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# Integrity testing of Planova™ BioEX virus removal filters used in the manufacture of biological products

Shinya Sekine [a], Masayasu Komuro [b], Takayuki Sohka [c, *], Terry Sato [c]

[a] Bioprocess Technology Development Department, Asahi Kasei Medical MT Corporation, 5-4960 Nakagawara, Nobeoka, Miyazaki 882-0031, Japan
[b] Medical Material Laboratory, Medical Products Development Division, Asahi Kasei Medical Co., Ltd., 5-4960 Nakagawara, Nobeoka, Miyazaki 882-0031, Japan
[c] Technical Marketing Department, Bioprocess Division, Asahi Kasei Medical Co., Ltd., 1-105 Kanda Jinbocho, Chiyoda-ku, Tokyo 101-8101, Japan

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ABSTRACT

Confirmation of virus filter integrity is crucial for ensuring the safety of biological products. Two main types of virus filter defects may produce inconsistent and undesirable performance in virus removal: improper pore-size distribution across the membrane; and specific damage, such as tears, broken fibers, or pinholes. Two integrity tests are performed on each individual filter manufactured by Asahi Kasei Medical to ensure the absence of these defects prior to shipment. In this study, we verified that typical usage of Planova™ BioEX filters would not improperly shift the pore-size distribution. Damage occurring during shipment and use (e.g., broken fibers or pinholes) can be detected by end-users with sufficient sensitivity using air–water diffusion based leakage tests. We prepared and tested filters with model pinhole defects of various sizes to develop standard acceptance criteria for the leakage test relative to porcine parvovirus infectivity logarithmic reduction values (LRVs). Our results demonstrate that pinhole defects at or below a certain size for each effective filter surface area have no significant impact on the virus LRV. In conclusion the leakage test is sufficiently sensitive to serve as the sole end-user integrity test for Planova™ BioEX filters, facilitating their use in biopharmaceuticals manufacturing.


## 1. Introduction

Fractionated plasma products and biopharmaceuticals, such as recombinant proteins and monoclonal antibodies, are playing an increasingly important role in human medicine [1,2]. However, the starting material for these products carries the risk of contamination by viruses and other pathogens. In order to ensure patient safety, regulations and guidelines for removing viruses have been enacted and proposals have been made for the implementation and validation of virus removal and inactivation methods [3–8]. The respective strengths and limitations of various virus inactivation and removal methods have been the subjects to continuous discussion among both regulators and manufacturers of biopharmaceuticals in the last few years. Virus filtration, also known as nanofiltration, is a gentle, nonspecific, and widely applied process that removes virus particles from solutions based on size exclusion properties that do not alter the functional and antigenic characteristics of most proteins [9–12]. Planova™ BioEX filters (manufactured by Asahi Kasei Medical, Co., Ltd., Japan) are composed of membranes of hollow fibers made of hydrophilic modified polyvinylidene fluoride (PVDF). PVDF is used to produce membranes for ultrafiltration [13], nanofiltration [14], and hemodialysis and is recognized for its mechanical strength [15].

Performing integrity tests (ITs) on virus filters both before and after nanofiltration is very important for confirming filter integrity and for supporting claims of virus removal. Several IT methods have been developed for virus filters [10,11,16–19]. For example, in the case of Planova™ cellulose filters, a post-use test using gold colloid particles can be used to assess improper shifts in membrane pore-size distribution. Although highly effective, this gold particle test (GPT) has the drawback of being destructive, and therefore, it cannot be performed prior to using the filter in the process setting. Thus, other testing strategies that are both accurate in assessing

*Abbreviations:* BP, bubble point; GPT, gold particle test; IT, integrity test; LRV, logarithmic reduction value; Lv, leak value; PLT, Planova™ Leak Tester; PPV, porcine parvovirus; PVDF, polyvinylidene fluoride; SD, standard deviation; $TCID_{50}$, 50% tissue culture infectious dose.

* Corresponding author. Tel.: +81 3 3296 3783.
*E-mail address:* souka.tb@om.asahi-kasei.co.jp (T. Sohka).

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membrane pore-size distribution and are efficient, robust, and user friendly must be considered.

Virus filters are verified as having the specified pore-size distribution and to be free from other defects before shipment to end-users. Several pore-size distribution ITs, such as the bubble point (BP) method, are conducted through routine sampling during the spinning of hollow-fiber membranes. In addition, each Planova™ BioEX filter is subjected to a pressure hold test and a leakage test [10,16]. End-user ITs conducted before use of the filter in the process stream must be non-destructive and should not require specialized solutions or set-up that would compromise the quality of the biopharmaceutical product or be difficult to perform. ITs for detecting improper shifts in pore-size distribution may not be required if the filter has physical and chemical properties that are sufficiently robust to withstand the filtration conditions used. On the other hand, any damage occurring during shipment and use is likely to result in gross defects larger than the micron scale, including hollow fiber breakage. Pre- and post-use ITs performed by the end-user are needed to detect such damage. Typical large gross defects should be easily detectable by visual inspection; however, it is also important to consider the risk of hard-to-detect defects that may impact virus retention capacity.

Many approaches exist for detecting the presence of post-shipping gross defects. Methods using particles and bacteria to detect artificially produced and actual defects in the micrometer-scale range in ultrafiltration membranes have been reported [20,21]. For virus removal filters, original and modified leakage tests based on air–water diffusion are commonly used and regularly evaluated [10,16,19]. Methods involving binary gases and water/organic solvent mixtures have also been developed [22–24]. The approach we follow, known as the "single-point forward/diffusive flow test" [10,16,19], is relatively simple and can be used to detect micrometer-size gross defects.

