**Blood Protein Derivative Viral Safety: Observations and Analysis**

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The well-documented viral safety of albumin arises from several factors operating in concert, including virus removal during preparation, immune neutralization, serendipitous inactivation, virus sterilization through pasteurization. Safety with respect to HBV transmission was achieved even prior to the development of sensitive screening tests for HBsAg, as can be predicted given the initial virus load and the influence of factors affecting removal and inactivation. Coagulation factor concentrates, as traditionally prepared, are known to have transmitted the viral agents of hepatitis and AIDS with high frequency. Application of virucidal procedures to these concentrates, in some cases, appears to have eliminated virus transmission, raising the question as to whether absolute safety has now been achieved. Clinical proof of absolute safety is made difficult by the small number of eligible patients who can be monitored, lengthy and expensive monitoring procedures, and opportunity for transmission of virus by product-independent routes. Based on viral load analysis, modern coagulation factor concentrates are predicted to have the same probability of freedom from HIV, HBV, and HCV transmission as that exhibited by albumin.

**INTRODUCTION**

A number of articles reviewing the safety of blood derivatives have appeared recently [1–5]. For the most part, these have given an overview of the various methods currently employed to inactivate viruses together with a comparison of their effectiveness, based on animal and clinical study results. This paper will examine the issue from a different perspective, one that is more theoretical and statistical, in which predicted rates of infectivity of various products for blood-borne viruses are calculated based on information concerning viral loads and compared with the actual published incidence data. By doing so, it is hoped that we will be better able to judge whether the current generation of coagulation factor concentrates are truly safe with regard to the transmission of human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV; formerly non-A, non-B hepatitis virus), the viruses of principal concern in blood protein derivatives.

**ALBUMIN SAFETY**

As a consequence of pasteurization, combined with methods of purification which remove virus, albumin solutions have achieved an admirable record of virus safety [6]. The decision to prescribe and infuse albumin solutions today rests solely on issues of

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*Abbreviations: AIDS: acquired immunodeficiency syndrome  FFP: fresh frozen plasma  HBsAg: hepatitis B surface antigen  HBV: hepatitis B virus  HCV: hepatitis C virus  HIV: human immunodeficiency virus  ID50: the amount of virus causing infection in 50 percent of cases  NANBH: non-A, non-B hepatitis virus*

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medical need and cost, and not on issues of safety, which is as it should be. Pasteurized albumin is considered to be so innocuous that it is frequently used as the placebo in clinical trials of other protein solutions, and as a formulation aid. Understanding why albumin is as safe as it is will place into context the new methods of virus inactivation applicable to far more labile molecules than albumin.

Analysis of virus safety requires an appreciation of the initial viral load, the extent to which virus is removed during processing, serendipitous inactivation which occurs during processing and/or storage, the efficacy of the inactivation method employed, and host susceptibility factors. Provided we are willing to accept a degree of uncertainty, an estimate of each for HBV is provided in Table 1 for susceptible hosts [1,7-12]. Prior to implementation of screening assays for HBsAg, both the proportion of units containing infectious HBV and the titer of HBV contained in infectious units was substantially higher than that observed today. Under this circumstance, the probability that a vial of albumin would contain infectious HBV is calculated at 3 \times 10^{-6}. Phrased differently, one vial per 300,000 is predicted to contain infectious HBV. This calculation is in good agreement with the occasional, but rare, outbreak of hepatitis B arising from albumin prepared from unscreened donors. With inclusion of HBsAg screening, the number of vials/ID_{50} exceeds 10^9. This corresponds to the well-documented belief that albumin is safe [6].

While this analysis of safety may appear to be esoteric, it should be accepted with the same confidence as is recognized when assessing methods of bacterial sterilization, such as by autoclaving, sterile filtration, ultraviolet irradiation, and the like. In the case of albumin manufacture and HBV transmission, it is important to understand that albumin's large margin of safety arises from a combination of factors operating in concert (Fig. 1). The safety margin is sufficiently high that the U.S. Food and Drug Administration does not generally demand a product recall following the accidental inclusion of plasma positive for HBsAg into a plasma pool.

SAFETY OF COAGULATION FACTOR CONCENTRATES

Traditionally Manufactured Concentrates

Coagulation factor concentrates, as traditionally prepared, transmit HIV and HCV [6,14-16]. Based on a combination of published evidence and assumptions concerning HCV properties during fractionation, a viral load analysis similar to that provided for albumin can be made.

