

GUIDE FOR THE ASSESSMENT OF **CLOTTING FACTOR CONCENTRATES**

3rd edition

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for the World Federation of Hemophilia



WFH

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INTRODUCTION

*NB: **Bolded** terms are defined in a glossary in Appendix 4*

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 Agency (EMA). These agencies are dedicated to assessing products and granting **marketing authorizations**. 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.

Previous editions of this Guide have focused on the safety of hemophilia products. This is because, particularly in the well-regulated environments, there has been no question about the efficacy of factor VIII (FVIII)¹ concentrates to prevent and stop bleeding. Nonetheless, it is important to obtain assurance regarding efficacy and **potency**, especially for products from suppliers outside the main regulatory jurisdictions. Efficacy can be assessed against the scale of response to infusion in Table 1. In general, in excess of 90% of responses to treatment, for both on-demand and prophylaxis protocols, should be *excellent*, and no more than 2% of responses should be *moderate*. *Poor* responses should not occur in patients without inhibitors.

Table 1: Scale of response to on-demand and prophylactic treatment

Outcome	On-demand (treatment of bleeding episodes)	Prophylaxis (prevention of bleeding)
Excellent	Abrupt pain relief and/or unequivocal improvement in objective signs of bleeding within approximately 8 hours of a single infusion	<0.75 spontaneous bleeding episodes per month
Good	Definite pain relief and/or improvement in signs of bleeding within approximately 8 to 12 hours of an infusion, requiring up to two infusions for complete resolution	Between 0.75 and 1 spontaneous bleeding episodes per month
Moderate	Probable or slight beneficial effect within approximately 12 hours of the first infusion, requiring more than two infusions for complete resolution	Between 1 and 1.5 spontaneous bleeding episodes per month
Poor	No improvement within 12 hours, or worsening of symptoms, requiring more than two infusions for complete resolution	>1.5 spontaneous bleeding episodes per month

Hemophilia treatment products are of two main types, those made from plasma donated by human blood donors and those made using recombinant technology. One feature of this Third Edition of the Guide for the Assessment of Clotting Factor Concentrates is that some guidance will be offered regarding recombinant factors. As more of these products appear on the market, their availability in developing countries has become possible. 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 human immunodeficiency virus (HIV) and hepatitis, it

¹ In this Guide, clotting factors are referred to by their abbreviation, F, together with the appropriate Roman numeral, e.g., FVIII, FIX.

is very important to ensure that products being considered for purchase are safe and free from viral infection. This historical risk has abated as regulatory- and industry-driven measures have ensured the exclusion and elimination of pathogens from the blood supply.

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. As the pathogen safety risk has decreased, increased focus on the most serious current hazard—the generation of inhibitors to clotting factors following replacement therapy—has occupied authorities and manufacturers alike. This Guide will also discuss this risk.

Systems for the regulation and control of pharmaceutical medicinal products are well established in the United States of America (U.S.A.) and the European Union (EU). The approaches used in these countries may be helpful for countries that want to develop their own framework for assessing and selecting products. North American and European practices are summarized with comment in Section 2. It is important to note that these arrangements are complex and may not be appropriate in a country that is establishing new regulatory arrangements. However, it is fair to assume that products licensed by these authorities have undergone a high level of scrutiny for their safety and efficacy. This should be borne in mind when assessments are made by other agencies.

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 these products. It also explores aspects of finished product testing, and the potential contributions (and limitations) of such testing to evaluating the safety of individual batches of product with regard to infectious risks. The plethora of new products released in recent years has prompted the World Federation of Hemophilia (WFH) to include advice on the assessment of product efficacy. Features related to recombinant products are assessed in Section 3, as is the issue of inhibitors.

Drawing on the principles outlined in previous sections, Section 4 offers a model for the evaluation of products by decision makers in countries without established regulatory agencies. It discusses the minimum requirements that must be met in order for a product to be considered, and explores example scenarios of product evaluation.

Section 5, which in previous editions discussed locally produced cryoprecipitate, has been removed from the Third Edition. Although advances have been made in the safety of such products [1, 2], the WFH reiterates its position that the products of choice for the treatment of hemophilia are industrially manufactured concentrates; these are the only products that can satisfy the necessary principles of pharmaceutical **Good Manufacturing Practice (GMP)**.

The appendices to this Guide include various materials to help authorities assess products. Appendix 1 introduces the *WFH Online Registry of Clotting Factor Concentrates*². An electronic listing of currently available hemophilia treatment products, it includes available information for each on plasma source, serological tests performed on donor plasma, and viral reduction procedures. It also provides information on the manufacturers of the products.

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.

2 Available online at <http://elearning.wfh.org/resource/online-cfc-registry/>

Often the language and acronyms used by regulators and government officials can be difficult to understand. Appendix 3 provides a list of abbreviations and acronyms, Appendix 4 is a glossary defining terms used to describe processes in the manufacture and control of hemophilia treatment products (glossary terms are bolded at their first instance in the Guide text), and Appendix 5 is a list of other WFH resources particularly relevant to the assessment of clotting factor products.

This Guide was written with hemophilia treatment products specifically in mind; however, many of the principles discussed apply to all biological drugs, including other 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 pathogen safety of therapeutic products derived from biological sources

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

These principles are also applicable to the products of recombinant manufacture. The relevant cell lines and culture fluids must be selected from low-risk sources, and screened for potential pathogenic contaminants when possible. Pathogen elimination techniques, introduced as steps in the manufacture dedicated to this purpose, are mandatory [3].

Of these three principles, the elimination of pathogens through the manufacturing process has enhanced the safety of hemophilia treatment products the most. This is because, particularly for products made from large numbers of individual plasma donations, the level of clearance achieved through a validated manufacturing process significantly exceeds the level possible through **donor selection** and screening procedures [4].

Measures to enhance the pathogen safety of plasma products include:

- Selection procedures that ensure the exclusion of donors with high-risk behaviour
- Mandatory serological testing on all plasma donations for the main transfusion-transmitted viruses—HIV, hepatitis B (HBV), and hepatitis C (HCV)
- Plasma inventory hold and exclusion based on post-donation information
- Nucleic acid testing (NAT) of minipools for HCV, HIV, HBV, human parvovirus B19, and hepatitis A virus (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.

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. In the well-regulated markets of North America and Europe, infections from these agents have been excluded from the hemophilia community for the past 25 years [5]. Some residual concerns exist regarding the **non-enveloped viruses** [6], due to the higher level of resistance to pathogen elimination methods of these viruses; however, fractionated plasma products manufactured by today's processes, and with attention to GMP, are among the lowest risk therapeutic products in use. Since their introduction in the late 1980s, no recombinant clotting factor concentrate (CFC) has been associated with pathogen transmission.

Please note that the following sections detail features particular to the assessment of plasma-derived clotting factor products

Plasma quality

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

- 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)
- Donor epidemiology (e.g., viral infection, prion disease)
- 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 a history of:

- Blood-borne infections
- Intravenous drug use
- High-risk sexual behaviour (e.g., men who have sex with men, prostitution)
- Receiving human biological materials (e.g., blood, tissue)
- Risky behaviour (e.g., tattoos, piercing)
- Medical procedures (e.g., certain illnesses, surgery)

As for 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 that removes only the donor's plasma. When collected and processed with steps that exclude and inactivate or eliminate **enveloped viruses** (e.g., HIV, HCV, and HBV), both recovered and source plasma have the same level of viral safety in the derived products.

