEDURES IN COUNTRIES WITHOUT WELL-ESTABLISHED REGULATORY SYSTEMS

Introduction

National regulatory authorities (NRAs) operating without well-established systems for licensing, regulation, and control of plasma products must act – and must be seen to act – in a way which safeguards public health without artificially restricting the availability of products and without unnecessarily escalating the cost.

The establishment and maintenance of a complex regulatory environment is beyond the capacity of most healthcare systems in the developing world. However, despite the lack of such an infrastructure, most countries can develop an appropriate decision-making framework for assessing and choosing hemophilia treatment products.

There are some obstacles that may get in the way when assessing and choosing products, including:

- Limitations of the NRAs themselves
  - Lack of experience
  - Lack of resources
- Plasma products supply is not a level playing field
  - Several generations of product (e.g., for protein purification and viral elimination methods) are typically still available and the benefits of a particular product are not always clear
  - Variability in the quality of the plasma used for manufacture
  - Variability in manufacturing standards employed
  - Local distributors may lack sufficient information on product specifications
- Decision makers need to respond to changing circumstances
  - Product availability and price will be driven by events elsewhere
- Perceptions of quality may not reflect reality

Recommended measures and pitfalls to avoid

To ensure the most control over the selection of treatment products, NRAs should try to incorporate some or all of the following measures into their approach:

- Build alliances with other purchasers to maximize resources
- Build a direct, managed relationship with suppliers or manufacturers, where possible
- Whenever possible, select products licensed with an established NRA
- Get information on plasma and the manufacturing process in advance using a pre-contract questionnaire (See model product assessment questionnaire in Appendix 2)

- Audit potential suppliers who meet the NRA's safety and quality requirements, focusing on plasma supply (especially donor selection, testing, and traceability) and manufacturing and distribution processes
- Use pre-shipment samples to support selection, but not as the basis for choice (available test methods are unlikely to be validated for the product, and batch pre-selection limits the time to expiry of selected batches)

Potential pitfalls decision makers and regulators should avoid include:

- Allowing the supplier or product to be selected on the basis of price alone. For example, low product price may be a result of non-conformance with (expensive) quality control measures, such as ensuring that donors are coming from low-risk populations through appropriate selection procedures. If a manufacturer is able to access large volumes of plasma from paid donors of low socio-economic status, strict selection measures are mandatory to ensure the product's safety
- Allowing supplier and product selection to be driven by political expediency.
- Creating dependence on third-party suppliers (brokers/agents), which can limit communication on key quality matters
- Relying on finished product testing to assure fitness-for-purpose

Using distributors of imported products

Since many countries attempting to access hemophilia products lack a domestic plasma fractionation capacity, local distributors or agents for the manufacturer are often used. They undertake a sponsorship role for the product and organize its presentation to the government authorities, arrange its distribution once products are approved, and handle liability issues, etc. Using agents is generally less preferable than dealing directly with manufacturers, because they are rarely familiar enough with the specialized products used for hemophilia treatment. As agents tend to change periodically, and sometimes represent more than one manufacturer, it can be difficult to maintain a level of continuity and consistency in product choice processes. This is particularly the case when there is no established NRA, because then there is similar instability on both sides.

If distributors are used, NRAs should establish procedures to ensure that distributors offer the following minimum standard features in their procurement of hemophilia products:

- Evidence that the distributor is the sole agent for that particular manufacturer in the country in question, through a statement from the relevant manufacturer
- Demonstrated capacity to provide the required infrastructure, particularly adequate volumes of refrigerated storage
- Demonstrated capacity to ensure product traceability to the end-users, and to carry out product recalls or withdrawals when required
- All other features specified in the requirements of the model product assessment questionnaire included in Appendix 2 of this guide

Whether the government agency interacts with a manufacturer directly or through an agent, it is extremely desirable to establish a contact at the manufacturer, preferably with the regulatory affairs department. All details regarding such a contact including records of all past correspondence should be included in the documentation generated for each procurement, in order to maximize continuity.

The role of end-product testing

Manufacturer testing of end product to a pre-determined specification is an essential feature of product quality control leading to release on the market. Regulatory authorities such as the FDA and EMEA generally conduct some form of independent oversight of this process by routinely reviewing manufacturer test release results and/or conducting tests themselves in official medicine control laboratories (OMCL). This testing of products before official release is called batch release testing (BRT). It is not a universal practice among regulators, some of whom consider that there is little value added to assuring product quality by duplicating the manufacturer's release testing. Product quality depends on ensuring that testing methods and release criteria are approved as part of the overall review process and are subject to all the requirements of good manufacturing practice. It is important to emphasize that the overall process is what builds quality and safety into a product; it is not possible to ensure product quality by testing in the absence of these features.

If national authorities feel that end-product testing allows them a level of assurance on quality and safety, they should use (or adapt) the approach used by established regulators or the batch release protocol from the European Directorate for the Quality of Medicines (EDQM)[10]. However, end-product testing should not be a mandatory requirement to measure the safety of hemophilia products for the authorities to whom this guide is directed. Whatever approach is adopted regarding end-product testing, it should not replace the review process detailed in this guide.

Can end products be tested for pathogens?

All final product batches are tested for sterility to minimize the risk of bacterial infection. These sterility tests are designed for testing pharmaceutical products and are validated for this purpose for individual products such as hemophilia concentrates.

It is important to note also that end-product testing cannot be used to assure viral safety. The testing used for screening plasma for viral agents, whether performed on donations or pools, and whether serologic or molecular, is not designed or validated for testing end products. Using these tests for end products is highly inappropriate and adds nothing to the assurance of safety to the products. In particular, experience shows a high level of false positivity when using these tests for this purpose. Its application may lead to incorrect assessments of product quality and safety and hold up product release. Two main points require recognition:

- Even assuming that some virus has ended up in the final product despite the various measures to exclude this, any such virus would be found in low amounts. Therefore, statistical considerations predict that the possibility of such a low amount of virus remaining undetected is high[11]. This is shown in Figure 1 where two viral particles are present in a 10-ml volume of product. This amount of virus may well prove infective, but the analysis shows that the probability of it remaining undetected, irrespective of the features of the test used, is 82%.

- Even if the tests were able to detect viruses in products, and even if the limitations in sample size described above could be overcome, finding a positive test for a viral marker does not mean that actual live virus is in the product. It has been shown that, for example, solvent-detergent treatment, which inactivates HCV infectivity very reliably, has no effect

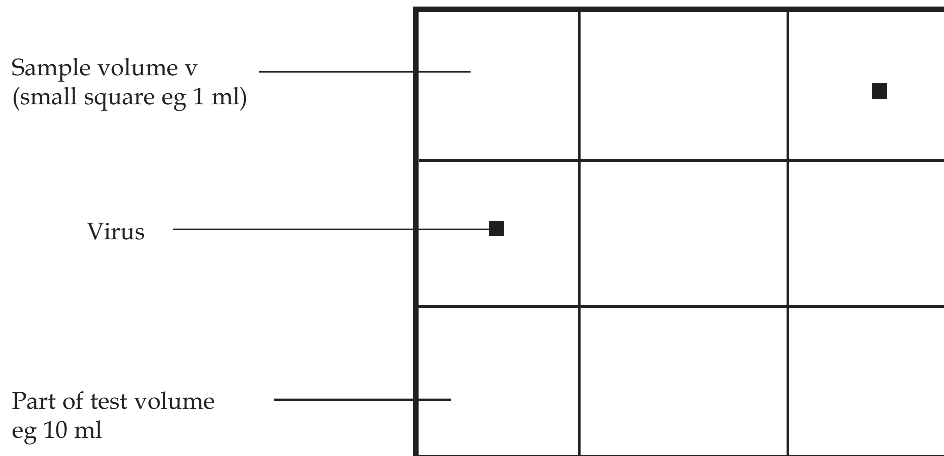
[10] Accessible from http://www.pheur.org/site/download.php

[11] Willkommen H, Lower J. Theoretical considerations on viral inactivation or elimination. Dev Biol Stand, 1993; 81:109-16.

on HCV reactivity in product samples as measured by nucleic acid detection[12]. Therefore, detecting a positive sample using this technique on the final product would lead to the erroneous conclusion that the product was infectious. It is known, through retrospective testing of batches of albumin that many such batches were reactive for HIV nucleic acid in the 1980s; had such testing been available and used to prevent product release a crisis in albumin supply would have ensued. Albumin has never transmitted HIV despite the virus' wide prevalence in the plasma pool because the viral inactivation processes destroyed infectivity, while allowing the retention of reactivity to tests.

The safety of plasma products is assured through adherence to standards and good manufacturing practice. No amount of product testing for viruses can substitute for these crucial requirements.

Figure 1: Distribution of a small amount of virus in a relatively large volume



Virus concentration $c = 2/10\ \text{ml} = 200/\text{l} = 10^{2.3}/\text{l}$
Probability p_ of obtaining a negative test result (Poisson distribution):

$P_{-} = e^{-cv}$
$P_{-} = e^{-200*0.001} = e^{-0.2} = 0.82$

Source: Willkommen H, Lower J. Theoretical considerations on viral inactivation or elimination. Dev Biol Stand, 1993; 81:109-16.

SUMMARY

- Regulatory agencies in countries with no established arrangements for regulation of plasma products should ensure the safety and quality of plasma products by:
  - Forming alliances with similarly placed NRAs
  - Working directly with manufacturers, not through brokers or agents
  - Considering products licensed through established NRAs first
  - Establishing arrangements for pre-selection and audit of suppliers
  - Focusing on evidence of plasma quality and secure manufacture rather than on testing finished product
  - Consulting with independent institutions or experts.

- End-product testing for pathogenic agents such as viruses is inappropriate in most instances and can affect patient safety and product supply. It can never be a substitute to adequate oversight of the whole manufacturing cycle.

[12] Transfusion. 1997 Sep; 37(9):935-40

SECTION 4

EVALUATING CLOTTING FACTOR CONCENTRATES

Introduction

When it comes to assessing which product to purchase, there is no universally right answer. There are certain minimum requirements that should be met, but authorities must assess each product on its own merits and weigh carefully the relative features of each product in making a decision. This section focuses on the evaluation process, first outlining the key information that must be gathered from the manufacturer, and second summarizing the basic requirements that must be met for a product to be considered safe. Several scenarios are given in this chapter to provide examples of the assessment process.

