Millistak+® Pod Disposable Depth Filter Performance Guide

Innovative, high-performance Pod filters ideal for primary and secondary clarification at lab, pilot and process-scale

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Introduction

The Millistak+® Pod Disposable Depth Filter Performance Guide is a reference document to provide assistance in evaluating and validating Millistak+® Pod depth filters for clarification applications. This guide includes general guidelines on various performance aspects of Millistak+® Pod devices, as well as a number of applications and case studies. These studies have been designed or selected for inclusion in this Performance Guide to provide a diverse overview of the device performance.

Results are intended as general examples and are not to be construed as product claims or specifications. The results included in this guide summarize outcomes and observations obtained in applications studies conducted with the specific model stream and detailed experimental conditions. Therefore, all test results should be confirmed by the end user using feed stream and process conditions representative of the specific application. Additional studies are available. Please contact Technical Service for more information on Millistak+® filters, or visit our website.

Millistak+® Pod Disposable Depth Filters
Millistak+® media in process-scale Pod format.

Summary of studies

Millistak+® media in µPod® format.

Scalability and Improved Product Recovery

Review of data showing scalability of Millistak+® formats, including Pod filters and lenticular stacks. Also discussed is the uniform plug flow behavior in the Pod format, which results in improved product recovery and reduced buffer usage over lenticular devices.

Reduced Water Usage

Description of alternative flushing procedures to reduce the amount of flush water and flow rates required for Millistak+® media prior to use.

Pre- and Post-Use Sanitization Methods

Evaluation and performance of Millistak+® Pod filters after each of three sanitization methods, including hot water at 80 ºC, Sodium Hydroxide and autoclaving in a Pod holder.

Initial Bioburden Level Evaluation

Measurement of the initial bioburden levels in Millistak+® Pod devices after using the prescribed flushing procedures, and three days after the initial flushing procedure is implemented.

Scalability and improved product recovery

Millistak+® F0HC scalability study: Device and lot variability

The capacity of a set of media lot-matched Millistak+® F0HC filter devices was measured in triplicate in four device formats using a CHO cell culture that had been acid-precipitated and then centrifuged, in order to assess the scalability of the depth filter devices. Two additional lots of F0HC media were simultaneously tested in triplicate in the lab-scale Pod format in order to assess the variability in capacity between various depth filter media lots.

Filters

Catalogue numbers and lot designations for each of the depth and sterile filter devices are listed in **Tables 1** and **2**. Millistak+® F0HC devices were specially manufactured from a single lot of each layer of the media constituting the filter, designated "Lot A" in this report. Lab-scale Pods were also manufactured with two additional lots of F0HC media, as listed in **Table 1**.

Table 1. Millistak+® F0HC Devices Used for the Scalability Study

Lot Designation	Millistak+® F0HC Device	Area (m^2)	Cat. No.
Lot A	µPod®	0.0023	**MF0HC26CL3S**
Lot A	Lab-scale Pod	0.027	**MF0HC027H1**
Lot B	Lab-scale Pod	0.027	**MF0HC027H1**
Lot C	Lab-scale Pod	0.027	**MF0HC027H1**
Lot A	Process-scale Pod	0.11	**MF0HC01FS1**
Lot A	Lenticular stack*	0.45	**LF0HCH6S6**

*Lenticular devices were made in two device lots containing the same media lots.

Table 2. Millipore Express® SHC Devices Used for the Scalability Study

Millipore Express® SHC Devices	Area (m^2)	Cat. No.
OptiScale® 25	0.00035	**SHGEA25NB6**
OptiScale® (2 per lab-scale Pod)	0.00354	**SHGEA47HH3**
Opticap® XL 150 capsule	0.014	**KHGES015FF3**
Opticap® XL 600 capsule	0.059	**KHGES006FF3**

Methods

The Millistak+® F0HC filters were evaluated under constant flow conditions (150 LMH) according to the P_{max}™ method. The F0HC filters were run in-line with the Millipore Express® SHC filters at an area ratio of approximately 7:1, F0HC:SHC (see **Table 3**).

Before the start of filtration, Millistak+® F0HC devices were flushed with at least 100 L/m² Water for Injection (WFI). Lenticular stacks and process-scale Pods were flushed at a flux of 150 LMH, and µPod® and lab-scale Pods were flushed at a flux of 600 LMH. After flushing, the lenticular stacks were gravity-drained to minimize product dilution.

Turbidity of the centrate was taken periodically during the study. Over the course of the filtration runs, the feed turbidity increased from 136 NTU to 140 NTU.

The LMH Formula

$$\frac{L}{m^2 \times H}$$

L = Liters
m^2 = Surface area
H = Hours

Table 3. Clarification Trains for the Scalability Study

Millistak+® F0HC Device		Millipore Express® SHC Device				
Device	**Area (m^2)**	**Device**	**Area (m^2)**	**Filter Area Ratio**	**Flux* (LMH)**	**Flow rate (L/min)**
µPod®	0.0023	OS 25	0.00035	6.57	150	0.00575
Lab-scale Pod	0.027	2x OptiScale®	0.00354	7.63	150	0.0675
Process-scale Pod	0.11	Opticap® XL 150	0.014	7.64	150	0.275
Lenticular stack	0.45	Opticap® XL 150	0.059	7.68	150	1.125

*Flux based on F0HC filter area

Results and Discussions

The pressure drop across the Millistak+® F0HC filter was calculated and analyzed according to the P_{max}™ methodology. **Figure 1** displays the resistance versus throughput curves for the Millistak+® F0HC filters from the study, with the targeted resistances indicated on the graph. Turbidities of lab-scale Pod filtration pools were measured at the end of the runs. The turbidities of the pools from these nine runs varied between 2.24 and 3.11 NTU.

Tables 4 and 5 contain a list of average capacities by device format and media lot, with standard deviations and coefficients of variation within a device and different sets of devices. The Millistak+® F0HC filter capacities were calculated at resistances of 0.133 psi/LMH and 0.100 psi/LMH, equivalent to 20 psi and 15 psi at 150 LMH. At 0.133 psi/LMH, capacities for all devices varied from 389 to 491 L/m², with an average of 443 L/m², a standard deviation of 35 L/m² and a coefficient of variation of 7.8%. At 0.100 psi/LMH, capacities for all devices varied from 327 to 416 L/m², with an average of 373 L/m², a standard deviation of 33 L/m² and a coefficient of variation of 8.8%.

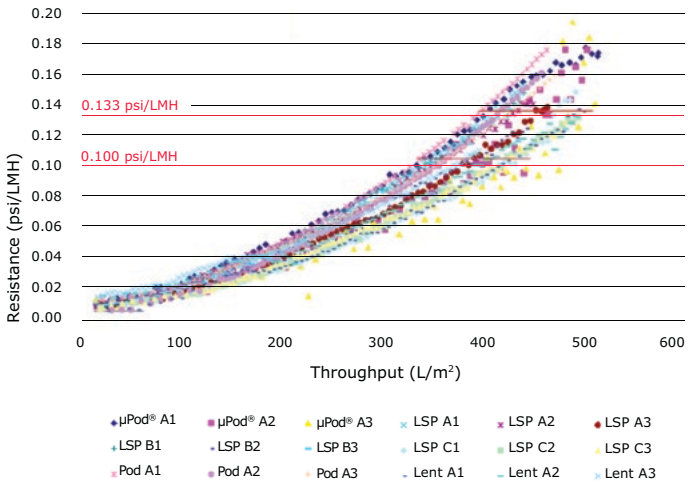


Figure 1. Resistance versus throughput for the Millistak+® F0HC filters.

Table 4. Millistak+® F0HC Device Throughputs and Related Statistics at a Resistance of 0.133 psi/LMH

Lot Designation	Millistak+® F0HC Device	Run No.	Throughput (L/m²) at 0.133 psi/LMH	Average Throughput (L/m²)	Standard Deviation (L/m²)	Coefficient of Variation (%)
Lot A	µPod®	1	407			
		2	449	441	31	7.1%
		3	468			
Lot A	Lab-scale Pod	1	404			
		2	425	427	24	5.6%
		3	452			
Lot B	Lab-scale Pod	1	412			
		2	449	425	21	4.9%
		3	415			
Lot C	Lab-scale Pod	1	473			
		2	491	487	13	2.6%
		3	497			
Lot A	Process-scale Pod	1	389			
		2	410	406	15	3.8%
		3	420			
Lot A	Lenticular stacks	1	486			
		2	491	472	28	5.9%
		3	440			
	All devices	-	-	443	35	7.8%
	Lot A devices	-	-	437	33	7.6%
	Lot A Pod devices	-	-	425	26	6.1%
	Lab-scale Pod devices	-	-	447	35	7.8%

Table 5. Millistak+® F0HC Device Throughputs and Related Statistics at a Resistance of 0.100 psi/LMH

Lot Designation	Millistak+® F0HC Device	Run No.	Throughput (L/m²) at 0.100 psi/LMH	Average Throughput (L/m²)	Standard Deviation (L/m²)	Coefficient of Variation (%)
Lot A	µPod®	1	331	378	43	11.2%
		2	390			
		3	414			
Lot A	Lab-scale Pod	1	336	355	22	6.3%
		2	350			
		3	380			
Lot B	Lab-scale Pod	1	340	355	20	5.9%
		2	379			
		3	345			
Lot C	Lab-scale Pod	1	413	413	1	0.2%
		2	413			
		3	414			
Lot A	Process-scale Pod	1	327	342	13	3.8%
		2	349			
		3	349			
Lot A	Lenticular stacks	1	416	397	24	5.9%
		2	404			
		3	370			
	All devices	-	-	373	33	8.8%
	Lot A devices	-	-	368	32	8.8%
	Lot A Pod devices	-	-	358	30	8.3%
	Lab-scale Pod devices	-	-	374	33	8.8%

Study results are summarized in **Figure 2**. Analysis of the scalability between Millistak+® F0HC devices containing Lot A media revealed no statistical difference in capacity between all Pod devices. All Pod formats had capacities within 10% of the overall average capacity for lot-matched devices, with Lenticular stacks capacity approximately 11% higher than the Pod devices. **Table 6** demonstrates media lot-matched Millistak+® devices scaled within 11% of capacities achieved with µPod® and lab-scale Pod small-scale devices.

The variability in capacity within a device format also was different among Pods and Lenticular stacks. The estimated percent variation within devices containing lot A media was approximately 6%.

The variability contribution from depth filter media lot was higher than that from device format for the Pod devices at approximately 8% to 9%. Including media and device format variation, the overall percent standard deviation found in this study for Millistak+® F0HC filters with a single harvest lot was 9% to 12%.

Table 6. Resulting Change in Capacity for Scale-up Device

Scale-down Device	Lab-scale Pods	Process-scale Pods	Lenticular Stacks
µPod®	-3.2%	-7.9%	+7.0%
Lab-scale Pods	N/A	-4.8%	+10.6%

Difference in capacity achieved when scaling up media lot-matched Millistak+® devices, based on results achieved from sizing at 0.133 psid/LMH.

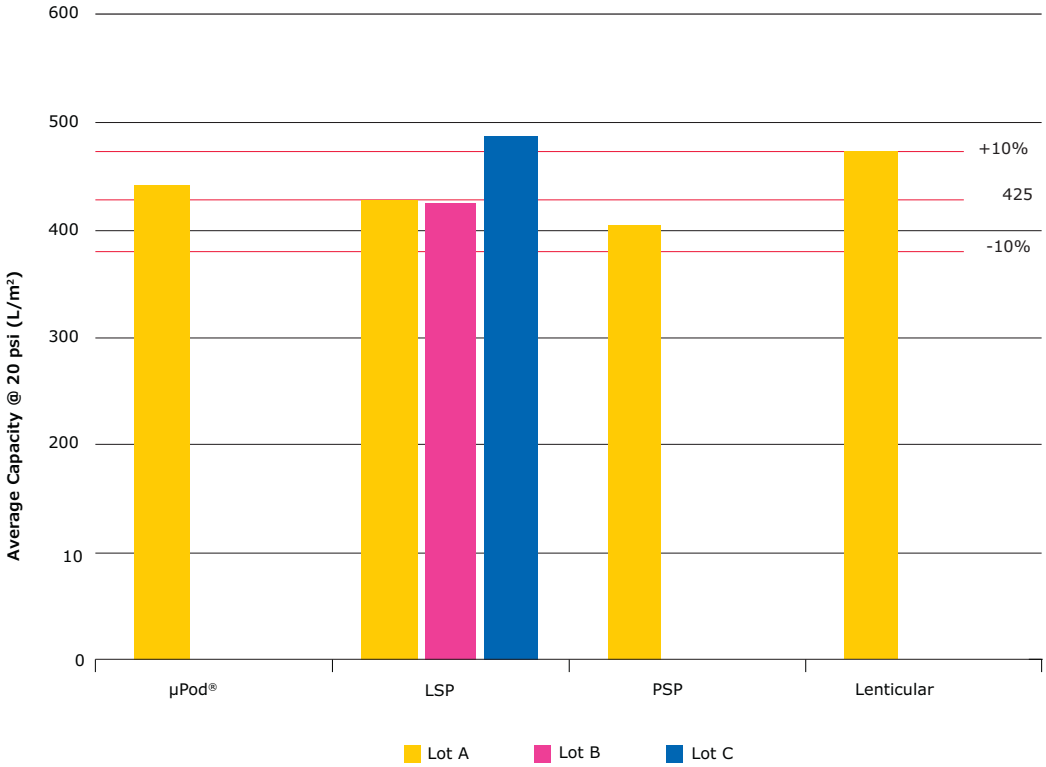


Figure 2. Summary of Millistak+® F0HC capacity for acid-precipitated CHO centrate at a pressure drop of 20 psi (0.133 psi/LMH).

