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# Efficacy of viral clearance methods used in the manufacture of activated prothrombin complex concentrates: focus on AUTOPLEX® T

G. HORWITH* and D. R. REVIE†
**Vice President, Clinical Medical Affairs, Nabi, 12280 Wilkins Avenue, Rockville, MD 20852, USA, and †Process Development Manager, Nabi, Immunotherapy Division, 5800 Park of Commerce Blvd NW, Boca Raton, FL 33487, USA*

**Summary**. Various methods are described for the elimination of infectious viruses from activated prothrombin complex concentrates (aPCCs) and for the analysis of the final products (AUTOPLEX® T and FEIBA® VH). Viruses of concern in human plasma-derived products are enveloped (hepatitis B and C, cytomegalovirus, Epstein–Barr virus, and human immunodeficiency virus [HIV]) and nonenveloped (hepatitis A and parvovirus B19). Donated blood used for AUTOPLEX® T is screened for antihepatitis C, HBsAg, anti-HIV types 1 and 2, and p24 antigen. Plasma pools utilized for raw materials are also tested by PCR for HIV and hepatitis C virus. Partial virus inactivation and partitioning are achieved by purification of the aPCC. Further reduction of virus infectivity is accomplished by lyophilization and dry-heat treatment. Each step undergoes virus elimination validation studies in which a relevant sample is 'spiked' with the appropriate virus or model virus. The total reduction in virus from raw material to final product can then be calculated. For AUTOPLEX® T the cumulative $\log_{10}$ reduction factors for several viruses vary from 4.2 to 14.3. This ensures an exceptionally high margin of safety. Definitive evidence for product safety was obtained by clinical observation of treated patients. The viral inactivation process of AUTOPLEX® T involves a four-tier viral safety program, including Cohn alcohol fractionation and dry-heat treatment, in place of the two-stage vapour-heating process for FEIBA®.

**Keywords:** activated prothrombin complex concentrates, AUTOPLEX® T, coagulation factor concentrates, FEIBA®, methodology, viral inactivation.

## Introduction

Optimization of the viral safety of plasma-derived products for the treatment of clotting disorders entails a multimodal approach including donor screening, testing of donated blood for viral markers, partitioning of virus from biologically active proteins during the alcohol fractionation procedure and virus removal or inactivation process steps utilized specifically to enhance product safety. Virus removal and inactivation are important considerations in the manufacture of all blood products. Beyond their ability to clear virus, these processes are selected to minimize the effect on the integrity of active clotting factor proteins. As a result, some virus-kill compromise is accepted in order to avoid complete denaturation of the clotting proteins [1].

Viruses of concern in human plasma-derived products may be divided into two categories: enveloped and nonenveloped. Among the enveloped viruses are hepatitis B virus (HBV), hepatitis C virus (HCV), cytomegalovirus (CMV), Epstein–Barr virus (EBV) and human immunodeficiency virus (HIV). Among the nonenveloped viruses are hepatitis A virus (HAV) and parvovirus B19, the latter being associated with mild or inapparent and self-limited disease.

Virus removal is often accomplished through partitioning of viruses from clotting concentrates with cryoprecipitation or immunoaffinity chromatography, ultrafiltration, or nanofiltration. In contrast, virus inactivation is most often accomplished through physical–chemical means as a result of exposure to alcohol, lyophilization, dry-heat or a steam-heat technique. The efficiency of both these types of mechanisms is dependent on a host of processing parameters. Exposure to alcohol may efficiently inactivate labile enveloped viruses such as HIV but is ineffective for inactivating most nonenveloped viruses. For example, there have been recent reports of hepatitis A transmission [2,3] as well as of parvovirus B19 transmission in patients who have received blood concentrates prepared by methods without a specific step for virus clearance [4]. Dry-heat inactivation of concentrates at temperatures of

Correspondence: Gary Horwith, MD, Vice President, Clinical Medical Affairs, Nabi, 12280 Wilkins Avenue, Rockville, MD 20852, USA. Tel.: +1 301 770 3099; fax: +1 301 770 0155.