Product information from the manufacturer

To properly assess a product, national regulatory agencies must have information on:

1. The quality of plasma raw material, including:
    - Regulatory status of the plasma supplier
    - Donor epidemiology
    - Donor exclusion criteria
    - Screening tests done on the blood/plasma
    - Quality assurance measures
    - Inventory hold
    - Plasma pool size
    - Testing of the plasma pool

2. The manufacturing process, including:
    - Crucial manufacturing steps and related in-process controls
    - Viral inactivation and/or removal steps
    - Process consistency
    - Batch release specification

3. The final product, including:
    - Potency of the product and shelf life
    - Other markets where product is available
    - Product history
    - Clinical studies demonstrating the product's efficacy

This information can be gathered from the manufacturer using the model product assessment questionnaire in Appendix 2.

Basic requirements

There are a number of requirements which should be met in a satisfactory fashion for a product to be considered safe. These include:

1. **The manufacturer must have full confidence in the safety and quality of the plasma raw material through adequate contractual arrangements with the plasma supplier.**

   The plasma supplier should be licensed by the relevant national health authority. The manufacturer must specify the measures used to ensure that donors are selected on the basis of low risk for the transmission of blood-borne viruses, including questionnaires that identify high-risk behaviour, exclusion of collecting sites from high-risk areas such as prisons, and attempting to build up a base of repeat and accredited donors. While plasma inventory hold and repeated donor qualification are viewed as very desirable features, they are not always possible, particularly when the plasma is recovered from whole blood donations. The manufacturer's confidence can best be acquired by performing audits of the collection centres based on these and other features of good manufacturing practices. These audits should be performed by the manufacturer, although reference to audits performed by a national regulatory authority are satisfactory, as long as they occur within the period of contract between the supplier and the manufacturer.

   Under no circumstances should authorities accept product where the source of the raw material is unknown and unspecified, even if the manufacturer claims that the blood has been tested or the product is viral inactivated. In this regard, the use of uncharacterized plasma from the "spot" market is not recommended.

2. **Blood testing should include screening at the individual donation level for the serological markers of HIV, HBV, and HCV.**

   Screening should be done using test kits for the latest generation of the relevant test, preferably in a format registered by a licensing authority. While the technologies used for detecting infection during the serological window, such as NAT, are also desirable to increase viral safety margins, it is improbable that they will be critical to ensure the viral safety for products sourced from serologically screened plasma which are subjected to robust viral inactivation steps. This also applies for serological and/or NAT testing of the plasma pool by the manufacturer. Confidence in the quality of the serological screening tests is therefore crucial. For this reason, **a quality assurance system for ensuring the performance of viral screening tests is essential.**

3. **Viral inactivation and/or removal in the form of deliberate, well-validated manufacturing steps is essential for the safety of hemophilia products.**

   While a number of viral inactivation steps have been shown to enhance greatly the safety of hemophilia products, **solvent-detergent treatment is the current gold standard for safety from the highly infectious enveloped viruses, and should be seriously considered as the option of choice when assessing products. Similarly, nanofiltration is the option of choice when considering non-enveloped viruses, and also has the potential to decrease the risk of vCJD.**

   Solvent-detergent treatment is not effective at inactivating non-enveloped viruses, which are also resistant to other viral inactivation techniques, and therefore additional steps specifically targeting such viruses are highly advisable. Nanofiltration is an option for factor IX and other smaller plasma proteins; however, it may not be the best option for factor VIII concentrates until new membranes are introduced and validated. For factor VIII concentrates, heating in solution and, to a lesser extent, dry-heat treatment have been shown to contribute to the elimination of non-enveloped viruses.

Another advantage of the solvent-detergent and nanofiltration procedures is the low risk of induction of protein neoantigens.

Any incidental elimination of viruses during the manufacturing procedure that contributes to the overall safety of the product should be welcomed. However, any such contributions through the manufacturing process should be viewed as supplementing rather than substituting for a deliberate viral elimination step.

Given the repeated demonstration that enveloped viruses including HCV, HIV, and HBV are the biggest threat to the hemophilia population, authorities should focus on products with proven safety records against these viruses through well-validated and controlled viral inactivation mechanisms.

4. **Other measures to enhance safety from non-enveloped viruses, including vaccination of people receiving blood products where such vaccines are available (e.g., for hepatitis A virus) and decreasing the viral load of the plasma pool to levels not associated with infection through testing (e.g., NAT) are recommended.**

NAT has been shown to contribute to enhancing the safety from infection by human parvovirus B19. Manufacturers have started to incorporate such testing, and authorities may want to require NAT for specific viruses, known to be prevalent in the donor population contributing to the product. With validated viral inactivation procedures for disease-causing enveloped viruses, such plasma pool testing is probably more beneficial for unscreened non-enveloped viruses. In combination with nanofiltration, NAT may reduce the risk of small non-enveloped viruses such as B19 in the plasma pool, although this has yet to be confirmed in clinical studies.

5. **An assessment of efficacy is an important feature of regulatory oversight, and should be included even for products which are reportedly similar to other, better-characterized products.**

Some emerging countries are known to be purchasing products from manufacturers who have acquired processes through technology transfer. These processes are therefore intended to deliver products which are very similar to the products from the parent technology, which would have been fully characterized for physico-chemical and clinical properties. Clinical trials are expensive and difficult with small patient populations such as hemophilia. There are therefore understandable reservations on the part of manufacturers and authorities from these countries to engage in the rigorous clinical trial framework mandated by the EMEA. While this framework may be modified according to the particular circumstances, it is inadvisable to abandon totally any assessment of efficacy. At the very least, an evaluation of the pharmacokinetics of the product should be performed in order to ensure normal recovery and half life compared to historical controls. An assessment of the correction of bleeding in controlled clinical circumstances should also be attempted, with as many patients as reasonably possible. Once a country is able to resource access to products at whatever level, these modest measures should not be unattainable.

6. **Monitoring of the product's performance after approval to enter the market should be performed, in order to detect possible adverse events.**

The considerations outlined in point 5 for assessment of efficacy apply here as well.

Given that small changes in the manufacturing process for biologicals such as hemophilia concentrates can affect in vivo in ways which are not always predictable from pre-approval assessment, it is important to monitor the product's history in the market. Currently, the onus of concern for hemophilia products has shifted from viral safety to inhibitors. Regular monitoring of heavily exposed patients should be a feature of hemophilia care for countries that can afford some level of

treatment, and therefore samples should be drawn and analyzed to assess patient viral status and measure inhibitor levels. The documents from the EMEA may provide guidance on this and individual authorities may tailor these guidelines to suit their particular circumstances. The relevant laboratory tests are similar to those used to assess the quality of the plasma for viral status and factor levels. It may be possible to enter into contractual agreements with the relevant manufacturers to perform these analyses as part of any supply arrangement.

7. **People receiving factor IX for the treatment of hemophilia B should be given concentrates specifically enriched in this factor and purified to remove other coagulation factors. Highly purified concentrates of factor VIII and factor IX are preferable, when possible, as long as the manufacturing processes are secure in relation to known viral agents and do not cause products to form inhibitors, and as long as purchase of such concentrates does not result in restriction of treatment due to excess cost.**

Example scenarios

To illustrate the application of these principles, examples of the types of choices facing authorities are provided.

Example 1

As a result of a tendering process, the following factor VIII products have been offered:

Product A: A concentrate made from plasma about which the manufacturer has little knowledge except the country of origin. The manufacturer claims to test for the serological markers of the transfusion transmitted agents HIV, HCV, and HBV on the plasma pools after plasma has been thawed for manufacture. The manufacturer also performs NAT testing for HCV on these pools, and the final product is viral inactivated with solvent-detergent and dry-heat treatments. Limited viral inactivation studies have been generated by the manufacturer for the conditions and plasma source specific to the product. The product is the cheapest of those offered.

Product B: A concentrate made from plasma which is characterized by a fully documented quality system incorporating the tenets of the European plasma master file concept. The product is subjected to solvent-detergent treatment. The manufacturer has validated this process for inactivation of viruses in accordance with the requirements of the EMEA's Committee for Human Medicinal Products (CHMP).

Product C: A concentrate made from plasma which is characterized by a fully documented quality system incorporating the tenets of the European plasma master file concept. The product is highly purified on monoclonal antibody affinity columns and solvent-detergent treated. The product is stabilized with albumin; the cost of the product is double that of the next most expensive product.

Product D: A concentrate made from plasma collected by centres under contract to the manufacturer. A full quality system is not evident but the manufacturer has data on donor viral epidemiology and selection protocols to exclude high-risk groups. The product is manufactured using two ion-exchange purification steps which have been demonstrated in literature studies to eliminate significant levels of infectious material including vCJD-like agents. The product undergoes two viral inactivation steps: solvent-detergent and pasteurization. The manufacturer has limited clinical studies and has offered literature-based evidence for efficacy.

Product E: A concentrate made from plasma which is characterized by a fully documented quality system incorporating the tenets of the European plasma master file concept. The product is an intermediate-purity concentrate incorporating terminal dry heat at 80°C for 72 hours in its manufacture. The plasma pool is

tested for HCV and HIV using NAT. The product as manufactured by the company has a long history of safety with appropriately designed clinical studies.

Evaluation

Some of the considerations when evaluating the products in this scenario should include:

1) There is a total lack of knowledge about the plasma quality of Product A. The manufacturer's use of pool testing is not an acceptable substitute for a fully documented quality system. Despite the use of well-accredited viral inactivation steps, the manufacturer's limited ability to validate these is a deficiency. This product, despite its favourable price, should not be considered further.

2) Product B is singly inactivated using solvent-detergent treatment, the best method for eliminating the most highly infectious viruses. However, the lack of any other viral inactivation step is a disadvantage, and regulatory authorities should further consider other products.

3) Product C is very highly purified and is solvent-detergent treated, two attractive features. However, its cost-effectiveness against the other products is probably low. Other products should be considered.

4) Product D has attractive features but the manufacturer should perform its own validation studies on the elimination of the infectious agents from which it claims product safety. The company's contract for plasma supply should be rigorously assessed for its adherence to the crucial features of the plasma master file requirement. Although a full clinical trial may not be required, some evidence of normal pharmacokinetics and efficacy would be desirable.

5) Product E is satisfactory in all features except in the lack of a second viral inactivation step. This does not reflect best practice, but the product's good clinical safety record makes it worthy of consideration. Some evidence exists that dry heating may decrease the risk of transmission of non-enveloped viruses. The manufacturer should be asked for details of its validation process for the inactivation of viruses other than HCV, HIV, and HBV. It should also be asked to comment on the capacity of the manufacturing process to eliminate vCJD-like agents, and for details regarding its plans to move to a double viral-inactivated product.

