TABLE 2. Validated viral elimination when processing fibrinogen: Omrix process

		Log kill or removal				
	Virus:	HIV-1	BVDV	PRV	HAV	CPV
	Model for:	HIV	HCV	HBV	HAV	Parvovirus B19
Step	Enveloped virus?:	Yes	Yes	Yes	No	No
Cryoprecipitation + Al(OH) treatment		ND	ND	ND	1.5	1.5
S/D treatment		>4.4*	>4.4†	>4.4†	ND	0
Pasteurization		>4.4‡	>5.5‡	ND	>5.8‡	1.3
	Sum:	>8.8	>9.9	>4.0	>7.3	2.8

* No infectivity after 5 minutes. Treatment is for 4 hours.
† No infectivity after 10 minutes, the first time point taken.
‡ 9 to 10 hours were required to achieve reported kills. Treatment is for 10 hours.
Al(OH) = aluminum hydroxide; CPV = canine parvovirus; ND = not done.

TABLE 3. Validated viral elimination when processing thrombin: Omrix process

		Log kill or removal						
	Virus:	HIV-1	BVDV	Sindbis	PRV	EMCV	CPV	MMV
	Model for:	HIV	HCV	HCV	HBV	HAV	Parvovirus B19	Parvovirus B19
Step	Enveloped virus?:	Yes	Yes	Yes	Yes	No	No	No
Cryo removal		ND	ND	ND	ND	ND	ND	ND
Anion-exchange chromatography		ND	ND	ND	ND	ND	ND	ND
S/D treatment		>5.8*	>4.7†	>5.3‡	>4.3†	ND	0	ND
Cation-exchange chromatography		ND	ND	ND	ND	ND	ND	ND
Nanofiltration		>4.4	ND	>5.3	>5.5	7.0	5.9	5.8
	Sum:	>10.2	>4.7	>10.6	>9.8	7.0	5.9	5.8

* No infectivity after 5 minutes. Treatment is for 6 hours for thrombin.
† No infectivity after 10 minutes, the first time point taken.
‡ No infectivity after 15 minutes, the first time point taken.
EMCV = encephalomyocarditis virus; MMV = mouse minute virus; ND = not done.

its sole, dedicated virus inactivation step; S/D treatment has been added recently. Another difference is that its thrombin component is isolated starting with Baxter's activated prothrombin factor complex.

The FDA and other applicable regulatory authorities demand that formal viral inactivation and/or removal studies be performed and that these adhere to international standards as they relate to the selection of viruses to be used, the conduct of these studies under Good Laboratory Practice guidelines and the calculations provided. We need not reiterate those guidelines here, except to say that the model viruses selected were chosen to represent multiple viral types and, in particular, the viruses of concern for products derived from human blood. Thus, viral elimination studies typically use HIV, bovine viral diarrhea virus (BVDV; model for HCV), pseudorabies virus (PRV; model for HBV), HAV or another picornovirus such as encephalomyocarditis virus, and canine parvovirus (or another model for human parvovirus B19).

The results from these formal studies for the fibrinogen and thrombin components of Omrix's and Baxter's fibrin sealant products are given in Tables 2 and 3 and Table 4, respectively (see product package inserts, with updates from manufacturers; see Acknowledgments). The clearance factors for enveloped viruses and the models for HIV, HCV, and HBV exceed the challenge dose for each of the dedicated viral elimination steps (i.e., S/D, pasteurization, nanofiltration, and vapor heating). Consequently, when the same virus has been studied in each of the two dedicated steps, the validated clearance factors exceed 9 log, and where higher doses of virus have been used or more steps validated, clearance factors as large as 18 log have been reported. The validated clearance of nonenveloped viruses is significantly less than for enveloped viruses since only one of the two dedicated viral elimination methods is effective against these viruses. Parvoviruses are a special case since they are especially heat-stable, and only 1 to 2 log of animal parvoviruses are inactivated by either pasteurization or vapor heating. It should be noted, however, that human parvovirus B19 may be more heat-sensitive than the models used here.[49] Nanofiltration is significantly more effective, and Omrix has shown for its thrombin preparation that nanofiltration removes approximately 6 log of parvoviruses.