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60–68 °C for short periods of time has also been associated with transmission of HIV, HAV and HCV [5,6]. Two cases of acute HCV infection closely related to clotting factor administration were reported from the Bonn Haemophilia Center in 1991 (Case I) and 1989 (Case II) [7]. Case I involved the administration of a total of 7000 units of factor VIII produced by the blood transfusion centre of a German university clinic. The protocol employed in preparing the clinical material included pasteurization at 68 °C for 72 h. Case II involved the administration of pasteurized Haemate HS cryoprecipitate from Behringwerke, Marburg, Germany. Although other routes of infection could not completely be excluded in these two cases, the close temporal conjunction between the administration of clotting factors and the development of acute hepatitis gives rise to suspicion that the risk of transmitting viral infections by clotting factor concentrates is not completely abolished by pasteurization protocols utilizing 72 h or less heat.

In order to improve the efficiency of viral clearance, additional procedures have been implemented such as higher-temperature dry-heat treatment, longer periods of heat treatment, pressurized steam (or vapour) treatment, solvent/detergent or a combination (i.e. multimodal) of techniques. Vapour heating for 10 h has been reported to effectively eliminate infectivity of HAV [8], HBV, HCV and HIV, when analysed by log reduction factors and clinical follow-up [1,9]. Biesert *et al*. have reported laboratory findings which demonstrate the efficacy of a combination approach to decrease virus infectivity. In addition to pasteurization for 10 h at 63 °C, the product was also subjected to alcohol fractionation, which resulted in virus partitioning. These *in vitro* validation studies demonstrated that the manufacturing process provided effective virus inactivation of HIV, herpes virus (HSV-1), HBV and HCV [10].

In contrast to the viruses mentioned above, human parvovirus B19 appears to be particularly difficult to inactivate because of its small size (18–20 nm) and lack of an envelope. In one study, dry-heat treatment of plasma-derived factor concentrates at 100 °C for 30 min had no apparent effect on the viability of human parvovirus [11]. Transmission of parvovirus has also been reported by other investigators who treated patients with both dry- and steam-heated blood products [12].

Activated prothrombin complex concentrates are used in the treatment of haemophiliacs who have developed inhibitors to either factor VIII or IX replacement products. Viral inactivation of the two activated prothrombin complex concentrates used in the United States – AUTOPLEX® T (Nabi, Boca Raton, FL, USA) and FEIBA® VH (Baxter Healthcare, Glendale, CA, USA) – is accomplished by two different processes. The inactivation process for AUTOPLEX® T is accomplished by augmenting the alcohol fractionation used in production with a terminal lyophilization/dry-heat treatment, whereas FEIBA® entails vapour heating. Although the process is not unique, the efficacy and ultimate safety of AUTOPLEX® T's dual viral inactivation technique has been documented by viral clearance studies, the results of which are reported here. Additional assurance of the safety of AUTOPLEX® (marketed from 1979 to 1987) and AUTOPLEX® T (marketed since 1987) is corroborated by the absence of reports of virus transmission during the clinical use of these products for over 20 years.

## Virus clearance of AUTOPLEX® T

Many steps in the production process of plasma-derived products contribute to their ultimate safety, from the initial collection of blood to manufacturing methodology to lot testing and clinical postmarketing surveillance [13]. The safety of blood products is increased when the blood donor pool is refined to eliminate as much potentially infectious material as possible. In an effort to reduce the potential viral load of pooled plasma used in the manufacture of AUTOPLEX® T, blood is collected from paid donors screened for anti-HCV, HBsAg, anti-HIV types 1 and 2, and p24 antigen. As of December 1997, plasma pools utilized as raw materials must test negative for HIV and HCV by polymerase chain reaction (PCR). In addition, donors who are initially demonstrated to have no evidence of hepatitis B infection at any time are immunized against HBV before their blood is collected. Such neutralizing antibodies can be an important barrier against the spread of HBV [14]. The product then undergoes fractionation with 20% cold alcohol utilizing the Cohn–Oncley plasma fractionation process up to Cohn Fraction $IV_1$. The product then undergoes terminal lyophilization and dry-heat treatment for $151 \pm 0.5$ h at $57 \pm 2$ °C, which was found to balance the objective of reducing the risk of viral infectivity with the need to provide a clinically efficacious product similar to unheated products.

Nabi-conducted studies of the Cohn–Oncley cold alcohol fractionation process with virus-spiked cryo-poor plasma indicated that both virus inactivation by alcohol and partitioning are effective in removing HIV-1 and partially effective in removing HAV, bovine virus diarrhoea (BVD) and porcine parvovirus. The effectiveness of the 6-day heating step in reducing viral infectivity was then assessed by additional *in vitro* viral validation studies.

