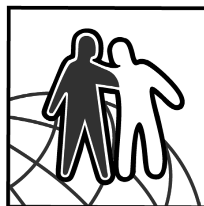
Facts and Figures Monograph Series **No. 7** **November 1999**

Transmissible Agents and the Safety of Coagulation Factor Concentrates

Jerome Teitel, MD, FRCPC
Toronto and Central Ontario
Comprehensive Hemophilia Program
St. Michael's Hospital
Toronto, Canada



WORLD FEDERATION OF HEMOPHILIA
FÉDÉRATION MONDIALE DE L'HÉMOPHILIE
FEDERACION MUNDIAL DE HEMOFILIA

Published by the World Federation of Hemophilia.

This publication is accessible from the World Federation of Hemophilia's web site at www.wfh.org, under WFH Library. Additional copies are also available from the WFH at:

World Federation of Hemophilia
1425 René Lévesque Boulevard West, Suite 1010
Montréal, Québec H3G 1T7
CANADA
Tel. : (514) 875-7944
Fax : (514) 875-8916
E-mail: wfh@wfh.org
Internet: www.wfh.org

The Facts and Figures series is intended to provide general information on factor replacement products and the administration of hemophilia care. The World Federation of Hemophilia does not engage in the practice of medicine and under no circumstances recommends particular treatment for specific individuals. Statements and opinions expressed here do not necessarily represent the opinions, policies, or recommendations of the World Federation of Hemophilia, its Executive Committee, or its staff.

Transmissible Agents and the Safety of Coagulation Factor Concentrates[1]

Jerome Teitel, MD, FRCPC

Viral Transmission by Factor Concentrates

The major remaining barrier to the safe transfusion of blood components is the transmission of micro-organisms that cause disease. From the point of view of replacement therapy for hemophilia and related diseases, viruses are the primary concern. The removal or inactivation of every virus particle in coagulation factor concentrates is a worthy goal in theory, but in practical terms it may be unnecessary and unattainable. In any event, absolute viral removal cannot be proven, as only a sample of the entire product is tested. From a practical perspective, the goal is to reduce pathogenic viral contamination to residual levels, where the virus is not infectious.

Separating viruses from blood components is difficult because of their size. Viral particles are smaller than other pathogens (disease-causing agents). In addition, some viruses are relatively resistant to inactivation techniques. Finally, new viruses periodically cross the species barrier undetected, and may enter the human blood supply. Once this occurs, subsequent global distribution is likely, given the degree of human mobility in the modern world. New disease-causing viruses would not be identified by monospecific screening tests, and they could be resistant to the viral reduction strategies in use.

Major Pathogenic Viruses Transmissible by Factor Concentrates

The major transmissible viruses that are present in plasma and cause serious and/or chronic disease are HIV, hepatitis B (HBV), and hepatitis C (HCV).

Other viral pathogens that can be transmitted by factor concentrates are of lesser concern. Parvovirus B19 is commonly transmitted by plasma-derived factor concentrates, but in adults, at least, it generally causes mild disease that is without symptoms and is not considered clinically recognizable. Factor VIII concentrate has been implicated as the source of several limited outbreaks of infection with the hepatitis A virus (HAV). HAV also generally causes mild or subclinical disease, and it is not associated with a chronic hepatitis or with a persistent carrier state. In Addition, effective vaccines are available to protect susceptible individuals. Both parvovirus B19 and HAV are small and lack a lipid envelope, characteristics that make them difficult to remove from plasma-derived products. They are also resistant to chemical inactivation by solvent-detergent reagents. They can, therefore, be considered “sentinel viruses,” which could indicate the presence of other potentially dangerous viruses with similar physical properties.

Some transfusion-transmitted viruses, notably cytomegalovirus and HTLV-I, are not present in plasma, but only in the cellular matter of blood, and are therefore not relevant to coagulation factor concentrates.
Some characteristics of viruses transmitted by factor concentrates are shown in Table 1.

[1] This monograph is an updated edition of Teitel J. *Viral Safety of Coagulation Factor Concentrates*. Facts and Figures series, number 4. World Federation of Hemophilia, 1997.