Conclusions

The device scalability and media variability of Millistak+® F0HC filters used for the secondary clarification of acid-precipitated CHO centrate has been assessed. With lot-matched Millistak+® devices (µPod®, lab-scale Pod, process-scale Pod and Lenticular stacks), differences in capacity were statistically insignificant, and all device formats scaled within 11% of the median capacity of the devices. Variability within a device format was approximately 6% for all devices. No significant fouling was noted on the Millipore Express® SHC devices evaluated during testing.

Variability in capacity between media lots assessed on lab-scale Pod devices was approximately 8% to 9%. Including media and device format variation, the overall percent standard deviation found in this study for Millistak+® F0HC filters with a single harvest was 9% to 12%.

Constant Flow: P_{max}™

When a constant flow test is performed and size exclusion is the primary method of particle removal, P_{max}™ is the preferred test method. The capacity of the filter is determined by a pressure endpoint. The P_{max}™ sizing method involves determining the filter resistance to flow as a function of throughput. Based on these two parameters, filter sizing can then be easily calculated in the P_{max}™ sizing spreadsheet. The advantages to this method are that it provides a basis for filter train selection and is independent of plugging model. The main disadvantage to this method is that it requires potentially longer test times and larger test fluid volumes.

P_{max}™ Sizing Tool Summary

Advantages

- Experimentally determine filter/fluid performance
- Provides basis for filter train selection
- Independent of plugging model

Disadvantages

- Requires longer test times close to process time

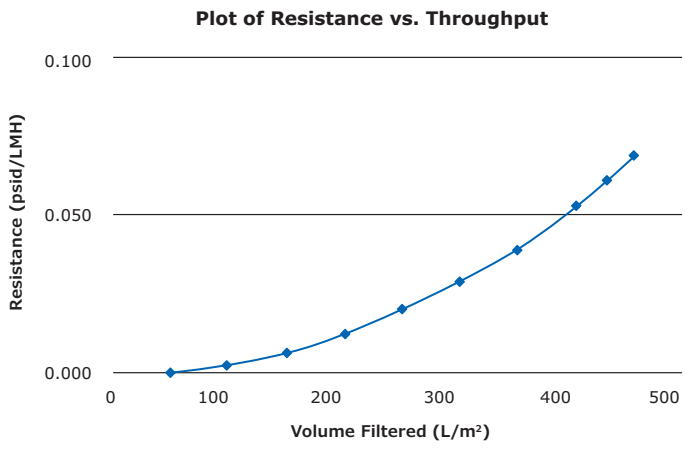
Uses

- Polygard® CR cartridges
- Millistak+® Pods

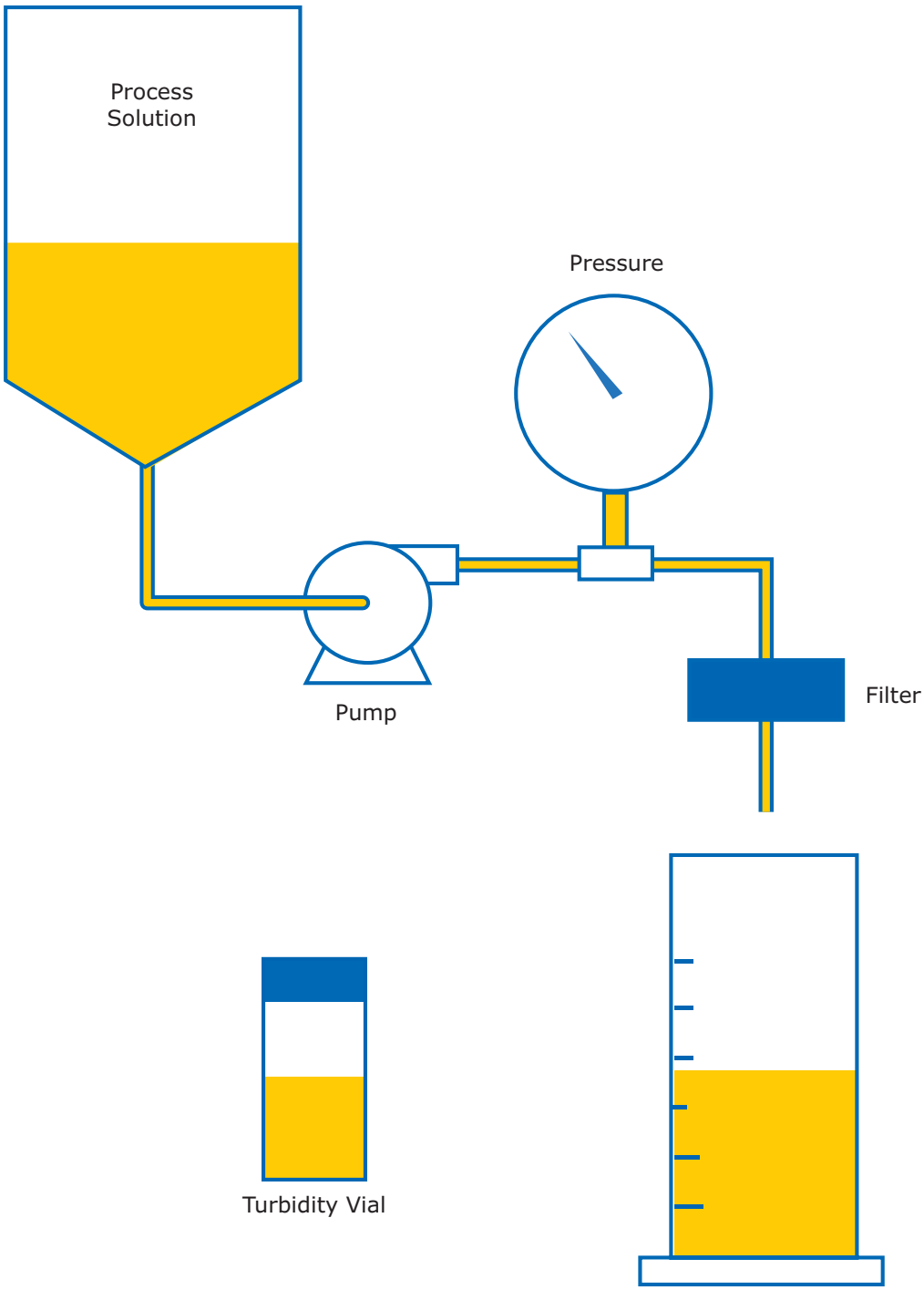
P_{max}™ Sizing Spreadsheet

We have a sizing spreadsheet available to assist with P_{max}™ calculations.

- Plots Data
- Sizing Calculations
- Sizing Recommendations
- Reporting Features



Constant Flow Test



Reduced water usage

Reduced Flushing of Millistak+® Pod Filters

Conventional cellulosic depth filters not only require high flush volumes at high flow rates to wet the filters, but also to reduce the level of extractables inherent in this technology. Standard preparation protocols for Millistak+® filter media call for a clean water flush of 100 L/m^2 of installed surface area at a flow rate of 600 LMH. For a large installation in biopharmaceutical production, this volume of Water for Injection (WFI) presents a considerable operating expense, and the high flow rate required can create equipment difficulties. At these larger scales, system process designs and pump capabilities cannot readily accommodate the lower flow rates for actual processing (a range of 50 to 200 LMH), and also the higher (600 LMH) flows for flushing and wetting with the same pump. Several studies have been undertaken since the introduction of the Millistak+® Pod format to minimize the amount of flushing volume required, in addition to lowering the flow rate at which the flush water is introduced to the filters.

Single-Pass Flushing

The recommended flushing protocol for Millistak+® Pod devices requires flushing with WFI at 600 LMH for 10 minutes. A fully loaded, 30 filters (33 m^2), three-rack process-scale holder results in a flow rate of 330 L/min. This implies that a separate pumping skid would be required to accommodate the flow rates and volumes required for flushing, since typical operating flux rates are approximately 100 ± 50 LMH, a flow rate of 27.5 to 82.5 L/min for 33 m^2 filter area.

To reconcile this issue, a flushing study was conducted on Millistak+® A1HC media to determine the potential to decrease the flushing volumes and flow rates required. The study was conducted on a fully loaded Millistak+® process-scale Pod three-rack system at 270 LMH for 20 minutes. The flux rate of 270 LMH was chosen to represent the maximum flow rate that might be realistically obtained from a pump designed to operate at 100 ±50 LMH. Samples from the flush water effluent and RO feed were taken at intervals of 2.5, 5, 10 and 20 minutes and analyzed for TOC. **Figure 3** compares the TOC values obtained with the modified protocol with those obtained from flushing three Millistak+® Pod filters in a pilot holder according to the recommended protocol of 600 LMH for 10 minutes.

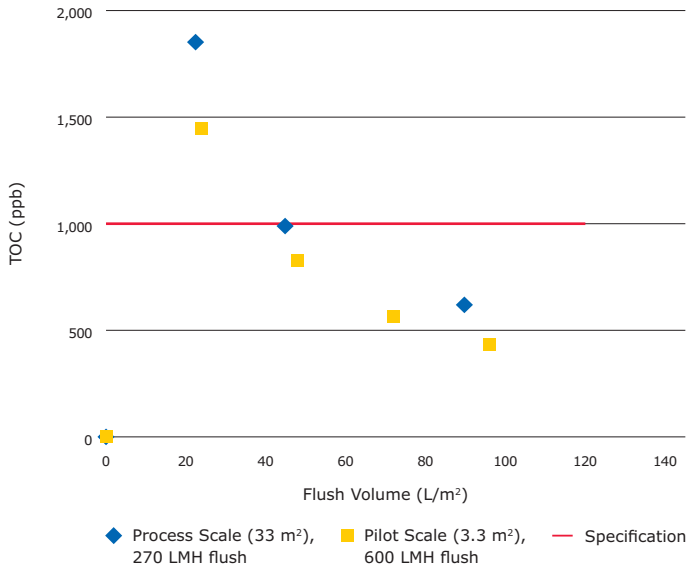


Figure 3. Comparison of flux rates used during the pre-use flushing of Millistak+® A1HC media.

All of the TOC levels shown in **Figure 3** have been normalized to account for the TOC of the RO water utilized for flushing. **Figure 3** clearly demonstrates that similar TOC levels can be achieved when flushing the Millistak+® A1HC media at either 270 or 600 LMH. TOC levels of less than 1000 ppb can be achieved at flush volumes of 40 to 50 L/m^2.

Recirculation Test Method

Alternative methods for volume reduction were investigated for media grades with higher DE content. Initial test results showed that the solubility of the extractable material in water is much higher than the flush water can reach in a single pass. However, simply soaking the depth filters in WFI is not an effective methodology for extractables removal within a reasonable time frame. At the standard flux rates prescribed (600 LMH), the flush water does not reach the saturation limit of any or all of the extractable components.

It then becomes apparent that recirculating a fixed volume of water could achieve the same effect as a single-pass flush without consuming large quantities of water.

Procedure

All testing was performed with Millistak+® X0HC 270 cm^2 lab-scale Pod filters. The equipment setup is shown in **Figure 4**. All glassware was depyrogenated and triple-rinsed with WFI both before and after use. **Table 7** lists the Millistak+® Pod filters used for the flushing study, Catalogue No. MX0HC027H1. Nine devices were used spanning three different device lots. These filters were chosen because they represent the most challenging flushing requirements for Millistak+® filters.