Virus clearance studies of the production process can provide a high level of confidence that specific manufacturing steps enhance the viral safety of the product [15]. The six basic steps involved in the validation studies of AUTOPLEX® T are: (1) scaling down the production process; (2) selecting appropriate viruses based on product raw materials and manufacturing methods; (3) accurately

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assessing cytotoxicity and viral interference; (4) virus 'spiking' at various production steps and measuring the degree of physical removal or inactivation by the manufacturing technique; (5) evaluating the overall log reduction of infectious viral particles across at least two experiments; and (6) the sum of these steps.

Because cell cultivation of certain viruses, such as HCV and HBV, is not yet possible *in vitro*, it is necessary to use model viruses that are similar in specific characteristics to demonstrate virus clearance (Table 1). Notwithstanding the fact that model viruses may exhibit subtle differences from the pathogenic viruses they represent, the Committee for Proprietary Medicinal Products (CPMP) issued suggested guidelines for the use of model viruses for the purposes of virus clearance studies [16]. As defined by the guidelines, the objective of validation is to estimate quantitatively the overall level of virus reduction at various stages of purification or virus inactivation. This is achieved by 'spiking' viruses to aliquots of the crude material and fractions obtained during the purification stages to measure virus removal or inactivation. These CPMP guidelines were used in the following virus validation studies of the AUTOPLEX® T manufacturing process.

## Virus validation methods

AUTOPLEX® T bulk was a Fraction $IV_1$ precipitate prepared by a combination of precipitation and therapeutic moiety activation steps. The bulk was sterile filtered, aseptically filled and lyophilized. The individual bottles containing AUTOPLEX® T were subjected to a heat-treatment cycle for 144–153 h at 60.2–60.9 °C. The goal of these studies was to evaluate the effectiveness of lyophilization and the heat-treatment step in inactivating viruses.

The Anti-Inhibitor Coagulant Complex bulk was spiked with the viruses listed in Table 1 prior to lyophilization and heat-treatment in order to mimic conditions of the manufacturing process. During the course of the lyophilization and heat-treatment, samples were collected and assayed for viruses (Fig. 1).

The subsequent $\log_{10}$ reductions in virus titres achieved by Cohn–Oncley fractionation and heat-inactivation are shown in Table 1. The virus-inactivation is most robust for enveloped viruses, with a 5–21 $\log_{10}$ reduction in infectivity. Nonenveloped viruses are, nonetheless, partially inactivated with a 4–12 $\log_{10}$ reduction in

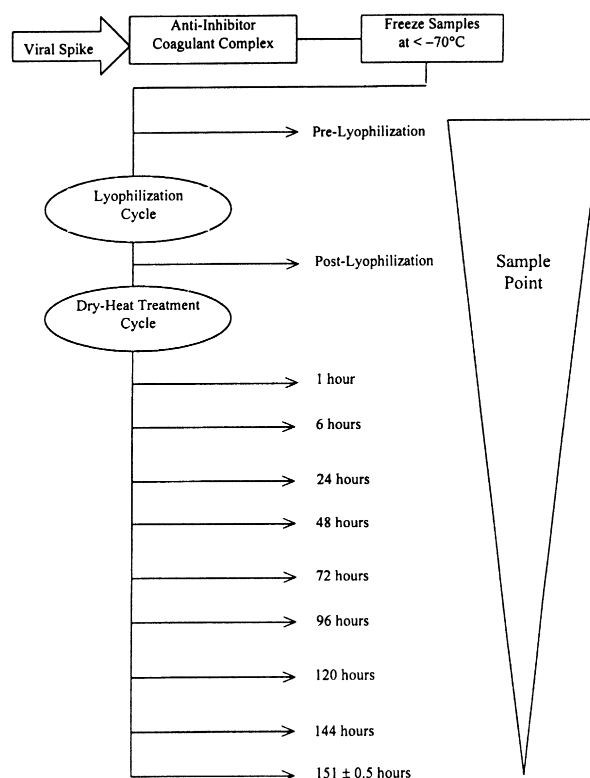


**Fig. 1.** Lyophilization/heat-treatment process for AUTOPLEX® T.

**Table 1.** Results of viral inactivation studies of AUTOPLEX® T

| Virus group & virus | Model for | Reduction ($\log_{10}$) with Cohn–Oncley fractionation | Reduction ($\log_{10}$) with heat treatment | Cumulative ($\log_{10}$) reduction |
|---|---|---|---|---|
| Enveloped | | | | |
| Sindbis | HCV | 4.8 | 5.0 | 9.8 |
| BVD | HCV | 1.2 | 4.3 | 5.5 |
| PRV* | HBV/herpes* | 4.6 | 3.4 | 8.0 |
| HIV | HIV | 8.2 | 6.1 | 14.3 |
| Nonenveloped | | | | |
| EMC | HAV | 5.1 | 7.1 | 12.2 |
| HAV | HAV | 1.9 | 2.3 | 4.2 |