Table 1:
Major Viruses Transmitted by Clotting Factor Concentrates

VIRUS	SIZE (nm)	GENOME	ENVELOPE
HIV-1	**90-100**	**RNA**	**YES**
HBV	**40-45**	**DNA**	**YES**
HCV	**40-60**	**RNA**	**YES**
HAV	**25-30**	**RNA**	**NO**
B 19	**18-20**	**DNA**	**NO**

New, Emerging, or Potential Viral Threats from Factor Concentrates

Hepatitis C infection accounts for most, but not all, cases of post-transfusion non-A, non-B hepatitis. This has naturally prompted a search for the causes of the remaining cases. The first credible candidate was hepatitis G virus (HGV). This virus has probably been transmitted via factor concentrates. It is likely that HGV is susceptible to the viral inactivation strategies that are effective for other enveloped viruses, although this remains to be established. Even if it is confirmed, the effectiveness of these procedures could be compromised if there are high initial viral levels in unscreened plasma pools. In any event, HGV's ability to cause disease as well as its tropism for liver cells remains uncertain. It is now considered unlikely that HGV is the cause of post-transfusion non-A, non-B hepatitis.

The prosaically named transfusion-transmitted virus (TTV) is a non-enveloped DNA virus. It was recently identified as a possible causal agent of some cases of post-transfusion acute and chronic hepatitis in Japan. TTV appears to be common around the world, and, although it seems clear that it is a frequent contaminant of blood products, the concentration of virus in blood appears to be low. This may account for the much lower positivity rate for TTV than for HCV in screening studies of recipients of non-viral-inactivated factor VIII concentrates. Furthermore, even solvent-detergent treatment appears to inactivate this non-enveloped virus in factor concentrates, possibly again owing to its low concentration. TVV's ability to cause disease (clinically important liver disease, in particular) has not yet been established. Like HGV, TTV may not be clearly associated with liver disease.

Very recently, another candidate non-A, non-B, non-C hepatitis virus was identified, named SEN-V. The implications of this virus for transfusion recipients in general, and for people with hemophilia in particular, will need to be established.

Non-human viruses are a potential concern, but their ability to cause disease in humans is often unclear. For example, porcine factor VIII concentrate was recently found to be contaminated with porcine parvovirus (PPV). PPV is highly endemic in pig herds, but it is not known to be transmissible to humans. Laboratory and clinical data on recipients of porcine factor VIII have failed to demonstrate that it poses a health risk to humans. Attempts to exclude or inactivate PPV are complicated by its small size, and by the fact that porcine factor VIII is a fragile molecule that does not withstand the vigorous procedures needed to inactivate PPV. Therefore, screening of small plasma pools became the practical approach to prevent contamination of porcine factor VIII concentrate with PPV.

General Principles for the Optimization of Viral Safety

A multifaceted approach that includes safety measures at every stage of production is needed to minimize the risk of viral transmission by coagulation factor replacement products. It includes screening donors, testing of donated blood, removal of viruses from therapeutic components, and inactivating viruses. Incorporating complementary safety measures further reduces the viral load entering the plasma pool and provides protection against manufacturing errors or oversights in any one of the steps. Responsibility for performing these steps lies with the manufacturers. Regulators are responsible for promulgating guidelines for viral safety (such as those of the Paul Ehrlich Institute or the Committee for Proprietary Medicinal Products) and for releasing the products for distribution. Treaters and consumers bear joint responsibility for ensuring that susceptible individuals are immunized against HBV and HAV, and that plasma-derived replacement products are used appropriately.

Limiting Exposure to Products with Potential Viral Contamination

The risk of viral transmission can be limited by using products that are not derived from human plasma in situations where it is safe and effective to do so. Unfortunately, most hemophilia treaters can cite anecdotes in which treatment was based on misdiagnosed coagulation disorders, or concentrates were administered overly aggressively or for questionable indications, or outmoded and relatively unsafe replacement products were used. The use of clotting factor replacement therapy when pharmaceuticals such as desmopressin or anti-fibrinolytic agents may have been effective is also a common occurrence.