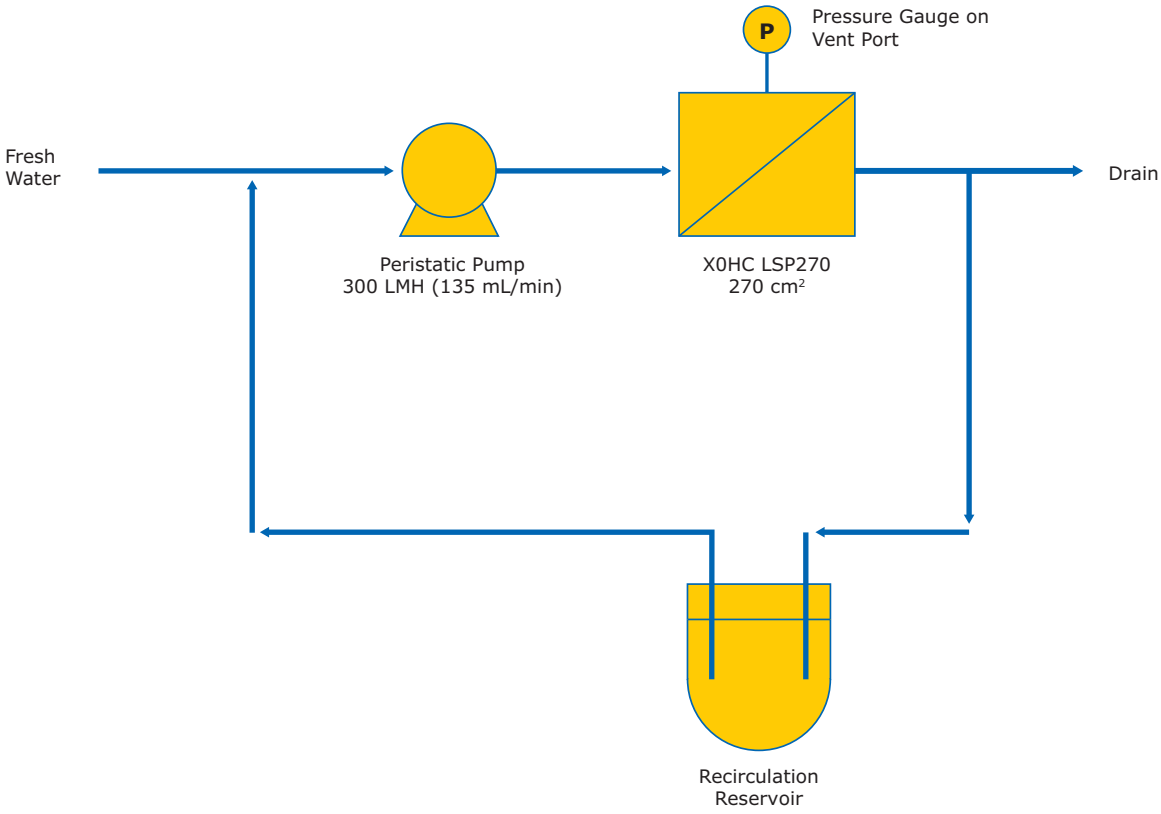


Figure 4. Equipment setup for reduced volume media flushing by recirculation.

Table 7. Millistak+® X0HC Devices Used for Recirculation Flushing Studies

Lot No.	Serial No.	Lot Device Designation	Test Setup	Test Day
CP0HA85352	19	1–1	1	1
	12	1–2	2	1
	25	1–3	1	2
CP9PN79849	02	2–1	2	1
	03	2–2	1	1
	04	2-3	2	2
CP9NN79374	05	3–1	1	1
	03	3–2	2	1
	08	3–3	1	2

The Millistak+® X0HC lab-scale Pod filter reduced flushing was performed according to the following procedure:

1. At the start of each day, take a 5 mL sample of WFI for TOC analysis (in glass vial) and a 10 mL sample of WFI for conductivity analysis of baseline values.
2. Install new size 15 silicone tubing on the inlet, outlet and vent lines of the 270 cm^2 lab-scale Pod.
3. Attach a pressure gauge to the vent port to monitor inlet pressure and attach a luer vent valve at the end of the vent line.
4. Direct the feed line to a clean reservoir with 45 L/m^2 water (1215 mL).
5. Set the peristaltic feed pump to 135 mL/min (300 LMH).
6. Flush the lab-scale Pod filter to drain with 25 L/m^2 water (675 mL), venting initially to remove air and fully wet out the media. Time = 5 minutes.
7. Direct the filtrate line to the feed container and recirculate residual 20 L/m^2 water (540 mL) through the lab-scale Pod filter; volume includes device holdup of 11.5 L/m^2 (310 mL). Time = 1 hour. Take a 5 mL sample for TOC analysis (in glass vial) and a 10 mL sample for conductivity analysis from the filtrate line after 30 minutes and 60 minutes of recirculation.
8. Stop the pump.
9. Direct the filtrate line to drain.
10. Direct the feed line to a clean reservoir with 1.5 L fresh water.
11. Flush the lab-scale Pod filter to drain with 50 L/m^2 water (1350 mL), venting if necessary to remove any air in the feed line. Time = 10 minutes. Take a 5 mL sample for TOC analysis (in glass vial) and a 10 mL sample for conductivity analysis from the filtrate line after 10, 20, 30, 40 and 50 L/m^2 (every 270 mL or 2 minutes).
12. Stop the pump.
13. Measure the conductivity samples and record the values.
14. Repeat on remaining lab-scale Pod filters; use new tubing sets on each.

Total flushing time was 1.25 hours per lab-scale Pod, not including setup, breakdown and sample analysis.

Results

The TOC and conductivity values shown in **Figures 5** and **6** are actual reported sample values and have not been adjusted. The baseline WFI for TOC and conductivity are shown on each figure for reference and represent an average of the measurements obtained on the two test days.

Figure 5 shows the average TOC profiles for each of the three lots during the reduced flushing. Each curve represents an average of the TOC measurements from three individual lab-scale Pod filters within the specific lot; error bars represent the intralot standard deviation. For the Millistak+® X0HC media, the recommended final extractable levels are ≤3 ppm TOC and ≤40 µS/cm conductivity. By the end of the 50 L/m² fresh WFI flush to drain, the average TOC values for Lots 1, 2 and 3 were 1.52 ppm, 1.39 ppm and 1.46 ppm, respectively.

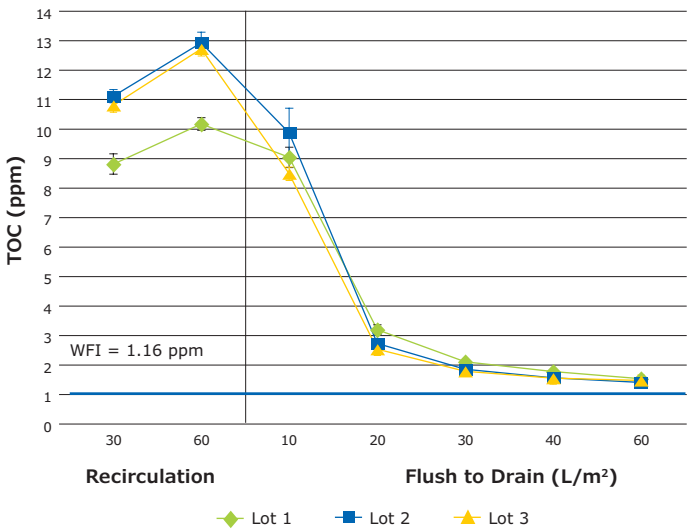


Figure 5. Millistak+® X0HC media TOC profiles by device lot for recirculation flushing method.

Figure 6 shows the average conductivity profiles for each of the three lots. As with the TOC profiles, each curve represents an average of the conductivity measurements from the three individual lab-scale Pod filters within the specific lot; error bars represent the intralot standard deviation. By the end of the 50 L/m² fresh WFI flush to drain, the average conductivity values for Lots 1, 2 and 3 were 13.0 µS/cm, 23.2 µS/cm and 25.3 µS/cm, respectively.

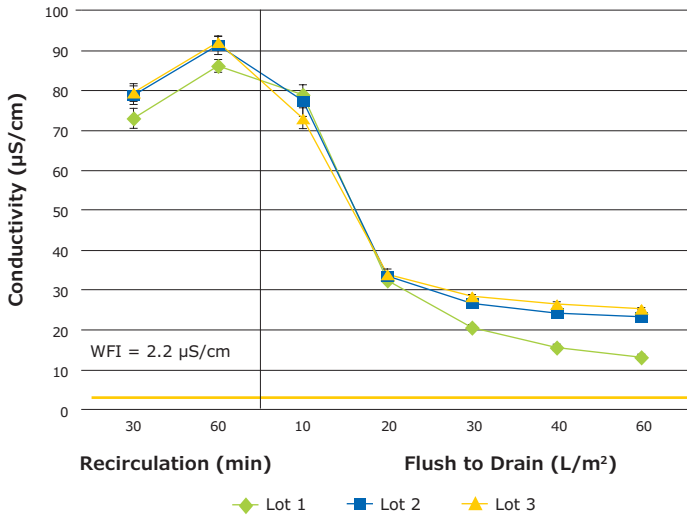


Figure 6. Millistak+® X0HC media conductivity profiles by device lot for recirculation flushing method.

Discussion

In general, a TOC value of 1.5 ±0.1 ppm and a conductivity value of 20.5 ±5.7 µS/cm can be obtained using a total WFI flush volume of 75 L/m² for the Millistak+® X0HC media, well below the recommended pre-use extractables levels of ≤3 ppm TOC and ≤40 µS/cm conductivity. The 270 cm² lab-scale Pod filters used in this study had a holdup volume of 310 mL (11.5 L/m²), so a total of 20 L/m² WFI were used for the recirculation step to prevent air entrainment. This value may be adjusted based on the holdup volume of the desired Pod installation. Any adjustments will likely affect the TOC values during the recirculation step, but should not affect the final TOC value obtained at the end of the fresh WFI flush to drain.

Conclusions and Recommendations

In general, the recommended pre-use extractables levels for Millistak+® X0HC devices can be met at a total WFI flush volume of 75 L/m² using the recirculation method detailed above. It is recommended that users determine the appropriate volumetric endpoint for their flush based on the desired final TOC value. For example, if a TOC ≤3 ppm and ≤40 µS/cm conductivity is determined to be acceptable, as recommended in the validation guide, then the final flush volume may be reduced to 30 L/m², resulting in a total WFI flush volume of 55 L/m².

Reduced Pre-use Flushing Flow Rate and Volume for Millistak+® Filters in Series

Introduction

Before their use in a biopharmaceutical process, depth filters are commonly flushed with purified water or buffer in order to accomplish two goals: removal of extractables and wetting of the media. For commercial or other large-scale operations, the total volume and flow rate for the pre-use flush may be limited by the availability of purified water and the capacity of pumps. In these cases, it is desirable to minimize the recommended volume and flow rate required to meet the filter vendor's recommended threshold for extractables removal.

One strategy for reducing flush volume and flow rates for depth filters is to flush two or more stages of filters in series. In effect, this allows for "re-use" of the flush water from an earlier stage in the series to remove extractables from the downstream stages. Each stage of depth filter could consist of the same media as the previous stage or a different media. By arranging the filters in series, and limiting the number of filters that are in parallel, the effective flux through the filters is maximized, while reducing the total volume of purified water.

For this study, two scenarios were examined. In the first, optimal strategies for flushing primary and secondary depth filters, Millistak+® D0HC and X0HC filters, in series were examined. In the second, strategies for flushing a single media grade, the Millistak+® X0HC filter, through multiple stages in series, were examined.

Materials and Methods

Materials

- Millistak+® D0HC lab-scale Pod: Cat no. MD0HC27H1, lot nos. CP1AA91425, CP1JA97042, CP1JA97458 and CP1KA98448
- Millistak+® X0HC lab-scale Pod: Cat no. MX0HC27H1, lot nos. CP0PA90523, CP1EA95929 and CP1JA97471

Methods

Table 8 summarizes the recommendation from the Millistak+® Pods Validation Guide for the pre-use, purified water flush of Millistak+® Pod filters. Extractables levels in the filter effluent are generally measured using conductivity and Total Organic Carbon (TOC). The values for extractables in the Millistak+® Pods Validation Guide establish the baseline against which all flushing trials were compared. For trials in which D0HC and X0HC filters were flushed, in series, the extractables limits for X0HC filters were used as an endpoint for flushing. The Millistak+® X0HC media represents the greatest challenge to extractables flushing, due largely to the increased diatomaceous earth content relative to other Millistak+® HC grades.

Flushing studies were performed using lab-scale Pod devices with 270 cm² filter area. These devices have been shown to be a reliable scaling device to process-scale Pod devices (data shown in previous studies). For the first set of trials, Millistak+® D0HC and Millistak+® X0HC Pods were arranged at a filter area ratio of 3:1. This mimics a common filter area ratio used for whole cell culture clarification, where the D0HC filter performs the primary clarification stage and X0HC performs the secondary clarification. Initially, the two filter grades were flushed individually at 300 LMH to determine if the use of a lower-than-recommended flux could still meet the extractables limits established in the Millistak+® Pods Validation Guide.