A more complete estimate of safety margin needs to take into account the contribution of the other steps in the process that contribute to safety despite not being formally validated. It is commonly accepted that immune neutralization contributes to HAV and parvovirus B19 safety and that the neutralization capacity of antibodies to

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

		Log kill or removal				
	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

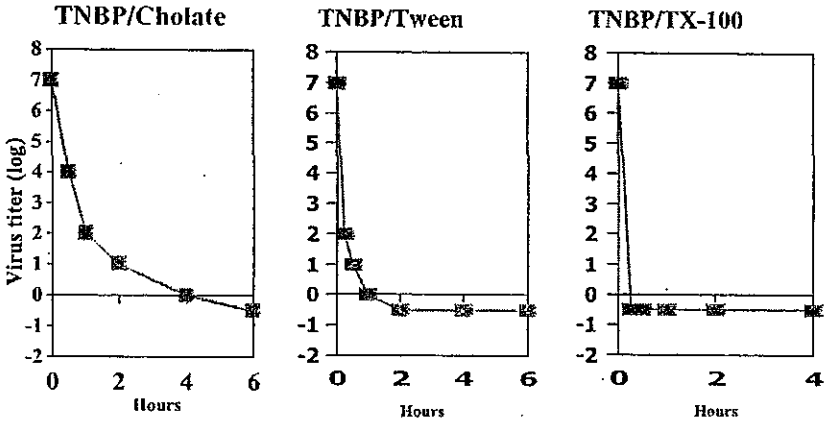

Fig. 2. S/D inactivation of vesicular stomatitis virus added to an antihemophilic factor concentrate.

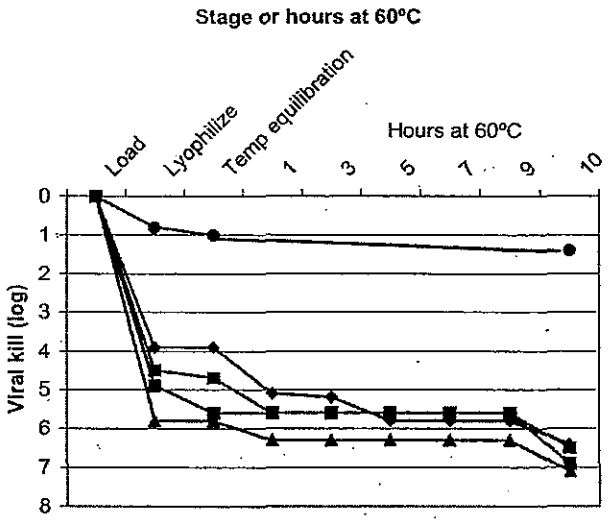

Fig. 3. Validated viral kill on vapor heating of fibrinogen (currently used Baxter process). BVDV (■) and PRV (▲) were undetectable upon reaching 60°C, and HIV (◆) and HAV (■) were undetectable after 5 and 1 hour, respectively, at 60°C. (●) Mouse minute virus.

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 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

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^{-5}
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^{9}	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^{-6}§
Adjusted risk/vial (all lots)†	4×10^{-21}	8×10^{-22}	2×10^{-19}	3×10^{-19}	7×10^{-6}-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.