The total quantity of HIV, HBV, and HCV in a plasma pool used in the manufacture of a traditional AHF concentrate is estimated at 2 \times 10^3, 3 \times 10^5, and 4 \times 10^7 infectious doses (ID_{50}), respectively (Table 2) [3,6,14,18,20-28]. For HIV, cryoprecipitation and aluminum hydroxide adsorption have been reported to remove 10^3 ID_{50}, and lyophilization results in the inactivation of 10^1 ID_{50} [29]. Consequently, on filling, one out of four vials would be expected to contain infectious HIV. A similar calculation performed for both HBV and HCV predicts that 30,000 vials would contain one ID_{50} of HBV and 1/0.03 vials (i.e., 40 ID_{50}/vial) would contain infectious HCV. Based on these estimates, a hemophiliac treated for one year with 80,000 units of coagulant in the form of these concentrates would have a probability of being exposed to HIV, HBV, and HCV of, respectively, 100 percent, 0.3 percent, and 100 percent. Actual transmission rates are in accord with these calculated values [6,14,15,17-19]. Of interest, as
compared with a hemophiliac treated with the same amount of AHF in the form of single-donor cryoprecipitates, a hemophiliac treated with concentrate had a fourfold higher chance of exposure to HIV, a ninetyfold lower chance of exposure to HBV (as a consequence of antibody neutralization on pooling many units of plasma), and the exposure to HCV was virtually certain for each. Clinical experience is in line with these predictions [15–17].
Modern Coagulation Factor Concentrates

Laboratory and preclinical validation of virucidal methods indicate a wide variance in their effectiveness; some methods inactivate $<10^4$ ID$_{50}$ of HIV, HBV, and/or HCV (Table 3A), and other methods inactivate the highest levels of challenge available, $10^6$-$10^{11}$ ID$_{50}$ for HIV, $10^6$ ID$_{50}$ for HBV, and $10^5$ ID$_{50}$ for HCV (Table 3B). With 200 ID$_{50}$ of HIV and 0.03 ID$_{50}$ of HBV remaining in the fractionated pool (Table 2, line 7) application of even a modestly effective virucidal procedure, e.g., one that inactivates $10^3$ ID$_{50}$, is calculated to provide rates of infectivity which approach zero. This rate compares well with the observed transmission rates; 2/157 and 0/35 carefully monitored patients were infected with HIV and HBV, respectively (Table 3A). Application of the same virucidal procedure to the quantity of HCV estimated to be present, 40,000 ID$_{50}$, is not, however, sufficient to yield an HCV-safe product. This prediction is borne out by the observation that 29/67 of patients developed signs of HCV infection during the course of several months of treatment (Table 3A). In contrast, highly virucidal methods, defined as those shown or likely to inactivate $>10^5$ ID$_{50}$ of HIV, HBV, and HCV (Table 3B), are predicted to provide a product found to be safe in clinical transmission studies, and such has been the case. HBV and HCV transmission has occurred rarely and, where observed, may well represent transmission by community vectors and not by product.

What, then, is the predicted viral safety of modern coagulation factor concentrates?

Adoption of the solvent/detergent inactivation method [30,31] or another similarly potent virucidal procedure, when coupled with improvements in donor screening and product purification, is calculated to increase the vials/ID$_{50}$ to $>10^{16}$ for HIV, $>10^{13}$ for HBV, and to $>10^6$ for HCV (Table 4). This rate compares favorably with the predicted HBV safety of albumin (Fig. 2). Furthermore, it should be remembered that the values for vials/ID$_{50}$ in AHF concentrates are minimums, since, in the validation studies, virus kill was complete to the extent of challenge. The potential for virus kill can be substantially higher.

CONCLUSION

Substantial progress has been made toward eliminating virus transmission by blood derivatives. Albumin has long been considered a virally safe product, and clinical
TABLE 2

Viral Load of Traditional AHF Concentrates*

| Line | HIV       | HBV       | HCV       |
|------|-----------|-----------|-----------|
| 1    | Number of units/batch | 24,000    | 24,000    | 24,000    |
| 2    | % with virus | 0.04      | 0.05      | 2.00      |
| 3    | Virus in infectious unit (ID$_{50}$/mL) | $1 \times 10^3$ | $1 \times 10^3$ | $3 \times 10^2$ |
| 4    | Virus in pool (ID$_{50}$) | $2 \times 10^3$ | $3 \times 10^3$ | $4 \times 10^3$ |
| 5    | Removal during processing | $1 \times 10^1$ | $1 \times 10^2$ | $1 \times 10^2$ |
| 6    | Inactivation during processing | $1 \times 10^1$ | $1 \times 10^2$ | $1 \times 10^2$ |
| 7    | Residual virus in pool (ID$_{50}$) | $2 \times 10^2$ | $3 \times 10^{-2}$ | $4 \times 10^4$ |
| 8    | Vials filled | 900       | 900       | 900       |
| 9    | ID$_{50}$/vial | $3 \times 10^{-1}$ | $3 \times 10^{-3}$ | 40        |
| 10   | Vials/ID$_{50}$ | 4         | 30,000    | 0.03      |
| 11   | Annual probability of exposure | 1.0       | 0.003     | 1.00      |
| 12   | Annual probability of exposure with single donor cryoprecipitate | 0.23      | 0.27      | 1.00      |

