

# TSE Clearance During the IGIV-C Filtration Process

*Chris Stenland  
Jarrett Terry  
Kang Cai  
Mark Nelson*

*Randal Hartwell  
Richard Rubenstein  
Michael Fournel  
Stephen Petteway*

Recent work with animal models (Rohwer et al, Houston et al) demonstrated the presence of transmissible spongiform encephalopathy (TSE) infectivity in rodent and ovine blood. Transmission of TSE infectivity by human blood or blood components has not been established, but remains a theoretical risk. TSE agents are resistant to standard methods of pathogen inactivation. Current methods that can reduce TSE infectivity titers (e.g., treatment with strong base or autoclaving) destroy the biological activity of therapeutic proteins. Thus, increasing the margin of safety for biologicals regarding TSE transmission relies heavily on clearance methods. The initial filtration steps employed in the manufacture of a new intravenous immune globulin produced by caprylate virus inactivation and column chromatography, IGIV-C, were evaluated for their ability to remove spiked TSE infectivity and the pathogenic prion protein. The bench scale model was characterized by biochemical analysis and found to operate similarly to the larger scale process. Resuspended II + III paste, the starting material for the production of IGIV-C, was spiked with 1% final concentration hamster scrapie brain homogenate and the filtration steps were performed. The input and output fractions were evaluated for PrP<sup>Sc</sup> content by Western blot and TSE infectivity by animal bioassay. More than 10 logs of TSE infectivity removal were demonstrated during the filtration steps.

Presented at the 44th American Society of Hematology Annual Meeting  
Philadelphia, Pennsylvania, December 6–10, 2002  
Publication in *Blood*, Volume 100, Issue 11, a2799

# TSE Clearance During the IGIV-C Filtration Process

C. STENLAND,<sup>1</sup> J. TERRY,<sup>1</sup> K. CAI,<sup>1</sup> M. NELSON,<sup>1</sup> R. HARTWELL,<sup>1</sup> R. RUBENSTEIN,<sup>2</sup> M. FURNEL<sup>1</sup> AND S. PETTEWAY<sup>1</sup>

<sup>1</sup> Department of Pathogen Safety Research, Bayer Corporation, P.O. Box 13887, Research Triangle Park, North Carolina 27709  
<sup>2</sup> Department of Virology, New York State Institute for Basic Research in Development Disabilities, Staten Island, NY 10314

## ABSTRACT

Recent work with animal models (Rohwer et al, Houston et al) demonstrated the presence of transmissible spongiform encephalopathy (TSE) infectivity in rodent and ovine blood. Transmission of TSE infectivity by human blood or blood components has not been established, but remains a theoretical risk. TSE agents are resistant to standard methods of pathogen inactivation. Current methods that can reduce TSE infectivity titers (e.g., treatment with strong base or autoclaving) destroy the biological activity of therapeutic proteins. Thus, increasing the margin of safety for biologicals regarding TSE transmission relies heavily on clearance methods. The initial filtration steps employed in the manufacture of a new intravenous immune globulin produced by caprylate virus inactivation and column chromatography, IGIV-C, were evaluated for their ability to remove spiked TSE infectivity and the pathogenic prion protein. The bench scale model was characterized by biochemical analysis and found to operate similarly to the larger scale process. Resuspended II+III paste, the starting material for the production of IGIV-C, was spiked with 1% final concentration hamster scrapie brain homogenate and the filtration steps were performed. The input and output fractions were evaluated for PrP<sup>Sc</sup> content by Western blot and TSE infectivity by animal bioassay. More than 10 logs of TSE infectivity removal were demonstrated during the filtration steps.

## INTRODUCTION

Minimizing the risk of transmission of infectious diseases is of primary importance in the manufacture of products derived from the human plasma resource. A novel chromatography-based intravenous immunoglobulin (IGIV-C; Gamunex™ 10%) manufacturing process was developed to inactivate lipid-enveloped viruses, as well as increase the yield and purity of the final IgG product and generate a product that more closely reflects the IgG subclass distribution found in plasma. Transmissible spongiform encephalopathies (TSEs) are a group of lethal mammalian neurodegenerative diseases, and infectivity is associated with the pathogenic isoform of the prion protein.<sup>1,2</sup> Human TSEs include kuru, sporadic and variant Creutzfeldt-Jakob disease, Gerstmann-Sträussler-Scheinker syndrome and fatal familial insomnia. To date no epidemiological evidence for a TSE transmission by human plasma-derived biologicals has been observed, although experimental evidence in rodent, sheep, and primate models suggests the presence of low level infectivity in various blood fractions.<sup>3-7</sup> Thus, a theoretical risk for transmission of TSEs by human plasma-derived biologicals exists. Methods that can reduce TSE infectivity titers (e.g., treatment with strong base or autoclaving) destroy the biological activity of therapeutic proteins. Increasing the margin of safety for biologicals regarding TSE transmission relies heavily on clearance methods. Using spiking studies, the potential of the IGIV-C manufacturing process to remove TSE-related pathogens was demonstrated.