In the next series of trials, the D0HC and X0HC filters were flushed in series in two configurations. The first configuration maintained three D0HC filters in parallel flow with the X0HC filter in series, as would commonly be arranged during a harvest process. The second configuration placed each D0HC filter in series, followed by the X0HC filter, allowing the flush water to pass sequentially through each of the four filters. In the next set of flushing trials, the X0HC filters were flushed as a single grade using either two filters or five filters in series.

Recommended Process Conditions and Extractables Limits for Pre-use Flush of Millistak+® Pod Filters

Filter	Flush Volume (L/m²)	Flush Flux (LMH*)	Final TOC (ppm)	Final Conductivity (µS/cm)
Millistak+® D0HC Pod	100	600	≤1	≤10
Millistak+® X0HC Pod	100	600	≤3	≤40

*Flux values given in liters/meter²/hour.

Table 8.

Results

I. Single-stage flushing of D0HC and X0HC at 300 LMH

Single lab-scale Pods of D0HC and X0HC were separately flushed at a flux of 300 LMH (135 mL/min for the 270 cm² filters), as shown in **Figure 7**, to observe the flushing effectiveness of the lower flow, as compared to the original standard of 600 LMH. **Figures 8** and **9** present the conductivity and TOC measurements for a single-stage flush at 300 LMH, along with data measured at 600 LMH during validation testing of Millistak+® HC media. For both media grades, the recommended extractables limits were met at a flush volume of approximately 80 L/m² at 300 LMH, indicating that a change in flush flux from the 600 LMH does not increase the required water volume.

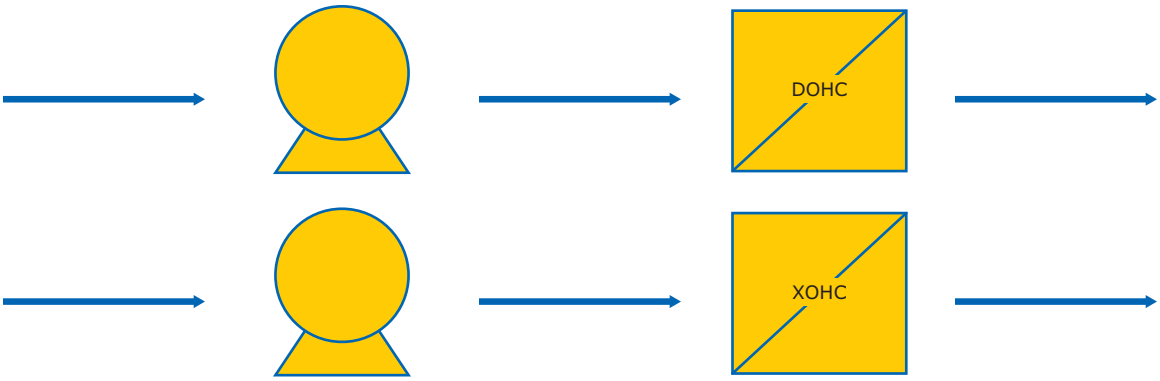


Figure 7. Diagram of Millistak+® D0HC and X0HC Pod filter single-stage flushing.

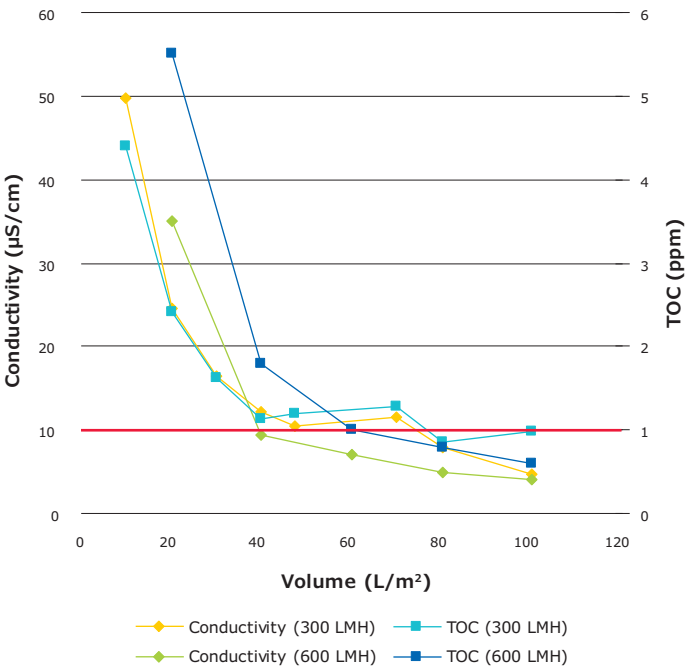


Figure 8. Millistak+® D0HC Pod filter flushed at 300 and 600 LMH.

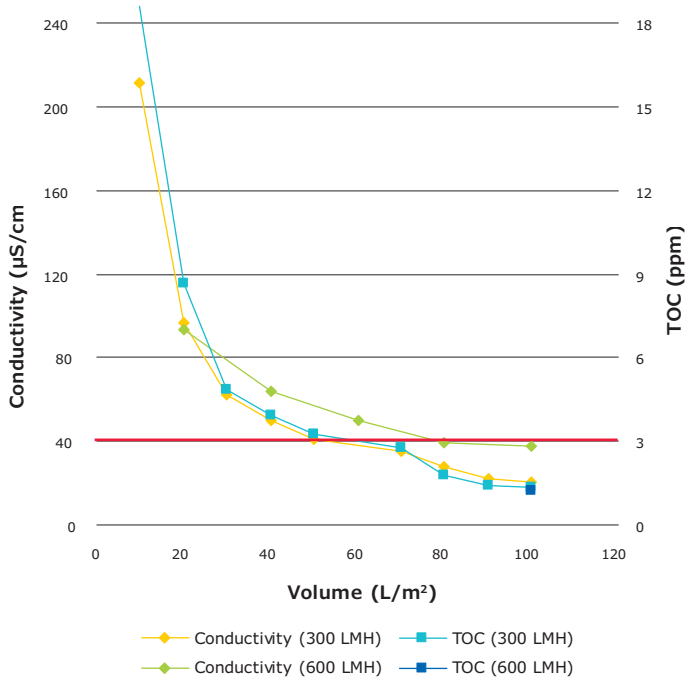


Figure 9. Millistak+® X0HC Pod filter flushed at 300 and 600 LMH.

II. D0HC and X0HC flushing in series

The D0HC and X0HC filters were flushed in series in the configuration indicated in **Figure 10**. Two filter trains were flushed at 600 and 100 LMH based on the D0HC area, providing 1800 and 300 LMH, respectively, to the X0HC Pod. **Figure 11** illustrates the extractables flushing curves for the filter train effluent, as measured at the outlet of the X0HC filter. Flushing at higher flux did appear to reduce the TOC and conductivity more quickly than the lower flux. The lower flux run (100 LMH on the D0HC filter) was halted before the targeted levels were met (final data point at 26.3 L/m^2 total filtration area was 49.3 µS/cm and 3.5 ppm). At the higher flux—600 LMH for the D0HC—the extractables limits were met at 30 L/m^2, while at the lower flux—100 LMH for the D0HC—the extractables limits appear that they would have been met at 40 L/m^2.

For the next trials, the D0HC and X0HC Pod filters were flushed with each of the four filters in series at 300 LMH, as outlined in **Figure 12**. This experiment was performed in triplicate, and the results are illustrated in **Figure 13**. The TOC and conductivity limits were met at ≤25 L/m^2. TOC samples from Trial 3 were improperly processed, and insufficient volume remained for retesting; results are not shown.

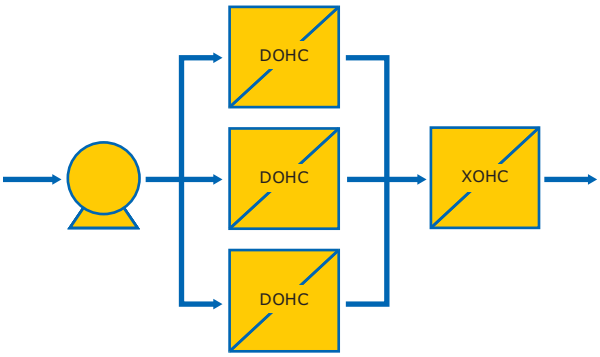


Figure 10. Diagram of Millistak+® DOHC and XOHC Pod filter flushing in parallel.

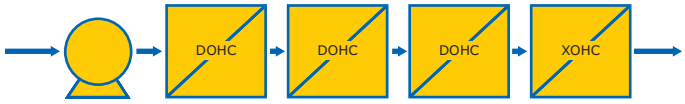


Figure 12. Diagram of Millistak+® D0HC and X0HC Pod filter flushing in series.

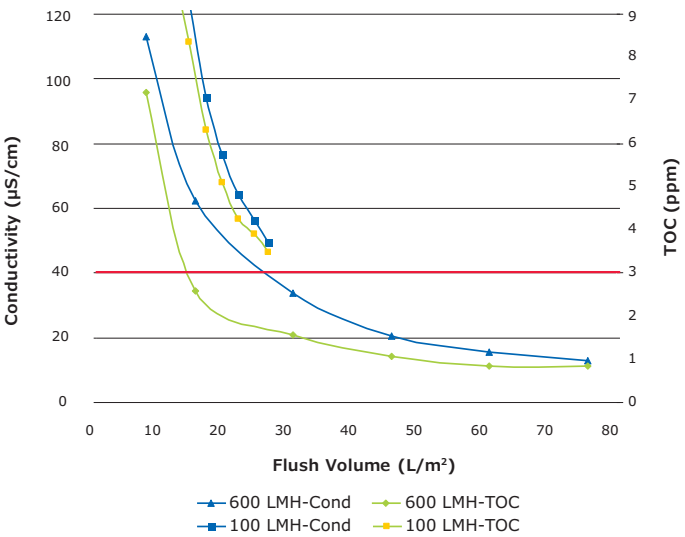


Figure 11. Conductivity and TOC vs. flush volume for D0HC and X0HC filters flushed with D0HC filters in parallel. Reported fluxes are for the D0HC filters. Conductivity and TOC measured at the outlet of the X0HC filter.

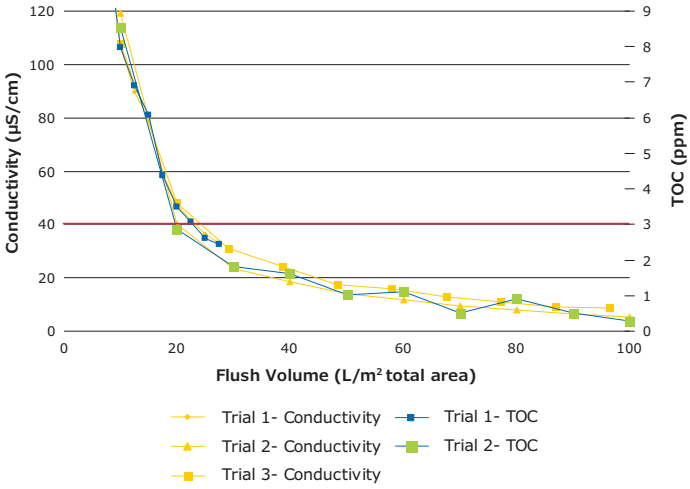


Figure 13. Conductivity and TOC vs. flush volume for D0HC and X0HC filters flushed in series at 300 LMH. Conductivity and TOC measured at the outlet of the X0HC filter.

III. X0HC flushing series

Two X0HC Pod filters were flushed in series at 110 LMH, and 5 X0HC Pod filters were flushed in series at 150 LMH, as shown in **Figure 14**. **Figure 15** shows the conductivity and TOC flushing curves for these trials. The filters met the required limits for TOC and conductivity at a flush volume of 40 L/m^2. No decreased flush volume per area was seen when increasing the number of filters flushed from two to five filters. While no further decrease in volume was achieved, placing more filters in series during the flush would still have the impact of decreasing the required flow rate, while increasing the operation time, for a fixed filtration area. The influence of increasing the flux during flushing was not examined.

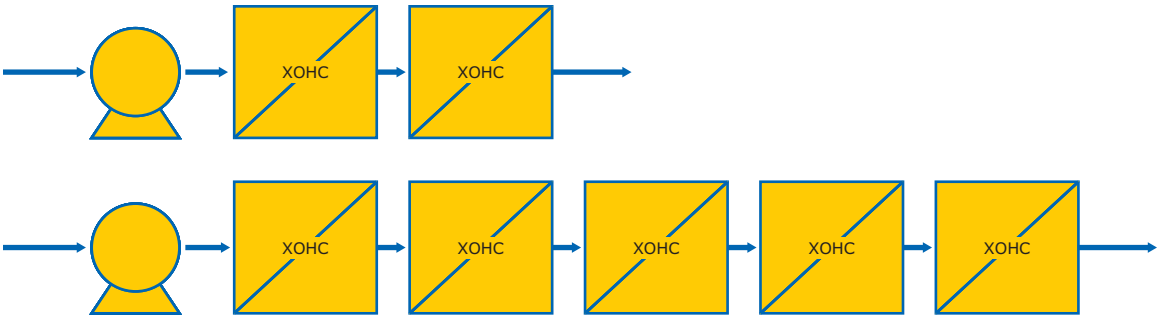


Figure 14. Diagram of Millistak+® X0HC Pod filter flushing in series.