Prior to the introduction of regulation in the blood products sector, plasma for **fractionation** from paid donors was considered to constitute a higher risk of viral infection than plasma from voluntary donors drawn from the same population. This is no longer the case in the developed blood systems of North America and Europe because of the strict regulatory regimens and the introduction of similarly strict industry standards in these regions. The performance of NAT on plasma for fractionation in these systems has greatly reduced the viral load of HIV and HCV from all donor types. An equivalent safety cannot be assumed for other donor populations, and authorities must assess each plasma source against the safety factors described in this Guide, regardless of whether it comes 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, such as inventory hold and donor qualification, has made this plasma potentially a safer raw material 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. Unpaid donors in countries with high endemic infectious disease rates are frequently less safe than paid donors in developed countries where these rates are lower. 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. **Donor screening** is currently available for HBV, HCV, and HIV. All plasma donations should be tested for these three viruses.

Tests that detect 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 the case of 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, nonetheless the window period also exists for this viral infection. With NAT, this period is shortened by detecting the viral genome, which appears in the blood before the immunological markers. The introduction of NAT has decreased the viral load of plasma pools and therefore increases the margin of safety should viral reduction procedures break down.

Table 2: Donor screening tests for blood-borne viruses

TEST	RECOMMENDED	MANDATORY *
Anti-HIV	Yes	Yes
Anti-HCV	Yes	Yes
HbsAg (HBV)	Yes	Yes
HIV-RNA [†] (NAT)	Yes	No
HCV-RNA (NAT)	Yes	No
HBV-DNA [†]	Yes	No
Parvovirus B19 DNA	Yes	No
HAV RNA	Yes	No

* While NAT is not mandatory in many countries, it represents the best blood safety practise and is mandatory in some well-regulated countries (e.g., the U.S. FDA).

† DNA and RNA constitute the essential elements of the genetic code.

DNA, deoxyribonucleic acid; HAV, hepatitis A virus; HbsAg, antigen associated with HBV; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; NAT, nucleic acid testing; RNA, ribonucleic acid.

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 infection. 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 plasmapheresis donors can donate more frequently, which may result in more donations during the infectious window period. The particular features of an inventory hold vary among organizations. It is most effective when donations that are not re-tested remain unused, 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 when 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 ascertain 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 obtaining it from their own centres or from centres subscribing to their own standards. Such spot plasma will not be subject to the same level of safety and regulatory control as 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 rendered pooled hemophilia treatment products safe from HCV before the introduction of viral testing increased the safety of normal blood transfusions and single-donor cryoprecipitate from HCV transmission. Authorities tasked with assessing measures that are essential for ensuring safe products—as opposed to measures which, while enhancing safety, are nonessential—must bear 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 (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 (i.e., 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 (e.g., HAV, parvovirus B19), and this concern can be extended to unknown viruses and infectious agents.

There are a number of different viral reduction methods available, including solvent-detergent, heat treatment (e.g., pasteurization, dry-heat, steam heat), and nanofiltration. The advantages and limitations of these are outlined in Table 3. Relative to the highly pathogenic nature of blood-borne viruses (i.e., HIV, HCV, and HBV) the unbroken safety record of factor concentrates treated with solvent-detergent [7] is a strong argument for making this viral-reducing method a mandatory component in the manufacture of such products.

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 key to viral elimination. Process **validation**, and the systems at the heart of GMP—traceability, segregation of product manufacturing steps to avoid cross-contamination, training, documentation, change control, and deviation reporting—are the keys to the reliable manufacture of safe and 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 (e.g., against HAV) of people receiving plasma concentrates on a life-long basis, should also be used. For known non-enveloped viruses, several manufacturers have established schemes involving **limit testing** of the plasma pool using NAT with the aim of restricting the level of viral contamination to a very low maximum level of viral contamination, rather than absolute elimination. In the absence of validated viral reduction in-process steps, this likely offers the current best general approach for reducing the viral burden of the plasma pool and, therefore, reducing the transmission potential for viruses detected via NAT.

In recent years, the development of methods to produce high-titer preparations of parvovirus B19 in the laboratory has permitted the validation of many elimination processes against this virus, including some forms of heat treatment and **nanofiltration**. Uncertainty remains regarding the transmission of parvovirus B19 via concentrates manufactured in the era of NAT viral reduction [6], but this may be due to the use of less well-accredited processes during the earlier years of surveillance [8]. There have been recent concerns regarding hepatitis E virus (HEV) and there is uncertainty surrounding the extent of HEV infection in the hemophilia community. Nonetheless, viral-inactivation processes utilized during the manufacture of concentrates appear to minimize infection [9].

While nanofiltration is generally very effective for reducing non-enveloped viruses in plasma products this process is more suitable for the preparation of FIX concentrates [10]. It should be a mandated feature of concentrates for hemophilia B. Nanofiltration has been successfully applied to FVIII concentrates manipulated to dissociate FVIII from von Willebrand factor (VWF) [11], and to concentrates containing the smaller B-domain-deleted recombinant FVIII molecule [12]. Overall, non-enveloped viruses continue to pose a greater challenge than enveloped viruses, as a universal technology for their inactivation, such as solvent-detergent treatment for enveloped viruses, has yet to be developed. The technologies in hand, coupled with the diminution in viral load through the application of NAT, have been well validated for the known non-enveloped viruses.

Importance of geographical source of products

A number of blood-borne viruses reported in recent years have not been evident in hemophilia communities in countries with well-established regulatory systems. These include West Nile virus (WNV), Chikungunya, Zika, and other agents transmitted primarily by mosquito bites. In some instances these viruses have been transmitted by blood transfusion, but the viral-inactivation processes used in clotting factor manufacture have eliminated them as a risk for hemophilia patients. However, the situation is different in developing countries. It is clear that the classical transfusion-transmitted viruses are still present in the blood supply of some countries and that the rudimentary hemophilia therapies available, such as plasma and cryoprecipitate, continue to constitute a vehicle of infection for people with hemophilia [13-15]. Similarly, newly emerged agents such as WNV and Zika may well be transmitted to hemophilia patients exposed to non-viral-inactivated components. Countries with under-resourced regulatory systems should focus on the issues outlined in this Guide regarding the basic principles of GMP, in light of recent incidences of fraudulent source materials [16, 17]. Authorities should accept hemophilia products solely from companies with a well-accredited adherence to GMP.

Variant Creutzfeldt-Jakob disease (vCJD)

Experiments using various animal species have shown that diseases known as transmissible spongiform encephalopathies (TSEs) are indeed transmissible through blood, plasma, and plasma fractions. These experiments demonstrated that a considerable portion of the infectivity was localized to the plasma and could be carried in plasma fractions when processed into therapeutic products. However, depending on the fraction studied and the manufacturing technique used, much of the infectivity could be removed or cleared from the therapeutic product as a result of the manufacturing process.

Transmission of TSE through infected blood and blood products has been confirmed in humans. There have been four cases of human TSE—variant Creutzfeldt-Jakob disease (vCJD)³—in recipients of whole

3 vCJD is the human form of bovine spongiform encephalopathy (BSE), a disease in cattle that affected animals in the United Kingdom (U.K.) and European continent, and is thought to have entered the human food chain through the consumption of contaminated meat products. Current information on the prevalence of BSE in different countries is available at <http://www.oie.int>.

blood or red blood cells from donors who developed the disease. vCJD is, therefore, a blood safety risk and is addressed through the same combination of measures used to minimize the risk of viral transmission. The only donor selection measure that can minimize the risk of the pathogen entering the plasma pool is the deferral of individuals who have been exposed to bovine spongiform encephalopathy (BSE) through travel to, 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 to, or residence in, the United Kingdom (U.K.) in the period 1980 to 1996, during which BSE entered the human food chain. Some countries also defer donors with a history of travel to, or residence in, other countries with minor BSE epidemics.