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 manufactured 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.[59]
	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.[60]
	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.[61]
	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.[62]
Chikungunya virus	IVIG	Pasteurization at 60°C for 10 hr	>5.2	1 hr	Uemura et al.[63]

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[56] and 2006[59]
Al(OH) extraction and filtration	>2	Omrix validated study
Oil extraction of S/D reagents	2	Omrix preliminary study
Hydrophobic chromatography	2	Foster, 1999[53]
Clearance capacity (log)	>7.6	
Safety margin (fold)	1995	
Adjusted risk/vial†	7×10^{-10}	
Thrombin		
Cryo removal	1	Foster, 2000[56] and 2006[59]
Filtrations	2	Foster, 2000[56] and 2006[59]
Anion-exchange chromatography	3	Foster, 2000[56] and 2006[59]
Cation-exchange chromatography	3	Foster, 2000[56] and 2006[59]
Nanofiltration	4.4 mean (1.6 to >5.9)	Foster, 2000[56] and 2006[59]
Clearance capacity (log 10)	13.4	
Safety margin (fold)	1.3×10^9	
Adjusted risk/vial†	1×10^{-16}	

* See also additional Foster references.[51,54,55,57] 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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REFERENCES

1. Key NS, Negrier C. Coagulation factor concentrates: past, present and future. Lancet 2007;370:439-48.
2. Abusriwil H, Stockley RA. Alpha-1-antitrypsin replacement therapy: current status. Curr Opin Pulm Med 2006;12:125-31.
3. Toubi E, Etzioni A. Intravenous immunoglobulin in immunodeficiency states: state of the art. Clin Rev Allergy Immunol 2005;29:167-72.
4. Solomon B. Intravenous immunoglobulin and Alzheimer's disease immunotherapy. Curr Opin Mol Ther 2007;9:79-85.
5. Laursen I, Houen G, Hojrup P, Brouwer N, Krogsoe LB, Blou L, Hansen PR. Second-generation nanofiltered plasma-derived mannan-binding lectin product: process and characteristics. Vox Sang 2007;92:338-50.
6. Lariviere B, Rouleau M, Picard S, Beaulieu AD. Human plasma fibronectin potentiates the mitogenic activity of platelet-derived growth factor and complements its wound healing effects. Wound Repair Regen 2003;11:79-89.
7. Poulle M, Burnouf-Radosevich M, Burnouf T. Large-scale preparation of highly purified human C1-inhibitor for therapeutic use. Blood Coagul Fibrinolysis 1994;5:543-9.
8. Jackson MR. Fibrin sealants in surgical practice: an overview. Am J Surg 2001;182(2 Suppl):1S-7S.