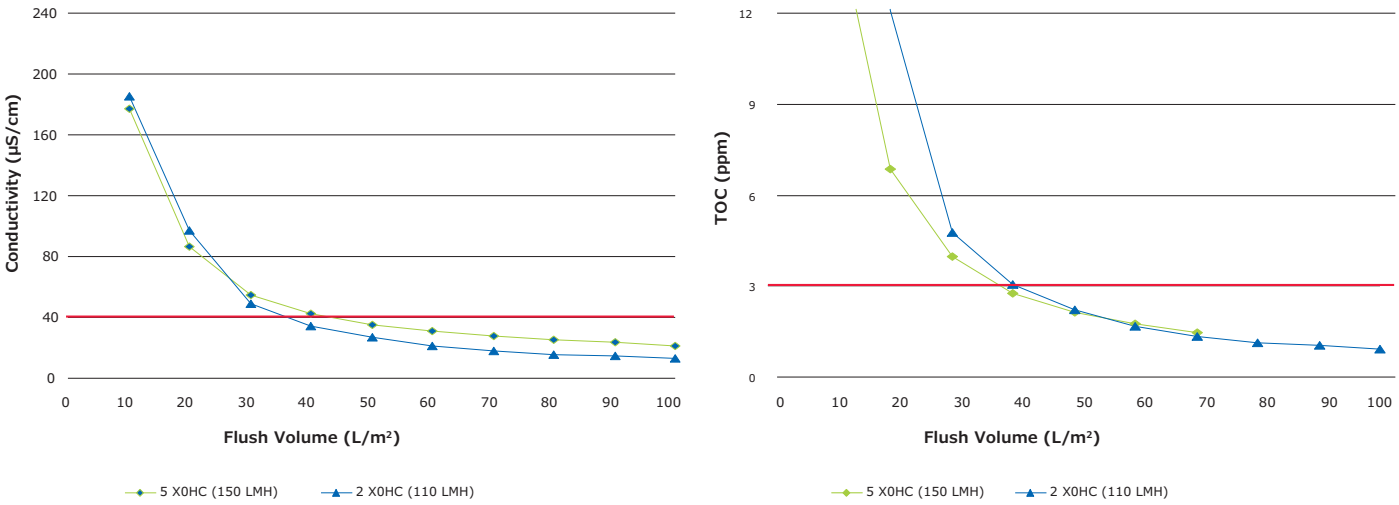


Figure 15. Conductivity and TOC vs. flush volume for multiple XOHC filters flushed in series.

Conclusions

Based on the results of this study, a significant reduction in water volume and flow rate can be achieved for the pre-use flushing of Millistak+® D0HC and X0HC media.

Flushing the D0HC and X0HC media in series was shown to significantly reduce the total water volume requirement during pre-use flushing. Flushing three D0HC devices in parallel to one X0HC device reduced the required water volume to approximately 30–40 L/m^2 at a flux of 100–600 LMH. In this configuration, operating at low flux did increase the required flush volume from 30 L/m^2 at 600 LMH to 40 L/m^2 at 100 LMH. Flushing three D0HC devices in series to one X0HC device reduced the required water volume to 25 L/m^2 at a flux of 300 LMH (based on D0HC and X0HC frontal area). **Table 9** summarizes each method tested, with the advantages and disadvantages of each.

Table 9. Example Flushing Volumes and Fluxes for Flushing of Millistak+® D0HC and X0HC Pod Filters

	Standard	Process-style	Full Series
Description	Flush through single media grade to drain D0 then X0	Flush D0HC in parallel directly to X0HC in series	Flush each rack/level of D0HC in series into X0HC
Advantages	1. Straightforward 2. Matches Millistak+® Pods Validation Guide	1. Lower water use 2. Reduced flow rate 3. Same piping as process	1. Lowest water use 2. Lowest flow rate
Disadvantages	Highest water volume and flow rate	None	Significant change in piping to perform series flow
Water use for: 33 m^2 D0HC into 11 m^2 X0HC[1]	4,400 L at 300 LMH (100 L/m^2) 165 L/min D0HC 55 L/min X0HC	1,760 L at 100 LMH (30–40 L/m^2) 55 L/min	1,100 L at 300 LMH (25 L/m^2) 55 L/min

[1]This configuration represents the area contained in a single three-level holder of Millistak+® D0HC Pods into a single one-level holder of Millistak+® X0HC Pods, a common configuration for whole cell culture harvest.

For multiple filters of the same media in series, flushing two X0HC filters and five X0HC filters in series reduced the required flush volume to 40 L/m^2 at 110–150 LMH. Table 10 summarizes the X0HC multi-stage flushing results and compares this method with the standard flushing method and the recirculation flushing method described in this Guide.

Table 10. Example Flushing Volumes and Fluxes for Multi-stage Flushing of Millistak+® X0HC Pods

	Standard	Recirculation	Series (≥ 2 filters)
Description	Flush through single media grade to drain	Flush out the hold-up and recirculate a fraction of the flush before flushing to drain	Multiple holder racks of single media are piped in series for flushing
Advantages	1. Straightforward 2. Matches Millistak+® Pods Validation Guide	1. Lower water use	1. Lowest water use 2. Lowest flow rate
Disadvantages	Highest water volume and flow rate	1. Re-piping, extra reservoir, system hold-ups 2. Adds complexity to flushing process	1. Re-piping after flushing 2. Fibers getting into the next filter 3. Good for larger area than smaller
Water use for: **165 m^2 X0HC[2]**	16,500 L at 300 LMH (100 L/m^2) 825 L/min	9,900 L at 300 LMH (60 L/m^2) 825 L/min	Flushing 5 stages in series: 6,600 L at 110-150 LMH (40 L/m^2) 60.5 – 82.5 L/min

[2]This configuration represents the area contained in five three-level holders of Millistak+® X0HC Pods, a large installation of depth filter that could be used for secondary clarification.

Pre- and Post-use Sanitization Methods

Millistak+® Pod filters are currently used at various points in the bioprocess template, including primary and secondary clarification, post-affinity chromatography haze removal, and as a prefilter for virus filtration. While other studies conducted with Millistak+® media demonstrated very low levels of initial bioburden, as well as the effectiveness of the recommended flushing procedures, a need has been identified for pre-use sanitization guidelines. Several studies were conducted to evaluate the feasibility to implement sanitization methods commonly used in the bioprocess industry. These methods include the following:

- Hot water at 80 ºC
- 0.5 N and 1.0 N Sodium Hydroxide
- Autoclaving of Pod filters installed into a holder (Please note: modified hardware is required for this option)

Sodium Hydroxide (NaOH) Sanitization

This study aims at understanding and confirming the impact of 0.5 N and 0.1 N NaOH sanitization at room temperature. The effect of NaOH sanitization on Millistak+® Pod filters, as well as performance of the clarification train, was studied. The following parameters were used as study metrics:

1. Depth filters capacity
2. Sterile filter capacity
3. TOC levels in flush samples after sanitization
4. Conductivity levels in flush samples after sanitization

Materials and Test Method

Control and sanitized filters were used from the same lot for each sanitizing condition (**Table 11**). The mixture of whey solution and CHO cells was prepared in the lab to mimic the typical turbidity observed in cell culture harvest. Different concentrations of ingredients were used to make loading samples for Millistak+® A1HC and X0HC filters based on targeted loading of 300 L/m^2. Feed composition was changed for 0.5 N NaOH sanitization study based on the plugging observed in 0.1 N sanitization study. The formulations are shown in **Table 12**.

Table 11. Material Information

Test Material	Lot No.	Catalogue No.
Millistak+® Pod A1HC 0.027 m^2	CP9NN79372	**MA1HC027H1**
Millistak+® Pod A1HC 0.027 m^2	CP9PN79809	**MA1HC027H1**
Millistak+® Pod X0HC 0.027 m^2	CP9NN79374	**MX0HC027H1**
Millistak+® Pod X0HC 0.027 m^2	CP9CN73418	**MX0HC027H1**
Durapore® CVGL 13.8 cm^2	R6AN42605	**GVWP04700**
whey	115K0037	**W1500-2**
PBS	059K8215	**P4417-100TAB**

Table 12. Feed Formulations

NaOH Concentration	A1HC	X0HC
0.1 N NaOH	5 g/L whey	5 g/L whey
	5 g/L PBS	5 g/L PBS
	20 mL/L CHO cells	20 mL/L CHO cells
Turbidity	81.5 NTU	44.5 NTU
0.5 N NaOH	10 g/L whey	2.5 g/L whey
	5 g/L PBS	5 g/L PBS
	20 mL/L CHO cells	20 mL/L CHO cells
Turbidity	170 NTU	50.9 NTU

Test Method

1. Wet media at 600 LMH (270 mL/min for 270 cm^2) for 100 L/m^2 (2.7 L for 270 cm^2)
2. Perform sanitization at 300 LMH (135 mL/min for 270 cm^2). Recirculate for 1 hour at room temperature.
3. Flush filter with room temperature water at 300–600 LMH until a filtrate conductivity of <10 µS/cm is achieved. Take samples every 100 L/m^2 for TOC and conductivity analysis.
4. Perform P_{max}™ capacity experiment at 150 LMH (67.5 mL/min for 270 cm^2) using whey and CHO cell solution to a final pressure of 20 psid (1.38 bar).
5. Perform V_{max}™ capacity experiment on the Millistak+® Pod filtrate using Durapore® 0.22 µm membrane (47 mm disk holder).

Filters were run at a constant flux of 150 LMH using a peristaltic pump. Filters were evaluated with lab-scale Pod filters (270 cm^2) with pressure transducers upstream of each filter to monitor filter plugging. Pressure was monitored throughout the run using the our Data Acquisition system, and filtrate turbidity was measured using a Hach turbidity meter.

Results and Discussion

TOC and Conductivity

Figure 16 shows that after sanitization, the Total Organic Carbon (TOC) levels are similar for both control and sanitized filter flush samples. In **Figure 17**, a slightly higher TOC value is shown for flush samples from 0.5 M NaOH sanitized A1HC and X0HC filters. The TOC values of the water, which was used for flushing, was 0.506 ppm and 0.306 ppm in 0.1 N and 0.5 N sanitization, respectively.

Conductivity has historically been used to track the removal of extractables during the pre-use water flush. Since the sanitization solutions have a high conductivity, conductivity values seen in flush samples cannot be used to assess residual levels of extractables. Use of a buffer at the end of or for the entire pre-use flush should equilibrate the media to desired pH and conductivity before introduction of product.

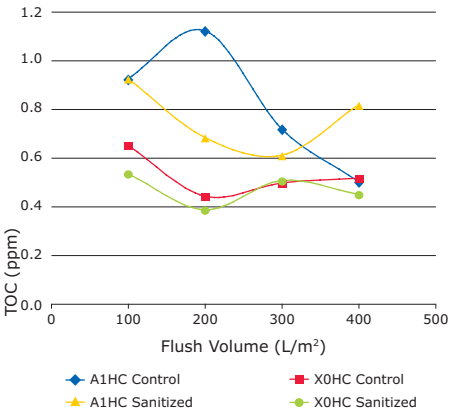


Figure 16. TOC levels in the post-0.1 M NaOH sanitization water flush.

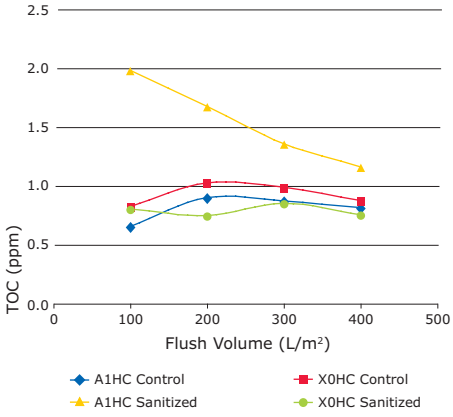


Figure 17. TOC levels in the post-0.5 M NaOH sanitization water flush.

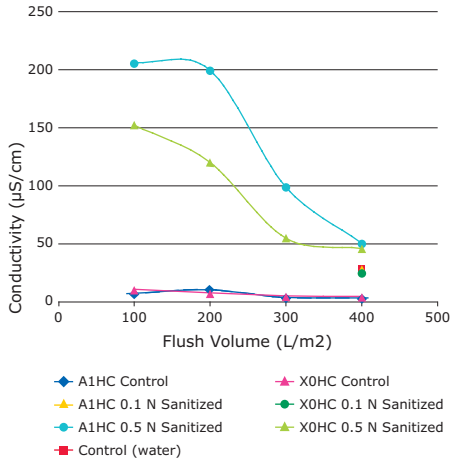


Figure 18. Conductivity levels in 0.1 N and 0.5 N NaOH post-sanitization flush.