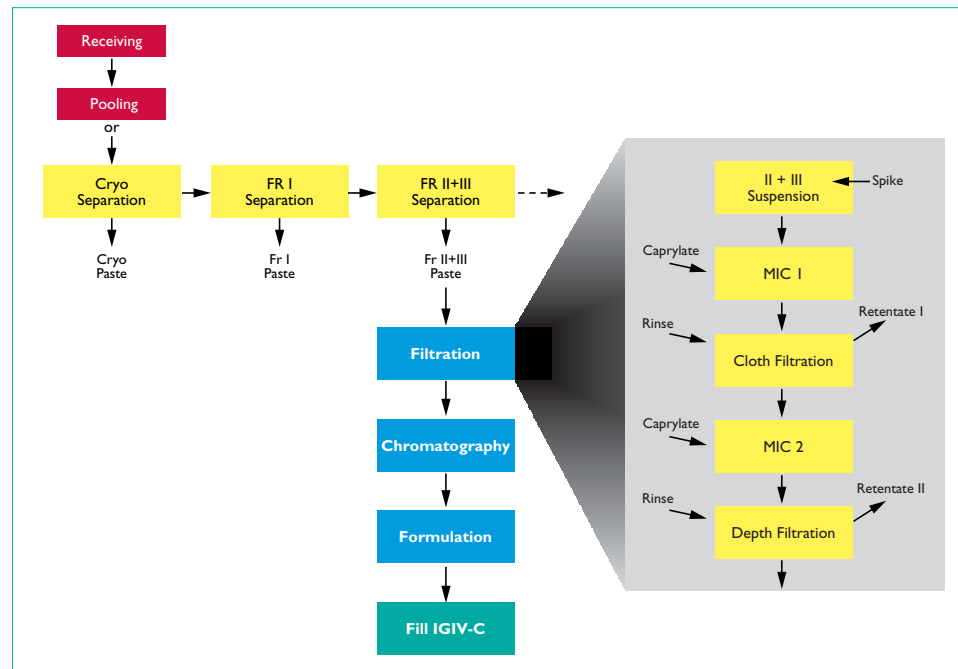
## OBJECTIVE

The objective of this study was to assess the TSE reduction in the IGIV-C (Gamunex™ 10%) manufacturing process.

## MATERIALS AND METHODS

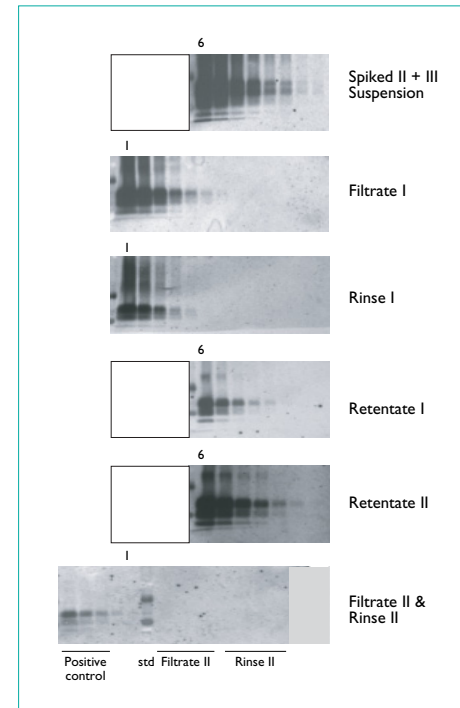
- The large-scale IGIV-C process was scaled down and characterized.
  - The small-scale process was shown to be a good model of the larger scale process (data not shown).
  - Next the TSE spike challenge was performed in triplicate.
- Fraction II+III Paste (Bayer, Clayton, NC) was resuspended and spiked with scrapie brain homogenate (Hamster 263K).

- Caprylate was added to this to form Material In Caprylate I (MIC I); impurity proteins are precipitated.
- Filter aid added and the suspension filtered.
- Caprylate was again added, and incubated for the MIC 2, a viral inactivation step.
- Filter aid added and the solution was depth filtered.
- Filtrates and retained portions were sampled and analyzed by Western blot<sup>8</sup> and animal bioassay<sup>9</sup> for prion clearance.