Example 2

As a result of a tendering process, the following factor IX products are offered:

Product X: A prothrombin complex concentrate manufactured from plasma which is characterized by a fully documented quality system incorporating the tenets of the European plasma master file concept. The product is subjected to dry-heat treatment at 80°C for 72 hours as the sole viral inactivation step.

Product Y: An intermediate-purity concentrate specifically enriched in factor IX by affinity chromatography. It is viral inactivated by solvent-detergent treatment and nanofiltration. The plasma source is satisfactorily characterized and the plasma pool is NAT tested for HCV and HIV.

Product Z: A concentrate made from a satisfactorily characterized plasma source containing factor IX purified to biochemical homogeneity (100% purity) by monoclonal antibody chromatography and treated with solvent detergent as the sole viral inactivation step.

Evaluation

Some of the considerations when evaluating the products in this scenario should include:

1) Use of prothrombin complex concentrates for the treatment of hemophilia B is unacceptable in current medical practice. Furthermore, the single purification step is not an effective method for eliminating vCJD-like agents. A single viral inactivation step can have some effect on the risk of viruses, but any TSE elimination has to come from the purification process, and may therefore be expected to be more effective with multiple purifying steps. The product may be considered for treatment of indications not associated with hemophilia, such as warfarin-reversal, etc.

2) Purity to the level of biochemical homogeneity of factor IX has not been demonstrated to have an effect on the safety of hemophilia B products.

3) Because nanofiltration partitions viruses from proteins of the molecular weight of factor IX, it is a recommended step for eliminating non-enveloped viruses, as well as contributing to the elimination of enveloped viruses.

SUMMARY

- National regulatory authorities (NRAs) must assess each product on its own merits and weigh carefully the relative features of each product in making a decision.
- To properly assess a product, NRAs must have information on:
  - Quality of the plasma raw material
  - Manufacturing process
  - Final product
- Certain minimum requirements should be met:
  - The manufacturer must have full confidence in the safety and quality of the plasma raw material.
  - Individual donations of plasma should be screened for serological markers of HIV, HBV, and HCV.
  - Manufacturing process must include deliberate, well-validated viral inaction and/or removal steps.
  - Other safety measures to enhance safety from non-enveloped viruses, such as vaccination of people who receive concentrates on a lifelong basis and decreasing the viral load of the plasma pool, are recommended.
  - Use of highly purified concentrates of factor VIII and especially factor IX are recommended, as long as it does not result in the restriction of treatment due to excess cost.

SECTION 5

SAFETY ASPECTS OF LOCALLY MADE SMALL POOL CRYOPRECIPITATE

Introduction

Some blood centres with links to the local hemophilia community produce coagulation factor concentrates in-house. This practice has largely ended in developed countries since large pharmaceutical plants have started manufacturing concentrates. However, it is still done in developing countries and provides the main source of product for treating hemophilia A in a number of countries, including Cuba and Thailand. For this reason it is relevant to assess such products in terms of the quality and safety issues which authorities need to be aware of. It should be reiterated that the products of choice for the treatment of hemophilia are concentrates manufactured by accredited pharmaceutical fractionators subject to the full range of regulatory and quality control measures.

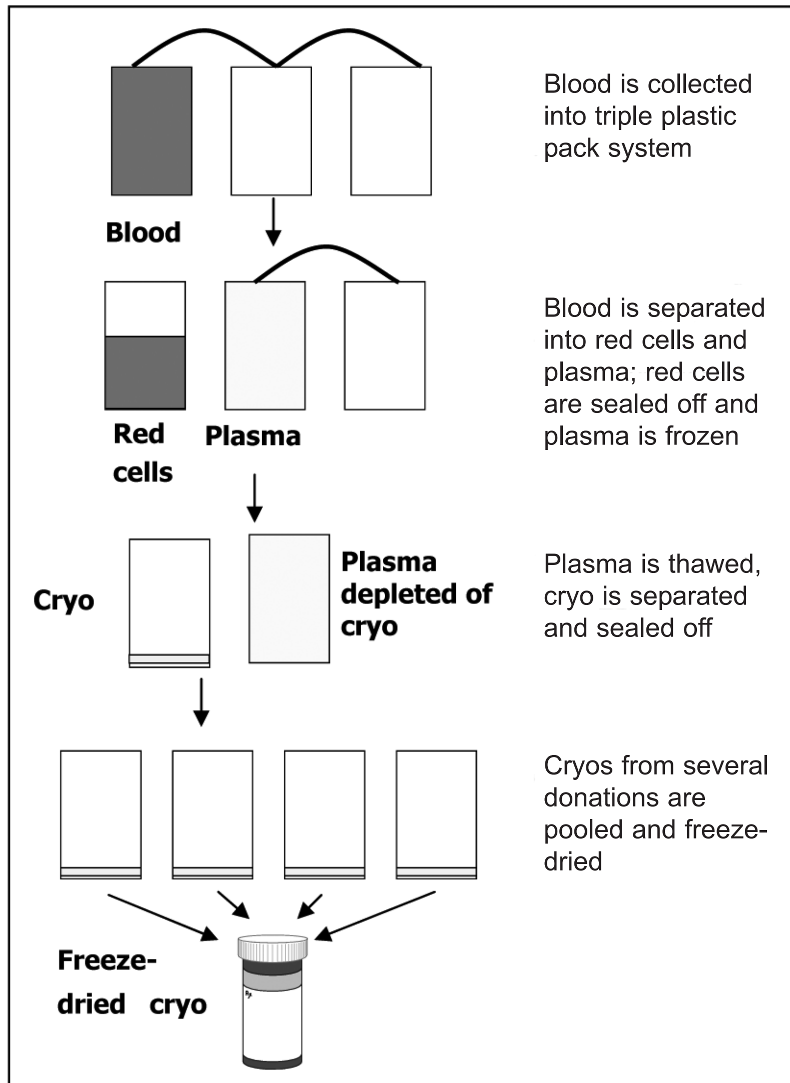
Products available

The most important product in this category is small pool cryoprecipitate for the treatment of hemophilia A. The technology required to produce this product is relatively modest, although it can become more complex and demanding as refinements are introduced. Cryoprecipitate forms when frozen plasma is thawed at temperatures which do not exceed approximately 4ºC. Under these conditions, a proportion of the plasma proteins remains insoluble and can be separated from the rest of the thawed plasma. The insoluble protein is called cryoprecipitate (cryo) and is mostly composed of the cold-insoluble proteins fibrinogen and fibronectin. When the plasma is frozen under conditions which preserve factor VIII activity, the cryoprecipitate is also enriched in factor VIII. This process is still used by pharmaceutical companies to extract factor VIII for the large-scale manufacture of factor VIII concentrates.

Cryoprecipitate produced in blood centres may be used for therapy without further modification. However, to make its use more convenient for patients, it may be subjected to further processing, such as freeze-drying. The production of cryoprecipitate is summarized in Figure 2. Whole blood and plasma collected by plasmapheresis can be used to produce cryo.

The production of cryo has been studied for many years. The variables which influence the amount of factor VIII present in the cryo (the yield) are well understood. With careful attention to maximize the amount of factor VIII in the cryo, about 600 units of factor VIII can be harvested from one litre of plasma. The final product is a freeze-dried concentrate which people with hemophilia can store in household fridges and reconstitute and administer themselves.

Figure 2: Production of cryoprecipitate



Quality and safety issues

Cryo is still manufactured in developed countries in small amounts, mostly for its fibrinogen content, but it is subject to different regulatory oversight processes than those to assure the quality and safety of concentrates produced on a large scale. However, if cryo is the mainstay of hemophilia A treatment, measures to assure its quality and safety are very important and should engage authorities in the same way as the oversight of concentrates.

Because of the nature of the product, some aspects of the assessment process regarding cryo are different than those for concentrates:

- Since cryo is produced using relatively low levels of processing, it is a crude product which will not meet many of the commercially acceptable criteria for high-purity plasma concentrates, such as potency, purity, and solubility. However, this is not a significant problem in terms of its safety.

- Since cryo is produced from small donation pools generally resulting in one or two vials of product from a pool, the ability to characterize the product through representative batch sampling is limited. In other words, it is not possible to label a vial of freeze-dried cryo for potency.
- Viral reduction techniques described in this guide are not easily applied to the manufacture of cryo. This is because they are based on technology not easily adapted to blood centres, and because the product's low purity prevents inactivation through heat.

Proposed approach to ensure the safety of small pool cryo

In spite of these limitations, it is possible to produce cryo of a sufficient standard of safety and quality in blood centres if the following principles are recognized and followed:

1) Although convenient and desirable, product purity is not a crucial safety issue for factor VIII concentrates. As long as dried cryo can be reconstituted in a volume which allows adequate replacement therapy, the purity levels achievable by blood centre production are entirely adequate. Imposing the potency and purity standards of developed countries in this context is inappropriate.

2) Since the assessment of potency on final product is not possible, quality assurance for these products depends on rigorous process validation before the product is introduced into the therapeutic environment. This validation must show, through analysis of product through different stages of the process, that the process is capable of delivering, in a consistent manner, product which falls within certain specified limits of crucial parameters, such as potency. Since the assurance of these limits will not be possible through actual assay in the production phase, substitutes for measurable in-process controls which are known to affect quality must be developed. For example, the production laboratory must be able to deliver, with each batch of dried product, temperature data showing that the freezing of plasma, the thawing of plasma, the freeze-drying, etc., were all done within limits which are known to be associated with the optimal preservation of factor VIII as confirmed by assays done in the development phase.

   This validation process is laborious and will result in the consumption of valuable product which cannot be used to treat patients. However, it is important that producers and authorities alike recognize the importance of this process if a consistently good quality product is to be delivered. The WFH will provide advice to individual producers of freeze-dried cryo on the optimization and validation of their processes.

3) Because of the limitations specified above, cryo cannot be subjected to the same level of viral elimination as concentrates. Modifications to the production method may allow dried cryo to be subjected to heat treatment. However, in the blood centre setting, heat treatment cannot exceed 60°C, a temperature which will not inactivate HCV. It may be possible to adapt other technologies to the treatment of cryo, and the WFH will assess examples of these over the coming years.

   In the immediate future, however, the mainstay for cryo safety must be the appropriate selection and testing of low-risk blood or plasma donors. These are of course, mandatory principles for the safety of any blood product, but with cryo they are all the more important as viral inactivation – the safety measure which has, above all others, made concentrates safe – is of limited applicability. Therefore, the producer and the overseeing body alike must optimize selection and screening procedures to minimize any viral contamination of the starting plasma.