9. Dorion RP, Hamati HF, Landis B, Frey C, Heydt D, Carey D. Risk and clinical significance of developing antibodies induced by topical thrombin preparations. Arch Pathol Lab Med 1998;122:887-94.
10. Ortel TL, Mercer MC, Thames EH, Moore KD, Lawson JH. Immunologic impact and clinical outcomes after surgical exposure to bovine thrombin. Ann Surg 2001;233: 88-96.
11. Schoenecker JG, Johnson RK, Lesher AP, Day JD, Love SD, Hoffman MR, Ortel TL, Parker W, Lawson JH. Exposure of mice to topical bovine thrombin induces systemic autoimmunity. Am J Pathol 2001;159:1957-69.
12. Su Z, Izumi T, Thames EH, Lawson JH, Ortel TL. Antiphospholipid antibodies after surgical exposure to topical bovine thrombin. J Lab Clin Med 2002;139:349-56.
13. Streiff MB, Ness PM. Acquired FV inhibitors: a needless iatrogenic complication of bovine thrombin exposure. Transfusion 2002;42:18-26.
14. Lawson JH, Lynn KA, Vanmatre RM, Domzalski T, Klemp KF, Ortel TJ, Niklason LE, Parker W. Antihuman factor V antibodies after use of relatively pure bovine thrombin. Ann Thorac Surg 2005;79:1037-8.
15. Kamoda S, Ishikawa R, Kakehi K. Capillary electrophoresis with laser-induced fluorescence detection for detailed studies on N-linked oligosaccharide profile of therapeutic recombinant monoclonal antibodies. J Chromatogr A 2006; 1133:332-9.
16. Hepner F, Cszasar E, Roitinger E, Lubec G. Mass spectrometrical analysis of recombinant human growth hormone (Genotropin® reveals amino acid substitutions in 2% of the expressed protein. Proteome Sci 2005;3:1.
17. Brand CM, Leadbeater L, Bellati G, Marotta F, Ideo G. Antibodies developing against a single recombinant interferon protein may neutralize many other interferon-alpha subtypes. J Interferon Res 1993;13:121-5.
18. Oberg K, Alm G, Magnusson A, Lundqvist G, Theodorsson E, Wide L, Wilander E. Treatment of malignant carcinoid tumors with recombinant interferon alfa-2b: development of neutralizing interferon antibodies and possible loss of antitumor activity. J Natl Cancer Inst 1989;81:531-5.
19. Berrini A, Borromeo V, Secchi C. Monoclonal antibodies can reveal immunoreactivity differences between pituitary and recombinant bovine growth hormone. Hybridoma 1994;13:485-9.
20. US Food and Drug Administration. Guidance for industry: Q5A viral safety evaluation of biotechnology products derived from cell lines of human or animal origin. September 1998. Available from: URL: http://www.fda.gov/cder/guidance/q5a-fnl.pdf
21. Schreiber GB, Busch MP, Kleinman SH, Korelitz JJ. The risk of transfusion-transmitted viral infections. The Retrovirus Epidemiology Donor Study. N Engl J Med 1996;334:1685-90.
22. Dodd RY, Notari EP, Stramer SL. Current prevalence and incidence of infectious disease markers and estimated window-period risk in the American Red Cross blood donor population. Transfusion 2002;42:975-9.
23. Janssen MP, Over J, Vanderpoel CL, Cuijpers HT, Vanhout BA. A probabilistic model for analyzing viral risks of plasma-derived medicinal products. Transfusion 2008;48: 153-62.
24. Mosesson MW. Fibrinogen and fibrin structure and functions. J Thromb Haemost 2005;3:1894-904.
25. Horowitz B. Blood protein derivative viral safety: observations and analysis. Yale J Biol Med 1990;63:361-9.
26. Hewitt PE, Llewelyn CA, Mackenzie J, Will RG. Creutzfeldt-Jakob disease and blood transfusion: results of the UK Transfusion Medicine Epidemiological Review study. Vox Sang 2006;91:221-30.
27. Burnouf T, Padilla A. Current strategies to prevent transmission of prions by human plasma derivatives. Transfus Clin Biol 2006;13:320-8.
28. Cohen MS, Pilcher CD. Amplified HIV transmission and new approaches to HIV prevention. J Infect Dis 2005;191: 1391-3.
29. Wawer MJ, Gray RH, Sewankambo NK, Serwadda D, Li X, Laeyendecker O, Kiwanuka N, Kigozi G, Kiddugavu M, Lutalo T, Nalugoda F, Wabwire-Mangen F, Meehan MP, Quinn TC. Rates of HIV-1 transmission per coital act, by stage of HIV-1 infection, in Rakai, Uganda. J Infect Dis 2005;191:1403-9.
30. Pilcher CD, Joaki G, Hoffman IF, Martinson FE, Mapanje C, Stewart PW, Powers KA, Galvin S, Chilongozi D, Gama S, Price MA, Fiscus SA, Cohen MS. Amplified transmission of HIV-1: comparison of HIV-1 concentrations in semen and blood during acute and chronic infection. AIDS 2007;21: 1723-30.
31. Fiebig EW, Wright DJ, Rawal BD, Garrett PE, Schumacher RT, Peddada L, Heldebrant C, Smith R, Conrad A, Kleinman SH, Busch MP. Dynamics of HIV viremia and antibody seroconversion in plasma donors: implications for diagnosis and staging of primary HIV infection. AIDS 2003; 17:1871-9.
32. Katayama K, Kumagai J, Komiya Y, Mizui M, Yugi H, Kishimoto S, Yamanaka R, Tamatsukuri S, Tomoguri T, Miyakawa Y, Tanaka J, Yoshizawa H. Titration of hepatitis C virus in chimpanzees for determining the copy number required for transmission. Intervirology 2004;47:57-64.
33. Busch MP, Caglioti S, Robertson EF, McAuley JD, Tobler LH, Kamel H, Linnen JM, Shyamala V, Tomasulo P, Kleinman SH. Screening the blood supply for West Nile virus RNA by nucleic acid amplification testing. N Engl J Med 2005;353:460-7.
34. Glynn SA, Wright DJ, Kleinman SH, Hirschkorn D, Tu Y, Heldebrant C, Smith R, Giachetti C, Gallarda J, Busch MP. Dynamics of viremia in early hepatitis C virus infection. Transfusion 2005;45:994-1002.
35. Yoshikawa A, Gotanda Y, Minegishi K, Taira R, Hino S, Tadokoro K, Ohnuma H, Miyakawa K, Tachibana K, Mizoguchi H; Japanese Red Cross NAT Screening Research

