TABLE 4. Validated viral elimination when processing fibrinogen and thrombin: Baxter process

	Virus:	HIV-1	BVDV or TBEV	PRV	HAV or ERV	MMV
	Model for:	HIV	HCV	HBV	HAV	Parvovirus B19
Process and step	Enveloped virus?:	Yes	Yes	Yes	No	No
Fibrinogen						
Cryoprecipitation + wash		2.6	1.3	1.5	1.8	ND
Lyophilization + vapor heating		>6.2	>6.8	>7.1	>6.5	1.5
S/D treatment		>6.6	>6.5	>6.7	NA	NA
	Sum:	>15.4	>14.6	>15.3	>8.3	>1.5
Thrombin						
Cryoprecipitation + wash		1.4	ND	1.1	ND	ND
Anion-exchange chromatography		2	ND	3.1	ND	ND
Lyophilization + vapor heating		>5.3	>5.9	>7.0	>4.7	1.0
S/D treatment		>5.2	>6.0	>6.9	NA	NA
	Sum:	>13.9	>11.9	>18.1	>4.7	1.0

MMV = mouse minute virus; NA = not applicable; ND = not done.

TABLE 5. Assignment of additional virucidal activity based on reserve capacity

Time required for complete kill (% of total)	Estimated minimal additional cidal power (log)
>100	0
76-100	1
51-75	2
26-50	3
≤25	4

these viruses is at least 3 to 4 log.[50] Since fibrinogen is purified by simple precipitations, it, like intermediate-purity FVIII preparations, likely benefits from the copresence of antibody in the final preparation.[51] Ion-exchange chromatography typically removes 2 to 3 log of virus.[52-55] Finally, some contribution to the calculation of safety margins should be ascribed to the "reserve capacity" of the viral inactivation method(s) employed, defined as the ability to achieve complete virus kill in a fraction of the treatment time allotted. While numerous publications make clear that linear extrapolation of virus inactivation curves overstates inactivation potential,[56,57] assigning no benefit to reserve capacity when calculating safety margins clearly underestimates inactivation capacity. Unless data indicate otherwise, we propose adopting the scheme described in Table 5. Although seemingly arbitrary, this scheme has the value of simplicity. Its use is supported by the dozens if not hundreds of times results with S/D and heat treatment methods have been reproduced, thereby increasing the quantity of virus subjected to challenge. Also, for S/D treatment methods, viral kill has been shown to be complete even when using tri-(*n*-butyl)phosphate (TNBP) together with sodium cholate, a combination that provides far slower kill kinetics than the more frequently employed TNBP-Tween 80 or TNBP-Triton X-100 combinations (Fig. 2), and the fact that reagent concentration can be halved without affecting viral kill (data not shown). We have not made reserve capacity estimates for vapor heating

since much of the loss in viral infectivity occurs before initiation of the heat cycle (Fig. 3).

Taking these factors into account, along with published information on the inactivation of HIV, HCV, and HBV,[58] for fibrinogen, we estimate that 15 to 17 log of enveloped viruses, 10 to 11.5 log of HAV or most other nonenveloped viruses, and 7 log of parvovirus are eliminated. For thrombin, we estimate that 17 to 22 log of enveloped viruses, 11 to 13 log of HAV, and 7 to 10 log of parvoviruses are eliminated (Table 6).

Calculation of safety margin

The calculated margins of safety are given in Table 7. For enveloped viruses, safety margins are exceedingly large, estimated at about 100 billion-fold for fibrinogen and 1 trillion-fold for thrombin. Although lower, the safety margins for HAV for both fibrinogen and thrombin exceed 1 million-fold. Owing to the potentially significantly higher content of parvovirus and its greater resistance to inactivation, fibrinogen enjoys only a small safety margin while that for thrombin is approximately 2000-fold. Even still, when expressed as risk of transmission per vial, a calculation typically required by regulatory authorities, the risk with fibrinogen is calculated at 1 in 500,000 vials and that with thrombin is approximately 1 in 100 million vials or less. Thus, parvovirus transmission should still be an infrequent event.