The use of recombinant clotting factor concentrates does not completely eliminate the risk of viral infection. Some recombinant products are formulated with albumin derived from human plasma. Albumin is produced using ethanol separation, and is pasteurized. The long experience with albumin as a volume expander attests to its safety, although it has not been subjected to the degree of scrutiny applied to clotting factor concentrates. More recently, recombinant factor VIII and factor IX concentrates are being formulated with saccharide (sugar) stabilizers, removing the theoretical risk of albumin. However, even without albumin in the final formulation, these products could theoretically transmit human or non-human mammalian viruses. These could be introduced via the parent cell lines, or by human or animal proteins contained in the liquid media used to freeze or grow the cells.

Reducing the Initial Viral Load

The viral burden entering the plasma pool can be limited by careful donor selection and by testing individual donations for anti-viral antibody or for viral antigen (see Table 2). There is a common perception that the safest donor pool is one composed of altruistic volunteers. In fact,

Table 2: Prevention of Virus Entry into the Plasma Pool

Donor selection

- Self-deferral
- Deferral by centre

Screening of individual donor units

- Surrogate assay
- Antibody positivity
- Viral antigen

Screening of pool

- Viral nucleic acid

Donor re-testing

- Quarantine donor unit pending result

evidence shows that blood from selected repeat paid donors is less likely to have viral contamination. This illustrates the principle that the health of the donor population is the source of the viral problem, not the payment of donors for their blood. The selection process must be accompanied by strict criteria for the re-entry of deferred donors into the pool, and by a registry that effectively ensures that plasma from deferred donors is not inadvertently released.

Specific viral screening tests are necessary in the production of plasma-derived concentrates, because subsequent viral removal or inactivation procedures can fail in the face of a heavily contaminated pool. However, given the limitations discussed below, it is evident that screening tests are not sufficient to insure optimal viral safety.

Traditionally, the most effective screening tools have been monospecific antibody or antigen detection tests for individual pre-selected virus targets. These tests should be periodically updated as technology improves. The priority of these tests is sensitivity, so that the ratio of true positive to false positive results should be low in selected (i.e., low prevalence) donor populations. In order to exclude false positives, all reactive sera should be subjected to a repeat screening test, followed by confirmatory testing. Nevertheless, the clinical significance of true antibody positivity can be problematic, as antibody is protective in some cases. This principle delayed the introduction of anti-HCV screening in the United States until 1991. There was real concern that removal of anti-HCV reactive units might compromise safety by eliminating protective antibody.

Viral antigen testing is available for HBV and HIV. Screening for HBV surface antigen (HBSAg) is extremely sensitive because large amounts of viral protein are synthesized early in the course of HBV infection. Still, HBV can be infectious at levels below the limit of detection by HBSAg tests. Furthermore, the sensitivity of the test may be reduced in the presence of antibody. In contrast to HBV, only small concentrations of HIV and HCV antigens circulate during early phases of infection, thus reducing their potential value as screening tests. In a large prospective American study, no positive HIV P24 antigen results were found in donor units that tested negative for HIV antibody. However, it is possible that in countries where there is a high incidence of newly acquired HIV infection, antigen testing may be useful in identifying some patients in the "window period," the early phase of infection before HIV can be detected by tests.

In the absence of clerical error or failure of quality control, false negative results are caused by sub-threshold viral contamination. This may occur during the window period early in the course of infection, or else in a late chronic carrier stage when the presence of the virus in the bloodstream or the host serological response has declined. Even though false negative results are rare, their significance may be greatly magnified, as a single virus positive unit can potentially contaminate a pool used to produce a large batch of factor concentrate.

One approach to reduce the risk of contamination during the window period is "donor re-testing," whereby frozen plasma is quarantined for sufficient time (e.g., three months) to allow re-testing of donors who initially test negative. Another approach is the detection of viral genetic material by nucleic acid testing (NAT), which typically uses amplification techniques such as the polymerase chain reaction (PCR). Manufacturers of clotting factor concentrates have introduced NAT in recent years. The power of NAT is exemplified by the sensitivity of PCR testing for HBV, which is about six orders of magnitude (that is, about one million times) greater than that of antigen testing. However, NAT is applied not to individual units but to the final pool, so viral genome could be diluted below the detection limit of even this technique.