In this report, we demonstrate the resistance of Planova™ BioEX filters to improper shifts in pore-size distribution and the maintenance of virus removal capacity even after harsh conditionings. We also discuss the use of an air/water diffusion–based leakage test as an IT for detecting gross defects associated with pinhole damage to the membrane, and we demonstrate the relationship between pinhole size and detection using air/water diffusion-based leakage tests and virus removal. Finally, the use of the leakage test as the only end-user IT for Planova™ BioEX filters is discussed.

## 2. Material and methods

### 2.1. Harsh conditioning of Planova™ BioEX filters

Planova™ BioEX (0.001 $m^2$ or 0.01 $m^2$) filters were conditioned by filtering 1000-mL solutions of either low pH (pH 2: hydrochloric acid aqueous solution) or high pH (pH 10: sodium hydroxide aqueous solution) at high temperature (40 °C) under a filtration pressure of 392 kPa (3.92 bar, 56.9 psi) for 12 h (the solution was recirculated and filtered multiple times during the experimental period), after which the filter was flushed with an excess of purified water (100 L/$m^2$). Control filters were used without any of the harsh conditioning described above.

In this report, all pressures are indicated as the filter inlet gauge pressure (above ambient atmospheric pressure), and the permeate side would have ambient atmospheric pressure only.

### 2.2. Virus propagation and virus removal study

Porcine parvovirus (PPV, 90HS strain, Japanese Association of Veterinary Biologics, Tokyo, Japan) was propagated in PK-13 cells (ATCC #CRL-6489) at 37 °C in Dulbecco's modified Eagle's medium (DMEM) supplemented with 3% (v/v) fetal bovine serum. At first sign of the death of inoculated host cells, the medium was exchanged with serum-free DMEM. The supernatant (containing a high titer of serum-free PPV) was collected, centrifuged at 1710 × *g* for 20 min at 4 °C, and then filtered through a 0.45-μm filter to remove the cell debris. This virus stock was titrated and stored at −80 °C until use [25,26].

The PPV stock was spiked into human polyclonal IgG (Venoglobulin®-IH, Japan Blood Products Organization, Japan). For harshly conditioned filters (0.001 $m^2$), the final virus-load sample was comprised of 0.5% (v/v) PPV stock suspension in 30 mg/mL human IgG/0.1 M sodium chloride aqueous solution. For pinhole filters, the final virus-load sample was comprised of 0.5% (v/v) PPV stock suspension in 30 or 1 mg/mL human IgG/0.1 M sodium chloride aqueous solution. Due to experimental limitations, the experiments using filters with a large effective surface area (1.0, 4.0, and 0.1 $m^2$) were conducted at the lower human IgG concentration, whereas tests of filters with 0.1-$m^2$ pinholes were conducted at both IgG concentrations and analyzed as a series of experiments. As a clear relationship between virus removal rate and a pinhole size of 0.1 $m^2$ was observed at both IgG concentrations, it was determined that protein concentration does not affect the virus removal rate and filtration flux.

After pre-filtration with Planova™ 35N filters (Asahi Kasei Medical, Ltd.) at 50 kPa (0.50 bar, 7.3 psi) for the purpose of obtaining mono-dispersed PPV particles, virus filtration experiments were conducted under 196-kPa (1.96 bar, 28.4 psi) dead-end, constant-pressure filtration at 25 °C. The virus titer was determined by hemagglutinin assay and was quantified as the 50% tissue culture infectious dose ($TCID_{50}$), determined by the method of Reed and Muench using PK-13 cells [27]. For the harshly conditioned filters (0.001 $m^2$), the initial titer of the virus load solution (after pre-filtration with Planova™ 35N) was set between $10^{5.7}$ and $10^{6.0}$ $TCID_{50}$/mL, and the filtration volume was 105 L/$m^2$, with permeate collection for analysis at the 100–105 L/$m^2$ fraction. For the pinhole filters (0.1, 1.0, and 4.0 $m^2$), the initial virus titer of the virus load solution (after pre-filtration with Planova™ 35N) was set to $10^{6.2}$, $10^{6.0}$, or $10^{6.7}$ $TCID_{50}$/mL, and the filtration volume was 5 L/$m^2$, with permeate collection for analysis at the 2–3 L/$m^2$ fraction. The $TCID_{50}$ was measured using the same method. Virus (PPV) removability was expressed as the logarithmic reduction value (LRV), or PPV LRV.

### 2.3. Gold particle test

Harshly conditioned Planova™ BioEX (0.01 $m^2$) filters and control filters receiving no pretreatment were subjected to the GPT [28,29]. Test solution containing colloidal gold particles (diameter of approximately 20 nm; Asahi Kasei Medical, Ltd.) was prepared as directed and filtered using 98-kPa (0.98 bar, 14.2 psi) dead-end constant-pressure filtration at 25 °C. The filtration volume was 1.0 L/$m^2$, and the permeate (0.5–1.0 L/$m^2$ fraction) was collected and the absorbance was assayed at 530 nm (UV–VIS spectrophotometer, UV2450, Shimadzu, Japan). Gold particle removability was expressed as LRV.

### 2.4. Model pinhole filters

A krypton fluoride (KrF) excimer laser was used to make single pinholes in individual fibers, and this work was performed by L.P.S. Works Co., Ltd. (Tokyo, Japan). Briefly, a Planova™ BioEX hollow fiber was positioned such that irradiation by the KrF excimer laser would penetrate through one surface of the BioEX hollow fiber and produce a single pinhole. Each pinhole fiber was then crafted into a filter of 0.001 or 0.01 $m^2$ effective surface area using normal hollow