Supporting Information

Figure S1 Resistance to proteolysis of different prion strains in sheep. PrPres from brain homogenates of sheep infected with classical scrapie, experimental cBSE, or atypical Nor-98 scrapie, and of an uninfected control sheep. Samples were purified using low (odd lanes) or high (even lanes) concentrations of proteinase K, and visualized with monoclonal antibodies that recognize either the core region (Panel A) or the octapeptide region (Panel B) of the protein. With the lower concentration of PK used in the purification step (in order to maximize test sensitivity) of one widely utilized BSE screening test [13], all three strains gave a positive result with both the anti-core and anti-octapeptide antibodies (odd lanes). Using a higher concentration of PK (even lanes) did not alter the positivity with either antibody for classical scrapie, but the cBSE strain no longer reacted with the anti-octapeptide antibody while Nor98 did not react with either antibody. Thus, by using the higher concentration of PK and two different antibodies, it is possible to discriminate between all three strains.

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Figure S2 Lesion profiles in cBSE- and BASE-infected macaque, and in MM2 sporadic CJD patients. The lesions were scored from 0 to 4 (negative, light, mild, moderate, and severe) for the different following gray matter regions: frontal (FC), temporal (TC), parietal (PC) and occipital (OC) neocortices, hippocampus (HI), parasubiculum and entorhinal cortex (EC), neostriatum (ST) (nuclei caudatus and putamen), thalamus (TH), substantia nigra (SN), midbrain periventricular gray (PG), locus ceruleus (LC), medulla (ME) (periventricular gray and inferior olive), and cerebellum (CE). Scoring for MME sCJD patient was issued from Parchi et al. [7].

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Author Contributions

Conceived and designed the experiments: CC CIL. Performed the experiments: NLE SF DM FA. Analyzed the data: EEC NLE MMR SF JPD. Contributed reagents/materials/analysis tools: CC GZ MMR MC. Wrote the paper: EEC GZ PB JPD. Participated to the final reviewing of the manuscript: CC SM NS MC PL CIL.