Clinical experience

Tisseel has been marketed in the United States since 1998, and in Europe it was introduced clinically more than a decade earlier. Evicel and, except for a formulation change, its identical predecessor product Crosseal have been marketed in the United States since 2003 and were available in Europe several years earlier. Throughout this use, there have been no known cases of hepatitis or HIV

TNBP/Cholate
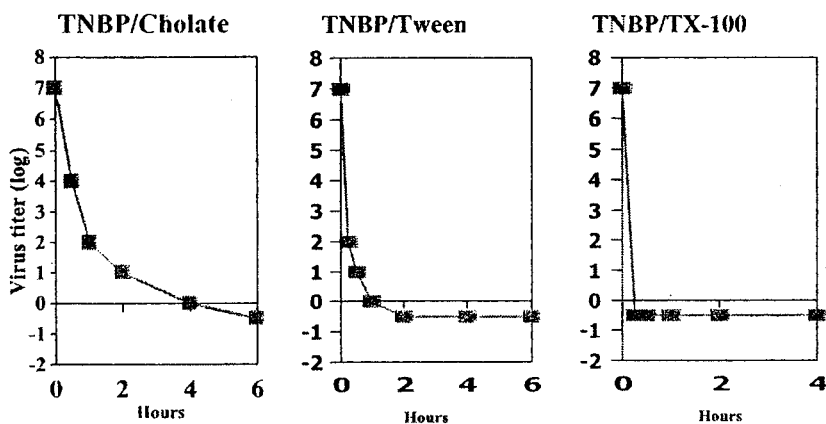

TNBP/Tween

TNBP/TX-100

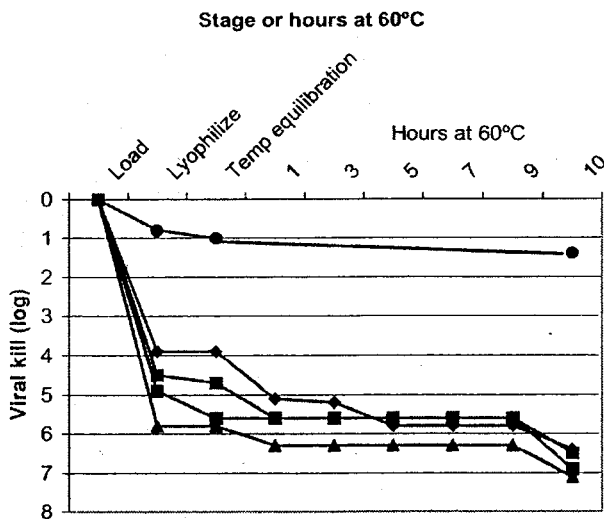Fig. 2. S/D inactivation of vesicular stomatitis virus added to an antihemophilic factor concentrate.

Fig. 3. Validated viral kill on vapor heating of fibrinogen (currently used Baxter process). BVDV (■) and PRV (▲) were undetectable upon reaching 60°C, and HIV (◆) and HAV (■) were undetectable after 5 and 1 hour, respectively, at 60°C. (●) Mouse minute virus.

starting with 10,000-fold higher parvovirus loads. Experience with S/D-plasma indicates that reducing the quantity of parvovirus DNA to no more than 10^4 geq per mL (10^6 geq/patient exposure) eliminates parvovirus transmission as measured by DNA replication or seroconversion when the patient also receives product containing parvovirus antibody.[61] Additional clinical studies are needed to show whether the fibrin sealants manufactured today can still transmit parvovirus B19 or one of the newly described, human blood-borne parvoviruses.[62]

New viral threats

In the past 5 to 8 years, three new pathogens, WNV, Chikungunya virus, and vCJD, have emerged as potential threats to the blood supply. Other infectious agents, like H5N1 influenza virus, the strain of corona virus that causes severe acute respiratory syndrome (SARS); dengue virus; and vaccinia virus are potentially transmissible by blood and blood products. With the exception of vCJD, all are enveloped viruses and would be expected to be completely cleared by the processes now in place for manufactured blood products like fibrin sealant or thrombin. As shown in Table 8, WNV, H5N1 influenza virus, SARS-associated corona virus, and Chikungunya virus were all inactivated completely to the extent of challenge by the methods of viral inactivation discussed above.