Some measures which should be included in a program are:

- Donors should be selected from low-risk populations. This can only be done using the kind of epidemiological data that can be obtained using tools such as the plasma master file model (see page 15).
- Once collected, plasma should be quarantined until the donor has been recalled and re-tested for markers of infection. All such testing should be done with the most sensitive tests possible. If the donor does not return for re-testing the plasma should not be used. Re-testing should be done using intervals which allow donors to seroconvert for the transfusion-transmitted viruses in case they were infected at the time of donation. The quarantine period should be related to the window period of infection for the relevant virus. This will depend on whether NAT screening is used.
- Testing for viruses using molecular technology should be introduced. Using NAT, the viral window period for the important viruses can be substantially decreased. This measure probably has minimal effect on the safety of plasma derivatives in developed countries because of the effect of viral inactivation; however, its use on products, such as cryo, which cannot undergo such inactivation would enhance safety considerably.
- Producers and authorities may be able to further enhance the safety and quality of product through other means. For example, optimization of factor VIII yields may allow some level of viral inactivation to be achieved, since the loss of yield due to viral inactivation may be tolerated if production yields are high. Pools of dedicated plasma donors, carefully selected and repeatedly tested, can in time become a source of very safe raw material, compared to first-time donors. The pharmacological stimulation of donors to produce more factor VIII may be worth considering as a means of improving yields.

Conclusion

The production of safe cryoprecipitate is a feasible option for countries without access to plasma concentrates. It requires a high level of commitment both in the generation of safe raw material and in the technical production of the product. It should be pointed out that the safety features outlined in this chapter require policies and investment which would benefit all aspects of blood safety. This level of investment will increase the cost of cryoprecipitate. The technical expertise gained in optimizing cryo production should also be viewed as a long-term benefit for when the country's economic development makes plasma fractionation more feasible

SUMMARY

- Small pool freeze-dried cryoprecipitate is a viable option for the treatment of hemophilia A for countries lacking access to fractionation facilities.
- Careful attention to technique can allow sufficient product to be generated to deal with most everyday treatment requirements.
- Safety of the product cannot be assured through viral inactivation; therefore, donor selection and screening measures are all the more important.

CONCLUSION

Choosing appropriate products for the treatment of hemophilia is not an easy task. It depends on the resources and unique circumstances of each country. However, the principles and information given here can provide guidance to regulatory authorities when making decisions about the purchase of hemophilia treatment products.

The WFH updates this guide regularly, and welcomes comments for improving it.
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APPENDIX 1

REGISTRY OF CLOTTING FACTOR CONCENTRATES

Eighth Edition, 2008

by Mark Brooker

Introduction

The Registry was created in 1997 by Meirione Costa e Silva of Brasilia and Dr. Carol Kasper for the International Society on Thrombosis and Haemostasis. Its purpose is to help medical personnel identify available concentrates and stay abreast of pharmaceutical company changes.

The registry provides an overview of available products and clarifies differences among them. It also helps doctors and pharmacists identify products that patients are offered during their foreign travels or those they may bring home with them, or have sent to them. Similarly, patients traveling abroad may bring along their own concentrates, which may not be familiar to local healthcare personnel.

Agencies contemplating bulk purchase of concentrates are advised to consult the WFH's publication *Guide to National Tenders for the Purchase of Clotting Factor Concentrates* by Brian O'Mahony.

Plasma obtained from donations of whole blood is called recovered plasma. Plasma obtained by apheresis is called source plasma. Donors of whole blood are not paid any substantial amount inany of the countries listed in this registry. Donors of apheresis plasma are paid in most countries.

Several national fractionation centres produce concentrate from domestic recovered plasma for domestic use. A few fractionators (for example, CSL in Australia, Grifols in Spain, Sanquin in The Netherlands) accept plasma from small countries, fractionate it separately, and return it as concentrate to the donor country, a process called contract or toll fractionation. Several fractionators use source plasma from countries permitting paid apheresis. Such plasma may be blended with smaller amounts of unpaid recovered plasma.

Within the tables, concentrates are grouped first according to method of fractionation, then according to method of viral inactivation or degree of purification from lowest to highest. Fractionators cite the purification level of clotting factors as specific activity, or the amount of the desired clotting factor per milligram of total protein, minus any added albumin (SA s Alb). Specific activity may actually be measured or may be an approximation. Retention of plasma after donation and before processing to ascertain further information about a donor is called inventory hold or quarantine.

Tables 1A and 1B describe measures that help ensure the safe use of plasma. The array of serologic tests varies slightly from country to country. More sensitive nucleic acid tests that directly detect viruses are becoming commonplace.

Table 2 lists FVIII concentrates made by techniques generally associated with a lesser level of purification. Many contain von Willebrand factor (VWF), which stabilizes FVIII and is needed for treatment of von Willebrand disease (VWD). Table 3 lists FVIII concentrates made by techniques allowing higher level

of purification, including recombinant FVIII concentrates. Prothrombin complex concentrates, which are not highly purified, are described in Table 4.

Table 5 lists concentrates primarily intended for use in patients with inhibitors. At the present time, all are activated concentrates (bypassing agents). Concentrates of factor IX alone are described in Table 6. Concentrates for rare clotting factor deficiencies are listed in Table 7. They are not widely available, and there are some deficiencies for which no concentrate is made. NovoSeven®, the recombinant activated factor VII concentrate, is increasingly used for patients with congenital deficiency of factor VII and now is licensed for that use in the U.S.A. The FX/IX-concentrate Factor X P Behring® provides an up to two-fold higher content of FX compared to FIX and no FII or FVII. Therefore it is almost only used for patients with factor X-deficiency.

There is one concentrate of VWF virtually alone, LFB's Wilfactin®, Table 7. Another LFB product, Wilstart®, available only in France, combines 1000 IU of Wilfactin® and 500 IU of Factane®, for use in acute bleeding in VWD or as the initial surgical dose. Octapharma's Wilate®, in Table 2, was developed for use in VWD as well as in hemophilia A. Several other products, in Tables 2 and 3, also contain VWF and are used in VWD as well as in hemophilia.

Concentrates for deficiencies of antithrombotic factors are listed in Table 8. The only plasminogen available is combined with streptokinase in the clot-lysing product, Eminase® (Roberts Pharmaceutical Corporation, New Jersey).

No shortages of factor VIII and factor IX concentrates were reported in the United States in 2007, nor were there any reports of breaches of safety. No HIV has been transmitted by any American concentrate since 1987. No hepatitis A, B, or C has been transmitted by American concentrates since the Centers for Disease Control and Prevention began its broad surveillance of persons with hemophilia in 1998.

TABLE 1A: SEROLOGIC TESTS ON INDIVIDUAL DONOR PLASMA

PLASMA SOURCE	Syphilis	HIV 1-2	p-24 antigen	HTLV-1	HTLV-2	HBcAb	HBsAb	HBsAg	HCVAb	ALT[1]	B-19 parvovirus
US paid apheresis (Talecris, Grifols, others[4])	Yes[2]	Yes	No [3]	No	No	No	No	Yes	Yes	Yes	No
United States, recovered, unpaid	Yes[2]	Yes	No[3]	Yes	Yes	Yes	No	Yes	Yes	Yes	No
Baxter BioScience: United States, Austria, Germany	Yes[2]	Yes	No	No	No	No	No	Yes	Yes	Yes	No
CSL Behring: Austria, Denmark, Germany, United States	Yes	Yes	No	No	No	No	No	Yes	Yes	No	No
Biotest: Austria, Belgium, Germany, United States	Yes	Yes	No	No	No	No	No	Yes	Yes	Yes	No
Intersero: Austria, Belgium, Germany	Yes	Yes	No	No	No	No	No	Yes	Yes	Yes	No
Germany unpaid	Yes	Yes	No	No	No	No	No	Yes	Yes	Yes	No
Octapharma: Sweden, Austria, Germany	Yes	Yes	No	No	No	Yes[5]	No	Yes	Yes	No	No
American Community Blood Centers, unpaid (Octapharma)	Yes	Yes	No	Yes[5]	Yes[5]	Yes[5]	No	Yes	Yes	No	No
Finnish Red Cross BS: Finland, unpaid	Yes	Yes	No	1st donation & q 3 yrs	1st donation & q 3 yrs	No	No	Yes	Yes	No	No
Sanquin: The Netherlands	Yes	Yes	No	Yes	Yes	No	No	Yes	Yes	No	No
LFB: France	Yes[6]	Yes	No	Yes	Yes	Yes[7]	Yes [8]	Yes	Yes	No	No
Grifols: Spain, Czech Republic, Slovakia	Yes	Yes	No	No	No	No	No	Yes	Yes	Yes	No
Kedrion: Italy	Yes	Yes	No	No	No	No	No	Yes	Yes	Yes	No
National Bioproducts Institute: South Africa[11]	Yes	Yes	No	No	No	No	No	Yes	Yes	No	No
Australian Red Cross Blood Service [9]	Yes	Yes	No	Yes	Yes	No	No	Yes	Yes	No	No
New Zealand Blood Service [9]	Yes	Yes	No	1st donation	1st donation	No	No	Yes	Yes	No	No
Centre for Transfusion Medicine, Singapore [9]	Yes	Yes	No	No	No	No	No	Yes	Yes	No	No
National Blood Center, Malaysia [9]	Yes	Yes	No	No	No	No	No	Yes	Yes	No	No
Hong Kong Red Cross BTS [9]	Yes	Yes	No	Yes	Yes	No	Yes	Yes	Yes	No	No
Japan	Yes	Yes	No	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes
Korean Red Cross: South Korea [10]	Yes	Yes	Yes	No	No	No	No	Yes	Yes [10]	Yes	No
Shanghai RAAS Blood Products Co: China	Yes	Yes	No	No	No	No	No	Yes	Yes	Yes	No

1. ALT testing is not required for release of plasma in the USA. The requirement in Europe is country-specific.
2. Performed every 4 months in accordance with the US Code of Federal Regulations.
3. Not required if a US FDA licensed HIV-1 NAT test, approved as an alternative to HIV-1 p24 Ag testing, is used..
4. US paid apheresis source plasma is used by several European fractionators, as indicated; US unpaid plasma (recovered from whole blood donations) is used by fractionators in other countries. US recovered plasma originates from the American Red Cross and other licensed US blood banks.
5. Only relevant for transfusion blood products but not for plasma for fractionation.
6. Not required for apheresis plasma intended only for fractionation.
7. Only in first time donors or for hepatic assessment after seroconversion.
8. Only performed when screening test for Hc Ab is positive.
9. CSL Bioplasma in Australia fractionates plasma on a contract (toll) basis for the Australian Red Cross Blood Transfusion Service, the New Zealand Blood Service, the Hong Kong Red Cross Blood Transfusion Service, the Center for Transfusion Medicine of Singapore and the National Blood Center of Malaysia, Kuala Lumpur.
10. NAT for HCV is performed on individual donations in Korea.
11. Since 2005, NAT tests for HCV, HBV and HIV-1 have been performed on individual donations of plasma supplied by SA Blood Transfusion Services