Table 3: Advantages and points to consider when selecting viral reduction methods for factor concentrates. Adapted from [18, 19]

Method	Advantages	Points to consider
<p>Solvent-detergent (SD) Treatment with a mixture of chemicals—solvents and detergents—that inactivates viruses through the removal of the lipid envelope that coats some types of viruses. This method is ineffective against non-enveloped viruses.</p>	<ul style="list-style-type: none"> ▪ Extremely efficient against enveloped viruses ▪ Requires relatively simple equipment ▪ Non-denaturing effect on proteins ▪ High recovery of protein functional activity 	<ul style="list-style-type: none"> ▪ Requires a subsequent manufacturing step to eliminate the SD agents ▪ Ineffective against non-enveloped viruses (e.g., HAV, parvovirus B19)
<p>Pasteurization A generic term for the 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 on fragile proteins, such as clotting factors, the solution must include protective chemicals to preserve the proteins; however, these chemicals may also preserve viruses. Each process must be evaluated on the basis of the data submitted by the manufacturer.</p>	<ul style="list-style-type: none"> ▪ Potential to inactivate enveloped and non-enveloped viruses, including HAV ▪ Requires relatively simple equipment 	<ul style="list-style-type: none"> ▪ Dependent on conditions ▪ Protein stabilizers may protect viruses ▪ Does not inactivate parvovirus B19 ▪ Low recovery of fragile clotting factors ▪ Potential generation of neoantigens
<p>Vapour-heat Currently restricted to one manufacturer.</p>	<ul style="list-style-type: none"> ▪ May inactivate enveloped and non-enveloped viruses, including HAV 	<ul style="list-style-type: none"> ▪ Possible risk of transmission of HCV and HBV ▪ Does not inactivate parvovirus B19
<p>Terminal dry-heat 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 to which the product is exposed. Conditions described by manufacturers include:</p> <ul style="list-style-type: none"> ▪ 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 <p>Each process must be evaluated on the basis of the data submitted by the manufacturer. For example, 60°C is known to be less effective than 80°C, when applied for similar lengths of time.</p>	<ul style="list-style-type: none"> ▪ May inactivate enveloped and non-enveloped viruses, including HAV ▪ Treatment applied on the final container 	<ul style="list-style-type: none"> ▪ Does not inactivate parvovirus B19 ▪ Results in 10%–20% loss of clotting factor activity ▪ Requires strict control of residual moisture content

Method	Advantages	Points to consider
Nanofiltration through 15-nm membranes	<ul style="list-style-type: none"> ▪ Elimination of viruses based on size-exclusion effect ▪ Eliminates all major viruses, including HAV and parvovirus B19 ▪ May eliminate prions ▪ Integrity and removal capacity of the filter can be 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 	<ul style="list-style-type: none"> ▪ Not applicable to high molecular weight protein concentrate (without significant protein loss)
Nanofiltration through 35-nm membranes	<ul style="list-style-type: none"> ▪ Similar to the advantages associated with nanofiltration through 15-nm membranes ▪ Applicable to some FVIII and VWF concentrates 	<ul style="list-style-type: none"> ▪ Incomplete elimination of small viruses

Authorities faced with making a decision about excluding donors at risk of having been exposed to vCJD need to assess carefully the effect such deferral measures may have 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 of other more established risks, such as HIV and HCV infection, the deferral of well-accredited repeat donors because of a possible vCJD risk may mean that new donors with a higher prevalence of these established infections are used instead. As a group, new donors will have higher viral marker rates than repeat donors, and authorities in developing countries, where selection and screening procedures may not be optimized, must ensure that in attempting to avoid uncertain risks (i.e., vCJD), known risks (i.e., HIV and HCV) are not increased.

Since the publication of the Second Edition of this Guide, the vCJD epidemic in the U.K. has abated, with speculation that the disease will be undetectable by 2020. However, there is speculation that a second-wave of the epidemic may occur. A subset of the population exposed to contaminated beef when it initially entered the food chain have not yet developed the disease despite evidence of the presence of the pathogen [20]. This appears to be due to genetic variations. A second-wave of the epidemic may be far in the future [21], the attention of the blood industry remains on this illness.

A number of tests have been developed that show that prions can be detected in the blood of individuals with vCJD in both the symptomatic and pre-symptomatic phases of the disease [22, 23]. These tests are not yet available in a format capable of blood screening on the scale required for the safety of the mainstream blood supply, although development efforts are underway. Previous experience with viral infections suggests that the exclusion of infectious donations, to the extent that pooled plasma products are rendered safe, is probably not achievable through selection and testing measures alone.⁴ This leaves the clearance of infectivity through the manufacturing process as the main route for minimizing the risk of vCJD from plasma

4 Infections such as HIV and HCV continued to be transmitted through hemophilia treatment products after the introduction of selection and testing measures, as these are generally of insufficient sensitivity to exclude infected donations from plasma pools constituted from thousands of units.

products. The level of clearance attained by different processes is considerable and has probably contributed to the absence of overt vCJD disease in recipients of plasma products, including people with hemophilia, observed to date.

It is known that plasma from donors who subsequently developed vCJD has been used to manufacture diverse products, including CFCs. Assessments estimating the risk posed by these products have been developed by regulatory authorities including the FDA,⁵ using principles also described by the WFH. Several relevant publications are available on the WFH eLearning Platform.⁶

All the estimates concur that the risk is strongly dependent on the level of clearance achieved by the manufacturing process, and that higher **purity** products pose lower levels of risk as the infective agent is more effectively removed. This should be considered when assessing the safety of concentrates.

There have been no cases of vCJD transmitted by plasma products, including CFCs, but asymptomatic infection has been shown in a patient receiving a low purity FVIII concentrate. The post-mortem of a person with hemophilia A, treated with products manufactured from the plasma of donors who subsequently developed vCJD, showed evidence of infection in his tissues. He died of other causes and did not develop any vCJD symptoms up to the time of his death [24]. This justifies the conclusion by U.K. authorities that patients treated with FVIII concentrate have an enhanced risk of developing vCJD compared to the general U.K. population [25]. The manufacturing process of the FVIII concentrate involved is known to have a low capacity for clearance of prions. Post-mortem studies of patients exposed to immunoglobulin products manufactured from similarly infectious plasma [26] did not reveal parallel vCJD cases, highlighting the importance of prion clearance during manufacture. This clearance was considerably higher for the immunoglobulin production processes than for the implicated FVIII [27].

In summary, it is important to reiterate that there have been no cases of vCJD transmitted by plasma products, including CFCs, but asymptomatic infection has been shown in a patient receiving a low purity FVIII concentrate. Bleeding continues to be the major cause of mortality and morbidity for people with hemophilia, and it is important to retain access to products that prevent it. Regulatory authorities and manufacturers are now fully aware of the risk of vCJD from plasma derivatives 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 of CFCs.

Purity versus safety

Purity refers to the percentage of the desired ingredient (e.g., FVIII) in concentrates, relative to other ingredients present. Concentrates on the market vary widely in their purity (see *the WFH Online Registry of Clotting Factor Concentrates* in Appendix 1). Generally, products that are produced at higher purity tend to be associated with lower manufacturing yields, due to the presence of less VWF (the protein that naturally carries FVIII). As a consequence, higher purity products are more expensive.

For some products, higher purity leads to a clinical benefit. For example, high purity FIX concentrates lacking factors II, VII, and X are preferable to the mixture of factors contained in prothrombin complex concentrates for the treatment of hemophilia B, as the risk of thromboembolic complications is decreased. On the other hand, as long as adequate viral elimination measures are in place, increasing the purity of FVIII concentrates does not provide a clear clinical benefit. However, concerns regarding the currently unknown risk of vCJD have prompted manufacturers to validate their processes for the potential to eliminate

5 <https://www.fda.gov/downloads/BiologicsBloodVaccines/SafetyAvailability/BloodSafety/UCM095104.pdf>

6 Available from <http://elearning.wfh.org>

vCJD-like agents from the final product. These studies have shown that several processes for manufacturing FVIII and FIX products are capable of eliminating significant levels of contaminating vCJD-like agents. In general, the greater the purity of the product, the higher the level of such elimination.

