Viral Inactivation of Blood Products: A General Overview

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Introduction

In the early 1970’s in the United States, approximately 10% of recipients of single donor blood products, i.e. Whole Blood, Red Blood Cell Concentrates (RBCC), Platelet Concentrates (PC), and Fresh Frozen Plasma (FFP), inadvertently were exposed to HBV or HCV. In the early 1980’s, as many as one in 1,000 recipients of these same products was exposed to HIV. Seventy percent of severe hemophiliacs in the United States have been infected with human immunodeficiency virus (HIV), which contaminated the coagulation factor concentrates they used in their treatment largely between the years 1979 and 1985. World-wide, most severe hemophiliacs are infected with hepatitis B virus (HBV) and/or hepatitis C virus (HCV). The emergence of HIV in the general population served to mobilize action at all levels of government, industry and academia. Through scientific inquiry and understanding, creativity, and perseverance, each of the risks described above has been reduced substantially. The risk of HIV, HBV or HCV exposure from a single donor blood product is estimated at 1/225,000, 1/200,000, and 1/3,300 to 1/6,000, respectively, per unit infused. Coagulation factor concentrates prepared from plasma pools no longer transmit HIV and seldom transmit HBV or HCV. Had those of us charged with the safety and provision of blood to hospitals and physicians not responded adequately to the presence of HIV in donor blood, our industry would be decimated today. To the extent we were slow to respond, or perceived to have responded slowly, we suffer the consequences. It is the perception amongst many that it did not have to happen, and that inaction was the result of an insular community and, in some cases, avarice. It is the perception that government was a co-conspirator of inaction. Importantly, the public perceives that the dangers of the 1980’s continue today. Since patient concern has never been higher, the medical community must know the facts as distinguished from the perceptions and must act to regain the public’s confidence.

Over the past decade, blood banking and blood processing procedures and the practice of transfusion medicine have changed substantially. Today, we are more aware of the dangers of blood transfusion and of steps to reduce if not eliminate these dangers. Blood donors are examined and questioned more closely than ever before in an attempt to eliminate donors who are more likely to harbor an infectious, blood-borne virus. Every donation is tested by new and more sensitive blood tests, and in some cases, blood screening tests are introduced even before benefit is established. Donor histories and test results have been computerized, and the error prone manual transcription of critical information is being eliminated. Manufactured blood products are more highly purified than ever before, and purification procedures have been modified to more consistently reduce viral load. Virus inactivation technology is in wide-spread use in the preparation of coagulation factor concentrates, and validated virus inactivation methods are beginning to be applied to all blood protein solutions including immune globulins and FFP. One could not fathom introduction of a new blood protein product today if it was not virally inactivated. Regulatory oversight has increased and standards common in the pharmaceutical industry are beginning to be applied to blood. Where feasible,
autologous transfusions have replaced homologous, and to eliminate unnecessary blood exposure, hospital use committees regularly examine transfusion practice.

While it has and continues to be a challenging and difficult time, we each have much to be proud of and can take some comfort in the statement that “blood has never been safer”.

Nonetheless, our work needs to continue. The public has demanded the safest possible blood supply and we should do everything in our power to achieve it. To do otherwise will result in continued criticism, law suits, and inquiries into blood banking practices. Moreover, patient distrust can lead to donor distrust, eroding the underpinning of voluntary blood donor systems.

With respect to viral safety, the data are clear—the only way to achieve absolute safety is through viral inactivation, and numerous advantages accrue on adoption of virus inactivation processes. The window period will no longer be of concern, errors in testing or the inadvertent release of a blood unit which tests positive no longer will result in viral transmission, new viruses or new viral serotypes will be eliminated even before their presence is recognized, and tests for rare viruses need not be deployed.