In principle, the potential utility of amplification techniques has been demonstrated in areas of high prevalence for HBV, where PCR testing has been able to identify positive (potentially infectious) individual donors who are HBSAg negative or antibody positive. With respect to HIV, PCR data have been reassuring to date. They suggest that donors with indeterminate antibody results, and donors who are at high risk but seronegative, are

highly unlikely to be infectious. In practice, the benefit of adding NAT for HIV in factor concentrates will be difficult to demonstrate. Fewer than 30 cases of HIV transmission by antibody negative donor units were reported in the United States in the first decade after the introduction of testing. Even though this reported figure is likely to be an underestimate, the denominator is close to 150,000,000 units transfused. In clotting factor concentrates, this tiny residual risk is virtually eliminated by viral inactivation steps. Therefore, the incremental advantage of NAT would appear to be negligible.

Reverse transcriptase-PCR testing could be beneficial for HCV detection, in the absence of an antigen assay. Parvovirus B19 is also a realistic target for NAT, given its relative resistance to viral inactivation methodologies.

The question of pool size often generates controversy, as it is dictated by considerations of commercial cost-effectiveness. Over time, most people with severe hemophilia will be exposed to factor concentrates produced from many different pools. On the one hand, the probability of viral contamination in a pool is directly proportional to the number of donors it includes. On the other hand, the concentration of virus introduced by a contaminated donation is inversely proportional to the pool size. Dilution to a lower viral titre might reduce the risk of transmission, and can also enhance the efficiency of viral inactivation.

Removal or Inactivation of Viruses

Techniques for viral removal or inactivation are not specific for individual agents, although their effectiveness may be partially or completely restricted to certain classes of viruses. Therefore, in contrast to the screening tests described above, it is not necessary to perform specific viral reduction steps in order to eliminate each known pathogen. In addition, viral removal or inactivation techniques can potentially reduce the risk of transmitting viruses whose presence in the donor pool may not have been known or suspected. However, the effectiveness of viral removal or inactivation is has a limit. Some degree of viral kill must be compromised to avoid altering the clotting factor protein excessively. Therefore, these techniques complement donor selection and screening tests, but cannot replace them. Approaches for excluding and inactivating viruses in factor concentrates are summarized in Table 3.

Physical separation of viruses from clotting factor concentrates occurs incidentally during their purification and formulation. Steps such as cryoprecipitation, chromatographic separation (in particular immunoaffinity chromatography), and lyophilization remove substantial amounts of virus.

There has been renewed interest in the application of filtration techniques to deliberately exclude viruses. In particular, the factor IX molecule is small enough to pass through ultrafiltration and nanofiltration membranes, which retain even HAV and parvovirus B19, the smallest disease-causing viruses. These filters are now being used in the production of some factor IX concentrates, and are being investigated for factor VIII concentrates.

Specific viral inactivation procedures are applied to all plasma-derived coagulation factor concentrates. Heat treatment is a widely used process, as viruses have varying sensitivity to

Table 3:
Removal or inactivation of virus in the plasma pool

- Incidental removal during purification of protein of interest
- Specific viral removal by filtration
- Heat inactivation
  - 80-100°C x 0.5-72 hr
  - Pasteurization, 60°C x 10 hr
  - Heat under pressurized steam (60-80°C x 30-72 hr: OBSOLETE)
- Chemical inactivation
  - Solvent-detergent
  - Sodium thiocyanate
- Photochemical inactivation
  - Beta-propiolactone/UV
  - Ultraviolet-C

heat. Unfortunately so do proteins, many of which (notably factor VIII) are readily altered in solution at 60°C, the temperature used in pasteurization protocols. Unstable proteins are partially protected by the addition of chemical stabilizers, such as amino acids, citrate, or sugars, but losses of 10 to 15% of factor VIII activity are common. The effectiveness of heat as a treatment to inactivate viruses is a function of many factors, including time, temperature, physical state (dry or in solution), salt content, rate of temperature change, and nature and concentration of the stabilizers. In addition to pasteurization, heat is often applied to lyophilized concentrates at higher temperatures (80 to 100°C) for 0.5 to 72 hours. Heating such products to 60°C in hot vapour in an inert steam environment has an established safety record. With the exception of this process, temperatures below 80°C are relatively inefficient for inactivation of some model viruses in freeze-dried products.