Advances in recombinant clotting factor concentrates

The production of recombinant clotting factors became possible with the cloning and subsequent expression of functional proteins for both FVIII and FIX. Production of recombinant clotting factors in mammalian cell culture required overcoming significant challenges due to the complex post-translational modifications that are essential to their procoagulant function. Since their introduction, the recombinant versions of both FVIII and FIX have proven to be clinically similar to their plasma-derived counterparts. These recombinant products have gone through three generations since 1992. The first versions were produced in animal or human cell culture and stabilized with human serum albumin. The next generation was produced in animal or human protein containing cultures but with no albumin added to the formulation. The third generation is completely synthetic and free of all human or animal proteins. Notwithstanding these advances, adherence to GMP remains essential to ensure product safety.

The first hemophilia CFCs from recombinant sources were few in number and priced beyond the reach of most developing countries. As technology has advanced, more products have entered the market and prices have steadily decreased due to competition. Competitive tendering systems developed by several countries have exerted further downward pressure on prices, such that countries including the U.K., Australia, and Ireland now pay lower prices for recombinant CFCs than for plasma-derived products. The issue of price is beyond the scope of this Guide, but authorities charged with ensuring procurement and access to hemophilia therapies would do well to keep this in mind.

Modified recombinant clotting factor proteins

A number of products have been developed that feature modifications of the clotting factor protein molecule intended to achieve improved therapeutic properties. For example, certain products have been designed to demonstrate modified post-infusion **pharmacokinetics**, with a half-life that is longer than that of conventional products. These extended half-life (EHL) products offer the theoretical benefits of requiring less frequent infusion, and/or achieving higher clotting factor trough levels through a prophylactic regimen with a comparable infusion frequency to that of conventional products. The effect on the quality of care and quality of life of people with hemophilia has been widely discussed [28, 29].

This plethora of recombinant factors in the hemophilia treatment landscape, some of which are produced outside the mainstream plasma protein industry, and many of which can be produced in unlimited supplies, has the potential to increase access to care in countries where it is currently significantly limited.

In this context, some important issues must be considered by regulatory and other authorities:

- Underlying the distinguishing features of these modified products is their altered pharmacokinetics. Importantly, the pharmacokinetics of conventional FVIII vary significantly between individual patients infused with the same product. This patient-specific inherent variability suggests that a subset of patients may experience the benefits ascribed to the modified EHL recombinant factors when using conventional recombinant or plasma-derived CFCs [30]. One might also expect that the extent to which the modified products exhibit extended half-lives upon infusion might be impacted by patient-specific pharmacokinetics.

- The assessment of efficacy of any concentrate is an integral part of its approval process (see also the Introduction). In order to generate the evidence required by some regulatory agencies, sponsors of new recombinant CFCs now perform randomized studies comparing prophylaxis to on-demand treatment. Some reimbursement schemes in developed countries may also require this evidence. Authorities in developing countries must be aware that these randomized comparisons are often performed in their jurisdictions, and must insist upon measures to protect patients, as stipulated by the Declaration of Helsinki [31].
- Furthermore, if it is necessary to prove efficacy of a product for prophylaxis then the comparator population should be placed on a prophylaxis regimen with another product, previously approved for prophylaxis, rather than on-demand therapy with the product under review. This avoids the substantial burden associated with on-demand therapy [32].

The problem of inhibitors in hemophilia

This Guide has emphasized the measures required to evaluate CFCs for the risk of transmitting pathogens. Due to these measures, this threat has faded significantly in well-regulated environments, although constant vigilance is necessary. Meanwhile, the issue of the development of inhibitors to infused clotting factor has come to the forefront. This adverse event of replacement therapy is not new, it has existed for as long as replacement factor has been used in the management of hemophilia. Inhibitors are a known risk with any CFC and occur often in previously untreated patients (PUPs) treated with any FVIII product. Inhibitors (antibodies to replacement FVIII or FIX) are a well-**characterized** immunological response influenced by a number of intrinsic (patient-related) factors such as the presence of specific genetic mutations, a family history of inhibitors, or a specific ethnic background.

Much discussion has been devoted to the question of whether particular factor concentrates are associated with a greater risk of developing inhibitors. An enhanced risk has been established for plasma-derived FVIII concentrates subjected to certain viral-inactivation procedures that use specific heat treatments [33-35]. This finding prompted examination of the possibility that manufacturing processes can lead to molecular changes and neoantigenicity, or the development of new epitopes, in the FVIII molecule. Some *in vitro* systems have, in fact, detected such **neo-epitopes** in concentrates associated with enhanced antigenicity [11, 36].

Increasing use of recombinant products has led to investigation of whether aspects of their production might enhance the risk of inhibitor development. Some recombinant clotting factors, produced in rodent cell lines, differ from the protein found naturally in humans in several aspects of their glycosylation and other post-translational modifications (reviewed in [37]). Controversy surrounds the possible effect of these differences on product immunogenicity particularly in PUPs, whose likelihood of developing inhibitors is largely impacted by the multiple intrinsic factors mentioned above. In fact, the neoantigenicity of factor concentrates is best assessed in previously treated patients (PTPs) [38], as in the case mentioned above [33-35].

While several observational studies using meta-analyses have been inconclusive [39, 40], one prospective study has focused on this question, the Survey of Inhibitors in Plasma-Products-Exposed Toddlers (SIPPET) [41]. This study randomized hemophilia A PUPs, or minimally exposed patients, to receive plasma-derived FVIII concentrates containing VWF or recombinant FVIII concentrates, and assessed for inhibitors after 50 exposure days. A number of different products were used in each arm; all plasma-derived CFCs contained VWF, all recombinant products were raised in rodent cell lines, and none were of the newer EHL type. The study concluded that PUPs treated with the recombinant products had a higher incidence of inhibitors than PUPs treated with the plasma-derived products. This difference was observed to be statistically significant only for total inhibitors, not for high-titer inhibitors.

Following a thorough review of all available data, the EMA's Committee for Medical Products for Human Use (CHMP) released a statement in September 2017 concluding that there is no clear and consistent evidence of a difference in the incidence of inhibitor development between the two classes of FVIII medicines: plasma-derived and recombinant [42]. They recommend that, due to the different characteristics of individual products within the two classes, the evaluation of the risk of inhibitor development should be at the product level instead of at the class level. The risk for each individual product should continue to be assessed as more evidence becomes available.

The treatment of patients with established inhibitors involves bypassing agents, currently limited to one plasma-derived (FEIBA) and one recombinant product (NovoSeven® RT). Studies indicate a broadly equivalent therapeutic effect between the two agents [43]. The optimal therapeutic path for patients with inhibitors is obviation of the inhibitor through tolerization with clotting factor, a hugely expensive but, ultimately, cost-effective process [44]. The type of product (plasma-derived or recombinant) appears to have no significant effect on the achievement of tolerization [45].

Conclusions

Since the 1980s, various measures have been introduced to reduce the risk of viral transmission through fractionated plasma products. Not all practices are considered mandatory standards by regulatory agencies, and their use by fractionators must be assessed in the overall context of safety, availability, and cost. For example, while donor selection can offer significant benefits, other practices, such as NAT to narrow the window period and inventory hold, also reduce the risk of infectious units being pooled. Some measures may have only limited benefits for users of hemophilia treatment products, while possibly negatively affecting 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.