The infectious agent of vCJD is believed to be a protein that resists most methods of inactivation including all that are applied to manufactured plasma products. Although there is evidence that it can be found in blood (see above), despite years of surveillance there have been no reported transmissions by manufactured plasma products. Model studies indicate that significant quantities would be removed by the purification processes now in use, including cryoprecipitation, depth filtration with filter aids, nanofiltration, and ion-exchange or affinity chromatography.[25,63-69] Based mostly on published findings, compared with a total maximum load of about 4 log of vCJD per plasma pool, the fibrinogen and thrombin processes should remove greater than 7.6 and greater than 13 log, respectively (Table 9), providing large safety margins.

DISCUSSION

The safety of modern plasma-derived products with respect to HBV, HCV, and HIV has been proven clinically over the past decade or more, mostly using manufacturing procedures employing only one dedicated method of

transmission associated with commercial fibrin sealants.[59] This is notable since, for much of this time, the manufacturing process for Tisseel utilized only one dedicated viral elimination step while modern processes utilize two. On the other hand, epidemiologic evidence suggests that among patients who do not have parvovirus antibody at the time of fibrin sealant application, approximately one-fifth have reduced reticulocyte counts 12 to 20 days after surgery and develop parvovirus antibodies 12 to 48 weeks after surgery.[60] This finding is consistent with the calculations presented above since the study was performed using fibrin sealant prepared from plasma pools that were not screened by NAT for parvovirus, thereby potentially

TABLE 6. Estimated viral elimination when processing fibrinogen and thrombin: based on both Omrix's and Baxter's processes

Step		Log reduction				
		HIV	HCV	HBV	HAV	Parvovirus B19
Fibrinogen						
Cryoprecipitation + Al(OH) or wash		1.5	1.5	1.5	1.5	1.5
Immune neutralization					3*	3*
S/D treatment		>6	>6	>6	0	0
Heat treatment		>6	>6	>6	>5.8 to >6.5†	1.3
Greater heat sensitivity of B19 than CPV						1
Reserve capacity of virucidal methods‡		4	4	2	0	0
	Sum:	17.5	17.5	15.5	10.3-11.5	6.8
Thrombin						
Cryo removal		1	1	1	1	1
Immune neutralization		0	0	0	0	0
Initial fractionation (Baxter)§		2	2	2	2	2
Chromatographic purification		3	3	3	3	3
S/D treatment		>6	>6	>6	0	0
S/D reserve capacity		4	4	2	NA	NA
Heat treatment (Baxter)		>6	>6	>6	6.9	1.3
Nanofiltration (Omrix)		>4.4	>5.3	>5.5	7	5.9
	Sum:	18-22	19-22	17.5-20	11-13	7.3-9.9¶

* Virus neutralization is predicated on the fibrinogen containing antibody.
† The lower number applies to Omrix's fibrinogen and the higher number applies to Baxter's fibrin.
‡ The reserve capacity of vapor treating is estimated at zero since most of the reported viral kill takes place prior to initiating the heat cycle. The estimate of HBV reserve capacity with S/D treatment comes from studies with duck HBV added to whole plasma.
§ A mean of 2 log removal during cold alcohol fractionation is assumed.
¶ The lower number applies to Baxter's thrombin and the higher number applies to Omrix's thrombin.
NA = not applicable.

TABLE 7. Calculation of viral safety margins

	HIV	HCV	HBV	HAV	Parvovirus
Viral load (log; from Table 1)	5.7	6.0	4.4	2.9	6.6
Fibrinogen					
Viral clearance capacity (log)	17.5	17.5	15.5	10.3	6.8
Safety margin (fold)	6.3×10^{11}	3.2×10^{11}	1.3×10^{11}	2.5×10^{7}	1.6
Risk/vial (with virus at maximum load)*	3×10^{-16}	5×10^{-16}	1×10^{-15}	7×10^{-12}	1×10^{-4}
Adjusted risk/vial (all lots)†	1×10^{-19}	3×10^{-19}	5×10^{-17}	2×10^{-16}	2×10^{-6}
Thrombin					
Viral clearance capacity (log)	18	19	17	11	7.3-9.9
Safety margin (fold)	2×10^{12}	1×10^{13}	4×10^{12}	1×10^{8}	2×10^{3}
Risk/vial (with virus at maximum load)‡	8×10^{-18}	2×10^{-18}	4×10^{-18}	1×10^{-13}	3×10^{-5}-8×10^{-9}§
Adjusted risk/vial (all lots)†	4×10^{-21}	8×10^{-22}	2×10^{-19}	3×10^{-18}	7×10^{-8}-2×10^{-10}§

* Assumes 1 vial per L of plasma.
† The risk was adjusted to include lots without virus (calculated from Table 1, Column 4) and further assumes that the average viral load of contaminated lots is 1 log lower than the maximum load.
‡ Assumes 10 vials per L of plasma.
§ The larger number applies to Baxter's thrombin and the smaller number applies to Omrix's thrombin.

