Prion Clearance with Membrane Filters

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Pall, in collaboration with Q-One Biotech, has presented data for hamster-adapted scrapie (HSc) and Reovirus clearance (see Acknowledgments). The spiked challenge fluid was filtered directly through grades DV50 or DV20 filters or first prefiltered through the grade DV50 followed by the filtration of this effluent through the grade DV20 membrane. No signal was detected using the Western blot assay (3F4 antibody for detection of PrP) postfiltration through either the grades DV50 or DV20 membrane, demonstrating complete clearance of the HSc spike (>2.8 log titer reduction). The ability to make a higher clearance claim is restricted by the level of HSc spike used and the limited sensitivity of the Western blot assay relative to bioassays.

Routinely, from a process standpoint, a filter's performance in terms of bacterial/viral removal must be documented through a physical test, performed during manufacturing, which can be correlated to its performance. Physical nondestructive integrity tests commonly used include a "bubble point" or a "forward flow" air diffusion test. In general, the tighter the grade of membrane, the higher the bubble point. "Bubble point" type tests to confirm the integrity of small area filtration systems (e.g., 47 mm discs) are of limited value for virus removal grade membranes as the water-wet "bubble point" of these membranes is excessively high (>300 psi); consequently, such testing is logistically impractical. In view of the inability to conduct a physical integrity test with a small area (47 mm) disc assembly, a biological internal control, namely, Reovirus (sized at 60–80 nm), was included in the challenge testing. The grades DV50 and DV20 membranes have been rated to provide ≥6 log removal of viruses larger than 50 nm. As expected, complete clearance of the Reovirus spike was observed.

Transformation of an innocuous host cell protein into a pathological isoform (PrP^{sc}) confers on it unique properties, making prions resilient and difficult to clear by methods that would constitute "overkill" for conventional infectious agents. Validation studies to demonstrate prion clearance are difficult to design. Key issues to address include the detection method and the nature of the spiking agent to be used. While there is no clear agreement about what constitutes a single prion particle, theoretical work as well as experimental findings suggest that prions exist in an aggregated state. For evaluation of filtration for prion clearance, a crude (unpurified) spike preparation is not relevant because membrane association of the prion protein results in increase in the effective filtration size of the agent and, consequently, enhanced removal by the filtration system. Detergent-solubilized agent is the spike of choice in filtration studies, constituting a "worst case" challenge for filter membranes. Detergent reduces the size of the spiking agent and may, in fact, make the prion agent more likely to penetrate the pores of the filter.

The novel biology of prions makes their detection difficult. The Western blot assay is 2–3 logs less sensitive than a bioassay, but it serves as a significant tool for detection and preliminary evaluation of the capacity of a particular manufacturing process for prion clearance. Available data suggest a close correlation between the data obtained from Western blot assays and hamster bioassays. In this study, two "viral retentive" nanofiltration membranes, the Ultipor® VF grades DV50 and DV20 membranes, were evaluated for their prion clearance ability. The grade DV50 membrane provides >6 log reduction of viruses ≥50 nm in size and is being used in manufacturing processes for clearance of retroviruses and other specific and nonspecific model viruses. The grade DV20 membrane has been demonstrated to remove ≥3 logs of parvovirus and other small viruses and provides >6 log reduction for >50 nm viruses. Reovirus (sized at 60–80 nm) was included as an internal biological control in our study. As expected, both DV50 and DV20 membranes provided complete clearance of the Reovirus (based on $TCID_{50}$ bioassay) and HSc (as detected by the Western blot assay). Higher claims (>2.8 LTR) for HSc clearance could not be made because of the low input spike used and the limited sensitivity of the Western blot assay as compared with infectivity assays. Our data suggest applicability of the "virus grade" nanofiltration membranes for removal of not only conventional viral agents but also unconventional agents, such as TSE agents. These filters are especially suitable where an integrity testable filter is desired to ensure the safety of biologicals and biopharmaceuticals.

Calculation of Log Titer Reduction Factor: Log Titer Reduction (LTR) was calculated relative to the level of PrP or Reovirus in the spiked start material. Reovirus was assayed using the $TCID_{50}$ assay. Titers of the scrapie agent were calculated based on the end point dilution for samples after analysis by Western blotting. The end point dilution is the first dilution at which no PrP can be detected; the reciprocal of this dilution is taken as the titer of the agent in arbitrary units/mL. Where no PrP was detected at the highest concentration of sample tested, the reciprocal of the dilution was taken as 1, and reduction was expressed with a $\geq$ sign preceding the logarithmic (log 10) value.

Company Information

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Pall is the leading global provider of filtration, purification, and separation technologies to the diverse and rapidly expanding life sciences market. Its products are used from the earliest stages of discovery and development of new drugs through production and delivery of therapies for the prevention, diagnosis, and treatment of disease.

In the laboratory, Pall products help facilitate the drug discovery and development process to get innovative drugs to the market faster. In biopharmaceuticals, Pall products are applicable to laboratory and pilot-scale development, aseptic processing, biologicals, bioprocessing, fermentation, and downstream processing. In medical and transfusion medicine, Pall is the market leader in blood filtration systems to improve the quality and safety of the world's blood supply, and in medical filters that enhance the safety of cardiac surgery, drug delivery, intravenous feeding, and the accuracy of diagnostic tests.

Pall Life Sciences provides membrane and membrane devices to optimize detection and sample preparation in basic drug research, clinical diagnostics, combinatorial chemistry, and high-throughput screening, as well as in the growing genomics and proteomics markets.

In pharmaceutical and bioprocess manufacturing, Pall's direct and tangential flow filtration technologies are used for biomass removal, clarification, particulate and bioburden reduction, prefiltration, sterile filtration of liquids and gases, mycoplasma and virus clearance, endotoxin and DNA removal, and biomolecule purification and concentration. Products range from small laboratory-scale devices to large automated systems and filter test instruments.

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Pall's Scientific and Laboratory Services, and Validation Laboratory groups have been a cornerstone of support to our customers for more than 30 years. We employ more than 400 scientists, engineers, and technicians in fully equipped laboratories in the Americas, Europe, Australia, and Asia.

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Acknowledgments

A full report is available from Pall Corporation: *USTR2178, Demonstration of Scrapie Agent (Prion) Clearance by Hydrophilic PVDF Membrane Filters*. This work was a collaborative effort between Pall Corporation and Q-One Biotech. Ultipor is a registered trademark of Pall Corporation.