Group. Lengths of hepatitis B viremia and antigenemia in blood donors: preliminary evidence of occult (hepatitis B surface antigen-negative) infection in the acute stage. Transfusion 2007;47:1162-71.
36. Satake M, Taira R, Yugi H, Hino S, Kanemitsu K, Ikeda H, Tadokoro K. Infectivity of blood components with low hepatitis B virus DNA levels identified in a lookback program. Transfusion 2007;47:1197-205.
37. Komiya Y, Katayama K, Yugi H, Mizui M, Matsukura H, Tomoguri T, Miyakawa Y, Tabuchi A, Tanaka J, Yoshizawa H. Minimum infectious dose of hepatitis B virus in chimpanzees and difference in the dynamics of viraemia between genotype A and C. Transfusion 2008;48:286-84.
38. Busch MP, Murthy KK, Hirschkorn DF, Herring BL, Delwart EL, Racanelli V, Rehermann B, Alter HJ. Infectivity of donations from eclipse and ramp-up stages of HCV in chimpanzees. Abstract S39-030H. Amer Assn of Blood Banks Annual Meeting 2007, Anaheim, CA., Oct 2007. Transfusion 2007;47(9 Suppl):17A.
39. Ma M, Piatak M, Fritts L, Lu D, Lifson J, Busch MP, Miller CJ. Transmission of simian immunodeficiency virus (SIV) by plasma collected prior to detectable viremia, and infectivity of ramp-up versus chronic stages. Abstract S40-030H. Amer Assn of Blood Banks Annual Meeting 2007, Anaheim, CA, Oct 2007. Transfusion 2007;47(9 Suppl):17A-18A.
40. Hijikata M, Shimizu YK, Kato H, Iwamoto A, Shih JW, Alter HJ, Purcell RH, Yoshikura H. Equilibrium centrifugation studies of hepatitis C virus: evidence for circulating immune complexes. J Virol 1993;67:1953-8.
41. Brummelhuis HG, Over J, Duivis-Vorst CC, Wilson-de Sturler LA, Ates G, Hoek PJ, Reerink-Brongers EE. Contributions to the optimal use of blood. IX. Elimination of hepatitis B transmission by (potentially) infectious plasma derivatives. Vox Sang 1983;45:205-16.
42. Prince AM, Horowitz B, Baker L, Shulman RW, Ralph H, Valinsky J, Cundell A, Brotman B, Boehle W, Rey F, Piet M, Reesink H, Lelie N, Tersmette M, Miedema F, Barbosa L, Nemo G, Nastala CL, Allan JS, Lee DR, Eichberg JW. Failure of a human immunodeficiency virus (HIV) immune globulin to protect chimpanzees against experimental challenge with HIV. Proc Natl Acad Sci U S A 1988;85:6944-8.
43. Prince AM, Pawlotsky JM, Soulier A, Tobler L, Brotman B, Pfahler W, Lee DH, Li L, Shata MT. Hepatitis C virus replication kinetics in chimpanzees with self-limited and chronic infections. J Viral Hepat 2004;11:236-42.
44. Bruce ME, McConnell I, Will RG, Ironside JW. Detection of variant Creutzfeldt Jakob disease infectivity in extraneural tissues. Lancet 2001;358:208-9.
45. Llewelyn CA, Hewitt PE, Knight RS, Amar K, Cousens S, Mackenzie J, Will RG. Possible transmission of variant Creutzfeldt-Jakob disease by blood transfusion. Lancet 2004;363:417-21.
46. Peden AH, Head MW, Ritchie DL, Bell JE, Ironside JW. Preclinical vCJD after blood transfusion in a PRNP codon 129 heterozygous patient. Lancet 2004;364:527-9.