Viruses that have lipid coats (including HIV, HBV, and HCV) can be efficiently inactivated by exposure to an organic solvent, usually tri-(n-butyl)phosphate (TNBP), in the presence of a detergent, either Tween 80, sodium cholate, or Triton X-100. As is the case for heat treatment, the effectiveness of solvent-detergent (S/D) protocols is time and temperature dependent. S/D causes rapid and complete inactivation of lipid-coated viruses, and the safety record of S/D-treated plasma fractionation products with respect to these viruses is excellent. Pooled S/D-treated plasma has recently become available, and is an alternative to single donor fresh frozen plasma for bleeding disorders for which viral-inactivated concentrates are not available. The relative advantages of S/D-treated plasma versus plasma produced from large pools should be considered on a case-by-case basis. It should be remembered that S/D plasma is deficient in the largest, most active multimers of von Willebrand factor.

Recently, dual viral inactivation processes have been applied to factor concentrates, typically the addition of a terminal heat treatment step to S/D-treated products. This broadens the spectrum of viral inactivation to include non-enveloped viruses, while retaining the advantage of the potent activity of the S/D treatment. The experience to date with these dual-treated products has been good, with no evidence that the added manipulation has enhanced their likelihood to provoke an immune response. At least one manufacturer is doing tests to evaluate a triple viral reduction protocol, incorporating filtration with S/D and heat treatment.

Other approaches to viral inactivation are based on chemical or photochemical methods. Sodium thiocyanate, a so-called chaotropic agent, has been successfully applied to factor IX concentrate, which is sufficiently stable to withstand the treatment. Photochemical treatment using methylene blue plus visible light has been used for viral inactivation in plasma for several years in Europe. Similar techniques, using ultraviolet irradiation of various wavelengths with or without added chemical sensitizing agents, have been applied to factor IX concentrates. Most of these procedures cannot be applied to unstable proteins such as factor VIII. Exposure to ultraviolet-C is an exception, which could become a useful supporting viral inactivation technique.

Costs of Viral Inactivation

The application of viral reduction strategies adds costs to clotting factor concentrates, both financial and otherwise. These procedures increase the complexity of the manufacturing process and reduce the yield of clotting factor, leading to increased monetary costs. The chemical agents that are added to concentrates in procedures such as S/D treatment are potentially toxic. It is important to ensure their removal from the final product. These procedures may also alter the clotting factor proteins in such a way as to render them less effective and/or more likely to provoke an immune response. This is not entirely a theoretical concern. Revision of a viral inactivation process applied to a plasma-derived factor VIII product in the Netherlands gave rise to a well-documented epidemic of factor VIII inhibitors, most of which were thankfully of low level and transient.

Interpretation of Viral Safety Data

Hemophilia treaters must interpret viral reduction data critically. It must not be assumed that log reductions achieved by individual fractionation and inactivation steps are necessarily additive, although data are often presented in a way that implies that this is the case. In practice, each step is evaluated individually for its ability to remove or inactivate virus “spiked” into the starting material. This experimental design is necessary, as there must be sufficient virus at each step for its viral reduction capacity to be measurable. However, as a result, interactions that may occur among the different methodologies are obscured. For example, different steps may not provide additional benefit if they preferentially inactivate the same subset of viral particles. In addition, some spiking studies use “model viruses,” which may differ in subtle but important ways from the pathogens they are meant to mimic. Even when the authentic pathogens are used, cultured virus strains may behave differently from their wild-type counterparts. Finally, published viral reduction data are derived from small-scale experiments, and the results may not always be applicable to the larger production scale.