**Figure 1.** Overview of IGIV-C production and steps evaluated within the filtration process. IGIV-C starts from pooled human plasma and the immunoglobulin rich (Fraction II+III) paste isolated by the Cohn-Onclay purification method. The IGIV-C process consists of two purification phases: filtration and chromatography. Only the filtration steps were evaluated for the current study. The Fraction II+III paste is solubilized and

caprylate is added under conditions that precipitate unwanted proteins (MIC I). Filter aid is added and the solution is filtered through a cloth membrane. This filtrate is again treated with caprylate, a viral inactivation step, which also allows for clearance of TSE infectivity and PrP<sup>Sc</sup>. Filter aid is added after the caprylate incubation, and the solution filtered through a depth filter pad.



**Figure 2.** Prion Clearance in the IGIV-C Filtration Process. Representative Western blots from the TSE-spiked IGIV-C filtration are shown. The number above the blot is the first vial evaluated in the half log serial dilution. Blots are offset where necessary to compare the endpoints from each sample. If the first lane shown (i.e., 6) is positive for PrP<sup>Sc</sup>, then all earlier dilutions of sample were also positive for PrP<sup>Sc</sup> (not shown). The concentration of PrP<sup>Sc</sup> is directly proportional to the number of positive lanes counted in each blot. The panel at the bottom shows the final filtrate and rinse, with positive controls to the left of the molecular weight standard. The presence of the positive control contrasts the absence of PrP-specific signal in the filtrate and rinse portion, indicating PrP<sup>Sc</sup> is absent (or below the limit of detection) in the sample and not failure of the assay.

## DETERMINATION OF CLEARANCE

- Infectivity titer (LD<sub>50</sub>) was calculated by the method of Spearman and Karber.
- Clearance was calculated with the following formula:

$$\text{Log}_{10}(\text{RF}) = \text{Log}_{10} \left[ \frac{\text{Input PrP}^{\text{Sc}} \text{ Titer} \times \text{Input Volume}}{\text{Output PrP}^{\text{Sc}} \text{ Titer} \times \text{Output Volume}} \right]$$

**Table 1.** Prion clearance of IGIV-C filtration process.

Process Step	Log <sub>10</sub> Reduction of Hamster Scrapie Spike	
	PrP <sup>Sc</sup> Partitioning	Infectivity Partitioning
Caprylate Precipitation/Cloth Filtration	2.9	2.8
Depth Filtration	≥ 4.9	≥ 7.4
Global Reduction	≥ 7.8	≥ 10.2

## RESULTS AND CONCLUSION

- Caprylate precipitation/cloth filtration produces 2.8–2.9 log<sub>10</sub> clearance of prion protein and infectivity.
- Depth filtration shows clearance to the limit of detection for both prion protein (≥ 4.9 log<sub>10</sub>) and infectivity (≥ 7.4 log<sub>10</sub>).
- Global reduction by the IGIV-C (Gamunex™ 10%) filtration process is ≥ 7.8 log<sub>10</sub> for prion protein and ≥ 10.2 log<sub>10</sub> for infectivity.
- Both assays demonstrate significant reduction of TSE infectivity by filtration steps of the IGIV-C (Gamunex™ 10%) process.

## REFERENCES

- Kitamoto T, Tateishi J. Human prion disease and human protein disease. *Curr Top Microbiol Immunol* 1996;207:27–34.
- Prusiner S, Hsiao K. Human prion diseases. *Ann Neurol* 1994;35:385–95.
- Robinson MM. Transmissible encephalopathies and biopharmaceutical production. *Dev Biol Stand* 1996;88:237–41.
- Brown P. Transmission of spongiform encephalopathy through biological products. *Dev Biol Stand* 1998;93:73–8.
- Brown P, Rohwer RG, et al. The distribution of infectivity in blood components and plasma derivatives in experimental models of transmissible spongiform encephalopathy. *Transfusion* 1998;38:810–6.
- Brown P, Cervenkova L, et al. Further studies of blood infectivity in an experimental model of transmissible spongiform encephalopathy, with an explanation of why blood components do not transmit Creutzfeldt-Jakob disease in humans. *Transfusion* 1999;39:1169–78.
- Houston F, Foster JD, et al. Transmission of BSE by blood transfusion in sheep. *Lancet* 2000;356:999–1000.
- Lee DC, Stenland CJ, et al. Monitoring plasma processing steps with a sensitive Western blot assay for the detection of the prion protein. *J Virol Methods* 2000;84:77–89.
- Lee DC, Stenland CJ, et al. A direct relationship between the partitioning of the pathogenic prion protein and transmissible spongiform encephalopathy infectivity during the purification of plasma proteins. *Transfusion* 2001;41:449–55.