P_{max}™ (Capacity vs. Resistance) Testing

Figures 19 and **20** show there was no major difference between the resistance vs. capacity plots of any pair of control and sanitized filters. As shown in **Table 13**, the resistance curves of paired Millistak+® Pod filters matched closely throughout the filtration trial. The variation in the capacities of each pair of Millistak+® Pod filters (sanitized and control) was ≤15%. The filtrates from all the Pod runs were non-fouling on a 0.22 µm sterilizing grade filter. **Figure 21** shows that no significant difference was seen in the mean filtrate flux from feeds of sanitized or control Pods.

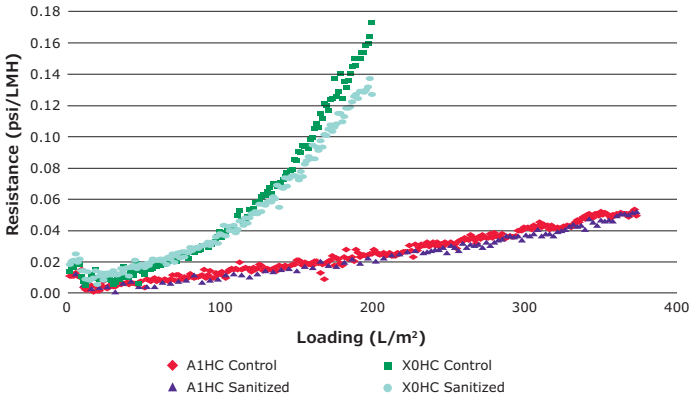


Figure 19. Resistance vs. capacity post-0.1 N NaOH sanitization.

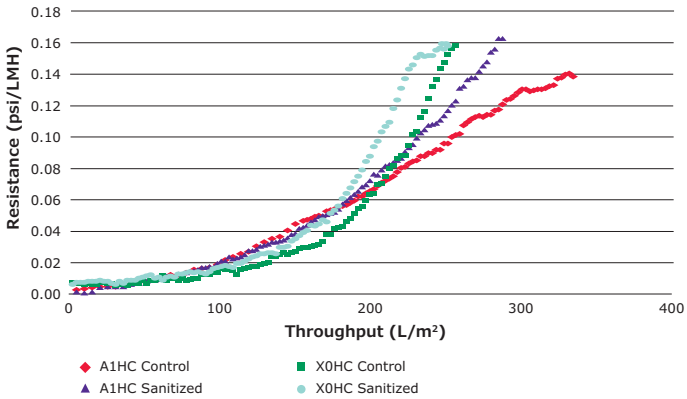


Figure 20. Resistance vs. capacity post-0.5 N NaOH sanitization.

Table 13. Pod Capacities at 20 psid (1.38 bar)

Millistak+® Filter	Capacity (L/m²) at 20 psid
A1HC control	373
A1HC 0.1 N NaOH sanitized	373
X0HC control	176
X0HC 0.1 N NaOH sanitized	190
A1HC control	306
A1HC 0.5 N NaOH sanitized	259
X0HC control	241
X0HC 0.5 N NaOH sanitized	220

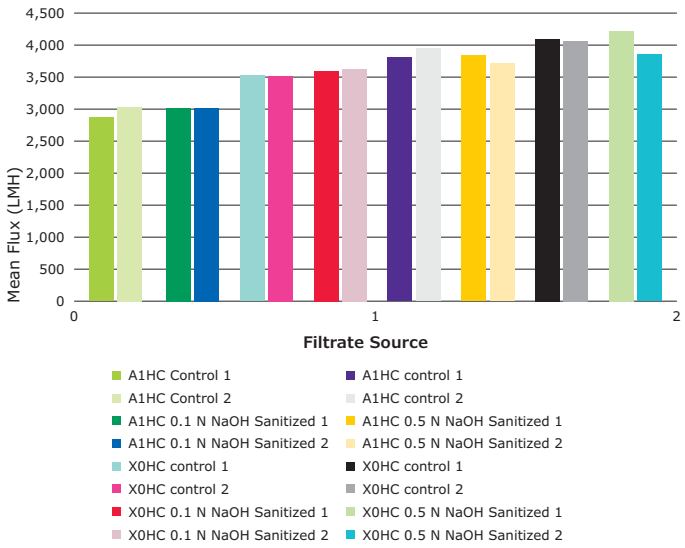


Figure 21. Mean filtrate flux for 0.22 µm sterile filtrate on Millistak+® Pod filtrates.

Conclusions and Recommendations

From the present P_{max}™ and V_{max}™ data, no significant effect of NaOH sanitization (0.1 N and 0.5 N) on Millistak+® A1HC and X0HC Pod filters was observed. Performance was similar for both Millistak+® Pod and Durapore® 0.22 µm sterile filters. As a result, we can conclude that 0.1 N NaOH at room temperature for 1 hour and 0.5 N NaOH at room temperature for 1 hour did not negatively impact the performance of the Millistak+® Pod filters based on the model feed stream utilized in this study. In this study, the tested Millistak+® media was flushed with water prior to sanitization. For a typical installation, it should be possible to proceed directly with the sanitization step without the need for prior water flush.

Autoclaving Millistak+® Pod in a Pod Holder

Pod devices are rated for one autoclave cycle of 60 minutes at 123 ºC; however, this claim is based on single devices being autoclaved alone (for example, in a Tyvek® bag) rather than on an autoclave procedure performed on devices installed in a holder. This study explores the feasibility to install devices in a holder, autoclave the entire assembly and then make an aseptic connection to a harvest system.

The purpose of this study is to:

- Determine suitable autoclave cycle parameters (cycle type, time and temperature) that can be used to sterilize Millistak+® Pod devices installed in a modified holder from which the hydraulics have been removed.
- Verify the feasibility of autoclaving Millistak+® Pod filters in a modified holder. “Feasibility” is demonstrated in two ways:
    1. Sterility can be achieved on a complete autoclaved Millistak+® Pod assembly.
    2. The autoclave cycle does not affect the integrity of the devices, as determined by an air-water pressure hold test.

Results of this study should be taken as demonstrating compatibility of the Millistak+® Pod filters with such a process; however, the results do not constitute a complete validation of the process. As with any autoclave procedure, the burden of validation rests with the end user.

Experimental Design

The experimental procedure was divided into three subsections:

1. Modification of the Millistak+® Pod holder hardware to render it compatible with autoclaving.
2. Temperature mapping experiments designed to identify “cold spots” or areas that lag behind the chamber temperature in the autoclave.
3. Spore kill experiments designed to show that heat-resistant bacteria can be rendered sterile in an autoclave cycle.

Millistak+® A1HC Pod devices were used in all experiments, because they contain the least permeable filtration media, providing the worst case for steam penetration.

Temperature mapping experiments were performed using a GE Kaye Instruments Validator® system. Millistak+® Pod devices were modified by drilling 0.313-inch diameter holes in four locations:

1. In the feed channel behind the blanked port located opposite the feed port.
2. In the permeate channel behind the blanked port opposite the permeate port.
3. Along the vertical centerline of the device at the lowest point, in the feed channel between plates 1 and 2.
4. Along the vertical centerline of the device at the lowest point, in the permeate channel between plates 4 and 5.

Thermocouples were installed into the holes, and the holes were then sealed using rubber stoppers. The stoppers were secured using autoclave tape.

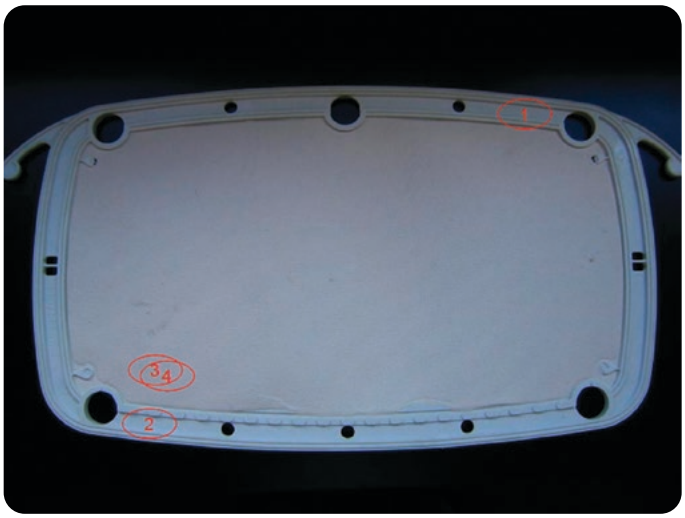


Figure 22. Location of spore strips inside Millistak+® Pod filter.

Figure 23. Millistak+® Pod pilot holder.

Spore kill experiments were performed on specially manufactured devices, which included spore strips located in four locations:

1. In the feed channel between plates 5 and 6 and behind the blanked port located opposite the feed port.
2. In the permeate channel between plates 5 and 6 and behind the blanked port located opposite the permeate port.
3. In the same corner as strip 2, but between the 60DE and 75DE layers.
4. In the same corner as strip 2, but between the 75DE and RW01 layers.

Table 14. Autoclave Cycle Parameters

Cycle Time	60 min
Cycle Temperature	123.1ºC
Pre-vacuum	80 ºC
Post-vacuum	Three pre-vacuum cycles

Temperature Mapping Experiments

Figures 24 and **25** show the results of the temperature mapping experiments performed on 1.1 m^2 and 5.5 m^2 installations, respectively. As seen in the temperature profiles, no significant lag was observed at any location in the device, regardless of the installation size.

From this data, it was determined that a standard validation approach would be suitable for the spore kill experiments without providing any additional cycle time for steam penetration. (See "Spore Kill Experiments" on page 32.)

From the 5.5 m^2 installation, the three unmodified devices were removed from the holder, individually flushed with water and then tested for integrity using an air-water pressure hold test. Flushing was performed at 600 LMH with 100 L/m^2 of water, and the pressure hold test was performed at approximately 3 to 3.5 psi (0.2 to 0.24 bar). Please note that flushing and testing were performed per the recommendations described in our Application Note, "Using Millistak+® HC Filters for Mammalian Cell Culture Clarification" (AN1100EN00). All devices passed the pressure hold test, indicating that the autoclave cycle did not damage the filter media.

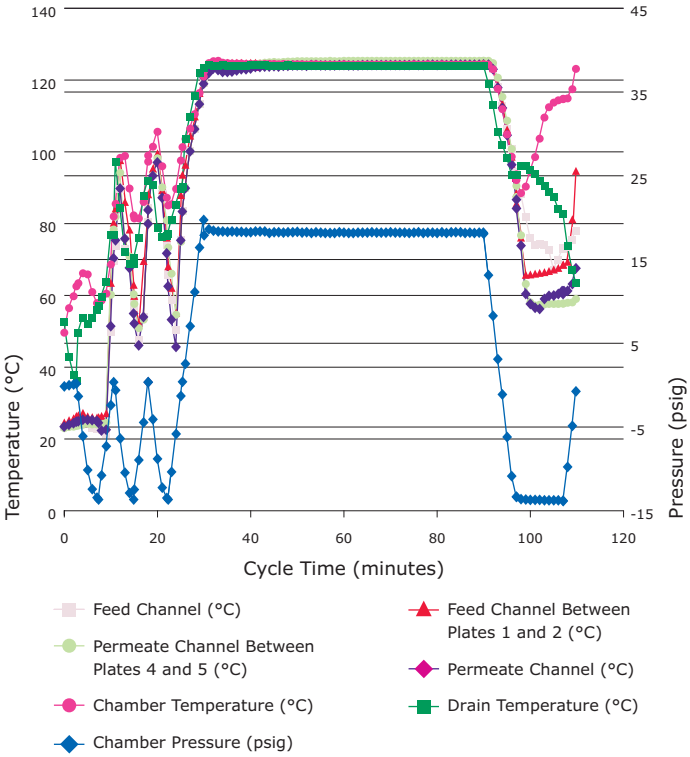


Figure 24. Single 1.1 m^2 Millistak+® Pod device temperature mapping during autoclave cycle.