Surveillance for Transfusion-transmitted Viral Disease

The recent impressive advances in the production of safe coagulation factor concentrates must not be allowed to create a sense of complacency in the hemophilia clinic. The ultimate proof of viral safety is not *in vitro* viral reduction data, but the demonstration that these concentrates do not transmit disease-causing viruses to susceptible individuals. Therefore, continued clinical and laboratory surveillance of the population receiving the concentrates is critical. This applies not only to the major known viruses but also to the less threatening blood-borne viruses, and to those of doubtful significance. Careful surveillance and a high degree of suspicion will also allow the timely recognition of clinical events that may signal the entry of new viruses into the blood supply.

Non-viral Pathogens: Creutzfeldt-Jakob and Related Diseases

Transmissible spongiform encephalopathies (TSEs) are progressive and fatal neurological degenerative diseases that occur in many species. The human TSE is called Creutzfeldt-Jakob disease (CJD). The consensus is that TSEs are caused by infectious particles called prions, which are abnormal forms of normal proteins. Although the prion theory was promulgated relatively recently, the diseases are not new; some of them having been recognized for centuries. CJD itself was first described in 1920, before blood transfusion became commonplace.

Human prions have been transmitted by ritual consumption of human brain, injection of a brain hormone into muscle, transplantation of human corneas or dura mater (the membrane that covers the brain), and implantation into the brain of contaminated needles or electrodes. All these routes have one thing in common: they involve the inoculation or implantation of tissue from the brain or of structures appended to it. Brain and related tissues and organs are in fact the major sites in which prions can be found. However, lower concentrations do appear in whole blood and blood fractions.

Although it is theoretically possible that CJD could be transmitted by blood, the available evidence suggests that this does not occur, or that it is so rare that it has not yet been detected. If CJD were a blood-borne illness, we would have expected to see cases of the disease in heavily transfused people, such as those with hemophilia or thalassemia. In fact, not a single case of CJD has been described in these groups. This is especially relevant because the estimated prevalence of asymptomatic “prionemia” (the presence of prions in the bloodstream) is up to 1 in 60,000, given the apparently prolonged incubation period of CJD. As the pools from which factor VIII and IX are fractionated may contain upwards of 60,000 donations, it is likely that most people with hemophilia who have received more than a few treatments with plasma-derived concentrates have been exposed to blood derivatives from affected donors. Many people in

the general population have likely been exposed to such donors as well, through sources such as albumin in vaccines. Yet the incidence of sporadic CJD has not increased over the course of the twentieth century.

These epidemiologic considerations are supported by surveillance data (including examination of brain tissue from people with hemophilia who died of neurological disease), cohort studies, and case-control studies, none of which support the theoretical possibility of blood-borne transmission of CJD. Animal models offer additional support. Whole blood and blood fractions from CJD patients have consistently failed to transmit the disease when injected either into the bloodstream or directly into the brains of non-human primates (monkeys and chimpanzees).

Prion diseases are generally difficult to transmit between species. One exception to this rule is BSE, the bovine TSE that is popularly known as mad cow disease. The BSE agent has entered the human food chain, and it appears that it has indeed caused a new illness in humans, referred to as "variant CJD" (vCJD). This disease has so far been identified in fewer than 30 people, almost all of them in the United Kingdom. No cases have occurred in North America. Reported cases of vCJD have been distinguished from sporadic CJD by both clinical and pathological characteristics.

Variant CJD is only known to be transmitted by the oral route. The possibility of its transmission by blood transfusion is hypothetical. Even experimentally, most of the infectivity of blood is in the cellular components, not in plasma. However, the BSE agent that gave rise to vCJD may be more transmissible than other prions; it has crossed the species barrier via the food chain to infect not only humans, but a variety of other domestic and wild animals. Also, since vCJD is a new disease in humans, we have not observed it for long enough to be reassured that it does not appear in transfusion recipients, as is the case for established diseases like sporadic CJD. Finally, although experts feel confident that they can distinguish cases of sporadic CJD from vCJD, it is possible that if more cases come to light we will find that vCJD is a more variable disease than we now appreciate, and that the distinction between the two TSEs is not always as unequivocal as it now seems to be.

TSEs are always fatal, and there are currently no methods to screen asymptomatic donors, or to detect or inactivate the agents that cause it. Therefore, although the risk is still only hypothetical, it is important to maintain a high degree of suspicion and very close surveillance to protect the blood supply from possible transfusion-transmitted prion disease.