Nomenclature Standardization for "Large Pore Size" Virus-Retentive Filters

by Kurt Brorson, Gail Sofer, and Hazel Aranha

What's in a label — or name or number for that matter? A great deal, apparently. Some of today's clothing manufacturers are intentionally labeling clothes a size or two smaller than they really are — just to cater to their customers' vanity. Unclear nomenclature is unacceptable, however, in the more serious world of biotechnology. If a filter is counted on to remove viruses from biotech products by a size exclusion mechanism, its rating or nomenclature must be crystal clear. For example, 18–26 nm parvoviruses are unlikely to be retained on/in a "large pore size" virus–retentive filter (designed to remove retroviruses and other larger viruses).

Virus filtration is a critical unit operation during the manufacture of recombinant proteins and plasma-derived biopharmaceuticals. At a conference earlier this year, Patrick Celis of the European Medicines Evaluation Agency (EMEA) remarked that virus filtration appears to be one of the most common virus clearance unit operations in bioprocessing based on their frequency in marketing authorization dossiers that he has reviewed (**1**).

Viruses vary widely in shape and size, and many types can potentially compromise the safety of biologicals. Virus filters vary as well. The PDA virus filter task force (**2**) has noted that a majority can be broadly grouped as targeting "large" (retroviruses, reovirus, herpes simplex virus) or "small" (parvovirus, hepatitis A) viruses. The situation is a bit more complex than that. "Small virus" removal filters can also be used to retain the intermediate size (hepatitis B and C) viruses as well, and some "large virus" filters can extend clearance to certain medium-size viruses also.

PALL CORPORATION (WWW.PALL.COM)

To date, the naming of virus filters has been vendor specific. Among the parameters used by virus filter manufacturers are

- average pore size measurement (Asahi Kasei Planova 35N, 20N, 15N)
- types of viruses retained (Millipore Viresolve NFR, NFP)
- size of virus retained (Pall Ultipor DV50 and DV20)
- nominal molecular weight cutoff (MWCO, according to dextran or protein measurement; Pall Filtron Omega 300K and 100K)
- MWCO of proteins (Millipore Viresolve 180 and 70) that can pass through the membrane (**3, 4**).

Some end users, particularly newer and less experienced professionals, may find those naming systems ambiguous and confusing. A single rating system would promote transparency by placing filters into groups where a minimum level of clearance of particles of defined size can be achieved. New entrants into the virus filter market would be generally expected by end users to achieve that level of performance.

In 2002, the Parenteral Drug Association (PDA, www.pda.org) convened a virus filter task force of biotech industry professionals, with representatives of regulatory groups and filter manufacturers. Their purpose was to develop a common nomenclature and standardized test methods for classifying and identifying viral-retentive filters. Additionally, the task force was charged with producing a technical report on virus filtration.

Based on a reported 53- to 64-nm diameter (**5–7**) and previous successful use in testing the size-exclusion properties of large-virus filters, the PDA virus filter task force arrived at a consensus that the coliphage PR772 could serve as a model to standardize nomenclature for large-pore-size virus filters.

After extensive discussion, a large-virus filter test method was drafted. The committee agreed that the purpose of this method was to provide a common nomenclature system for large-virus filters. The purpose was *not* to test filters at maximum or worst-case operating conditions, *nor* to compare filters from one manufacturer with another, *nor* to be a substitute for process validation for the purpose of regulatory compliance.

The general method applied to large-virus filters made by all filter manufacturers participating in the task force. It prespecified permissible ranges for relevant operating parameters based on industry practice, published literature, and filter manufacturer recommendations. Bracketed ranges were set to allow testing at conditions (recommended by the filter manufacturers) and to be realistic for commercial operations. The committee assumed that operating conditions for filters from one vendor would not be the same for those from others. When conducting tests of individual filters, manufacturers picked parameter set points (± reasonable limits) for their specific protocols from those bracketed acceptable operating ranges in the general document.

In Fall 2004, the filter method was prototyped in a third-party laboratory (CDER/FDA) in collaboration with three filter manufacturers: Pall Corporation, www.pall.com; Millipore Corporation, www.millipore.com; and Asahi Kasei, www.asahi-kasei.co.jp. Each vendor provided samples from three different QC-released filter lots intended for process-scale manufacturing.

The Model Works

Why use a bacteriophage instead of a mammalian virus for nomenclature standardization? When virus filters clear their targets primarily by size exclusion, their performance should depend almost entirely on spatial constraint. So it is logical that any virus (either mammalian or bacterial) in the same size range should be applicable as a surrogate for preliminary evaluation of the performance capabilities of filters.