**Viral Inactivation of Coagulation Factor Concentrates**

Nowhere can the value of virus inactivation be illustrated better than in the preparation of coagulation factor concentrates. Antihemophilic factor concentrate (AHF) and prothrombin complex concentrate manufactured without viral inactivation transmitted HIV, HBV, and HCV with high frequency\(^{12}-^{15}\). As late as 1985, essentially every vial of these concentrates were contaminated by HCV. With the advent of viral inactivation, HIV transmission was virtually eliminated. For example, in the United States there has not been a single documented case of HIV transmission associated with concentrate infusion since 1987\(^{4}\). While some of the first methods of viral inactivation failed to eliminate HBV and HCV transmission, currently employed methods have excellent records of safety also with respect to these viruses\(^{16}-^{21}\). Focussing on carefully constructed clinical studies in previously untreated patients, hepatitis has not been transmitted by products which have been pasteurized, vapor heated, dry heated at 80°C for 72 hours, or treated with solvent/detergent (Table 1)\(^{22}\). Substantial community use information supports a conclusion of safety. For example, with solvent/detergent treated products, used in the preparation of approximately two thirds of the world’s plasma derived coagulation factor concentrates, over 7 million doses have been infused without a single documented case of HIV, HBV, or HCV transmission (Table 2)\(^{23}\). While several reports indicate transmission of HBV or HCV with pasteurized\(^{24}\) or vapor heated product\(^{25}\), reports of transmission are rare and may be consistent with community and not product sources of virus. Based on probability determinations where viral load is compared with documented viral elimination, modern AHF concentrates prepared with solvent/detergent have a high probability of safety, calculated as less than one infectious unit of HIV, HBV, or HCV per \(10^{18}\), \(10^{12}\), and \(10^{8}\) vials of product, respectively\(^{26}\).

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Virucidal method</th>
<th>No. with hepatitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Behringwerke</td>
<td>Pasteurization</td>
<td>0/153</td>
</tr>
<tr>
<td>Immuno</td>
<td>Vapor heating</td>
<td>0/50</td>
</tr>
<tr>
<td>BPL</td>
<td>Dry heat, 80°C</td>
<td>0/38</td>
</tr>
<tr>
<td>NYBC, Biotransfusion, Aima,</td>
<td>Solvent/detergent</td>
<td>0/117</td>
</tr>
<tr>
<td>Baxter, Centro de</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hematologia Santa Catarina</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

adapted from Mannucci, 1993
The commonly employed viral elimination procedures are provided in Table 3, and each has distinctive features. Solvent/detergent acts by disrupting the viral lipid envelop, and a 12 year history of safety with respect to enveloped viruses supports its use. Virus kill is rapid and complete (Fig. 1). Because action is directed toward lipids, non-enveloped viruses and proteins (except for lipoproteins) are unaffected, and S/D can be applied equally and predictably with high recovery to complex mixtures such as plasma and to highly purified protein solutions. Safety with respect to HBV, HCV and HIV is supported by 13 independently run clinical trials, and by the preparation of HIVIG, a hyperimmune gamma globulin to the AIDS virus, prepared from donors, each of whom is infected with HIV.

Methods of thermal inactivation are advantageous in that all classes of virus are potentially susceptible, although non-enveloped viruses tend to be heat stable. Because thermal inactivation methods are not inherently specific, means of stabilizing proteins while achieving excellent virus inactivation had to be identified. With pasteurization, proteins are stabilized by addition of high concentrations of low molecular weight solutes, especially sugars and amino acids\(^{27-29}\). The rate of kill of selected viruses is given in Fig. 2. Although viruses are also stabilized, relatively good discrimination can be achieved, although at some cost in protein recovery\(^{30}\). Using the duck HBV as a model, S/D-treatment is more effective than pasteurization at killing HBV (Fig. 3)\(^{31}\). Additionally, many non-enveloped viruses, are also heat resistant. With dry heat, proteins are stabilized by reducing moisture content and process recovery can be high\(^{32}\). Unfortunately, lyophilized cakes are heterogeneous with respect to solute and moisture content both within the vial and between vials. Reproduc-

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**Table 2** SD-treated Product Usage 1985-March, 1994

<table>
<thead>
<tr>
<th>Product</th>
<th>Units</th>
<th>Doses (approx)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F VII</td>
<td>1.9MU</td>
<td>1,900</td>
</tr>
<tr>
<td>F VIIa</td>
<td>2.6MU</td>
<td>2,600</td>
</tr>
<tr>
<td>F VIII</td>
<td>6,085MU</td>
<td>6,085,000</td>
</tr>
<tr>
<td>F IX</td>
<td>333MU</td>
<td>333,000</td>
</tr>
<tr>
<td>Prothrombin complex</td>
<td>113MU</td>
<td>105,667</td>
</tr>
<tr>
<td>Fibrin glue</td>
<td>325,930mL</td>
<td>65,186</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>93,300g</td>
<td>23,300</td>
</tr>
<tr>
<td>IMIG &amp; IVIG</td>
<td>1,266,245g</td>
<td>253,249</td>
</tr>
<tr>
<td>MAB IgM</td>
<td>2,697vials</td>
<td>2,697</td>
</tr>
<tr>
<td>Anti-D IgG</td>
<td>83,702vials</td>
<td>83,702</td>
</tr>
<tr>
<td>Plasma</td>
<td>789,479units</td>
<td>197,400</td>
</tr>
<tr>
<td><strong>Sum</strong></td>
<td></td>
<td><strong>7,173,701</strong></td>
</tr>
</tbody>
</table>