TABLE 1-B. PLASMA INVENTORY HOLD AND NAT TESTING OF MINI-POOLS

COMPANY OR FRACTIONATOR	INVENTORY HOLD	MINI-POOL SIZE	MINI-POOL NAT TESTS	MANUFACTURING POOL NAT TESTS	NAT ON FINAL PRODUCT
CSL Behring: United States, Germany	60+ days	512 or fewer	HAV, HBV, HCV, HIV-1, B-19 parvovirus	HAV, HBV, HCV, HIV, B-19 parvovirus	No
Baxter BioScience: United States, Austria	60+ days	512	HAV, HBV, HCV, HIV-1, B-19 parvovirus	HAV, HBV, HCV, HIV 1-2, B-19 parvovirus	
Talecris: United States	60+ days	96 or 480	HBV, HCV, HIV 1, B-19 parvovirus	HBV, HCV, HIV-1, B-19 parvovirus	No
Grifols: United States, Spain, Czech Republic, Slovakia	60+ days	512 or fewer	HAV, HBV, HCV, HIV, B-19 parvovirus	HBV, HCV, HIV, B-19 parvovirus	
BPL, UK: US plasma used	60 days	512	HAV, HBV, HCV, HIV 1-2, B-19 parvovirus	European requirement[1]	
Biotest: Germany	60 days	960	HAV, HBC, HCV, HIV 1, B-19 parvovirus	HBV, HCV, HIV	
Intersero: Germany	60+ days	960	HAV, HBV, HCV, HIV-1, B-19 parvovirus	HBV, HCV, HIV	
German Red Cross BSO NSTOB	2 months	48	HAV, HBV, HCV, HIV-1, B-19 parvovirus	European requirement[1]	
Octapharma: Sweden, Austria, Germany, USA	2 months[6]	16 - 512	(HBV,B-19 parvovirus,HAV, HCV, HIV-1.	European requirement[1]	No
Finnish Red Cross BS: Finland		1 or 96	HBV, HCV, HIV (individual) HAV, B-19 parvovirus (mini-pool)	FRC BS does not make plasma pools	
Sanquin: The Netherlands		480	HCV, HIV, HBV (individual) B-19 parvovirus (mini-pool)	HAV, HBV, HCV, HIV, B-19 parvovirus	
LFB: France	80+ days [4]	(1) 300; (2) 1000	(1) B-19 parvovirus; (2) HAV, HCV [5]	HAV, HBV, HCV, HIV-1, B-19 parvovirus	
Kedrion: Italy	60+ days	480 or fewer	HBV, HCV, HIV, B-19 parvovirus (HAV if required)	European requirement[1]	
National Bioproducts Institute, South Africa		1[2] and 216	HCV, HIV, HAV, B-19 parvovirus	HCV, HIV, HAV	
CSL Bioplasma, Australia		480	HCV, HIV (except ARCBS and NZBS plasma)	HCV, HIV (ARCBS and NZBS)	
Australian Red Cross Blood Service Fractionated at CSL Bioplasma		1[3] or 24[3]	HCV,HIV		
New Zealand Blood Service Fractionated at CSL Bioplasma		1[3] or 16	HCV, HIV		
Hong Kong Red Cross BTS Fractionated at CSL Bioplasma		24 (ARCBS), 480 (CSL)	HCV, HIV (at both ARCBS and CSL Bioplasma)		
Centre for Transfusion Med, Singapore Fractionated at CSL Bioplasma		1[2] (Singapore) 480 (CSL)	HCV, HIV (CSL)		
National Blood Centre of Malaysia Fractionated at CSL Bioplasma		480 (CSL)	HCV, HIV (CSL)		
GreenCross: South Korea	45 days	< 450	HAV, HCV	HAV, HBV, HCV, HIV	HAV, HBV, HCV, HIV
Japanese Red Cross: Japan	6 months	20	HBV, HCV, HIV-1	HBV, HCV, HIV-1	HAV, HBV, HCV, HIV-1, B-19 parvovirus
Kaketsuken: Japan	6 months	(1) 50, (2) 500	(1) HBV, HCV, HIV-1, (2) HAV, B-19 parvovirus	HAV, HBV, HCV, HIV-1, B-19 parvovirus	HAV, HBV, HCV, HIV-1, B-19 parvovirus
Benesis, Japan	6 months	50	HBV, HCV, HIV-1	HBV, HCV, HIV-1	HAV, HBV, HCV, HIV-1, B-19 parvovirus
Shanghai RAAS Blood Products: China	60+ days	48	HBV, HCV, HIV-1	HBV, HCV, HIV-1	HBV, HCV, HIV-1

1.The European Pharmacopoeia requires HCV testing by NAT.
2. Since October, 2005, NAT tests for HCV HBV and HIV are performed on individual donations.
3. "1" indicates single-bag testing.
4. A minimal 80 day observation period between the day of collection and thawing, and a minimal 90 day observation period between the day of collection and the first step of the manufacturing process
5. These tests are not performed by LFB when they are already carried out by the local testing centre during the biological qualification of the donation.
6. 60 days inventory hold performed on US Plasma only.

TABLE 2. FACTOR VIII CONCENTRATES MADE BY PRECIPITATION (PPT), GEL PERMEATION OR ION EXCHANGE CHROMATOGRAPHY

BRAND	COMPANY	SITE OF MANUFACTURE	PLASMA SOURCE	EXPORT/ DOMESTIC	FRACTIONATION	VIRAL INACTIVATION	SA s alb FVIII IU/mg [1]	COMMENT
Factor 8 Y	BioProducts Lab.	Elstree, England	United States: paid apheresis	Both	Heparin/glycine ppt	Dry heat, 80° C, 72 hr	2.5 - 4	Contains VWF
Haemosolvate Factor VIII	National Bioproducts	Durban, South Africa	South Africa & United States: unpaid	Both	Heparin/glycine ppt	TNBP/ polysorbate 80	4-11	No albumin added; contains VWF
HEMORAAS SD plus H	Shanghai RAAS	Shanghai, China	China: paid and unpaid apheresis	Both	PEG/ glycine ppt	S/D, dry heat 100° C, 30 min	< 30	No albumin added, contains VWF
Haemate P (= Haemate HS)	CSL Behring	Marburg, Germany	United States, Germany, Austria: paid & unpaid	Both	Multiple precipitation	Pasteurization, 60° C,10 hr	38	Albumin added, contains VWF, ratio vWF/FVIII > 2.2
Humate P	CSL Behring	Marburg, Germany	United States: paid apheresis	To USA and Canada	Multiple precipitation	Pasteurization, 60° C,10 hr	38	Same as above
Conco-eight-HT	Benesis	Osaka, Japan	Japan: unpaid	Domestic	Glycine precipitation, gel filtration	TNBP/ polysorbate 80 & dry heat, 60° C, 72 hr	50	Albumin added
Koate DVI	Talecris	Clayton, NC, USA	United States: paid apheresis	Both	Multiple precipitation and size exclusion chromatography	TNBP/ polysorbate 80 & dry heat, 80° C, 72 hr	>20	Albumin added, contains VWF
BIOSTATE	CSL Bioplasma	Melbourne, Australia	Australia, New Zealand, Malaysia, Singapore, Hong Kong: unpaid; USA: paid	Both	Heparin/ glycine ppt, gel filtration chromatography	TNBP/ polysorbate 80 & dry heat, 80° C, 72 hr	50	Albumin added, contains VWF; SA without albumin & VWF = 180
HEMORAAS-IP, SD plus H	Shanghai RAAS	Shanghai, China	China: paid and unpaid apheresis	Both	PEG ppt & ion exchange chromatography	S/D; dry heat, 100° C, 30 min	< 100	No albumin added
HEMORAAS-HP, SD plus H	Shanghai RAAS	Shanghai, China	China: paid and unpaid apheresis	Both	PEG ppt & ion exchange chromatography	S/D; dry heat, 100° C, 30 min	> 100	No albumin added
GreenEight	GreenCross	Seoul, Korea	Korea: unpaid; United States: paid apheresis	Both	Ion exchange chromatography	TNBP/Triton X 100; Dry heat 100° C, 30 min	100+	No albumin added, contains VWF
Confact F	Kaketsuken	Kumamoto, Japan	Japan: unpaid	Domestic	Ion exchange chromatography	Dry heat, 65° C, 96 hr; 19 nm nanofiltration	50	Albumin added
Immunate	Baxter BioScience	Vienna, Austria	United States, Austria, Czech Republic, Germany, Sweden: mostly paid apheresis	Both	Ion exchange chromatography	Solvent-detergent ; vapor-heat, 60° C, 10 hr at 190 mbar	Mean 70, SD 30	Albumin added, contains VWF
Emoclot D.I.	Kedrion	Barga, Italy	Europe and United States: paid & unpaid	Both	Ion exchange chromatography	TNBP/polysorbate 80 & dry heat, 100° C, 30 min	> 80	No albumin added, contains 0.4 IU VWF:RCo per IU FVIII
Haemoctin SDH	Biotest	Dreieich, Germany	USA, Austria, Belgium, Germany: paid & unpaid	Both	Anion exchange chromatography	TNBP/polysorbate 80 & dry heat, 100° C, 30 min	100	No albumin added
Faktor VIII SDH Intersero	Intersero	Biotest, Dreieich, Germany	USA, Austria, Belgium, Germany; paid & unpaid	Domestic	Anion exchange chromatography	TNBP/polysorbate 80 & dry heat, 100° C, 30 min	100	No albumin added
Octanate	Octapharma	Vienna, Austria, Stockhom, Sweden & Lingolsheim, France	Sweden, Austria, Germany, United States	Both	Precipitations, ion exchange chromatography	TNBP/polysorbate 80 & Terminal dry heat, 100° C, 30 min at controlled residual moisture	> 100	No albumin added, contains VWF
Wilate	Octapharma	Vienna, Austria	Sweden, Austria, Germany, United States	Both	Precipitations, ion exchange and size exclusion chromatography	TNBP/Triton X 100, & terminal dry heat, 100° C, 120 min, at controlled residual moisture	>100	No albumin added; contains VWF and FVIII in the physiological ratio
FACTANE	LFB	France	Western European unpaid	Both	Adsorption on aluminium hydroxide gel Ion exchange chromatography	TNBP/ polysorbate 80 & 35-15 nm nanofiltration	> 100	No albumin added, contains VWF
Beriate P	CSL Behring	Marburg, Germany	USA, Austria, Denmark, Germany: paid & unpaid	Both	Ion exchange chromatography	Pasteurization, 60° C, 10 hr	170	No albumin, stabilized in amino acids & sucrose
Optivate	Bio Products Laboratory	Elstree, England	United States paid apheresis	Both	Cryoprecipitation, heparin and glycine precipitation, MPHS chromatography	Solvent-detergent and dry heat, 80°, 72 hr	800	Contains VWF