References

1. Will RG, Ironside JW, Zeidler M, Cousens SN, Estibeiro K, et al. (1996) A new variant of Creutzfeldt-Jakob disease in the UK. Lancet 347: 921–925.
2. Casalone C, Zanusso G, Acutis P, Ferrari S, Capucci L, et al. (2004) Identification of a second bovine amyloidotic spongiform encephalopathy: molecular similarities with sporadic Creutzfeldt-Jakob disease. Proc Natl Acad Sci USA 101: 3065–3070.
3. Biacabe AG, Laplanche JL, Ryder S, Baron T (2004) Distinct molecular phenotypes in bovine prion diseases. EMBO 5: 110–114.
4. Brown P, McShane L, Zanusso G, Detwiler L (2006) On the question of sporadic or atypical bovine spongiform encephalopathy and Creutzfeldt-Jakob disease. Emerg Infect Dis 12: 1816–1821.
5. Buschmann A, Gretzschel A, Biacabe AG, Schiebel K, Corona C, et al. (2006) Atypical BSE in Germany – Proof of transmissibility and biochemical characterization. Vet Microbiol 117: 103–116.
6. Capobianco R, Casalone C, Suardi S, Mangieri M, Miccolo C, et al. (2007) Conversion of the BASE prion strain into the BSE strain: the origin of BSE? PLoS Pathog; 3: e31. Doi:10.1371/journal.ppat.0030031.
7. Parchi P, Giese A, Capellari S, Brown P, Schulz-Schaeffer W, et al. (1999) Classification of sporadic Creutzfeldt-Jakob disease based on molecular and phenotypic analysis of 300 subjects. Ann Neurol 46: 224–233.
8. Collinge J, Whitfield J, McKintosh E, Beck J, Mead S, et al. (2006) Kuru in the 21st century – an acquired human prion disease with very long incubation periods. Lancet 367: 2068–2073.
9. Lasmézas CI, Deslys JP, Demaimay R, Adjou KT, Lamoury F, et al. (1996) BSE transmission to macaques. Nature 381: 743–744.
10. Lasmézas CI, Fournier JG, Nouvel V, Boe H, Marce D, et al. (2001) Adaptation of the bovine spongiform encephalopathy agent to primates and comparison with Creutzfeldt-Jakob disease: implications from human health. Proc Natl Acad Sci USA 98: 4142–4147.
11. Herzog C, Riviere J, Lescoutra-Etchegaray N, Charbonnier A, Leblanc V, et al. (2005) PrP^{TSE} distribution in a primate model of variant, sporadic, and iatrogenic Creutzfeldt-Jakob disease. J Virol 79: 14339–14345.
12. Herzog C, Salès N, Etchegaray N, Charbonnier A, Freire S, et al. (2004) Tissue distribution of bovine spongiform encephalopathy agent in primates after intravenous or oral infection. Lancet 363: 422–428.
13. Deslys JP, Comoy E, Hawkins S, Simon S, Schimmel H, et al. (2001) Screening slaughtered cattle for BSE. Nature 409: 476–477.
14. Deslys JP, Comoy E, Grassi J. Method for diagnosing a transmissible spongiform subacute encephalopathy caused by an unconventional transmissible agent strain in a biological sample. Patent N°99/14242, 12 November 1999.
15. Eloit M, Adjou K, Coulpier M, Fontaine JJ, Hamel R, et al. (2005) BSE agent signatures in a goat. Vet Rec 156: 523–524.
16. Gambetti P, Kong, Zou W, Parchi P, Chen SG (2003) Sporadic and familial CJD: classification and characterization. Brit Med Bull 66: 213–239.
17. Yutzy B, Holznagel E, Coulibaly C, Stuke A, Hahmann U, et al. (2007) Time-course studies of 14-3-3 protein isoforms in cerebrospinal fluid and brain of primates after oral or intracerebral infection with bovine spongiform encephalopathy agent. J Gen Virol 88: 3469–3478.
18. Kong Q, Zheng M, Casalone C, Qing L, Huang S, et al. (2008) Evaluation of the human transmission risk of an atypical bovine spongiform encephalopathy prion strain. J Virol 82: 3697–3701.
19. Benestad SL, Sarradin P, Thu B, Schonheit J, Tranulis MA, et al. (2003) Cases of scrapie with unusual features in Norway and designation of a new type, Nor98. Vet Rec 153: 202–208.
20. Le Dur A, Beringue V, Andreoletti O, Reine F, Lai TL, et al. (2005) A newly identified type of scrapie agent can naturally infect sheep with resistant PrP genotypes. Proc Natl Acad Sci USA 102: 16031–16036.
21. Brown P, Gibbs CJ Jr, Rodgers-Johnson P, Asher DM, Sulima MP, et al. (1994) Human spongiform encephalopathy: the National Institutes of Health series of 300 cases of experimentally transmitted disease. Ann Neurol 35: 513–529.
22. Williams L, Brown P, Ironside J, Gibson S, Will R, et al. (2007) Clinical, neuropathological and immunohistochemical features of sporadic and variant forms of Creutzfeldt-Jakob disease in the squirrel monkey (Saimiri sciureus). J Gen Virol 88: 688–695.
23. Krasnianski A, Meissner B, Schulz-Schaeffer W, Kallenberg K, Bartl M, et al. (2006) Clinical features and diagnosis of the MM2 cortical subtype of sporadic Creutzfeldt-Jakob disease. Arch Neurol 63: 876–880.
24. Puoti G, Giaccone G, Rossi G, Canciani B, Bugiani O, et al. (1999) Sporadic Creutzfeldt-Jakob disease: co-occurrence of different types of PrPSc in the same brain. Neurology 53: 2173–2176.
25. Polymenidou M, Stoeck K, Glatzel J, Vey M, Bellon A, et al. (2005) Coexistence of multiple PrP^{Sc} types in individuals with Creutzfeldt-Jakob disease. Lancet Neurol 4: 805–814.
26. Yull HM, Ritchie DL, Langeveld JPM, van Zijderveld FG, Bruce ME, et al. (2006) Detection of type 1 prion protein in variant Creutzfeldt-Jakob disease. Amer J Pathol 168: 151–157.