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virus inactivation.[70] The safety record of fibrin sealant products, composed of both fibrinogen and thrombin, matches the safety record of other manufactured plasma products.[57] This suggests that for these and other enveloped viruses, the safety margins of fibrin sealant or stand-alone thrombin should be much higher than required since both employ an additional, dedicated method of viral elimination and, indeed, our calculated safety margins for enveloped viruses are extremely high. As a consequence, when new threats from enveloped viruses (e.g., WNV, pandemic influenza, and dengue viruses) are identified, the procedures in place for manu-factured blood products are sufficient to ensure safety. This contrasts with the record of so-called labile blood components that have been shown to transmit, for example, WNV, at least until new screening tests are developed and deployed. From a patient safety perspective, it is also important to note that the safety margins for fibrin sealant and thrombin exceed those for labile blood components by many orders of magnitude. This is a direct consequence of the multiple improvements adopted by manufacturers of purified blood products over the past 20 years including deploying robust methods of virus inactivation.

TABLE 8. Inactivation of new viral threats

Virus	Preparation	Treatment	Log kill	First time point where infectious virus was not detected	Reference
WNV	α1-proteinase inhibitor	Pasteurization at 60°C for 10 hr	≥6.5	5 hr	Remington et al.[89]
	Antihemophilic factor concentrate	S/D (0.3% TNBP/1.0% Tween 80) at 28°C for 6 hr	≥5.9	1 hr	
WNV	FEIBA	Vapor heating (60°C for 10 hr and 80°C for 1 hr)	>7.6	6 hr	Kreil et al.[90]
	Antihemophilic factor concentrate	S/D (0.3% TNBP and 1% Triton X-100) at 20°C for 60 min	>6.0	<1 min	
H5N1 influenza virus	FEIBA	Vapor heating (60°C for 10 hr and 80°C for 1 hr)	>5.3	10 hr	Kreil et al.[91]
	IVIG	S/D (0.3% TNBP, 1% Triton X-100 and 0.3% Tween 80) at 18°C for 60 min	>4.7	<2 min	
SARS-associated corona virus	Haptoglobin, AT III, or IVIG	Pasteurization at 60°C for 10 hr	>3.3 to >6.5	1 hr	Yunoki et al.[92]
Chikungunya virus	IVIG	Pasteurization at 60°C for 10 hr	>5.2	1 hr	Uemura et al.[93]

AT III = antithrombin III; FEIBA = factor VIII bypassing activity produced by CSL Behring; H5N1 = the strain of influenza virus that causes SARS; IVIG = intravenous immune globulin.

Calculated safety margins for nonenveloped viruses are smaller since the manufacturing procedures for many plasma proteins, including both fibrinogen and thrombin, typically employ only one dedicated viral inactivation and/or removal method effective against these viruses, and parvoviruses are especially stable to thermal inactivation. There are no reports of HAV transmission by fibrin sealants even before adoption of NAT screening procedures. This is in accord with the finding that coagulation FVIII preparations did not transmit HAV provided they were either heat-treated or affinity-purified.[71] Additionally, given the modest processing fibrinogen undergoes and the known presence of immunoglobulin G in cryoprecipitate, it is reasonable to assume that fibrinogen is further protected by anti-HAV, present as a "contaminant."