1. Specific activity minus any added albumin

TABLE 3. FACTOR VIII CONCENTRATES: AFFINITY CHROMATOGRAPHY, OR RECOMBINANT

BRAND	COMPANY	SITE OF MANU-FACTURE	PLASMA SOURCE	EXPORT/ DOMESTIC	FRACTIONATION	VIRAL INACTIVATION	SA s alb FVIII IU/mg [1]	COMMENTS
Alphanate	Grifols	Los Angeles, CA, USA	United States: paid apheresis	Both	Heparin ligand chromatography	TNBP/polysorbate 80 & dry heat, 80° C, 72 hr	≥100	Albumin added, contains VWF; SA w/o alb & VWF = 1000-3000
Fanhdi	Grifols	Barcelona, Spain	1. USA: paid apheresis 2. Spain: unpaid recovered & apheresis 3. Czech Republic: recovered & apheresis 4. Slovakia, recovered & apheresis	1. Both 2.Domestic only 3. to Czech Republic 4. to Slovakia	Same as above	Same as above	≥100	Same as above
Monoclate P	CSL Behring	Kankakee, IL, USA	United States: paid apheresis	Both	Monoclonal Ab affinity chromatography	Pasteurization at 60° C, 10 hr	> 3000	Albumin added, no VWF
Hemofil M AHF	Baxter BioScience	Los Angeles, CA, USA	United States: paid apheresis	Both	Monoclonal Ab affinity & ion exchange chromatography	TNBP/ Octoxynol 9	Approx. 2000	Albumin added, no functional VWF
Replenate	Bio Products Laboratory	Elstree, England, UK	United States: paid apheresis	Domestic	Monoclonal Ab affinity & ion exchange chromatography	TNBP/ Triton X 100	> 2000	As above
Amofil	Sanquin OY	Sanquin, Amsterdam	Finland: unpaid recovered	To Finland	Same as above	Same as above	> 2000	As above
Octanativ-M	Octapharma	Stockholm, Sweden	Swedish unpaid recovered & apheresis	Both	Same as above	Same as above	>2000	As above
Aafact	Sanquin	Amsterdam, Netherlands	Netherlands: unpaid	Domestic	Same as above	Same as above	> 2000	As above
GreenMono	Greencross Corp	Seoul, Korea	Korea: unpaid	Domestic	Same as above	TNBP/ Triton X 100	> 2000	As above
Cross Eight M	Japanese Red Cross	Chitose City, Japan	Japan: unpaid	Domestic	Same as above	TNBP/ Triton X 100 & nanofiltration	> 2000	As above

1. Specific activity minus any added albumin

Recombinant factor VIII concentrates:

BRAND	COMPANY	SITE OF MANU-FACTURE	PLASMA SOURCE	EXPORT/ DOMESTIC	FRACTIONATION	VIRAL INACTIVATION	SA s alb FVIII IU/mg [1]	COMMENTS
Kogenate FS = KOGENATE Bayer (in EU)	Bayer	Berkeley, CA, USA	None, recombinant	Both	Recombinant: ion exchange & immunoaffinity chromatography	TNBP/ polysorbate 80	2600 - 6800	Full-length rFVIII; no VWF. Formulated with sucrose. Albumin not added during purification or formulation.
Helixate NexGen = Helixate FS	CSL Behring	Made by Bayer, Berkeley, CA	None, recombinant	Both	(Identical to Kogenate FS)	TNBP/ polysorbate 80	2600 - 6800	Same as above
Recombinate rAHF	Baxter BioScience	Thousand Oaks, CA, USA	None, recombinant	Both	As above		> 4000	Full length rFVIII, no functional VWF.: Human serum albumin added as stabilizer
Advate rAHF PFM	Baxter Bioscience	Neuchatel, Switzerland	None, recombinant	Both	Recombinant	TNBP/ polysorbate 80, Triton X 100	4000 – 10,000	Full-length rFVIII, no functional VWF: no addition of human- or animal-derived plasma proteins or albumin in the cell culture, purification or final formulation.
ReFacto	Wyeth	Stockholm, Sweden	None, recombinant	Both	Recombinant	TNBP/ Triton X 100	13,000	B-domain-deleted FVIII, no VWF. No albumin added in formulation.

1. Specific activity minus any added albumin

TABLE 4: PROTHROMBIN COMPLEX CONCENTRATES ("PCC"; concentrates of prothrombin and factors VII, IX and X)

BRAND	COMPANY	SITE OF MANU-FACTURE	PLASMA SOURCE	EXPORT/ DOMESTIC	FRACTIONATION	VIRAL INACTIVATION	SA s alb FIX IU/mg [1]	COMMENTS
Proplex – T	Baxter BioScience	Los Angeles, CA, USA	United States: paid apheresis	Both	Tricalcium phosphate absorption, PEG fractionation	Exposure to 20% ethanol; dry heat, 60° C, 144 hr	> 8	Heparin added; maximum 3.5 U factor VII per IU factor IX
Prothroraas	Shanghai RAAS	Shanghai, China	China, paid/unpaid apheresis	Both	PEG precipitation, DEAE sephadex	Solvent/ detergent, nanofiltration		
Beriplex P/N	CSL Behring	Marburg, Germany	United States, Austria, Germany paid/unpaid	Both	DEAE-sephadex	Pasteurization at 60° C, 10 hr, & nanofiltration	3.5 – 5	Contains protein C 700-900 IU per 500 IU factor IX; anti-thrombin III, heparin & albumin added
Haemosolvex Factor IX	National Bioproducts	Pinetown, South Africa	South Africa: unpaid	Both	DEAE-sephadex	TNBP/polysorbate 80	0.9	No albumin added; heparin added
Profilnine SD	Grifols	Los Angeles, CA, USA	United States: paid apheresis	Both	Double DEAE cellulose chromatography	Solvent/detergent	4	No albumin, heparin or antithrombin III added
Prothrombinex-VF	CSL Bioplasma	Melbourne, Australia	Australia, New Zealand, Hong Kong, Malaysia, unpaid	Both	DEAE cellulose absorption	Dry heat, 80° C, 72 hr Nanofiltration	1 – 5	No albumin added
Prothromplex-T	Baxter BioScience	Vienna, Austria	United States, Austria, Czech Republic, Germany, Sweden: mostly paid apheresis	Both	Ion exchange adsorption	Vapor heat, 60° C for 10 hr at 190 mbar, then 80° C for 1 hr at 375 mbar		Anti-thrombin III & heparin added
Bebulin VH	Baxter BioScience	Vienna, Austria	United States: paid apheresis	Export to USA	Same as above	Same as above		Heparin added
HT DEFIX	SNBTS	Edinburgh, Scotland	United States & Germany: unpaid	Both	Ion exchange chromatography	Dry heat, 80° C, 72 hr	2	Anti-thrombin III added
Octaplex	Octapharma	Vienna, Austria & Lingolsheim, France	Sweden, Austria, Germany & United States	Both	Ion exchange chromatographies	TNBP/ polysorbate 80 & nanofiltration	1 or more	Heparin added, no antithrombin or albumin added, low factor VIIa content
Facnyne	Greencross Corp	Seoul, Korea	Korea: unpaid	Domestic	Ion exchange chromatography	TNBP/ polysorbate 80	@ 6 – 7	No albumin added
Cofact	Sanquin	Amsterdam, Netherlands	Netherlands: unpaid	Both	DEAE ion exchange chromatography	TNBP/polysorbate 80 & 15 nm nanofiltration		Anti-thrombin III added
PPSB-human SD/Nano 300/600	German Red Cross NSTOB	Springe, Germany	Germany: unpaid	Domestic	DEAE-sephadex, ion exchange chromatography	TNBP/ polysorbate 80 & Two nanofiltration steps, 50 nm & 15-19 nm	1	Anti-thrombin III & heparin added; no albumin added
UMAN Complex D.I.	Kedrion	Barga,Italy	Europe & United States, unpaid & paid	Both	Anion exchange: DEAE-sephadex/ sepharose chromatography	TNBP/ polysorbate 80 & Dry heat, 100° C, 30 min	< 1.6	Anti-thrombin III & heparin added; no albumin added; factor II & factor X titration
KASKADIL	LFB	France	Western Europe, unpaid	Both	Ion exchange chromatography	TNBP/ polysorbate 80	≥0.6	Heparin added, no albumin or anti-thrombin III added

1. Specific activity minus any added albumin

TABLE 5: CONCENTRATES PRIMARILY INTENDED FOR USE IN PATIENTS WITH INHIBITORS: Activated Concentrates (Bypassing agents)

FEIBA VH	Baxter Bioscience	Vienna, Austria	United States, Austria, Czech Republic, Germany, Sweden; mainly paid apheresis	Both	Surface-activated PCC, batch-controlled	Vapor heat, 60° C, 10 hr, 190mbar then 80° C, 1 hr, 375 mbar	No heparin added
NovoSeven = Niastase (in Canada)	Novo Nordisk	Copenhagen, Denmark	None	Both	Recombinant Factor VIIa	None	Also licensed for use in congenital deficiency of factor VII, in United States