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研究報告の概要

本稿では，米国で最近上市された 2 種類の血液製剤（トロンビン及びフィブリノゲン）を用いて，パルボウイルス B19（B19V），A 型，B 型及び C 型肝炎，HIV ならびに変異型クロイツフェルト・ヤコブ病（vCJD）関連プリオンの病原体感染リスクを評価した。特に，A 型肝炎ウイルス（HAV）及び B19V は，エンベロープを持たないため不活化がより困難であることから本試験の対象とした。これら血液製剤の製造過程では 2 つの異なるウイルス除去工程が使われている。各病原体の安全域は，出発原料内の最大ウイルス量と製造工程の除去能との比較によって決定した。フィブリノゲン及びトロンビンは，ともに 1 バイアル当たりの病原菌が伝播する残遺リスクが極めて低く，A 型，B 型及び C 型肝炎及び HIV については 10^{15} 分の 1 未満と算出された。B19V については，トロンビンが 1000 万分の 1 未満，フィブリノゲンが 50 万分の 1 未満と算出された。同様に，vCJD の病原体伝播リスクも非常に低いと推定された。新型ウイルス（西ナイルウイルス，H5N1 型インフルエンザウイルス，重症急性呼吸器症候群（SARS）ウイルス又はチクングニヤウイルス―いずれもエンベロープを持つ）の脅威に関しては，現在の製造工程が完全な不活化をもたらすことがわかっている。したがって，血漿製造業界は，血液製剤の安全性を増強するために過去 10～20 年に実施された多くの改善点により，優良な安全性プロファイルをもつ製品の製造し，提供していると著者らは結論付けた。

報告企業の意見	今後の対応
著名な 2 研究機関が発表した本研究では，新たな脅威として現れたエンベロープウイルスに対する完全不活化法や非エンベロープウイルスやプリオンに対する実質的除去法など，病原体不活化法が常に改良され続けてきたことを確認している。したがって，実質的リスクが存在する血液製剤と違い，血漿分画製剤における病原体伝播リスクは極めて低いと考えられる。	現時点で新たな安全対策上の措置を講じる必要はないと考える。

使用上の注意記載状況・その他参考事項等

BYL-2008-0323

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Estimating the pathogen safety of manufactured human plasma products: application to fibrin sealants and to thrombin

Bernard Horowitz and Michael Busch

BACKGROUND: Plasma fractionators have implemented many improvements over the past decade directed toward reducing the likelihood of pathogen transmission by purified blood products, yet little has been published attempting to assess the overall impact of these improvements on the probability of safety of the final product.

STUDY DESIGN AND METHODS: Safety margins for human immunodeficiency virus (HIV), hepatitis C virus (HCV), hepatitis B virus (HBV), hepatitis A virus (HAV), parvovirus B19, and variant form of Creutzfeldt-Jakob disease (vCJD) were calculated for the two fibrin sealants licensed in the United States and for thrombin. These products were selected because their use in a clinical setting is, in most cases, optional, and both were relatively recently approved for marketing by the US Food and Drug Administration (FDA). Moreover, thrombin and fibrinogen both undergo two dedicated virus inactivation steps and/or removal steps in accord with the recommendations of regulatory agencies worldwide. Safety margins were determined by comparing the potential maximum viral loads in contaminated units to viral clearance factors, ultimately leading to the calculation of the residual risk per vial.

RESULTS: The residual risk of pathogen transmission per vial was calculated to be less than 1 in 10^{-15} for HIV, HCV, HBV, and HAV for both fibrinogen and thrombin. Owing to the greater quantities that can be present and its greater thermal stability, the calculated risk for parvovirus transmission was 1 in 500,000 vials for fibrinogen and less than 1 in 10^7 per vial for thrombin. Assuming that vCJD is found to be present in plasma donations, its risk of transmission by these purified and processed plasma derivatives would appear to be very low.

CONCLUSIONS: The pathogen safety initiatives implemented by plasma fractionators over the past 10 to 20 years have resulted in products with excellent pathogen safety profiles. Of the agents examined, parvovirus continues to have the lowest calculated margin of safety. Despite this, parvovirus transmissions should be rare. Manufacturers are encouraged to continue exploring processes to further enlarge parvovirus safety margins and to continue exploring ways of eliminating prions.