Table 1: Salient characteristics of bacteriophage PR 772

Physical Characteristics	• Icosahedral • Size per —Early TEM reports (Coetzee et al., 1979): 53 nm (probably inaccurate) —International Committee on the Taxonomy of Viruses (Bamford and Ackermann, 2000): 64 nm —Dynamic light scattering (Lute et al., 2004): 82 nm
Logistic Considerations	• *Escherichia coli* host used to propagate PR 772 is nonpathogenic so a Biosafety Level 1 laboratory is enough • Easily cultivated to high titers —Crude preparations: ~10^{10} pfu/mL —CsCl purification: ~10^{13} pfu/mL • Easy to enumerate using the agar-overlay plaque assay, with titers obtained after an 18–24 h incubation • Little loss of infectivity after storage at 4 °C for 2–4 months • Monodispersed after 2 months at 4 °C
Genetic Sequence	• Genome size: ~15 kb • 32 open reading frames of at least 40 codons each • 97% identity to the genome of *Tectiviridae* family prototype phage PRD1
Bioinformatic Analysis	• Overall gene order of PRD1 and PR 772 highly conserved (It was possible to assign putative functions to almost all gene products.) • No identifiably undesirable DNA sequences (e.g., virulence factor, antibiotic resistance): Phage–host system is suitable for routine laboratory work.
Other	• CsCl procedure eliminates almost all contaminating nucleic acids, a concern for QPCR assays. • Availability of genome sequence also provides a powerful tool for quality control of the phage preparations. • Distinguishable from other *Tectiviridae* phages by *Hae*III and *Rsa*I endonuclease digestions

From a practical standpoint, bacteriophages are far easier and safer to work with (Table 1).

For several decades, bacteriophages have been used as surrogates for mammalian viruses in size-based removal applications, such as the medical and environmental fields (**8**). Phages have also been used by filter manufacturers to evaluate the size exclusion properties of their viral removal filters (**9–11**).

Current regulatory expectations involve assessment of validation data with mammalian model viruses. However, a great deal of process development work must be done before a BLA submission. Process development groups at biopharmaceutical companies may perform feasibility studies to evaluate the potential performance of proposed viral clearance steps. Such studies are not part of any regulatory claim, so appropriate-sized bacteriophages can be considered for them. The PDA nomenclature will not exclusively serve as a regulatory claim, so the use of bacteriophages is warranted in this context.

Characterizing Bacteriophage PR772

PR772 had not been extensively characterized before its selection by the task force. Some TEM studies (**6, 7**), limited sequencing (**5**), and filtration studies (**9–11**) had been performed, but not enough to justify using this coliphage as a usiversal standard model for virus filters. To fill that gap, two members of the task force (Kurt Brorson and Hazel Aranha) decided to extensively characterize PR772, then published those findings (**12**). Key goals included the following:

- Develop standard and easy-to-perform preparation methods.
- Gain a better size estimate by dynamic light scattering (DLS) analysis.
- Determine aggregation status for various types of preparations (using DLS analysis).
- Determine prefilterability through 100-nm filters.
- Measure the stability of infectivity and monodispersion upon storage at 2–8 °C.

Table 2: Summary of prototype testing of the filter method at FDA/CDER in fall of 2004

Filter	n =	Integrity/ Installation	PR772 Retention (log_{10})	IVIG Passage
Planova 35N	9	+	>8.7	+
DV50	6	+	>9.2	+
NFR	6	+	>9.1 (5) 7.8 (1)	+

- Determine the genomic sequence of PR772.
- Develop a phage identity test.
- Determine stability after freezing and thawing.

In each case, PR772 proved suitable for its intended use. Cesium chloride (CsCl) gradient-purified preparations of this coliphage are stable, monodispersed, and easy to make at high titers. The hydrodynamic diameter of the virus was measured by DLS at 82 nm, a figure that is likely to be much more accurate than TEM measurements of 53 nm from the 1970s (**6, 7**), which are known to be artifact prone (**13**). Further, DLS measures the hydrodynamic behavior of particles in solution (**14**), a measurement that is more representative of actual viral behavior during filtration. Table 1 lists PR772s characteristics (**12, 15**).

Design of Experiments and Results

In Fall 2004, representatives of three filter manufacturers traveled to CDER/FDA (Bethesda, MD) to assist in prototyping the PDA task force's recommended test method. As detailed in PDA's TR41 (**2**), this method sets acceptance criteria for virus retention (LRV > 6 log_{10}), protein passage (>95% passage of IVIG), and integrity–installation testing (must pass vendor test). Three membrane lots or spinner series were tested, and each vendor's filters passed as described in Table 2. It should be noted that not only did the Viresolve NFR, Ultipor DV50, Planova 35N meet the PR772-LRV6 rating, but all filters exceeded the rating by an impressive 2–3 log_{10}.

Given this successful standardization of "large pore size" virus retention filters, the PDA/FDA task force will move on to standardize nomenclature of "small pore size" filters. Because of technical issues associated with small-virus filters, the task force assumes that a proscriptive approach of rigidly applying the current design as a template for its future small virus filter studies is not warranted. The committee also realizes that additional flexibility specific to the filter manufacturers may be needed in those future studies. The task force meeting first took place in Bethesda, MD, at a PDA/FDA joint conference in September 2005.

PDA and CDER have successfully developed and promulgated a large-virus filter nomenclature standard based on retention of the bacteriophage PR772. The virus filter task force is now working on standardization of "small pore size" virus-retention filters.

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