BVD = bovine virus diarrhoea; EMC = encephalomyocarditis virus; HAV = hepatitis A virus; HBV = hepatitis B virus; HCV = hepatitis C virus; HIV = human immunodeficiency virus; PRV = pseudorabies virus. *HBV and PRV are in different families; however, PRV is sometimes considered a model for HBV because both are enveloped DNA viruses. Data on file at Nabi.

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infectivity. These findings ensure an exceptionally high margin of safety.

## Postmarketing surveillance of AUTOPLEX® T

Although the efficacy of virus inactivation is validated by laboratory means, confidence in the virological safety of a plasma product ultimately revolves around clinical observation of treated patients. Postmarketing surveillance of patients receiving an estimated 11 924 infusions (a mean of 5250 FECUs per infusion) of heat-treated AUTOPLEX® T during the 5-year period 1993–1997 supports the safety of the product by the absence of reported cases of any virus transmission including HIV, HAV, HBV and HCV.

## Virus inactivation of FEIBA®

The virus-inactivation process of FEIBA® is a two-stage vapour-heating process known as STIM-4 involving the heating of bulk FEIBA® at 60 °C for 10 h at a pressure of 1190 mbar followed by 1 h at 80 °C at a pressure of 1375 mbar [1]. Virus-inactivation studies entail viral spiking with infectious units of HIV and model virus subjected to vapour-heat treatment. In these tests, the $\log_{10}$ reduction factor for HIV was 10.9, that for tickborne encephalitis virus (model for ss RNA viruses 40–60 nm) was $> 12.4$, that for pseudorabies virus (model for HBV) was $> 11.5$ and equine rhinopneumonitis virus (model for HAV) was $> 11.0$ [1]. Data are limited on the safety of FEIBA® in patients who are naive to blood product administration, since it is primarily used in inhibitor patients who have been exposed to other factor concentrates. There is no published report of transmission of HAV, HBV, HCV or HIV resulting from FEIBA® use.

## Discussion

The fractionation process originally used in the manufacture of AUTOPLEX® (the name was later changed to AUTOPLEX® T with the addition of thermal treatment) involved exposure of cryoprecipitate-poor plasma to 20% alcohol for at least 10 h. Even though it was unheated, AUTOPLEX® was found to have very low or no potential for transmission of HIV [17,18]. The efficacy of alcohol alone in removing virus from cryoprecipitate-poor plasma has been well established [18,19]. For example, in 1986, Gazengel and Larrieu reported that in a retrospective study conducted with patients who had received unheated AUTOPLEX® exclusively, none of the patients became positive for LAV/HTLV III [17]. The results of the virus inactivation due to the two-step process (Cohn–Oncley fractionation and dry-heat treatment), as well as continuing postmarketing surveillance studies, provide confidence in the extremely low likelihood of transmission of HIV, HAV, HBV or HCV with AUTOPLEX® T.

Although parvovirus B19 (PVB19) is generally associated with inapparent, mild or self-limited disease, there has been a report of life-threatening pancytopenia and septicaemia in an immunocompetent adult with PVB19 infection [20]. PVB19 poses some unique challenges because of its size ($\approx$ 20 nm) and extraordinary resistance to heat and chemical treatment, as well as its difficulty in removal by nanofiltration [21]. Nonetheless, increased awareness of PVB19 as a pathogen may warrant, in the future, additional virus inactivation steps directed against this pathogen.

Evaluation of virus inactivation involves a complex methodology. There is some suggestion that a large-volume assessment method as an enhancement of conventional titration methods (i.e. plaque assay and HIV syncytia formation assay) might improve detection of low levels of virus and thus increase the degree of product safety, particularly of those products relying on a single inactivation process [22].

## Conclusion

Overall, the combination of donor screening, fractionation by Cohn–Oncley alcohol precipitation, lyophilization, heat-treatment and supplemental PCR testing for HIV and HCV used in the manufacture of AUTOPLEX® T optimizes the safety of this product while maintaining functional activity. The specific manufacturing steps used in AUTOPLEX® T provide a high level of confidence of virus clearance. This is further corroborated by the absence of reports of virus transmission at any time in over 20 years of clinical use.

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