Products derived from human plasma have important therapeutic uses, including substitution therapy for hemophilia and primary immune deficiency disorders, plasma expanders after trauma and surgery, and as hemostatic agents.[1-3] Plasma proteins and their functions are so diverse that new applications for currently licensed plasma protein products continue to be investigated[4] and novel plasma protein products continue to be developed.[5-7] Consequently, there has been an increase in the quantity of plasma processed worldwide, and significant improvements have been made in manufacturing procedures and in plant design and operation. Many of these improvements were implemented with the goal of assuring safety of plasma derivatives from transfusion-transmissible pathogens. These include 1) improved selection of donors, 2) use of plasma only from "qualified" donors who repeatedly pass viral screening procedures, 3) use of nucleic acid amplification testing (NAT) methods to detect and eliminate virus before the pooling of donor units, 4) inventory hold policies that allow interdiction of "window-phase"

ABBREVIATIONS: BVDV = bovine viral diarrhea virus; HAV = hepatitis A virus; ID(s) = infectious dose(s); PRV = pseudorabies virus; SARS = severe acute respiratory syndrome; S/D = solvent/detergent (method of virus inactivation); TNBP = tri-(*n*-butyl)phosphate (the solvent in S/D treatment); vCJD = variant form of Creutzfeldt-Jakob disease that infects man and presumptively has arisen from the epidemic of bovine spongiform encephalopathy in cattle; WNV = West Nile virus.

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TRANSFUSION **;**:**-**.

units before pooling, 5) employment of purification procedures shown to remove virus or prions should they be present, 6) the use of two complementary or "orthogonal" methods of virus inactivation, and 7) the engineering and design of facilities so as to prevent contamination of downstream process streams with upstream fractions.

Products that promote hemostasis and tissue sealing following trauma and surgery are among the more recently licensed human plasma products in the United States. Two fibrin sealants, one from Omrix (New York, NY) and one from Baxter (Deerfield, IL), are licensed in the United States by the Food and Drug Administration (FDA), and Omrix also recently received approval of a topically applied thrombin. While they cannot be used in all surgical settings, such as to control high-pressure (arterial) bleeds, these products have been shown to improve surgical outcomes, reduce the time to hemostasis, reduce blood loss, and reduce surgical complications.[8] Substitutes for these human plasma-derived hemostatic agents have also been developed, including bovine thrombin and recombinant-derived human thrombin. Bovine thrombin is antigenically distinct from human thrombin and has been shown to elicit antibodies when used in man.[9] These antibodies, as well as antibodies elicited to bovine impurities in the product, especially antibodies to coagulation factor (F)V, have resulted in severe bleeding complications due to cross-reaction with their human counterparts.[10-13] Higher purification has reduced this complication, although a recent report[14] indicates that antibody formation still occurs. Products made by recombinant technology have their own, somewhat unique, issues. Depending on the gene construct used and the cell line chosen, the amino acid sequence may differ from that which occurs naturally, and differences in posttranscriptional processing often result in altered patterns of glycosylation or other molecular changes.[15-19] Consequently, immunogenicity is a potential problem that needs to be continually assessed. Also, depending on specific production details, manufacturing procedures must employ steps designed to inactivate and/or remove viral contaminants (and other potential pathogens) known to be present in the cell line and/or in the culture medium employed.[20] Additionally, in many circumstances, the higher cost associated with recombinant proteins limits their use.