*Prior to HIV and HCV antibody tests, virus sterilization, and extensive purification

Line 2: HIV—Of 1,027,786 units of donated blood tested following licensure of HIV antibody screening, 333 were western-blot positive, or 0.038 percent [40]. In addition, it has been estimated that from 1/40,000–1/250,000 (0.0004 percent–0.0025 percent) anti-HIV-negative donor bloods are infectious for HIV [18,21,41,42].

HBV—Refer to Table 1.

HCV—Reported incidence of hepatitis transmission with HBsAg-negative blood varies from 5.9–21 percent of recipients [21, a summary]. On a per-unit basis, the risk varied from 0.5 to 2.9 percent.

Line 3: HIV—The mean titer of HIV in filtered plasma from asymptomatic patients and in AIDS patients was reported to be $10^{1.4}$ and $10^{2.5}$ TCID$_{50}$/mL, respectively [43]. In a more recent study, HIV titers in plasma for patients with asymptomatic infections, AIDS, or ARC were, respectively, 30, 3,500, and 3,200 TCID$_{50}$/mL [25]. Viral titers of infectious plasma placed into fractionation might be higher than these values as a result of leukocyte contamination. For asymptomatic patients, 20 per million peripheral blood mononuclear cells had isolatable virus [25].

HBV—Refer to Table 1.

HCV—Chronic-phase plasma has been reported to contain infectious titers of $10^1$–$10^3$/mL [28]. Acute-phase plasma can contain $10^6$ ID$_{50}$/mL, but this has been rare [3].

Line 4: Line 1 x 250 mL/unit x line 2 x line 3

Line 5: 95 percent of HIV antigen added to plasma was found in cryoprecipitate, an intermediate fraction for AHF processing [44]. Aluminum hydroxide adsorption and subsequent purification by precipitation have been reported to remove/inactivate approximately 1 log each of other retroviruses [29].

Cryoprecipitation removes 1.9 log$_{10}$ of HBsAg [39].

The fractionation characteristics of HCV are unknown at this time.

Line 6: Serendipitous inactivation during processing is uncertain. Lyophilization inactivates approximately 1 log$_{10}$ of mouse retrovirus [29], and this finding is confirmed by our experience with HIV.

Considerable HBV is neutralized by excess anti-HBs [12], found in virtually every lot of final product. Serendipitous inactivation of HCV is estimated.

Line 7: Line 4 + (line 5 x line 6)

Line 8: Assumes recovery of 150 U/L and 1,000 U/vial

Line 9: Line 7 + line 8

Line 10: 1 + line 9

Line 11: Probability of infection = 1 – [ (1 – risk per vial) number of vials ]; assumes 80,000 units infused per year

Line 12: Assumes 125 units per cryoprecipitate and same assumptions as given for line 11
TABLE 3A
Low Virucidal Efficacy

| Procedure                                          | Validation (Log_{10} Kill) | Clinical Safety (Infected/Total) |
|----------------------------------------------------|----------------------------|---------------------------------|
|                                                    | HIV | HBV | HCV | HIV | HBV | HCV |
| Heated at 60° for 30 hours as lyophilizate         | 2   | <3.5| >3.5| 2/90| 0/2 | 2/2 |
| Heated at 60° for 72 hours as lyophilizate         | ~1  | <2.5| endog| 2/24| 0/12| 15/19|
| Heated at 68° for 72 hours as lyophilizate         | >2.8| NA  | >3.4| 0/6 | —   | 1/6 |
| Heated at 68° for 20 hours as lyophilizate, + heptane| >3.2| >2.7 to 4.0| 2.7 to <3.5| 0/37| 0/18| 8/37|
| Heated at 68° for 72 hours as lyophilizate, + chloroform| NA  | NA  | NA  | —   | 0/3 | 3/3 |