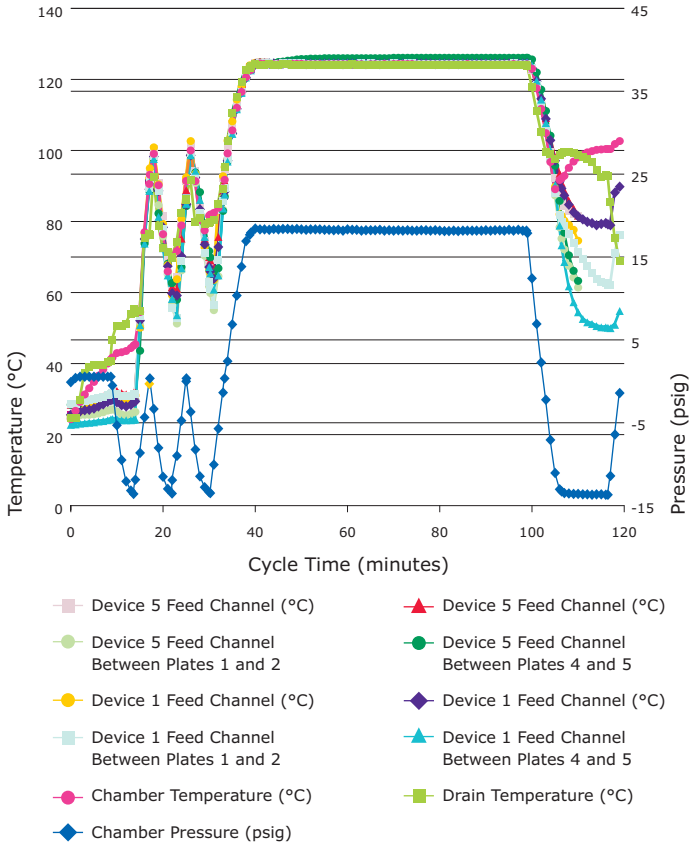


Figure 25. Five 1.1 m^2 Millistak+® Pod devices temperature mapping during autoclave cycle.

Spore Kill Experiments

Figures 26 and **27** show the temperature and pressure profiles of the autoclave cycle used during the spore kill experiments for 1.1 and 5.5 m^2 installations, respectively. Based on the results of the temperature mapping experiments, a 15-minute, 123.1 ºC cycle was chosen for the spore kill experiments. (Under a half-cycle validation approach, this cycle could be used to demonstrate the validity of a 30-minute, 123.1 ºC cycle under normal processing conditions.)

After autoclaving, the devices were removed from the holder and allowed to cool. The devices containing spore strips were autopsied to retrieve the strips, which were subsequently placed into tubes of growth media and incubated at 60 ºC. In addition to the spore strips from the autoclaved devices, spore strips were retrieved from a device that had not been autoclaved. These strips serve as a positive control to demonstrate that the manufacturing process did not render the strips sterile.

After 48 hours of incubation, the culture tubes were visually checked for growth. No growth was observed on any of the autoclaved samples, nor was growth observed on the negative control (i.e., growth media alone). All of the positive controls exhibited growth, indicating that the manufacturing process did not render the spores sterile. This result demonstrates that the autoclave cycle did achieve sterility inside the Millistak+® Pod devices, even at cycle times as low as 15 minutes.

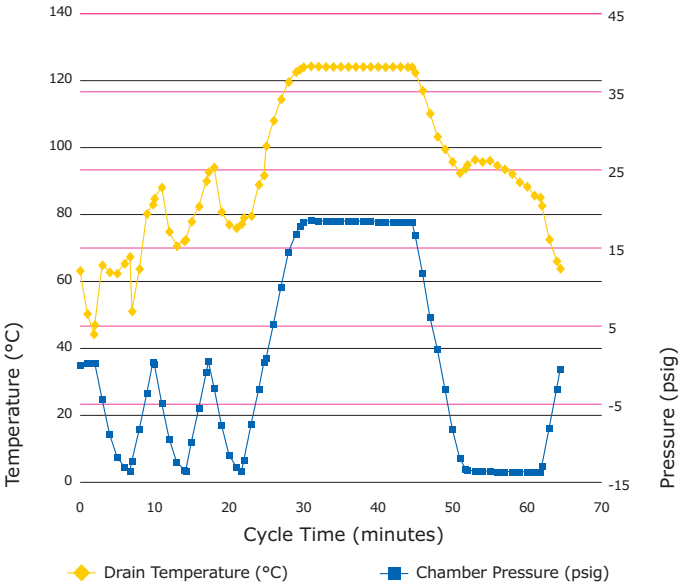


Figure 26. Single 1.1 m^2 Millistak+® Pod device spore kill autoclave cycle.

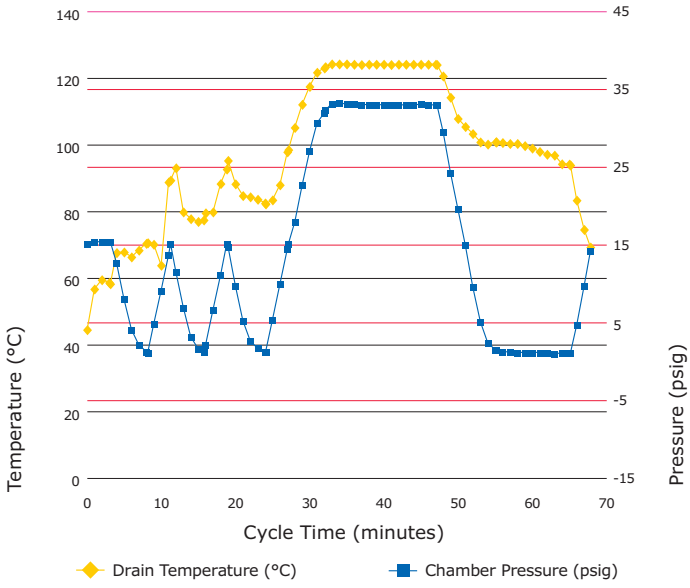


Figure 27. Five 1.1 m^2 Millistak+® Pod devices spore kill autoclave cycle.

Table 15. Spore Growth Results

Spore Location/Device ID	Experiment (Location)	Feed Channel	Permeate Channel	Between 60DE and 75DE	Between 75DE and RW01
CP7JN54563-02	1.1 m^2	–	–	–	–
CP7JN54563-05	5.5 m^2 (inlet)	–	–	–	–
CP7JN54563-03	5.5 m^2 (outlet)	–	–	–	–
CP7JN54563-48	Positive control	–	+	+	+
Culture media alone (negative control)	–				

– indicates no growth on spore strip (sterile result)

+ indicates growth on spore strip (non-sterile result)

Conclusions

Testing confirms that sterility can be achieved in Millistak+® Pod devices installed in a pilot-scale holder (up to 5.5 m^2 installations) using the autoclave conditions shown in **Table 16**.

In addition to demonstrating that sterility can be achieved, it was also shown that the device integrity is not compromised by the autoclave cycle. Six out of six (100%) devices tested remained integral after exposure to the above autoclave cycle.

Table 16. Autoclave Conditions

Pre-vacuum	Three pre-vacuum cycles
Cycle Time	15 min
Cycle Temperature	123.1 ºC
Post-vacuum	One 10 min post-vacuum cycle
Feed Port Termination	1 meter of .5 inch C-flex tubing terminated with Aervent®-50
Permeate Port Termination	1 meter of .5 inch C-flex tubing terminated with Aervent®-50
Vent Port Termination	1 meter of .5 inch C-flex tubing terminated with Opticap® XL2 capsule containing Aervent® membrane

Each series of thermoprofiles was run several times to provide an accurate picture of the heating and biological indicator results. The multiple cycles eliminated any single run variability, generated sufficient data for conclusions and allowed better trend analysis.

- Dry Millistak+® Pod A1HC individual devices can be sterilized at 123 ºC for 30 minutes with three pre-vacuum pulses (down to 1.4 PSIA).
- Wet individual Millistak+® Pod A1HC devices can be sterilized at 123 ºC for 30 minutes with three pre-vacuum pulses (down to 1.4 PSIA).
- Five wet Millistak+® Pod A1HC devices in a modified Millistak+® Pod pilot holder (5.5 m^2) can be sterilized at 123 ºC for 45 minutes with three pre-vacuum pulses (down to 1.4 PSIA).
- Wet Millistak+® Pod A1HC devices in a Millistak+® Pod pilot holder containing 11.0 m^2 (10 devices at 1.1 m^2) were not tested because of autoclave size limits.

Constant Pressure: V_{max}™

V_{max}™ is the preferred test method for a constant pressure test. In a V_{max}™ test, the challenge solution is filtered through the test device and cumulative volume is recorded as a function of time (typically for 10 minutes) at a selected differential pressure, usually 5–10 PSID. If a linear plot of t/v vs. t is obtained, it is assumed the solution follows the gradual pore-plugging model. V_{max}™ can then be calculated as the inverse slope of this graph. V_{max}™ represents the maximum volume of fluid that will pass through a filter before it is completely plugged. The advantages of this method are the smaller volumes of process fluid required and short testing times compared to traditional flow decay testing. These advantages over traditional flow decay testing help facilitate fast and efficient filter media selection. The disadvantage to V_{max}™ is that it does not accurately predict sizing when the fluid being tested does not follow the gradual pore-plugging model.

V_{max}™ Sizing Method Summary

Advantages

- Experimentally determine filter/fluid performance
- Provides basis for filter train selection
- Allows for rapid testing relative to traditional flow decay
- Requires smaller process fluid volume

Disadvantages

- Only applies to Gradual Pore Blocking mechanism

Uses

- Milligard®, Polysep®, Lifegard®, Polygard CN®, Durapore® filters

V_{max}™ Sizing Spreadsheet

We have sizing tools available to assist with V_{max}™ calculations.

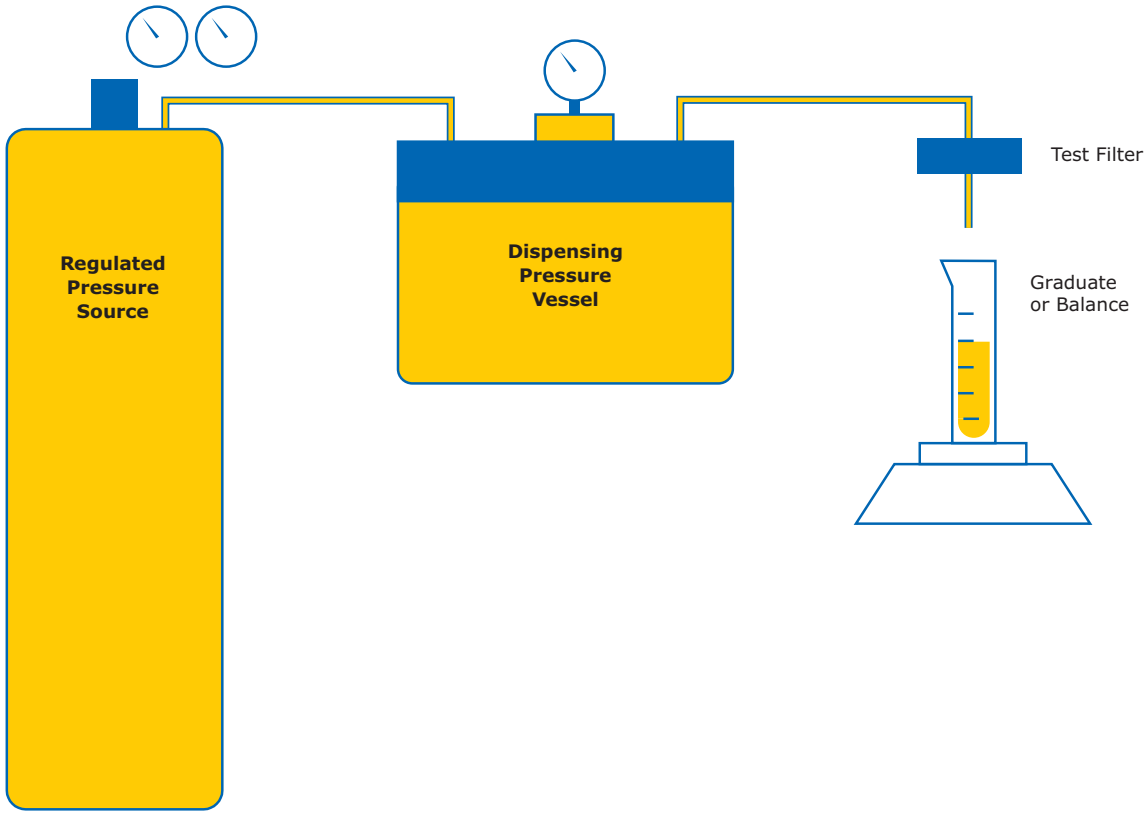
- Plots Data
- Sizing Calculations
- Sizing Recommendations
- Report of Data Analysis

Examples of spreadsheet output:

V_{max}™ Calculations

Slope	2.45 L^{-1}
Intercept	7.20 min/L
Corr. Coefficient	0.999
V_{max}™	0.408 L

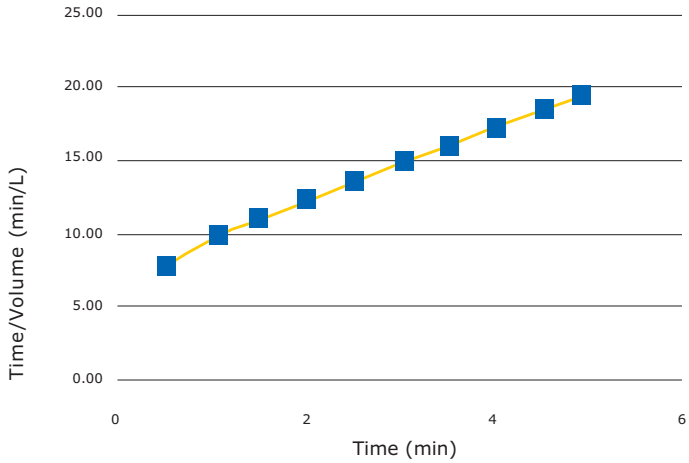
V_{max}™ Setup



Typical V_{max}™ Data

Time (min)	Volume (L)	Time/Volume (min/L)
0.5	.065	7.7
1.0	.106	9.4
1.5	.138	10.9
2.0	.164	12.2
2.5	.185	13.5
3.0	.203	14.8
3.5	.219	16.0
4.0	.232	17.2
4.5	.245	18.4
5.0	.256	19.5

V_{max}™ Graph



Initial Bioburden Level Evaluation

The purpose of this study is to follow the recommended pre-use flushing protocol when operating Millistak+® Pod filters, and to determine the initial bioburden levels in the flush samples and after three days of isolation of the Millistak+® Pod filters subsequent to flushing.