In the past decade, many estimates of the viral safety of transfused whole blood and its components (i.e., red blood cells, platelet concentrates, and fresh-frozen plasma) have been published, with each passing year showing improved viral and bacterial safety.[21,22] In the same time frame, aside from monitoring clinical outcomes and despite the aforementioned improvements, little has been published to assess the parallel increase in safety of manufactured plasma products. A recent publication by Janssen and colleagues[23] used a probabilistic, Monte Carlo model to estimate the risk of human immunodeficiency virus (HIV) and hepatitis B virus (HBV) in a hypothetical plasma derivative subjected to what appears to be a single method of viral inactivation. Based on their assumptions, they calculated that the risk per vial approximated 1 in 1 million. Given these improvements, the recent licensure of human plasma-derived topical thrombin, and the frequent surgical use of fibrin sealants, consisting of fibrinogen in addition to thrombin, it is timely to estimate their pathogen safety. These estimates are especially useful since the fibrinogen component of fibrin sealants is among the least processed blood derivatives, while the manufacturing procedures for thrombin, whether part of a fibrin sealant kit or used by itself, are typical of those employed with most newer plasma derivatives. Thrombin and fibrinogen both undergo two dedicated virus inactivation steps and/or removal steps. Fibrinogen and thrombin from each company are each treated by solvent/detergent (S/D). Additionally, Omrix pasteurizes its fibrinogen and nanofilters its thrombin, while Baxter vapor heats both components following lyophilization. The fibrinogen preparation cannot be nanofiltered without suffering large losses in fibrinogen and fibronectin due to their large size. The presence of fibronectin may be important since it contributes to cell adhesion.[24] For HIV, hepatitis C virus (HCV), and HBV, this report updates estimates made by one of us (BH) in 1990[25] using better information on viral loads than was available then and enlarges the pathogen list to include hepatitis A virus (HAV) and parvovirus, both of which are nonenveloped viruses, and the prion that causes variant form of Creutzfeldt-Jakob disease (vCJD). It is anticipated that the method of approach reported here can be applied to other existing or experimental blood protein products.

MATERIALS AND METHODS

Two distinct methods can be used to calculate pathogen safety. The first is to calculate safety margins by comparing the number of infectious units or doses of pathogen in the starting material to the clearance capacity of the manufacturing process. The second is to measure clinical outcomes, comparing the incidence of transmission to the quantity of product infused. Transmissions in a clinical setting should be considered the gold standard since they involve actually measuring what we want to know, and with this information, one can back-calculate clearance capacities of processes for known pathogen burdens. On the other hand, clinical studies of the type required can be extremely lengthy and expensive and the results possibly misleading. The former method has the advantage that estimates of safety can be made in advance of clinical testing. Moreover, the safety margins calculated for a wide range of viruses likely will also be applicable to unstudied and newly emerging viruses.

Product safety margins can be calculated by comparing potential viral loads with the viral clearance capacity by the formulas

$$VL = N \times C,$$

where VL is viral load, N is the number of units in a plasma pool containing infectious virus, and C is the concentration of virus in those units, and

$$\text{Safety Margin} = CC/VL,$$

where CC is the clearance capacity or the ability of the process to remove or inactivate the infectious agent being studied.

Viral load

The pathogens of interest for manufactured plasma products are largely viruses that are present in blood predominantly as cell-free virions (e.g., HBV, HCV, HIV, HAV, and parvovirus B19). Other examples include West Nile virus (WNV) and dengue viruses. The newly described vCJD agent, presumably a prion, is also a potential concern despite the absence of evidence that it is transmitted by purified plasma protein products.[26,27] Cell-associated viruses like cytomegalovirus, Epstein-Barr virus, and human herpes virus 8 are not a concern since infected cells are removed by the apheresis and filtration procedures in common use. Bacteria and fungi are also effectively removed by the terminal sterile filtrations applied to all biologic products, including plasma products, recombinant products, monoclonal antibodies, and so forth, and therefore will not be addressed here.

For the major transfusion-transmitted viral pathogens, the viral loads are typically measured as genome-equivalents (geq) per mL of plasma based on results of quantitative NAT. These loads vary dramatically during the progressive stages of infection with the highest viral loads seen transiently during the acute preseroconversion (i.e., so-called window period) stage of infection; moreover, infectiousness is also highest during this same period.[28-30] Subsequent to antibody seroconversion (and coincident with innate and adaptive cellular immune responses to infection), the agents are 1) eliminated from the body (e.g., eradication of infection, as occurs with WNV, HAV, and dengue); 2) cleared from plasma but with persistence of cell-associated virus in tissues (e.g., latent infections such as herpes viruses, parvovirus B19, and "occult" HBV infections); or 3) persistent at reduced concentrations in plasma (i.e., so-called set-point viremia after establishment of chronic HBV, HCV, and HIV infections). In addition to variations in viral load measured by NAT, as infections evolve the infectivity of viruses change profoundly.[31-39] For HIV (and its model agent simian immunodeficiency virus), HBV, HCV, and WNV, it is now well established that during the acute preseroconversion phase of infection (pre–ramp-up and ramp-up stages), virion particles in plasma are highly infectious, with 10 or fewer geq in the entire volume of plasma sufficient to transmit infection following parenteral injection. In contrast, viral particles present in plasma from the same infected individuals have significantly (10- to 1000-fold) reduced infectious potential weeks to years after seroconversion.[29-35,40-43] The reduced infectivity of plasma virus from postseroconversion phases of chronic infection is attributable to a combination of factors, including presence of endogenous neutralizing antibodies, generation of defective virions (i.e., lacking full genomes or other required infectivity factors), and immune selection of virions with reduced fitness. Hence, viral load distributions observed during acute versus chronic stages of infection need to be adjusted by a factor to account for the relative infectivity of virion particles to derive estimates for the functional viral load during each stage.