TABLE 3B
High Virucidal Efficacy

| Procedure                                          | Validation (Log_{10} Kill) | Clinical Safety (Infected/Total) |
|----------------------------------------------------|----------------------------|---------------------------------|
|                                                    | HIV | HBV | HCV | HIV | HBV | HCV |
| Heated at 80° for 72 hours and 80°C for one hour as lyophilizate| NA  | NA  | NA  | 0/32| 0/32| 0/32|
| Heated at 60° for 72 hours as lyophilizate, + vapor| >6.0| NA  | NA  | 0/109| 4/53| 0/58|
| Heated at 60° for ten hours in solution, + sucrose, glycine| >5.0| >5.6| >5.5| 0/237| 2/68| 2/95|
| Heated at 60° for 30 hours as lyophilizate, + high purity| >3.0| >5.5| >3.0| 0/19 | — | 0/19|
| TNBP/Detergent treated                              | >10.0| >6.0| >5.0| 0/200| 0/21| 0/106|
| TNBP/Detergent treated, + high purity              | >8.0| est | est | est | — | — |
| Beta-Propiolactone + UV                            | >14.0| est | est | 0/45 | — | 0/29|
|                                                    | >6.0| 6.9 | >4.5| 0/6 | 0/11| 0/11|

See Prince et al. [3] for most references. A more recent review [51] will also be available.

NA, not available; endog, endogenous

experience corresponds well with predicted rates of infectivity based on the known viral load and studies quantitating virus removal/inactivation during processing. During the past decade, no fewer than 12 different approaches directed toward improving the viral safety of coagulation factor concentrates reached the stage where product was placed into clinical evaluation. As a consequence, extensive information is available to permit a good estimate of initial viral loads and of the influence of factors affecting removal and inactivation. As shown in the accompanying analysis, there is a close correspondence between clinical experience related to the transmission of HIV, HBV, and HCV and the predicted probability of safety, thus increasing confidence in the predictions made. This confidence is especially important for HCV, because data are not available about its fractionation characteristics and assumptions had to be made.

Thus, through the development of improved donor and donor blood screening procedures, improved virus sterilization technology, and, in some cases, vigorous purification methodology, the calculated margin of safety for the best of the current
**TABLE 4**

Viral Load of Modern AHF Concentrates

| Line | HIV | HBV | HCV |
|------|-----|-----|-----|
| 1    | Vials/ID$_{50}$ | 4   | 30,000 | 0.03 |
| 2    | Inactivation by solvent/detergent | $1 \times 10^{11}$ | $1 \times 10^6$ | $1 \times 10^3$ |
| 3    | Improved donor select/screen | $4.0 \times 10^1$ | 5.0 | $1.5 \times 10^1$ |
| 4    | Removal during added purification | $1 \times 10^3$ | $1 \times 10^2$ | $1 \times 10^2$ |
| 5    | Final vials/ID$_{50}$ | $2 \times 10^{14}$ | $2 \times 10^{13}$ | $4 \times 10^4$ |
| 6    | Annual probability of exposure | $9 \times 10^{-15}$ | $5 \times 10^{-12}$ | $2 \times 10^{-5}$ |

*Following HIV antibody screening, virus inactivation by solvent/detergent or other similarly potent virucidal procedure, and immune affinity or other method of extensive purification.

Line 1: From Table 2

Line 2: Piszkiewicz et al. have demonstrated the inactivation of $\geq 10^{11}$ TCID$_{50}$ of HIV on treatment by solvent/detergent [34]. Virus kill is essentially instantaneous. In the chimpanzee model, we have shown the inactivation of $\geq 10^6$ CID$_{50}$ of HBV and $\geq 10^5$ CID$_{50}$ of HCV [45,46, unpublished results].

Line 3: HIV risk diminished as a consequence of donor screening from 40/100,000 to 0.4–2.5 per 100,000 (see line 2, Table 2). The overall improvement in donor selection, because of public education, donor questionnaires, and new screening tests must also reduce HBV viral load; fivefold is strictly an estimate. A combination of ALT and anti-HCV testing was predicted to decrease NANBH transmission by 60 percent [47]. When AHF is prepared from anti-HCV screened plasma, a further fivefold reduction might be expected.

HCV viral load is reduced by a combination of factors: institution of ALT and anti-HCV testing should reduce the per-unit risk by 60 percent [47], anti-HCV testing should cause (when performed) a reduction of 80 percent [32], and a further improvement should occur with improvements in donor selection and a decrease in virus titer associated with remaining infectious units.

Line 4: Highly purified concentrates are now beginning to appear. Enhanced purification has been reported to remove up to $10^4$ HIV [34], though lesser removal may be seen with other viruses, other methods, or in a production setting.

Line 5: Line 1 + (line 2 x line 3 x line 4)

Line 6: Assumes 80,000 units infused per year

coagulation factor concentrates approaches and may exceed that calculated for albumin. If correct, concerns of viral risk, at least for HIV, HBV, and HCV, should no longer hinder the clinical use of blood derivatives. This experience encourages the preparation of virally sterilized forms of the traditional single donor products, fresh

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frozen plasma (FFP) and cryoprecipitate, even if prepared from plasma pools. With the viral safety of the blood protein derivatives seemingly in hand, the final challenge in providing safe blood products will be the development of procedures to remove or inactivate virus from the cellular components.

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