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, in-process inactivation 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 reduced the risk of receiving an infected product to an extremely low level. Establishment and maintenance of GMP 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 (e.g., HBV, HCV, and HIV).
- Plasma products manufactured under current best practices, and manufactured with attention to GMP, 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 appropriately regulated, result in similar safety of manufactured products
 - Method of collection. In practice, all collection methods yield safe, effective products if processes are properly optimized and GMP is observed
- The inclusion in the fractionation process of one or more steps with validated capability to inactivate or remove relevant viruses, primarily enveloped viruses (e.g., 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 (e.g., HAV and parvovirus B19).
- The demonstration that vCJD can infect people with hemophilia through the infusion of replacement factor products means that manufacturing steps validated for prion clearance must be included in the production of all CFCs.
- Assessment of prophylaxis should be performed in head-to-head randomized comparisons with CFCs already approved for this purpose, and should not include the randomization of patients to on-demand treatment arms.
- Inhibitors are a known risk with any CFC and inhibitors occur often in PUPs treated with any FVIII product. Inhibitor development is caused by many risk factors not related to product type. Research in this area is ongoing. Following a thorough review of the SIPPET trial data and other relevant studies, including interventional clinical trials and observational studies, the EMA's CHMP stated in Sept 2017 that the PRAC concludes there is no clear and consistent evidence of a difference in the incidence of inhibitor development between the two classes of factor VIII medicines: those derived from plasma and those made by recombinant DNA technology [42].
- In the absence of evidence that switching products elicits inhibitors, treaters may elect to treat PUPs with plasma-derived FVIII concentrate containing VWF, if it is considered that a high risk for inhibitor development justifies a precautionary approach. These patients may be transitioned to other CFCs following 50 days of exposure to plasma-derived FVIII concentrate containing VWF. PTPs may be safely treated with either type of product. If a plasma-derived FVIII concentrate containing VWF is not available, treatment with a recombinant FVIII concentrate or a plasma-derived FVIII concentrate lacking VWF, that meet the requirements outlined in this guide, remains far superior to withholding treatment.
- Patients with inhibitors may be treated with either plasma-derived or recombinant bypassing agents, and, preferably, tolerized with a CFC, also either recombinant or plasma-derived.
- At the time of writing, clinical trials of new treatment avenues, such as bispecific antibodies that replace the function of activated FVIII in the clotting cascade [46], or RNAi-mediated knock-down of anticoagulant activity [47], suggest that novel therapies may soon provide alternative approaches to the management of hemophilia especially in the presence of inhibitors. The evaluation of such products would involve a different set of criteria as they are not based upon replacing the deficient factor (VIII or IX).

Section 2

LICENSING, REGULATION, AND CONTROL OF CLOTTING FACTOR CONCENTRATES IN THE UNITED STATES AND EUROPE

Introduction

Arrangements for the licensing, regulation, and control of medicinal products have been developed and formalized to ensure that the risk-to-benefit ratio, which is involved in any medical intervention, may be minimized to ensure 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, authorities in developing countries can benefit from an awareness 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 U.S. FDA and the EMA are outlined in this section, along with other approaches aimed at harmonization.

United States – FDA regulations and guidelines

The U.S. FDA is the largest regulatory body in the world, with wide responsibilities for assuring the quality of foodstuffs, medicines, and medical devices manufactured for sale and supply in the U.S.A. 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 Pharmacopeia (USP). The parts of 21CFR with specific relevance to CFCs, whether they are derived from plasma or recombinant sources, are⁷:

- Parts 210 and 211, which describe current GMP
- 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:

- FDA draft guidelines

⁷ Available from <http://www.access.gpo.gov>

- FDA inspection guides
- USP Sections 1000 and the following. It should be noted that these sections are not mandatory

Biologics, including plasma-derived and recombinant CFCs, 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

Most of the issues relating to CFCs are currently under the oversight of the CBER Office of Tissues and Advanced Therapies and the CBER Office of Blood Research and Review.

European regulations and guidelines

Regulatory provisions in Europe are defined through a comprehensive set of provisions contributed by the European Commission (EC), the EMA, and the European Directorate for the Quality of Medicines (EDQM; formerly known as the European Pharmacopoeia Commission). The series of Blood Directives of the EC specifies the requirements for the collection of blood and plasma, including plasma for fractionation⁸. The EMA approves products submitted for authorization through the centralized procedure, and also provides centralized oversight of the **plasma master file (PMF)**, which is a key component of the regulatory oversight of the raw material for plasma-derived products (See Plasma master file concept). The EMA also houses the Blood Products Working Party (BPWP) of the CHMP, which provides guidance on issues related to the safety and efficacy of hemophilia CFCs⁹. The EDQM provides a centralized laboratory for the generation of standards and other issues requiring testing, including the coordination of the Official Medicines Control Laboratory (OMCL) network for the batch release of products including CFC.

The EDQM publishes the *Guide to the Preparation, Use, and Quality Assurance of Blood Components* [48]¹⁰, which specifies the requirements for the manufacture of blood components as well as the key quality parameters of the individual components, including plasma. *The Good Practice Guidelines for Blood Establishment Required to Comply with Directive 2005/62/EC* (used for implementing standards and specifications for the quality system in blood establishments) [49]¹¹, which forms the first part of the EDQM's Guide, is strongly recommended as minimal requirements for ensuring blood components manufactured from blood and plasma donations are produced in an adequately controlled environment, adhering to GMP. These guidelines are particularly relevant when plasma is recovered from whole blood donations.

In addition, national member states of the EU oversee GMP in facilities that produce drugs, including CFCs marketed within their borders, and provide marketing authorization for drugs requiring national approval, as well as approval through the mutual recognition procedure of drugs to be exported.

In summary, the oversight of CFCs in Europe is more complex than that in the U.S.A., because of the different governance structures resulting from the EU. It is, concurrently, much more comprehensive and transparent than the provisions of the U.S. FDA, through the provision of detailed guidance on the key

8 Available from https://ec.europa.eu/health/blood_tissues_organ/key_documents_en#anchor0

9 Available from http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_000388.jsp&mid=WC0b01ac0580032ec8

10 Available for free download from <https://www.edqm.eu/en/news/new-19th-edition-blood-guide-now-available>

11 Available from https://www.edqm.eu/sites/default/files/goodpracticeguidelines-19th_edition_guide_preparation_use_qa_blood_components-december2016.pdf

issues requiring attention. The Guidelines of the BPWP¹² as well as the Good Practice Guidelines of the EDQM [49] are especially relevant to readers of this Guide. Several useful summaries of the European framework have been published [50, 51].

Plasma imported into the EU for the purpose of contract fractionation and re-export to the country of origin is captured in the European framework. This, therefore, requires that such plasma must meet EU standards of quality and safety. While these standards may be difficult to attain by developing countries wishing to use established European manufacturers for contract fractionation, the benefits of adherence for all parts of the blood system are clear. Satisfying the European requirements for plasma for fractionation ensures that the country's collection system is of a high enough standard to provide plasma for domestic manufacture once this capacity is attained [52].

Plasma master file concept

The concept of the PMF was developed by the EMA¹³. The PMF is a compilation of all of the required scientific data on the quality and safety of human plasma relevant to the manufacture of plasma-derived medicines. The purpose of the PMF is to allow the manufacturer to fully describe the source plasma used for different plasma products assuring adequate levels of quality and safety of the raw material.