All donated blood in the United States, whether for the preparation of components or for use in manufactured plasma products, is screened by serologic assays for HIV-1 and -2, HBV, HCV, and human lymphotropic virus-1 and -2 and by NAT for HIV and HCV. Donors are also excluded if they have certain risk factors that make their exposure to viruses or prions more likely. Additionally, plasma manufacturers screen donated plasma in a minipool format for HBV, HAV, and parvovirus by NAT. The use of NAT greatly reduces viral loads since positive units missed by serologic screening procedures typically have the highest concentrations of virus, which is also highly infectious. Consequently, with very rare exceptions of concordant testing errors in serology and NAT screening, only units that test both serologically negative (i.e., window-phase units) and that have relatively low titers of infectious virus (<500-5000 infectious doses [IDs]/mL) are pooled. Furthermore, manufacturing pools are retested by NAT before fractionation to assure that high-titer viremic units were not missed as a result of erroneous testing. As a result, the probability that a fractionation pool contains a significant level of virus is extraordinarily remote.

Pathogen infectious load estimates are given in Table 1. To estimate the number of positive units missed by the screening procedures employed, currently observed NAT yields, expressed as number of positive samples per million donations, were adjusted to account for the amount the window period is believed to be closed through the use of NAT. From this, we conclude that few plasma pools will contain HIV, HCV, or HAV while contamination by HBV and parvovirus B19 will be considerably more frequent. Based on the analytical sensitivity of the NAT assays, the dilution factors during assay, and the volume of an individual donor unit, we calculated the

TABLE 1. Viral load estimates

Virus	NAT yield (number/million donations)* (A)	Percent NAT closes window period† (B)	Number of positive units missed by NAT/million donations (C) = (A/B – A)	NAT analytic sensitivity‡ (geq/ml)	NAT operational sensitivity‡ (geq/mL in the donor unit)	Maximum genomic load (log geq/ fractionation pool)§	Ratio of IDs to geq¶	Maximum viral load (log ID/pool)
HIV	0.58	48% (11/23)	0.63	1.40	717	5.7	1:1	5.7
HCV	4.08	88% (50/57)	0.56	3.10	1,587	6.0	1:1	6.0
HBV	13	23% (10/43)	44	0.66	338	5.4	1:10	4.4
HAV	0.30	71% (5/7)	0.12	2.0	1,024	5.9	1:1,000	2.9
Parvovirus B19	50.5	71% (5/7)	20.6	22.6	5,120,000	9.6	1:1,000	6.6
vCJD						4.3	1:1	4.3

* For HIV, HCV, and HBV, a NAT yield unit is defined as an antibody- or hepatitis B surface antigen (HBsAg)-negative donation detected by RNA and/or DNA screening using pooled NAT systems. Rates presented are published rates from United States and European whole-blood donor screening programs.[75-78] Although NAT yield rates for these viruses among source plasma donors are higher, this is offset by source plasma policies that stipulate that only plasma from "qualified donors" be released for fractionation and that frozen units be held in inventory enabling interdiction of quarantined potential window-phase units when donors later test reactive for infectious disease markers or are deferred for other reasons.[79] For HAV and parvovirus B19, we use the rate of detection of high titer viremic donations by low sensitivity NAT screening of whole-blood and plasma donors, irrespective of serostatus of viremic units.[80,81]