TABLE 6: HIGHLY PURIFIED FACTOR IX CONCENTRATES

BRAND	COMPANY	SITE OF MANU-FACTURE	PLASMA SOURCE	EXPORT/ DOMESTIC	FRACTIONATION	VIRAL INACTIVATION	SA s alb FIX IU/mg [1]	COMMENTS
Berinin-P = Berinin HS	CSL Behring	Marburg, Germany	United States, Austria, Germany: paid & unpaid	Both	DEAE-sephadex, heparin affinity chromatography	Pasteurization at 60°, 10 hr	146	Anti-thrombin III & heparin added; no albumin added
Immunine	Baxter BioScience	Vienna, Austria	United States, Austria, Czech Republic, Germany, Sweden: mostly paid apheresis	Both	Ion exchange & hydrophobic interaction chromatography	Polysorbate 80 & vapor heat, 60° C, 10 hrs, 190 mbar then 80° C, 1 hr, 375 mbar	Approx. 100	
Hemo-B-RAAS	Shanghai RAAS	Shanghai, China	China: unpaid & paid apheresis	Both	Ion exchange & affinity chromatography	Solvent/ detergent, dry heat & nanofiltration	> 50	No albumin added
Octanine F	Octapharma	Vienna, Austria & Lingolsheim, France	Sweden, Austria, Germany & United States	Both	Ion exchange & affinity chromatographies	TNBP/ polysorbate 80 & nanofiltration	> 120	No albumin added
Nanotiv	Octapharma	Stockholm, Sweden	Sweden: Recovered & apheresis	Both	Ion exchange & affinity chromatographies	TNBP/ Triton X 100 & nanofiltration	150	No albumin added
MonoFIX-VF	CSL Bioplasma	Melbourne, Australia	Australia,New Zealand, Singapore, Hong Kong: Unpaid	Both	Ion exchange & heparin affinity chromatography	TNBP/ polysorbate 80 & nanofiltration	>50	Anti-thrombin III & heparin added, no albumin added
Christmassin -M	Benesis	Osaka, Japan	Japan: unpaid	Domestic	Ion exchange & immunoaffinity chromatography	TNBP/ polysorbate 80; dry heat, 60° C, 72 hr; 15 nm nanofiltration	Approx 170	Albumin added
Aimafix	Kedrion	Italy	Europe & United States: paid & unpaid	Both	Anion exchange, DEAE sephadex/sepharose & heparin affinity chromatography	TNBP polysorbate 80; dry heat 100° C, 30 min; nanofiltration 35 + 15 nm (registration pending for nanofiltration)	> 100	Anti-thrombin III & heparin added, no albumin added
BETAFACT	LFB	France	Western Europe, unpaid	Both	Ion exchange chromatography and affinity chromatography	TNBP/ polysorbate 80, 15 nm nanofiltration	110	No albumin added
Faktor IX SDN	Biotest	LFB, France	Western Europe, unpaid	Austria & Germany	As above	As above	110	No albumin added
Factor IX Grifols	Grifols	Barcelona, Spain	1. United States paid 2. Spain recovered and apheresis, unpaid	1. Both 2. Domestic	Precipitation & multiple chromatography (including metal chelate affinity)	Solvent detergent, 15 nm nanofiltration	> 150	No albumin added
AlphaNine SD	Grifols	Los Angeles, CA, USA	United States: paid apheresis	Both	Ion exchange and dual polysaccharide ligand chromatography	Solvent/detergent, nanofiltration	210	No albumin added
Mononine	CSL Behring	Kankakee, IL, USA	United States: apheresis paid	Both	Immunoaffinity chromatography	Sodium thiocyanate & ultrafiltration	> 190	No albumin added
Nonafact	Sanquin	Amsterdam, The Netherlands	The Netherlands: unpaid	Both	Immunoaffinity & hydrophobic interaction chromatography	TNBP/ polysorbate 80; 15 nm nanofiltration	200 or more	No albumin added
Novact M	Kaketsuken	Kumamoto, Japan	Japan: unpaid	Domestic	Immunoaffinity chromatography	Dry heat, 65° C, 96 hr; 15 nm nanofiltration	Approx 200	Albumin added
Replenine – VF	BioProducts Lab.	Elstree, England, UK	United States: paid apheresis	Both	Metal chelate chromatography	Solvent-detergent; 15 nm nanofiltration	200	No albumin added

1. Specific activity minus any added albumin

RECOMBINANT FACTOR IX CONCENTRATE

BRAND	COMPANY	MADE AT	EXPORT/ DOMESTIC	FRACTIONATION	VIRAL INACTIVATION	SA s alb FIX IU/mg [1]	COMMENTS
BeneFIX	Wyeth	Andover, MA; USA	Both	Recombinant	Nanofiltration	200 +	No human or animal proteins used in manufacture; no albumin added
BeneFIX	Baxter SA (Switzerland)	Wyeth, Andover, MA, USA	Europe	Recombinant	Nanofiltration	200+	No human or animal proteins used in manufacture; no albumin added

1. Specific activity minus any added albumin

TABLE 7: OTHER CLOTTING FACTOR CONCENTRATES

BRAND	COMPANY	SITE OF MANU-FACTURE	PLASMA SOURCE	EXPORT/ DOMESTIC	FRACTIONATION	VIRAL INACTIVATION	COMMENTS
Clottagen (fibrinogen)	LFB	France	Western Europe, unpaid	Both	adsorption on aluminum hydroxide gel, Ion exchange chromatography and affininity chromatography	TNBP/ polysorbate 80	
Fibrinogen HT	Benesis	Osaka, Japan	Japan: unpaid	Domestic	Ethanol fractionation, glycine precipitaion	TNBP / polysorbate 80; dry heat, 60° C, 72 hr; 35 nm nanofiltation	No albumin added
FIBRORAAS (fibrinogen)	Shanghai RAAS	Shanghai, China	China: paid & unpaid apheresis	Both	Multiple fractionation	TNBP/ polysorbate 80	
Haemocomplettan P = Haemocomplettan HS (fibrinogen)	CSL Behring	Marburg, Germany	United States, Austria, Germany; paid & unpaid	Both	Multiple precipitation	Pasteurization at 60° C, 20 hr	Albumin added
Factor VII	Baxter BioScience	Vienna, Austria	United States, Austria, Czech Republic, Germany, Sweden: mostly paid apheresis	Both	Aluminum hydroxide absorption	Vapor heat, 60° C, 10 hr at 190 mbar then 80° C, 1 hr at 375 mbar	
Factor VII	Bio Products	Elstree, England,	United States: paid apheresis	Both	Ion exchange chromatography	Dry heat, 80° C, 72 hr	S.A. 1.5 – 2 U/ mg protein
FACTEUR VII	LFB	France	Western Europe, unpaid	Both	Ion exchange chromatography	TNBP/ polysorbate 80	no albumin added
NovoSeven (=Niastase) (activated factor VII)	NovoNordisk	Copenhagen, Denmark	None	Both	Recombinant	Not applicable	Approved for congenital factor VII deficiency
Factor X P Behring	CSL Berhing	Marburg, Germany	United States, Austria, Germany; paid/unpaid	Both	DEAE-sephadex and precipitations	Pasteurization at 60° C, 10 hr	Contains high amount of factor X and some FIX, but no FII and FVII; antithrombin III and heparin added, no albumin added
Factor XI	Bio Products	Elstree, England, UK	United States: paid apheresis	Both	Affinity heparin sepharose chromatography	Dry heat, 80° C, 72 hr	Heparin, Anti-thrombin III added, S.A. 3- >5 U/ mg protein
HEMOLEVEN (Factor XI)	LFB	France	Western Europe, unpaid	Both	Ion exchange chromatography, depth filtration	TNBP/ polysorbate 80 15 nm nanofiltration	Heparin, Anti-thrombin III added, C-1 esterase inhibitor added
WILFACTIN (Von Willebrand Factor)	LFB	France	Western Europe, unpaid	Both	Adsorption on aluminum hydroxide gel Ion exchange chromatography and affininity chromatography	TNBP/polysorbate 80; 35 nm nanofiltration; dry heat 80° C, 72 hr	S.A. (before addition of albumin) : > 50 units ristocetin cofactor (VWF:RCo) per mg; Albumin added
Fibrogammin P (=Fibrogammin HS) (Factor XIII)	CSL Behring	Marburg, Germany	United States, Austria, Germany: paid & unpaid	Both	Multiple precipitation	Pasteurization at 60° C, 10 hr	Albumin added

TABLE 8. CONCENTRATES OF ANTI-THROMBOTIC FACTORS: (A) Anti-thrombin concentrates

BRAND	COMPANY	SITE OF MANU-FACTURE	PLASMA SOURCE	EXPORT/ DOMESTIC	FRACTIONATION	VIRAL INACTIVATION	COMMENTS
ACLOTINE	LFB	France	Western Europe, unpaid	Both	affinity chromatography, depth filtration	Pasteurization, 60º C, 10 hrs 20-15 nm nanofiltration	
Anbinex	Grifols	Barcelona, Spain	1.United States paid apheresis 2.Spain unpaid recovered and apheresis	1.Both 2. Domestic	Double heparin ligand chromatography	Pasteurization, 60º C, 10 hrs; 15 nm nanofiltation	Specific activity 7.9 ± 0.4 IU/mg
Anti-thrombin	GreenCross	Seoul, Korea	Korea unpaid and United States paid apheresis	Both	Ion exchange and heparin affinity chromatography	Pasteurization, 60º C, 10 hrs	Specific activity more than 8.3 IU/mg
ATIII	BPL	Elstree, England	United States paid apheresis	Both	Cryosupernatant; heparin absorption, sepharose chromatography	Pasteurization 60º C, 10 hrs and dry heat, 80º C, 72 hrs	
Kybernin P	CSL Behring	Marburg, Germany	United States, Austria, Germany; paid and unpaid	Both	Precipitations and affinity chromatography	Pasteurization, 60º C, 10 hrs	No heparin added
Neuart	Benesis	Osaka, Japan	Japan, unpaid	Domestic	Ethanol fractionation	Pasteurization, 60º C, 10 hrs and 20 nm nanofiltration	Specific activity 9-10 IU/mg
Thrombate-III	Talecris	Clayton, NC, USA			Ethanol fractionation	Pasteurization, 60º C, 10 hrs	
Thrombotrol-VF	CSL Bioplasma	Melbourne, Australia	Australia, New Zealand, unpaid	Both	Heparin, sepharose and gel filtration chromatography	Pasteurization, 60º C, 10 hrs and 15 nm nanofiltration	

(B) Protein C concentrates

Anact C (activated protein C)	Kaketsuken	Kumamoto, Japan	Japan, unpaid	Domestic	Affinity and ion exchange chromatography	Dry heat 65º C., 10 hrs, and 15 nm nanofiltration	
Ceprotin	Baxter	Vienna, Austria	United States & Europe	Both	Cryosupernatant; ion exchange and immunoaffinity chromatography	Detergent; vapor heat, 60° for 10 ht and 80° for 1 hr	Albumin added
PROTEXEL	LFB	France	Western Europe, unpaid	Both	Ion exchange chromatography and affininity chromatography	Solvent/Detergent (TNBP/polysorbate 80)	

APPENDIX 2

MODEL PRODUCT ASSESSMENT QUESTIONNAIRE

This questionnaire includes the minimum information needed to assess a product with a view to allowing it on the market. The manufacturer should be asked to provide all the information requested before any assessment of products begins.