Key elements of the PMF are:

- Requirement for a formal contract governing purchase and supply of plasma
- Description of the quality assurance system applicable to plasma supply and use
- Arrangements for donor selection (including population epidemiology)
- Requirements for testing of samples of individual 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. In conjunction with the data for the manufacturing process, this allows manufacturers and authorities alike to estimate the residual risk posed by the final product for different infectious agents. Since different manufacturers obtain their plasma from a dynamic environment in which companies change the source of their raw material according to convenience and market pressures, 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 PMF include:

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

While the PMF was developed for the European environment, it is an excellent model for assessing the safety of plasma, and can be adapted into a stand-alone document tailored to the particular needs of individual

12 Available from http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_000388.jsp&mid=WC0b01ac0580032ec8

13 Available from <http://www.ema.europa.eu/ema/>

countries. It includes all the information on plasma as a raw material that national authorities require 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 submission of a full PMF from manufacturers, and should ensure the exclusion of raw material providers whose safety profile does not conform to the PMF's requirements from the pool destined for hemophilia treatment concentrates. This is especially important in the context of today's globalized plasma industry, where some big companies deploy manufacturing plants in countries under EMA oversight and in others outside the EMA's mandate. Plasma not acceptable to the EMA might be incorporated, through manufacture in non-EMA regulated plants, into products destined for markets outside the EMA's regulation. This situation is undesirable in terms of patient safety. Despite the pivotal role that viral inactivation plays in the manufacturing process, if a manufacturer is unable to secure reliable supplies of safe raw material, as specified by the PMF, they are unlikely to be able to assure an authority of their capacity to manufacture safe and effective plasma products.

Common strengths of U.S. & EU regulatory provisions [53]

- Review of data in marketing authorization application:
 - Commitments on the quality and safety of the raw material—PMF
 - Process/batch consistency, including effectiveness of viral-inactivation/viral-removal steps
 - Safety, efficacy, and pharmacokinetic data
- Inspection and enforcement of:
 - Plasma donor base, collection facilities, and quality assurance systems
 - Manufacturing facility, process, and quality assurance 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

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.A., 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. Currently established guidance includes¹⁴:

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

¹⁴ Available from <http://www.ich.org/home.html>

Summary

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

SECTION 3

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

Introduction

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 that safeguards public health without artificially restricting the availability of products and without unnecessarily escalating costs.

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.

Some obstacles that may impede the assessment and selection of products, include:

- NRAs may face limitations
 - Lack of experience
 - Lack of resources
- The supply of plasma products is not a level playing field
 - Several generations of product (e.g., with respect to protein purification and viral elimination methods) are typically concurrently available and the benefits of a particular product are not always clear
 - Variability exists in the quality of the plasma used for manufacture
 - Variability exists in the manufacturing standards employed
 - Local distributors may lack sufficient information on product specifications
- Decision makers must 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 maximum 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
- Select products licensed with an established NRA, whenever possible

- Use a pre-contract questionnaire to obtain information on plasma and the manufacturing process in advance (refer to the 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

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 come 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 a dependence on third-party suppliers (brokers/agents), which can limit communication on key quality matters
- Relying on finished product testing to ensure fitness-for-purpose

Using distributors of imported products

Since many countries attempting to access hemophilia treatment products lack a domestic plasma fractionation capacity, local distributors or agents for manufacturers are often used. They undertake a sponsorship role for the product and organize its presentation to government authorities, arrange its distribution once products are approved, and handle liability issues, etc. In general, it is preferable to deal directly with manufacturers rather than using agents, because the latter are rarely adequately familiar 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 problematic in the absence of an established NRA, as this scenario involves instability on both sides.

If distributors are used, NRAs should establish procedures to ensure that the 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 NRA 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 this contact, including records of all 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 products to a pre-determined specification is an essential feature of product quality control leading to market release. Regulatory authorities such as the U.S. FDA and EMA 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. This testing of products before official release is called **batch release testing**. It is not a universal practice among regulators, some of whom consider there to be little product quality assurance value gained by duplicating the manufacturer's own release testing. Product quality ultimately 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 GMP. 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** will improve their assurance of quality and safety, they should use (or adapt) the approach of established regulators or the batch release protocol from the EDQM¹⁵. However, for the authorities to whom this Guide is directed, end-product testing should not be a mandatory requirement to measure the safety of hemophilia treatment products. 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 and validated for testing individual pharmaceutical products such as hemophilia treatment concentrates.

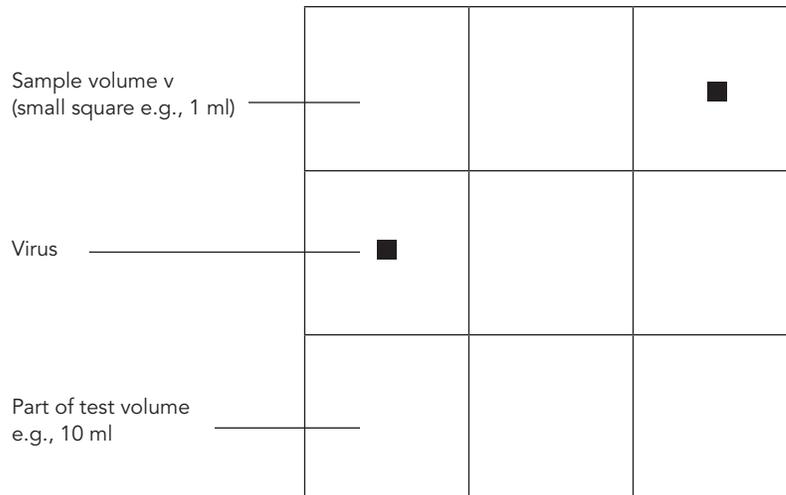
It is important to note that end-product testing cannot be used to ensure viral safety. The testing used to screen plasma for viral agents, whether performed on donations or pools, and whether serologic or molecular, is not designed or validated for testing end products. Using these tests for end products is highly inappropriate and adds nothing to the assurance of safety of these products. In particular, experience shows a high level of false positive results when using these tests for this purpose. This application may lead to incorrect assessments of product quality and safety and delay product release. Two important points to consider are:

- Even assuming the presence of some virus in the final product, despite the various measures to exclude this, any such virus would be present in low amounts. Statistical considerations predict that the probability of such a low amount of virus remaining undetected is high [54]. This is demonstrated in Figure 1 where two viral particles are present in 10 ml 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 tests were able to detect all viruses present in products, and even if the limitations in sample size described above could be overcome, a positive test for a viral marker does not necessarily mean that actual live virus is present in the product. For example, solvent-detergent treatment, which inactivates HCV infectivity very reliably, has no effect on the detection of HCV nucleic acid [55]. Therefore, a positive end-product testing result using this technique would lead to the erroneous conclusion that the product was infectious. Retrospective testing of batches of albumin has demonstrated that many such batches were reactive for HIV nucleic acid in the 1980s; had such testing been available and resulted in the halting of product release a crisis in albumin supply would have ensued. Despite a wide prevalence of HIV in the plasma pool, albumin products have never transmitted the virus because viral-inactivation processes destroy infectivity, while retaining test reactivity.

15 Available from <https://www.edqm.eu/en>

The safety of plasma products is ensured through adherence to standards and GMP. No amount of product testing for viruses can be a substitute for these crucial requirements.

Figure 1: Distribution of a small amount of virus in a relatively large volume [54]



Virus concentration $c = 2/10 \text{ ml} = 200/l = 10^{2.3}/l$
 Probability p_- of obtaining a negative test result (Poisson distribution):
 $P_- = e^{-cv}$
 $P_- = e^{-200 \times 0.001} = e^{-0.2} = 0.82$

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 the pre-selection and audit of suppliers
 - Focusing on evidence of plasma quality and secure manufacturing processes 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 for adequate oversight of the whole manufacturing cycle.

SECTION 4

EVALUATING CLOTTING FACTOR CONCENTRATES

Introduction

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

Product information from the manufacturer

To properly assess a product, NRAs must obtain information on:

1. The quality of plasma raw material, including:
 - Regulatory status of the plasma supplier
 - Donor epidemiology
 - Donor exclusion criteria
 - Screening tests performed 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 (IPCs)
 - Viral inactivation and/or removal steps
 - Process consistency
 - Batch release specification
3. The final product, including:
 - Product potency and shelf life
 - Other markets where the 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 that should be met in a satisfactory fashion for a product to be considered safe (See also Table 2).