Numerous reports describe the transmission of parvovirus B19 by coagulation factor concentrates[72] and its transmission by fibrin sealant has also been reported.[58] Frequent transmission results from the high concentration of virus that can be present in plasma pools containing units from donors with acute-phase viremia[73] and because parvovirus is not inactivated by S/D and is relatively stable to heat treatment methods. Beginning around 2001, commercial manufacturers of plasma products began employing NAT to screen incoming plasma units in a minipool format to limit viral loads. Originally, testing was performed to ensure that titers did not exceed 10^5 geq per mL; for Omrix's and Baxter's fibrin sealant products, a standard of not more than 10^4 geq per mL has been adopted for the minipool being tested. A recent article by Geng et al.[51] confirms the benefits of screening incoming plasma for parvovirus B19 by NAT. Despite this improvement, the maximum load of infectious virus that might be present remains considerable, and it would appear that the fibrinogen component might still transmit parvovirus B19, albeit at very low frequency. Clinical studies will be required to confirm this since the antibody content of fibrinogen might provide adequate protection, or parvovirus B19 might be more sensitive to heat treatment than the animal parvovirus models used, as has been suggested.[74] The greater safety margin calculated for the thrombin component arises from the use of more vigorous purification procedures and the overall effectiveness of nanofilters in removing parvovirus. Because of its size and shape, fibrinogen cannot be nanofiltered successfully.

All evidence to date indicates that vCJD is not transmitted by manufactured plasma products. Safety may result from the geographic restrictions that have been instituted to eliminate individuals who are at high risk of exposure, the very low levels in blood, and its removal by steps in common use including precipitations, filtrations, and column chromatography. If a vCJD contaminated unit was included in the plasma pool, the calculated safety margin for fibrinogen and thrombin is very high. Unlike viruses used in spiking studies, however, the structure of

TABLE 9. Clearance of prions: Omrix process

Prion load (log; from Table 1): 4.3

	Log removal	Reference*
Fibrinogen		
Cryoprecipitation	1.6 mean (0.6-2.6)	Foster, 2000[66] and 2006[69]
Al(OH) extraction and filtration	>2	Omrix validated study
Oil extraction of S/D reagents	2	Omrix preliminary study
Hydrophobic chromatography	2	Foster, 1999[63]
Clearance capacity (log)	>7.6	
Safety margin (fold)	1995	
Adjusted risk/vial†	7×10^{-10}	
Thrombin		
Cryo removal	1	Foster, 2000[66] and 2006[69]
Filtrations	2	Foster, 2000[66] and 2006[69]
Anion-exchange chromatography	3	Foster, 2000[66] and 2006[69]
Cation-exchange chromatography	3	Foster, 2000[66] and 2006[69]
Nanofiltration	4.4 mean (1.6 to >5.9)	Foster, 2000[66] and 2006[69]
Clearance capacity (log 10)	13.4	
Safety margin (fold)	1.3×10^5	
Adjusted risk/vial†	1×10^{-16}	

* See also additional Foster references.[61,64,65,67] It should be noted that the form that the infectious vCJD agent takes in plasma is unknown and that, should it be present in plasma pools, its behavior may differ from the materials used.
† We employed the same assumptions as used in Table 7 plus assumed risk of vCJD presence was the same as for CJD, i.e., 1 per 1 million donations, and mean load was same as maximum load.

the causative agent of vCJD is unknown and may differ significantly from the models in use. Because of this uncertainty and the devastating nature of the disease, the authorities in the United Kingdom have concluded that recipients of plasma-derived FVIII, F IX, and antithrombin prepared using donations from individuals who subsequently developed vCJD should be told that they may be at increased risk for developing the disease.[75] This emphasizes the importance of donor exclusion criteria implemented for all products licensed in the United States or Europe. With these exclusions taken into account, the risk for vialed product should remain well less than 1 in 1 million for fibrinogen and less than 1 in 1 trillion for thrombin.

Finally, we should comment about the methods employed in making these calculations. In addition to the validated studies presented to the FDA and other regulatory agencies, we examined other steps in the manufacturing process that were likely to contribute to safety together with published information from other related processes. We also have taken into account the benefit of using viral inactivation methods that have a large reserve capacity. Our intent in employing this approach was not to replace the stricter approach taken by regulatory authorities, but simply to more completely assess safety. Nonetheless, we acknowledge that our calculations are estimates and actual findings may differ. Acknowledging these shortcomings but based on our calculations, we encourage manufacturers and other interested parties to continue seeking ways to enlarge the safety margin, especially for nonenveloped viruses and to address the theoretical risk posed by vCJD

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