INFORMATION SUMMARY FROM CANDIDATE SUPPLIERS OF HEMOPHILIA PLASMA PRODUCTS

1) PLASMA RAW MATERIAL

(A) PLASMA SUPPLIER

Name of supplier	Source or recovered	% first time donors	% repeat donors

(B) DONOR EPIDEMIOLOGY

Name of supplier	HIV antibody positive donations		HCV antibody positive donations		HbsAg positive donations	
	per 10000 repeat donors	per 10000 new donors	per 10000 repeat donors	per 10000 new donors	per 10000 repeat donors	per 10000 new donors

(C) REGULATORY STATUS OF PLASMA SUPPLIERS

Name	Frequency of internal audits performed by supplier, if any	Frequency of external audits performed by manufacturer, if any	Frequency of external audits performed by government authority, if any	Any other certification by a competent body

(D) DONOR SELECTION – EXCLUSION CRITERIA (WHETHER CHECKED FOR AND WHAT ACTION)

Name	History of blood-borne infections (hepatitis/HIV, Etc.)	Intravenous drug abuse	High-risk sexual behaviour (male to male sex, prostitution, etc.)	Recipients of blood, tissues, etc.	Risky behaviour – tattoos, piercing, etc.	Medical procedures

(E) BLOOD/PLASMA SCREENING

Screening test	Name of kit – manufacturer	Regulatory status (USA/Europe)
HbsAg		
HCV antibody		
HIV antibody		
HCV NAT (if any)		
HIV NAT (if any)		

(F) QUALITY ASSURANCE OF TEST KITS

Describe any internal and external QA used by the collection agencies for their screening tests

(G) PLASMA MEASURES BY MANUFACTURER

Any inventory hold measures, etc.	Maximum number of donations in plasma pool	Testing of the plasma pool – serology, NAT, etc.	Estimate of viral load in plasma pool from viral incidence data		
			HIV	HCV	HBV

2) MANUFACTURING PROCESS

The manufacturer is to include a copy of the licence to manufacture issued by the country where the facility is located and any other authority

(A) CRITICAL STEPS

Here insert a flow chart of the manufacturing process, identify the crucial manufacturing steps and list their related in-process controls (IPCs)

(B) VIRAL REDUCTION

List dedicated viral reduction steps	
Validated $\log_{10}$ elimination for 1. HIV (actual virus) 2. HCV (specify model, e.g., BVDV, etc.) 3. HBV (specify model) 4. HAV (actual virus or specify model) 5. Parvovirus B19 (specify model)	
Estimated residual risk per vial of product from plasma pool viral load and validated viral elimination data, for 1. HIV 2. HCV 3. HBV	

(C) PROCESS CONSISTENCY

List in-process controls (IPCs) identified in 2(a) for three chronologically sequential batches of the product manufactured at the scale used for the marketed form manufactured within the last 18 months.

In-process controls	Batch – 01	Batch – 02	Batch – 03
IPC-1 IPC-2 IPC-3 IPC-4 IPC-5			

(D) STABILITY AND SHELF LIFE

Include the data for the potency (FVIII or FIX) of the product measured during the requested shelf life, at the temperatures sought in the application.

Potency IU/mL (mean+sd)	At release	3 months	6 months	12 months	24 months

Include the data for the product's potency after reconstitution as specified, at 2, 8, and 24 hours after reconstitution

3) FURTHER PRODUCT INFORMATION

(A) OTHER MARKETS

List the other markets where the product is available, its history in these markets, volumes supplied, and related marketing authorizations from licensing bodies.

(B) CLINICAL STUDIES

Summarize clinical trials used to demonstrate product efficacy, referring to the authorizations from other markets listed in 3(a). Manufacturers should comment on their endorsement or otherwise on the EMEA's *Note for Guidance on the clinical investigation of plasma derived FVIII and FIX products*, accessible from http://www.emea.eu.int/pdfs/human/bpwg/019895en.pdf.

(C) ADVERSE EVENTS

Describe manufacturer's system for receiving and reporting adverse events related to the product.

APPENDIX 3

GLOSSARY

Batch release testing: Testing of end products by regulatory authorities before official release to ensure that the product specification is met.

Characterization: Analytical measurements which allow detailed understanding of the composition and other attributes of a product.

Donor screening: Individual donations of blood are screened to ensure that blood-borne viruses do not enter the plasma pool. Screening is currently available for HBV, HCV, and HIV.

Donor selection: Procedures designed to identify and exclude donors at risk of being infected with viruses that can be transmitted by blood transfusion.

Enveloped/lipid enveloped viruses: The common transfusion transmitted viruses HIV, HCV, and HBV, which are all characterized by a lipid viral envelope and are highly infectious.

Finished product testing: Testing done on final product to allow manufacturers to characterize their products and to demonstrate compliance of every batch with the licensed specification.

Fractionation: The process of separating and processing human blood plasma into a range of products for therapeutic use.

Good manufacturing practices (GMPs): All the elements in established practice that will collectively lead to final products that consistently meet expected requirements as reflected in product specification. These include traceability, segregation of product manufacturing steps to avoid cross-contamination, training, documentation, change control, and deviation reporting.

Inventory hold: The retention in storage of plasma for fractionation while processes designed to assure donor safety are undertaken.

Limit testing: Testing of the plasma pool using nucleic acid testing (NAT) in which a maximum level of viral contamination, rather than an absolute elimination, is the aim.

Lyophilization: The process of isolating a solid substance from solution by freezing the solution and evaporating the ice under vacuum. Freeze-drying.

Marketing authorization: The formal permit from a regulatory authority allowing a manufacturer to market a product following that authority's scrutiny.

Minipools: Plasma samples pooled from several donations, and then tested for viral markers.

Nanofiltration: A process whereby protein solutions are passed over small pore filters which can remove viruses while allowing therapeutic proteins to pass through.

Non-enveloped /non-lipid enveloped viruses: Pathogenic viruses (for example, HAV or parvovirus B19) which lack a lipid envelope and therefore are not susceptible to viral inactivation techniques such as solvent-detergent treatment.

Nucleic acid testing (NAT): Testing for viral nucleic acid, thus allowing detection of a virus which may cause disease before the development of immunological markers of infection.

Pharmacokinetics: The action of drugs in the body over a period of time, including the processes of absorption, distribution, localization in tissues, biotransformation, and excretion.

Plasma master file: A dossier of information compiled according to European guidelines, which allows the manufacturer of plasma derivatives to fully describe the source material.

Plasma pool: Plasma from a number of donors used to make one lot of product.

Plasmapheresis: A method of collecting plasma from donors whereby only the donor’s plasma is removed. This method allows a donor to donate a larger volume of plasma per donation and donate more frequently than is possible when donating whole blood.

Potency: The biological activity which may be measured in the laboratory which is best related to a product's actual therapeutic effect.

Product specification: The properties of a product. They can be measured in the laboratory, allowing a manufacturer to assess and demonstrate fitness of purpose.

Purity: The proportion of the desired ingredient (e.g., factor VIII) in concentrates, relative to other ingredients present.

Quality assurance system: A mechanism for achieving, sustaining, and improving product quality.

Recovered plasma: Plasma collected as a by-product of donated whole blood. Recovered plasma is generally procured from unpaid donors.

Shelf life: The period of time during which a product may be stored under specified conditions and retain its characteristics.

Source plasma: Plasma collected from donors through a process known as plasmapheresis, which removes only the donor’s plasma. The majority of this plasma is obtained from paid donors.

Validation: The action of proving that any material, process, procedure, activity, system, or equipment used in manufacture or control can and will reliably achieve the desired and intended results.

Window period: The period between when a donor is infected with a virus or disease-causing agent and when infection can be detected by an immunological marker. During this period the donor is infectious but the infection is undetectable. With nucleic acid testing (NAT), the window period is shortened.

APPENDIX 4

WFH RESOURCES

Contract Fractionation (Spanish title: *Fraccionamiento por contrato*). World Federation of Hemophilia. Facts and Figures Monograph Series, No. 5. 1998, revised 2004, 2008.

Creutzfeldt-Jakob Disease and Hemophilia: Assessment of Risk (Spanish title: *La Enfermedad Creutzfeldt-Jakob y la hemophilia: Evaluación del riesgo*). Bruce Evatt. The Treatment of Hemophilia Monograph Series, No. 15. 1999. Revised 2000, 2004.

Guide to National Tenders for the Purchase of Clotting Factor Concentrates. Brian O'Mahony. 2006.

Key Issues in Hemophilia Treatment. Part 1: Products. Part 2: Organizing a National Programme for Comprehensive Hemophilia Care (Spanish title: *Temas clave en el tratamiento de la hemofilia: Productos y atención*). Facts and Figures Monograph Series, No. 1. 1998.

Predicting the Long-term Risk of HIV Exposure by Cryoprecipitate (Spanish title: *Estimación del riesgo a largo plazo de exposición al VIH por el críoprecipitado*). Bruce Evatt et al. The Treatment of Hemophilia Monograph Series, No. 20. 1999.

Registry of Clotting Factor Concentrates. Mark Brooker. Facts and Figures Monograph Series, No. 6. Eighth Edition, 2008.

Small Pool Heat Treated Intermediate Purity Factor VIII Concentrate. I. P. Petersen and A. R. Bird. Facts and Figures Monograph Series, No. 3. 1997, revised 2004.

The Preparation of Single Donor Cryoprecipitate (Spanish title: *La preparación de crioprecipitado de un solo donante*). Shân Lloyd. Facts and Figures Monograph Series, No. 2. 1997, revised 2004.

Transmissible Agents and the Safety of Coagulation Factor Concentrates (Spanish title: *Los agents transmisibles y la seguridad de los concentrados de factores de la coagulación*). Jerome Teitel. Facts and Figures Monograph Series, No. 7. 1999, revised 2004.

WFH Task Force on TSEs Bulletin 2: *Variant Creutzfeldt-Jakob Disease and Haemophilia: A Risk Assessment of Plasma-derived Products* (also in Spanish and French). Albert Farrugia. Revised 2001.

WFH Task Force on TSEs Bulletin 3: *Donor Deferral Measures* (also in Spanish and French). Albert Farrugia. 2001.

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