47. Wroe SJ, Pal S, Siddique D, Hyare H, Macfarlane R, Joiner S, Linehan JM, Brandner S, Wadsworth JD, Hewitt P, Collinge J. Clinical presentation and pre-mortem diagnosis of variant Creutzfeldt-Jakob disease associated with blood transfusion: a case report. Lancet 2006;368:2061-7.
48. Cervenakova LO, Yakovleva O, McKenzie C, Kolchinsky S, McShane L, Drohan WN, Brown P. Similar levels of infectivity in the blood of mice infected with human-derived vCJD and GSS strains of transmissible spongiform encephalopathy. Transfusion 2003;43:1687-94.
49. Yunoki M, Tsujikawa M, Urayama T, Sasaki Y, Morita M, Tanaka H, Hattori S, Takechi K, Ikuta K. Heat sensitivity of human parvovirus B19. Vox Sang 2003;84:164-9.
50. Terpstra FG, Parkkinen J, Tolo H, Koenderman AH, Ter Hart HG, von Bonsdorff L, Torma E, van Engelenburg FA. Viral safety of Nanogam, a new 15 nm-filtered liquid immunoglobulin product. Vox Sang 2006;90:21-32.
51. Geng Y, Wu CG, Bhattacharyya SP, Tan D, Guo ZP, Yu MY. Parvovirus B19 DNA in Factor VIII concentrates: effects of manufacturing procedures and B19 screening by nucleic acid testing. Transfusion 2007;47:883-9.
52. Einarsson M, Morgenthaler JJ. Removal of viruses from plasma fractions by chromatography. Curr Stud Hematol Blood Transfus 1989;56:138-45.
53. Burnouf T. Chromatography in plasma fractionation: benefits and future trends. J Chromatogr B Biomed Appl 1995; 664:3-15.
54. Lemon SM, Murphy PC, Smith A, Zou J, Hammon J, Robinson S, Horowitz B. Removal/neutralization of hepatitis A virus during manufacture of high purity, solvent/detergent factor VIII concentrate. J Med Virol 1994;43:44-9.
55. Griffith M. Ultrapure plasma factor VIII produced by anti-F VIII c immunoaffinity chromatography and solvent/detergent viral inactivation. Characterization of the Method M process and Hemofil M antihemophilic factor (human). Ann Hematol 1991;63:131-7.
56. Woese C. Thermal inactivation of animal viruses. Ann N Y Acad Sci 1960;83:741-51.
57. Tersmette M, deGoede RE, Over J, deJonge E, Radema H, Lucas CJ, Huisman HG, Miedema F. Thermal inactivation of human immunodeficiency virus in lyophilised blood products evaluated by ID50 titrations. Vox Sang 1986;51: 239-43.
58. Horowitz B. Specific inactivation of viruses which can potentially contaminate blood products. Dev Biol Stand 1991;75:43-52.
59. Joch C. The safety of fibrin sealants. Cardiovasc Surg 2003; 11(Suppl 1):23-8.
60. Kawamura M, Sawafuji M, Watanabe M, Horinouchi H, Kobayashi K. Frequency of transmission of human parvovirus B19 infection by fibrin sealant used during thoracic surgery. Ann Thorac Surg 2002;73:1098-100.
61. Brown KE, Young NS, Alving BM, Barbosa LH. Parvovirus B19: implications for transfusion medicine. Summary of a workshop. Transfusion 2001;41:130-5.