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 collection sites from high-risk areas such as prisons, and attempts 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 can best establish confidence by performing audits of the collection centres based on these and other features of GMP. These audits should be performed by the manufacturer, although reference to audits performed by an NRA 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 for which the source of the raw material is unknown and unspecified, even if the manufacturer claims that the blood has been tested or the product has been viral inactivated. 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 the latest generation of test kits for the relevant test, preferably in a format registered by a licensing authority. While technologies (e.g., NAT) used for detecting infection during the window period are also desirable to increase viral safety margins, it is improbable that they prove critical to ensuring the viral safety of products sourced from serologically screened plasma, which are subject to robust viral-inactivation steps. This is also the case 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 treatment products.

While a number of viral-inactivation steps have been shown to greatly enhance the safety of hemophilia treatment products, **solvent-detergent treatment is currently the gold standard for safety from the highly infectious enveloped viruses, and should be considered 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 the preparation of FIX and other smaller plasma proteins; however, it may not be the best option for FVIII (a much larger protein) concentrates

until new membranes are introduced and have been validated. For FVIII 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 solvent-detergent and nanofiltration procedures is the low risk of induction of protein neoantigens.

Any incidental elimination of viruses during the manufacturing procedure contributes to the overall safety of the product and should be welcomed. However, such contributions only supplement and should never replace a deliberate viral elimination step.

Given the repeated demonstration that enveloped viruses including HCV, HIV, and HBV are the biggest transfusion-transmitted threat to people with hemophilia, 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 HAV) 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 safety from infection by parvovirus B19. Manufacturers have started to incorporate such testing in their processes, and authorities may want to require NAT for specific viruses known to be prevalent in the donor population contributing to a product. With validated viral-inactivation procedures for disease-causing enveloped viruses, such plasma pool testing has the potential to be more beneficial for unscreened non-enveloped viruses. In combination with nanofiltration, NAT may reduce the risk of small non-enveloped viruses (e.g., parvovirus 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 conducted even for products which are reportedly similar to other, better-characterized products.**

Some developing countries purchase products from manufacturers who have acquired processes through technology transfer. These processes are intended to deliver products that are very similar to those from the parent technology, which would have been fully characterized for physicochemical and clinical properties. Clinical trials are expensive and difficult to conduct especially for rare disorders with small patient populations such as hemophilia. Understandably, manufacturers and authorities from these countries have reservations about engaging in the rigorous clinical trial framework mandated by the EMA. While this framework may be modified according to the particular circumstances, it is inadvisable to totally abandon all efficacy assessment. At the very least, an evaluation of the pharmacokinetics of the product should be performed 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. If a country is able access treatment products at any level, then these modest measures should also be attainable.

- 6. The product's performance, following approval to enter the market, should be monitored, in order to detect possible adverse events.**

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

Small changes in the manufacturing processes of biologicals such as hemophilia treatment concentrates can result in *in vivo* effects that cannot always be predicted from pre-approval assessment, therefore it is important to monitor the product following its introduction in the market. Currently, the onus of

concern for hemophilia treatment 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. Samples should be drawn and analyzed to assess patients' viral status and inhibitor levels. Documents available from the EMA may provide guidance on this [38], 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 relevant manufacturers to perform these analyses as part of any supply arrangement.

- 7. People receiving FIX for the treatment of hemophilia B should be given concentrates specifically enriched in this factor and purified to remove other clotting factors. When possible, highly purified concentrates of FVIII and FIX are preferable. Manufacturing processes must be secure in relation to known viral agents and not enhance the probability of products eliciting inhibitors. Furthermore, the purchase of such concentrates should not result in the restriction of treatment due to excess cost.**

Example scenarios

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

Example 1

As a result of a tendering process, the following FVIII 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 serological markers of the transfusion-transmitted agents HIV, HCV, and HBV on plasma pools after the plasma has been thawed for manufacture. The manufacturer also performs NAT 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 the plasma source specific to the product. The product is the cheapest of those offered.

Product B: A concentrate made from plasma, and characterized by a fully documented quality system incorporating the tenets of the European PMF 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 EMA's CHMP.

Product C: A concentrate made from plasma, and characterized by a fully documented quality assurance system incorporating the tenets of the European PMF concept. The product is highly purified via monoclonal antibody affinity columns and subject to solvent-detergent treatment. The product is stabilized with albumin. The product costs twice 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 assurance 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 that, according to the scientific literature, 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 but has offered literature-based evidence for efficacy.

Product E: A concentrate made from plasma characterized by a fully documented quality assurance system incorporating the tenets of the European PMF 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

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 assurance 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 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 its product to be safe. The company's contract for plasma supply should be rigorously assessed for its adherence to the crucial features of the PMF 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 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 FIX products are offered:

Product X: A prothrombin complex concentrate manufactured from plasma, and characterized by a fully documented quality assurance system incorporating the tenets of the European PMF 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 FIX by affinity chromatography. It is viral inactivated by solvent-detergent treatment and nanofiltration. The plasma source is satisfactorily characterized and NAT is applied to the plasma pool for HCV and HIV.

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

Evaluation

Considerations when evaluating the products in this scenario should include:

1. Whenever possible, the use of pure FIX concentrates (as opposed to prothrombin complex concentrates) is preferable for the treatment of hemophilia B. 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, which is expected to be more effective with multiple purifying steps. The product may be considered for the treatment of indications other than hemophilia, such as warfarin-reversal.
2. Purity to the level of biochemical homogeneity of FIX has not been demonstrated to have an effect on the safety of hemophilia B products.
3. Because nanofiltration separates viruses from proteins with molecular weights including that of FIX, it is a recommended step for eliminating non-enveloped viruses, as well as contributing to the elimination of enveloped viruses.

Example 3

In this example, we will assess a choice from the practical possibilities in the competitive market of 2017. While the primary focus of this Guide is on safety and efficacy, this example gives some consideration to cost.

A tendering process yielded the following FVIII products for consideration:

Product I: A recombinant FVIII manufactured using albumin to ensure product stability. This product meets all standard requirements as described in this Guide, and it is offered at a considerable discount relative to similar internationally available products.

Product II: A plasma-derived FVIII-VWF concentrate licensed for both hemophilia and von Willebrand disease (VWD). All product safety issues are met, as described in this Guide. The product is offered at a lower price than other products of this type in developed markets.

Product III: The manufacturers of this recombinant FVIII concentrate claim that it has an extended half-life. A clinical trial reported a 25% reduction in CFC consumption when used for prophylaxis by a group of people with hemophilia A. Most of the patients on prophylaxis with this product were able to achieve satisfactorily low bleeding rates with two infusions per week. Although it is offered at a discount relative to prices in developed countries; it is still the most expensive of the three contenders by a considerable margin.

Evaluation

Product I is manufactured using human albumin, which theoretically increases the pathogen safety risk, but albumin has not actually been associated with any pathogen transmissions in 70 years of use. Otherwise there is no issue with this product. It is clearly not indicated for the treatment of VWD.

Product II may be used to treat both hemophilia A and VWD.

Product III offers an attractive enhancement to patient convenience, but it is only useful in the prophylaxis context.

In this scenario, we can discern the benefits accrued by the current era marked by the availability of diverse products and technologies. These three contenders have the capacity, if chosen together in an appropriate pricing environment, to cover many aspects of modern hemophilia care. Product I can adequately address all the needs of patients on stable on-demand treatment. Product II also addresses the needs of hemophilia A patients, as well as those of VWD patients. Product III's requirement for less

frequent infusions would benefit parents of very young patients and offer convenience to older patients. Such a discerning therapeutic policy can capitalize upon many of the benefits of the different types of concentrates available.