† The percentage is determined by dividing the number of days NAT detects positive samples by the number of days from when a donor becomes infectious until there is sufficient antibody to be detected serologically (HIV, HCV, HBV) or there is sufficient antibody to render the donation noninfectious (HAV, parvovirus; see Busch et al.[82] for conceptual basis for this approach and Kleinman and Busch[83] for application of this approach to HBV). The residual infectious window periods are defined as the number of days from viremia reaching the minimal infectious threshold (set as 1 copy per 20 mL of plasma; Busch et al.[84]) to the level of viremia detected by pooled-sample NAT, using the viral doubling-times during the acute ramp-up phases established for each agent (20.5 hr for HIV, 10.8 hr for HCV, 2.6 days for HBV, and approx. 1 day for HAV and parvovirus B19). This yielded pre-NAT infectious window periods of 12 days for HIV, 7 days for HCV, 33 days for HBV, and 2 days for HAV and parvovirus B19. The NAT detection windows are based on time from reaching the 50 percent sensitivity of the NAT screening assays to the point of seroconversion for HIV (11 days), HCV (50 days), and HBV (10 days) or the duration of the estimated NAT yield window period for HAV (5 days) and parvovirus B19 (5 days).

‡ We assumed that the 50 percent sensitivity levels for assays used by source plasma donors are in the same range as those reported by the National Genetics Institute (NGI). NGI NAT assays are used by approximately 60 percent of the source plasma sector for all five viruses, as well as by the American Red Cross for HAV and parvovirus B19. For HIV-1, HBV, and HCV, the analytic sensitivity quoted is that of the assay itself without taking sample dilutions or pooling into account (Schreiber et al.[79]). Operational sensitivity takes these dilutions into account and refers to the maximum quantity that could be present in the contaminated donor unit. For parvovirus, Omrix's acceptance requirement for a pool of 512 units is less than 10,000 geq per mL, and thus for operational sensitivity we used $10,000 \times 512$ (the number of units in the minipool).

§ NAT operational sensitivity was multiplied by 700, the assumed volume of the donation. Based on the number of units missed by NAT per million donations and a pool size of 6000 L, we assume that only one positive unit will enter a fractionation pool. For vCJD, we assumed 30 ID per mL in a contaminated unit.

¶ For HIV and HCV, the infectious load is considered to be equivalent to the viral load expressed in geq or copies, given that we are restricting consideration to the acute preseroconversion viremic phase, which is known to be highly infectious (see text), and that seropositive units from other donors, which might contain neutralizing antibodies, have been detected by serologic screening and excluded from the manufacturing pools. For HBV, we similarly assume high-level infectivity of window-phase donations[35,85] but reduce this to a ratio of 1 in 10, in part, because of the likely presence of anti-HBsAg in the plasma pool. We used a ratio of 1:1000 for HAV and parvovirus. We believe this to be justified since the neutralization capacity of anti-HAV is well established. While the ratio of infectious units to geq for parvovirus is unknown, results from tissue culture infectivity studies indicate that the ratio is 1:5000 for genotype 1 and 1:260,000 for genotype 2[86] for products devoid of parvovirus antibody, the lowest ID that has been reported on infusion into a seronegative recipient is 2×10^4 geq,[70] and the infectivity of products containing parvovirus antibody has been shown to be reduced considerably.[83,87,88]