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**Table 3** Commonly Employed Methods of Virus Elimination

- Heat (pasteurization, dry heat, vapor)
- Solvent/detergent
- Beta-propiolactone/UV
- Acid
- NaSCN
- Filtration
- Extensive purification
- Combinations

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**Fig. 1** Solvent/Detergent Treatment of AHF
ible viral safety required that products be reformulated, use of 80°C in place of 60°C, and with vapor heating, introduction of additional moisture. A particular advantage of the dry heat method is that it can be performed on product in the final container, eliminating the possibility of post-treatment recontamination. With all other methods, re-contamination is prevented by separating pre- and post-virus inactivation areas, equipment, and personnel. Nonetheless, despite these differences, each method has eliminated HIV transmission by pooled plasma products, and HBV and HCV transmission has either been eliminated or greatly reduced.

More recently, the apparent transmission of hepatitis A virus (HAV) by an ion exchange purified, solvent/detergent treated AHF concentrate in several European countries raised concerns about non-enveloped viruses, first because they are not inactivated by solvent/detergent treatment and second because they tend to be heat stable. Viruses in this class include HAV and parvovirus B19. The general characteristics of these viruses are provided in Table 4. Consequently, manufacturers are examining newer viral elimination procedures in combination with established virucidal procedures. The advantage of combining methods which act by independent mechanisms is that both a broader spectrum and a higher quantity of viruses can be eliminated. As examples, antibody affinity purification validated as a virus removal method has been combined with either solvent/detergent or heat treatment, some products are now treated by both solvent/detergent and heat, other products have been processed through viral removal filters which have been developed recently and added to existing processes. New methods of virus inactivation under exploration include use of chaotropes such as sodium thiocyanate, short wavelength ultraviolet light in the presence of antioxidants, microwave heating, extraction with supercritical fluids, and of iodine. Given the extensive history of safety with respect to the principal viruses of concern achieved by currently employed methods and the limitations of laboratory and pre-clinical virus validation studies, the addition to rather than the replacement of existing processes seems likely. As an example, research...
at the New York Blood Center has shown that HAV and parvovirus are efficiently killed by UVC irradiation and that the presence of rutin had little or no effect on virus kill (Fig. 4). In contrast, the presence of rutin was important to protein recovery. On combining solvent/detergent with UVC irradiation, a wide variety of viruses including HBV, HCV, HIV, HAV, and parvovirus are killed (Table 5). Similar results were obtained when solvent/detergent was combined with filtration using a filter with 15 nm pore size. Unfortunately, 35 nm filters do not block passage of parvovirus and 15 nm filters cannot be used efficiently with large protein molecules or high protein concentration

**Other Purified Blood Protein Derivatives**

The success in applying viral inactivation methods to coagulation factor concentrates raises the question as to whether all blood protein products should be virally inactivated. This question is especially pertinent to immune globulin solutions which generally have a history of safety without use of a specific virus inactivation method. Recently, one intravenous immune globulin preparation was recalled world-wide because of a clear association with HCV transmission to over 100 recipients. Careful study of the fractionation characteristics of HCV suggests that this outbreak was caused by the recent adoption of generation 2 HCV antibody screening since the reduced level of anti-HCV antibody in the plasma pool resulted in more virus co-isolating with IgG during cold ethanol fractionation. It also seems probable that the lower titer of anti-HCV antibody in the final product reduced immune neutralization. Consequently, the United States FDA has recommended that all

![Image of inactivation graphs showing HAV and Parvovirus](image-url)