Summary

- NRAs must assess each product on its own merits and carefully weigh the relative features of each product when 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 must be met:
 - The manufacturer must have full confidence in the safety and quality of the plasma raw material
 - Individual donations of plasma must be screened for serological markers of HIV, HBV, and HCV
 - Manufacturing processes must include deliberate, well-validated viral inactivation and/or removal steps
 - Other safety measures to enhance safety from non-enveloped viruses, such as vaccination of people who receive factor concentrates on a life-long basis and decreasing the viral load of the plasma pool, are recommended
 - Use of highly purified concentrates of FVIII and especially FIX are recommended, as long as it does not result in the restriction of treatment due to excess cost

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 guide regulatory authorities making decisions about the purchase of hemophilia treatment products.

The WFH updates this Guide regularly, and welcomes comments for improving it. Please send any suggestions to:

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APPENDIX 1: WFH ONLINE REGISTRY OF CLOTTING FACTOR CONCENTRATES

Beta version launched on eLearning.wfh.org on July, 2016, updated on an ongoing basis

by Mark Brooker

The Registry¹⁶ was created in 1997 by Meirione Costa e Silva of Brasilia and Dr. Carol Kasper for the International Society on Thrombosis and Haemostasis (ISTH). Its purpose was to help medical personnel identify available concentrates and stay abreast of pharmaceutical company changes. In July 2016, the Registry was moved online to allow real-time updates and ease of use. It is intended for use by healthcare providers and officials, pharmacists, and people with bleeding disorders.

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 travelling abroad may bring along their own concentrates, which may not be familiar to local healthcare personnel.

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

Several national fractionation centres produce concentrate from domestic recovered plasma for domestic use. A few fractionators (e.g., CSL in Australia, Grifols in Spain, Biotest in Germany) 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 plasmapheresis. Such plasma may be blended with smaller amounts of unpaid recovered plasma.

In the Registry, concentrates are described according to method of fractionation, method of viral inactivation and degree of purification. 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. 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.

The array of serologic tests varies slightly from country to country. More sensitive NATs that directly detect viruses are becoming commonplace.

No HIV has been transmitted by any concentrates in the Registry since 1987. No hepatitis A, B, or C has been transmitted by these concentrates since the Centers for Disease Control and Prevention (CDC) began its broad surveillance of persons with hemophilia in 1998.

Manufacturers of CFCs can contact the WFH to have their products added to the Registry, and to revise and update information about products in the Registry.

16 WFH Online Registry of Clotting Factor Concentrates: <http://elearning.wfh.org/resource/online-cfc-registry/>

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 treatment products

1) PLASMA RAW MATERIAL (FOR PLASMA-DERIVED PRODUCTS ONLY)

(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	
(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 (U.S.A./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 quality assurance 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 (FOR PLASMA-DERIVED AND RECOMBINANT PRODUCTS)

The manufacturer must include a copy of the licence to manufacture issued by the country where the facility is located and any other authority.					
(A) CRITICAL STEPS Insert here a flow chart of the manufacturing process, identify the crucial manufacturing steps and list their related in-process controls (IPCs).					
(B) VIRAL REDUCTION					
For plasma-derived and recombinant products List dedicated viral reduction steps.					
For plasma-derived products only Validated log ₁₀ elimination for 1. HIV (actual virus) 2. HCV (specify model, e.g., BVDV) 3. HBV (specify model) 4. HAV (actual virus or specify model) 5. Parvovirus B19 (specify model)					
For plasma-derived products only 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 IPCs identified in 2(a) for three chronologically sequential batches of the product manufactured at the scale used for the marketed form within the past 18 months.					
IPCs	Batch – 01	Batch – 02	Batch – 03		
IPC-1 IPC-2 IPC-3 IPC-4 IPC-5					
(D) STABILITY AND SHELF LIFE					
Include data for the potency (FVIII or FIX) of the product measured during the shelf lives listed in the table, at the temperatures sought in the application.					
Potency IU/ml (mean±standard deviation)	At release	3 months	6 months	12 months	24 months
	2h:	2h:	2h:	2h:	2h:
	8h:	8h:	8h:	8h:	8h:
	24h:	24h:	24h:	24h:	24h:
Include data for the product's potency at 2, 8, and 24 hours after reconstitution.					

3) FURTHER PRODUCT INFORMATION (FOR PLASMA-DERIVED AND RECOMBINANT PRODUCTS)

(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 EMA's Guideline on the clinical investigation of recombinant and human plasma-derived factor VIII products [38], accessible at http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2015/06/WC500187409.pdf
(C) ADVERSE EVENTS Describe manufacturer's system for receiving and reporting adverse events related to the product.

APPENDIX 3: LIST OF ABBREVIATIONS AND ACRONYMS

21CFR	Title 21 of the Code of Federal Regulations
BSE	Bovine spongiform encephalopathy
BPWP	Blood Products Working Party
BVDV	Bovine viral diarrhoea virus
CBER	Center for Biologics Evaluation and Research
CDC	Centers for Disease Control and Prevention
CFC	Clotting (or coagulation) factor concentrate(s)
CFR	Code of Federal Regulations
CHMP	Committee for Medicinal Products for Human Use (EMA)
DNA	Deoxyribonucleic acid
EC	European Commission
EDQM	European Directorate for the Quality of Medicines
EHL	Extended half-life
EMA	European Medicines Agency
EU	European Union
FDA	U.S. Food and Drug Administration
FIX	Factor IX
FVII	Factor VII
FVIII	Factor VIII
FX	Factor X
GMP	Good Manufacturing Practice
HAV	Hepatitis A virus
HbsAg	Antigen associated with hepatitis B virus (HBV)
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HEV	Hepatitis E virus
HIV	Human immunodeficiency virus
ICH	International Conference on Harmonisation
IPC	In-process controls
ISTH	International Society on Thrombosis and Haemostasis

IU	International units
NAT	Nucleic acid testing
NRA	National regulatory authority
OMCL	Official Medicines Control Laboratory
PMF	Plasma master file
PTP	Previously treated patient
PUP	Previously untreated patient
RNA	Ribonucleic acid
RNAi	RNA interference
SD	Solvent-detergent
SIPPET	Survey of Inhibitors in Plasma-Products–Exposed Toddlers
TSE	Transmissible spongiform encephalopathies
U.K.	United Kingdom of Great Britain and Northern Ireland
U.S.A.	United States of America
USP	United States Pharmacopeia
vCJD	variant Creutzfeldt-Jakob disease
VWD	von Willebrand disease
VWF	von Willebrand factor
WFH	World Federation of Hemophilia
WNV	West Nile virus

APPENDIX 4: 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 that 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.

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

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.

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

Good Manufacturing Practice (GMP): Elements in established practice that 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 ensure donor safety are undertaken.

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

Lyophilized: The freeze-dried state of a preparation following lyophilization, the process of isolating a solid substance from solution by freezing the solution and evaporating the ice under vacuum.

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

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

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

Neoantigen/Neo-epitope: The formation of new epitopes—a component of a protein that functions as an antigenic determinant by binding antibodies.

Non-enveloped /non-lipid enveloped viruses: Pathogenic viruses (e.g., HAV, parvovirus B19) that 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 that 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 (PMF): 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. Plasma collected in this way is called source plasma or apheresis plasma.

Potency: The biological activity that is best related to a product's actual therapeutic effect and can be measured in a laboratory.

Purity: The proportion of the desired ingredient (e.g., FVIII) 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 5: WFH RESOURCES

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

<http://elearning.wfh.org/resource/contract-fractionation/>

Guide to National Tenders for the Purchase of Clotting Factor Concentrates, 2nd edition (Spanish title: *Guía sobre licitaciones nacionales para la compra de concentrados de factor de coagulación*). Brian O'Mahony. 2015.

<http://elearning.wfh.org/resource/guide-to-national-tenders-for-the-purchase-of-clotting-factor-concentrates/>

Creutzfeldt-Jakob (Spanish title: *La Enfermedad de Creutzfeldt-Jakob*) James W Ironside.

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