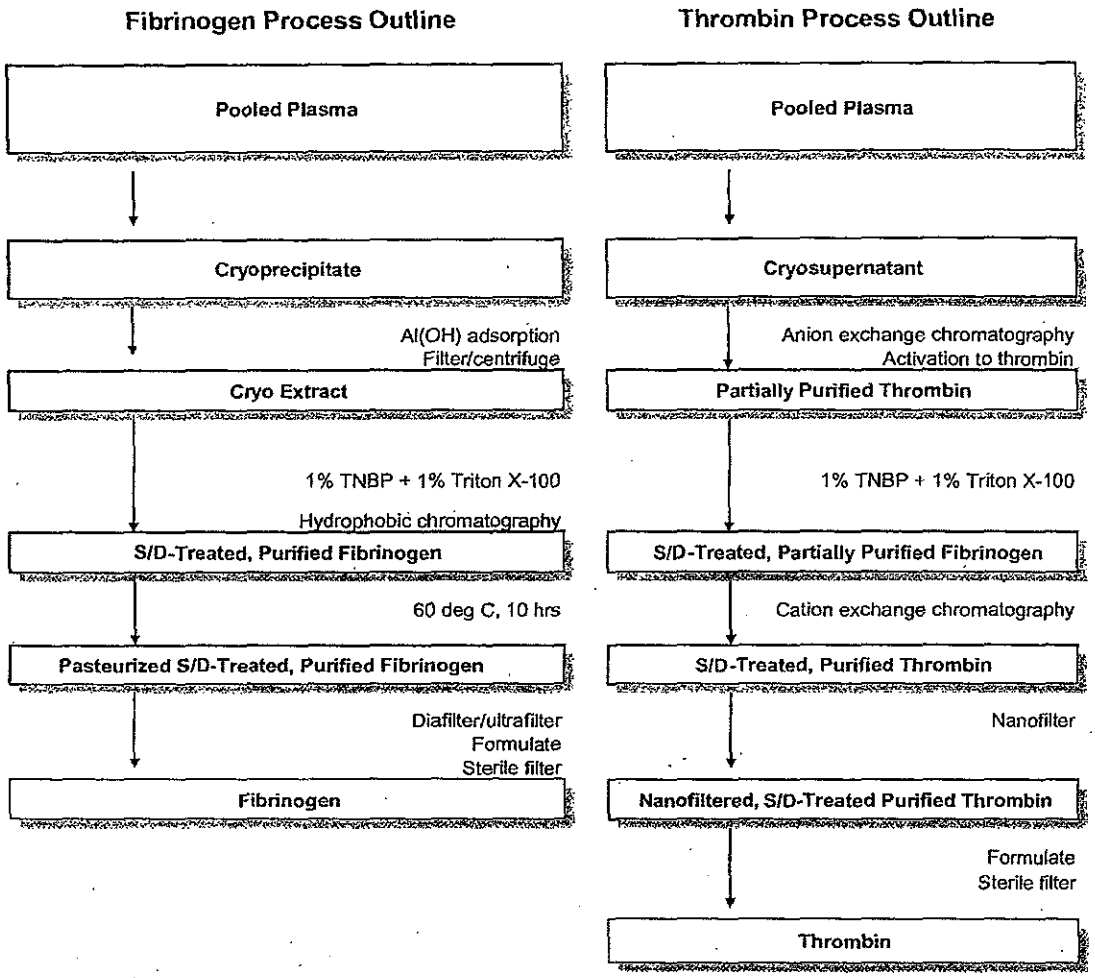

Fig. 1. Process outlines for fibrinogen and thrombin.

maximum genomic load likely to be present in a fractionation pool (Table 1, Column 7). This was adjusted downward for HBV, HAV, and parvovirus B19 to account for the reduced infectivity of virus that occurs as a result of neutralizing antibodies derived from other donors in the pool, since antibody screening is not performed for these prevalent agents. No adjustment was applied to HIV or HCV since later-stage infections with potential neutralizing antibodies are interdicted by the currently deployed serologic tests. Although attempts to transmit vCJD by human plasma have failed,[44] epidemiologic evidence supports its transmission by whole blood and blood components.[45-47] Based on animal models, its concentration is likely to be quite low, estimated at 20 to 30 IDs per mL.[48] With the use of this estimate, the maximum concentration in the plasma pool approximates 0.003 IDs per mL (3 IDs/L), and the total maximum load in a 6000-L plasma pool will approximate $10^{4.3}$ IDs (Table 1).

Clearance capacity

The clearance capacity for pathogens is a function of the extent to which pathogen is removed during steps designed to purify the protein of interest, the inclusion within the manufacturing process of dedicated viral inactivation and removal steps, the presence of neutralizing antibody in final product, and serendipitous inactivation that occurs. The process steps for fibrinogen and thrombin used by Omrix are outlined in Fig. 1. As is typical of modern plasma protein products, each process includes two dedicated, viral elimination steps: fibrinogen is treated with S/D and is pasteurized, and thrombin is treated with S/D and is passed through a purposefully designed, virus removal filter (so-called nanofiltration). Additionally, each chromatographic step and filtration in the presence of filter aid can contribute to pathogen removal. Baxter's Tisseel is processed similarly except, when first introduced in 1998, it utilized vapor heating as