**Table 5 Combination of SD and UVC**

<table>
<thead>
<tr>
<th>Virus</th>
<th>SD (log 10)</th>
<th>UVC (log 10)</th>
<th>Sum (log 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VSV</td>
<td>&gt;6.5</td>
<td>4.4</td>
<td>&gt;10.9</td>
</tr>
<tr>
<td>Sindbis</td>
<td>&gt;6.3</td>
<td>&gt;6.0</td>
<td>&gt;12.3</td>
</tr>
<tr>
<td>HBV</td>
<td>&gt;6.0</td>
<td>na</td>
<td>&gt;6.0</td>
</tr>
<tr>
<td>HCV</td>
<td>&gt;5.0</td>
<td>na</td>
<td>&gt;5.0</td>
</tr>
<tr>
<td>HIV</td>
<td>&gt;6.2</td>
<td>&gt;5.6</td>
<td>&gt;11.8</td>
</tr>
<tr>
<td>EMC</td>
<td>0</td>
<td>&gt;5.6</td>
<td>&gt;5.6</td>
</tr>
<tr>
<td>HAV</td>
<td>0*</td>
<td>&gt;5.3</td>
<td>&gt;5.3</td>
</tr>
<tr>
<td>Parvovirus</td>
<td>0</td>
<td>&gt;5.0</td>
<td>&gt;5.0</td>
</tr>
</tbody>
</table>
immune globulin preparations, including intramuscular, intravenous and hyperimmune gamma-globulins, be virally inactivated. Incorporation of a defined viral inactivation procedure into a manufacturing scheme will provide greater freedom when plasma collection or testing procedures or purification processes are modified in the future.

In conclusion, despite being prepared from plasma pools, today’s coagulation factor concentrates have proven to be safe from transmission of HBV, HCV and HIV. Virally inactivated concentrates are now safer than the individual units from which they were derived. Success with the sterilization of coagulation factor concentrates encourages research into the sterilization of blood components; i.e. Fresh Frozen Plasma, Red Blood Cell Concentrates and Platelet Concentrates.

**Viral Inactivation of Blood Components**

Before addressing the viral inactivation of blood components, we must ask if individual units of blood are already safe enough. As mentioned earlier, the risk of HIV, HBV and HCV on exposure to a single donor is 1/225,000, 1/200,000 and 1/3,300 to 1/6,000, respectively. However, since a single unit is seldom transfused, these risks must be multiplied by the number of donors, thus the risk of virus exposure for a patient receiving blood is better estimated at 0.1% to 1%. It is our belief that the goal should be to reduce viral risk to one per million or less, and that this goal can only be achieved through virus inactivation.

Transfusion Plasma—Our experience with solvent/detergent encouraged us to develop solvent/detergent-treated plasma (SD-Plasma) as a substitute for FFP. Briefly, units of FFP are combined, thawed, treated with 1% tri (n-butyl) phosphate (TNBP) and 1% Triton X-100 at 30°C for four hours, the reagents removed by hydrophobic chromatography, and the final product sterile-filtered, frozen and, optionally, lyophilized. Virus inactivation has been extensively validated. Under these conditions of S/D treatment, the rate of VSV and Sindbis virus killing exceeds that observed with AHF concentrates, treated either with TNBP/cholate or TNBP/Tween. We have also shown that ≥10^8 infectious doses (ID_{50}) of HBV, ≥10^5 ID_{50} of HCV, and ≥10^{-2} ID_{50} of HIV are killed, and that ≥10^{4.5} ID_{50} of HAV are neutralized. Because of pooling, a dose of SD-Plasma consistently has 30-times more anti-HAV antibody than a dose of intramuscular immune globulin, known to prevent HAV spread, and has approximately the same quantity of anti-parvovirus antibody as a dose of intravenous immune globulin, reported to be effective in the therapy of parvovirus infections. The coagulation factor content resembles that of the start pool, and is more consistent than that found in individual donor units. There is no evidence that coagulation factors are activated, and the level of other proteins is normal. Toxicology studies indicate that the tiny amounts of TNBP and Triton X-100 which remain are safe.

SD-Plasma has been extensively evaluated in the United States and Europe. In the U.S., over 20 clinical study sites took part. The principal efficacy endpoints were the correction of coagulation factor deficiencies, and the treatment of thrombotic thrombocytopenic purpura (TTP). In our own studies, 93 patients were treated on 504 occasions with 1,334 units of SD-Plasma. This included the successful treatment of 37 surgical episodes and 75 bleeding episodes in patients who were congenitally coagulation factor deficient and 9 successful uses to reverse warfarin therapy in advance of surgery. In patients with chronic or acute TTP, SD-Plasma was just as good as FFP in stimulating an increase in platelet count. Formal viral safety studies indicate that virus has not been transmitted, and this conclusion is supported by the published studies (cited above) and the more than one million units infused in Europe to date.

Blood Cell Concentrates—As compared with virus inactivation of blood protein products, sterilization of cellular products is more difficult because blood cells are more complex and fragile than
proteins, and multiple viral forms are present, including cell-free virus, virus which is adherent to cell membranes, actively replicating virus, and latently infected cells. Nonetheless, since erythrocytes and platelets do not replicate, methods that modify membranes or nucleic acid may prove useful.

Red Blood Cell Concentrates—While numerous methods have been investigated, including use of $\beta$-propiolactone, nitrogen mustards, aryl diol epoxides, ozone, and halogenated oxidizing agents\(^{56}\), the best results described thus far employ photodynamically active sensitizers and visible light\(^{57-61}\). Early work by Matthews and coworkers showed good virus kill with hematoporphyrin derivative. We have shown that by substituting phthalocyanine, which absorbs light where hemoglobin does not, virus kill is greatly improved.

It should be noted that with phthalocyanines as well as other dyes, like methylene blue or sapphrysins, oxygen is activated to its reactive forms and consequently the reaction mechanisms are complex. With phthalocyanine treatment of red cell concentrates, we have begun to analyze the reaction pathways through addition of quenchers of reactive oxygen species, the principle being that some compounds like mannitol and glutathione will quench oxygen radicals while other compounds like tryptophan and sodium azide principally quench singlet oxygen. Using this approach we have shown that virus kill is not mediated by oxygen radicals and is mediated by singlet oxygen (Figs. 5 and 6)\(^{62}\). This finding has practical importance since we can enhance reaction specificity by including quenchers of radicals at the time of light exposure. Thus, although AIPcS$_4$-treated baboon red blood cells circulate normally, rabbit red blood cells treated similarly had a reduced circulatory survival which could be improved through addition of type I quenchers. More recently, we have initiated structure/function studies with a number of phthalocyanine derivatives\(^{63}\). One compound, Pc4, is more effective than our original tetrasulfonate derivative in killing cell-free VSV, as well as various forms of HIV; thus studies at this point are focussing on Pc4.

Platelet Concentrates—Photodynamically active compounds such as those under evaluation in the treatment of RBCC reduce platelet aggregation response to collagen and to other agonists\(^{64,65}\). In contrast, encouraging results have been obtained with psoralen derivatives. Psoralens are naturally occurring furocoumarins found in many foods, and they have been used therapeutically since antiquity. The principal reaction of psoralens on exposure to long wavelength ultraviolet light is the

![Fig. 5 AIPcS4: Effect of Type I Quenchers on VSV](image-url)
cross-linking of nucleic acids. The initial report on the treatment of platelets with psoralens came from Dr. Corash’s Laboratory. Treatment of an oxygen depleted Platelet Concentrate with 8'-methoxypsoralen and UVA light was shown to inactivate ≥6.7 log₁₀ of E. coli, ≥6.9 log₁₀ of S. aureus, ≥7.3 log₁₀ PFU of phage fd, 2.5 log₁₀ of phage R17, and 5.1 log₁₀ of feline leukemia virus. When treatment was under deoxygenated conditions, platelet morphology, process recovery, and response to the aggregation agent A23187 were comparable to the untreated controls. If not first deoxygenated, aggregation response was adversely affected. We have overcome the requirement to deoxygenate has been overcome through the addition of quenchers of active oxygen species.

Additionally, new synthetic psoralens with increased reactivity with nucleic acids are being developed and may serve to enhance reaction specificity further. In a thrombocytopenic rabbit model, human platelets treated with aminomethyl-4,5', 8-trimethylpsoralen (AMT) were equally effective as the untreated control in shortening the bleeding time.

**Conclusion**

Blood and blood products have never been safer. However, the public’s continuing concern about blood viral safety and the differential safety profile between blood and other pharmaceuticals demand that we continually improve. The achievements of the past are laudable. Nonetheless, viral safety falls well short of a standard of less than one transmission per million units transfused, a realistic goal which we believe the transfusion community should adopt. Pooled blood products which have been virally inactivated meet this standard for most viruses, and use of a second viral elimination procedure which complements the first will further ensure safety of these products. For single donor blood products, improved screening systems may achieve this goal; however, screening systems alone will never eliminate the so-called window period, and screening tests cannot anticipate new viruses or viral serotypes. Incorporation of viral inactivation procedures into the manufacture of all blood products, including blood cell concentrates, overcomes the weaknesses of screening procedures, and the further development of virus inactivation methodology should continue to be encouraged.

**Selected References**

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44) Horowitz B: Unpublished data.