Experiment Protocol

1. Prior to the initiation of the study, all pipework, gaskets, clamps, valves and connectors were sterilized by autoclave at 121 ºC for 30 minutes.
2. The Millistak+® Pod pilot holder was disinfected with Ethanol.
3. After sterilization, the equipment was aseptically assembled (except the Millistak+® Pod filter) according to the schematic described in **Figure 28**.
4. One A1HC grade Millistak+® Pod filter (MA1HC05FS1, total area = 0.55 m²) was installed in the Millistak+® Pod pilot holder.
5. Three 20 L pressure tanks were filled with sterile Milli-Q® water. Milli-Q® water was sterile-filtered on Millipak® 0.22 µm disposable filters. Air pressure (800 mbar) was applied to the tanks and the Millistak+® Pod filters were filled at a flow rate of 5 L/min (corresponding to 600 LMH).
6. In total, 50 L of water were passed through each Millistak+® Pod filter at a flux of 600 LMH, in accordance with the recommended flushing procedure (100 L/m² at 600 LMH).
7. Once no bubbles were seen exiting the vent line, the outlet isolation valve was opened. Then the vent isolation valve was closed and flushing started.
8. During the flushing, 100 mL samples were collected using MicropreSure® On-Line Filtration Samplers at different locations.

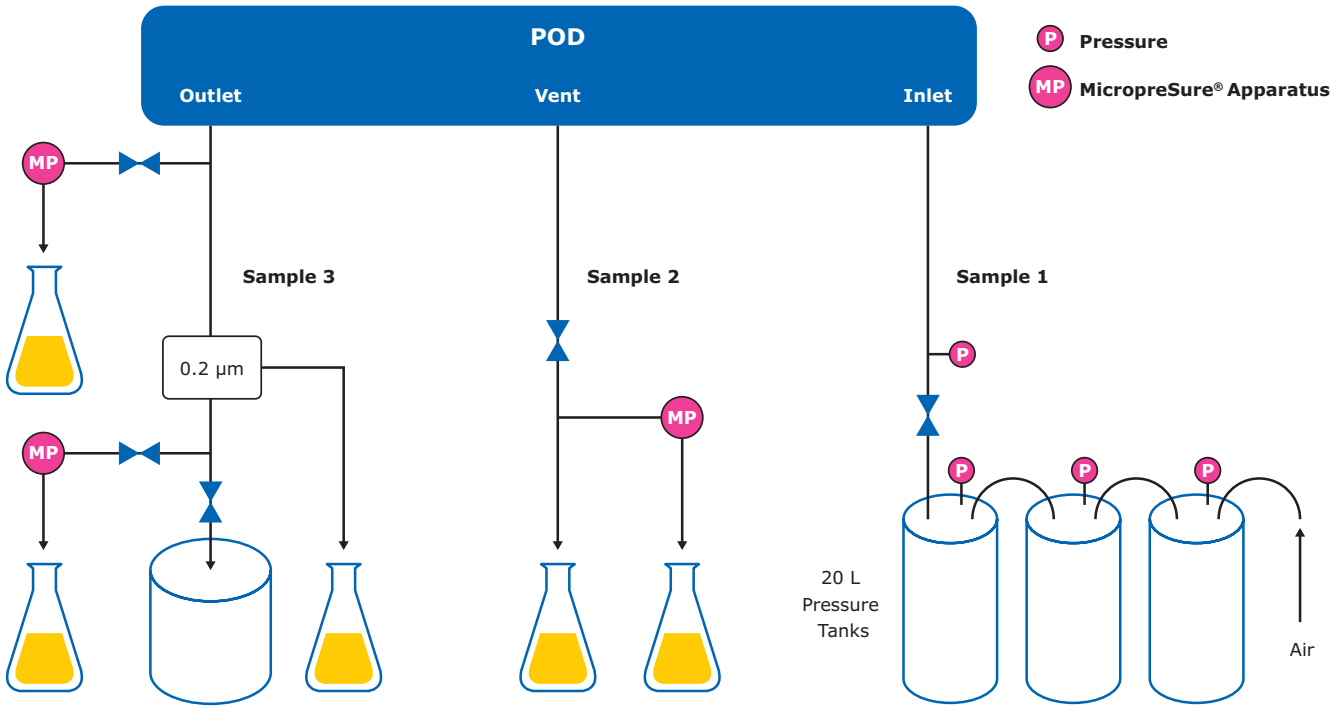


Figure 28. Test method: Millistak+® Pod filter flushing.

Table 17 shows the tabulated samples collected during the entire 72-hour flushing period. At the end of each flushing period, samples were collected and assayed for bioburden. The flushing was stopped and the holder isolated (closing the inlet and outlet isolation valves).

After 24 hours, the isolation valves were opened and the Millistak+® Pod devices were flushed with 5 L of Milli-Q® water. Samples were collected (**Table 17**) and assayed for bioburden. The flushing was stopped and the holder isolated (closing the inlet and outlet isolation valves).

After 48 hours, the isolation valves were opened and the Millistak+® Pod filters were flushed with 5 L of Milli-Q® water. Samples were collected (**Table 17**) and assayed for bioburden. The flushing was stopped and the holder isolated (closing the inlet and outlet isolation valves).

After 72 hours, the isolation valves were opened and the Millistak+® Pod filters were flushed with 5 L of Milli-Q® water. Samples were collected (**Table 17**) and assayed for bioburden. The flushing was stopped and the holder isolated (closing the inlet and outlet isolation valves).

The analytical 0.45 µm collection filters were used to check for the presence of any microorganism in the filtrate sample. The MF filters were aseptically deposited on Trypticase Soy Agar (TSA) plates and incubated at 32 ±2.5 ºC for 2 to 7 days.

The testing was carried out in our laboratory. This study was performed three times with three different lots of A1HC Millistak+® Pod filters.

Table 17. Sample Bioburden Assay

Samples		Milli-Q® Water from Tank (cfu/100 mL)	Vent (cfu/100 mL)	Outlet (cfu/100 mL)	After Opticap® 0.2 µm (cfu/100 mL)
Time (hours)	Flush (L)				
0	0–5	0	0	0	0
	20–25	N/A	0	0	0
	45–50	N/A	0	0	0
24	5	0	0	0	0
48	5	0	0	0	0
72	5	0	0	0	0

Table 18. Lot No. 1: Bioburden Assay

Samples		Milli-Q® Water from Tank (cfu/100 mL)	Vent (cfu/100 mL)	Outlet (cfu/100 mL)	After Opticap® 0.2 µm Filter (cfu/100 mL)
Time (hours)	Flush (L)				
0	0–5	0	0	3 (+1 mold)	0
	20–25	N/A	0	2	0
	45–50	N/A	0	0	0
24	5	0	0	0	0
48	5	0	0	220	0
72	5	0	0	225	0

Table 19. Lot No. 1: Microorganism Identification

The microorganisms observed were identified according to Biolog identification tools.

Samples		Microscopic Description	Microorganism Identified	Possible Origin
Time (hours)	Flush (L)			
0	0–5	• *Cocci Gram* + / catalase –	• *Micrococcus lylae*	• Mammalian skin
		• *Cocci Gram* + / catalase –	• *Micrococcus luteus*	• Mammalian skin
		• *Bacillus Gram* + / catalase +	• *Curtobacterium pusillium*	• Plants and oil brine
	20–25	• *Bacillus Gram* – / oxydase –	• No identification	
		• *Bacillus Gram* – / oxydase –	• No identification	
48	5	• *Bacillus Gram* – / oxydase +	• CDC group EO-2	• Air and soil
		• *Bacillus Gram* – / oxydase –	• No identification	
72	5	• *Bacillus Gram* – / oxydase –	• No identification	• Air and soil
		• *Bacillus Gram* – / oxydase +	• CDC group EO-2	• Soil and fresh water
		• *Bacillus Gram* + / catalase +	• *Rhodococcus* rubber	
		• *Bacillus Gram* – / oxydase –	• No identification	

Table 20. Lot No. 2: Bioburden Assay

April 21, 2008

Samples		Milli-Q® Water from Tank (cfu/100 mL)	Vent (cfu/100 mL)	Outlet (cfu/100 mL)	After Opticap® 0.2 µm Filter (cfu/100 mL)
Time (hours)	Flush (L)				
0	0–5	0	0	6 (+2 mold)	0
	20–25	N/A	0	9	0
	45–50	N/A	0	0	0
24	5	0	0	37 (+1 mold)	0
48	5	0	0	49	0
72	5	0	0	70	0

Table 21. Lot No. 2: Microorganism Identification

The microorganisms observed were identified according to Biolog identification tools.

Samples		Microscopic Description	Microorganism Identified	Possible Origin
Time (hours)	Flush (L)			
0	0–5	• *Cocci Gram* + / catalase +	• *Staphylococcus arlettae*	• Soil and water
		• *Bacillus Gram* – / oxydase –	• No identification	
	20–25	• *Bacillus Gram* – / oxydase –	• No identification	• Soil and water
		• *Cocci Gram* + / catalase +	• *Staphylococcus arlettae*	
24	5	• *Bacillus Gram* – / oxydase –	• No identification	• Soil and water
		• *Cocci Gram* + / catalase +	• *Staphylococcus arlettae*	
48	5	• *Bacillus Gram* – / oxydase –	• No identification	• Soil and water
		• *Cocci Gram* + / catalase +	• *Staphylococcus arlettae*	
72	5	• *Bacillus Gram* – / oxydase –	• No identification	• Soil and water
		• *Cocci Gram* + / catalase +	• *Staphylococcus arlettae*	

Table 22. Lot No. 3: Bioburden Assay

May 19, 2008

Samples		Milli-Q® Water from Tank (cfu/100 mL)	Vent (cfu/100 mL)	Outlet (cfu/100 mL)	After Opticap® 0.2 µm Filter (cfu/100 mL)
Time (hours)	Flush (L)				
0	0–5	0	0	6	0
	20–25	N/A	0	0	0
	45–50	N/A	0	1 mold	0
24	5	0	0	17	0
48	5	0	1	40	0
72	5	0	28	112	0

Table 23. Lot No. 3: Microorganism Identification

The microorganisms observed were identified according to Biolog identification tools.

Samples		Microscopic Description	Microorganism Identified	Possible Origin
Time (hours)	Flush (L)			
0	0–5 Outlet	• *Bacillus Gram* + / catalase +	• No identification	• Environment and soil • Skin flora
		• *Cocci Gram* + / catalase +	• No identification	
		• *Bacillus Gram* + / catalase –	• *Microbacterium spp.* (CDC. A-5)	
		• *Cocci Gram* + / catalase +	• *Staphylococcus lentus*	
		• *Bacillus Gram* – / oxydase +	• *Haemophilus actino.*	
24	Outlet	• *Cocci Gram* + / catalase –	• No identification	• Skin flora, soil and water • Environment and soil
		• *Cocci Gram* + / catalase +	• *Staphylococcus xylosus*	
		• *Bacillus Gram* + / catalase +	• *Curtobacterium pusillum*	
		• *Bacillus Gram* + / catalase +	• *Microbacterium spp.* (CDCA-5)	
		• *Cocci Gram* + / catalase +	• No identification	
		• *Bacillus Gram* + / catalase –	• No identification	
		• *Cocci Gram* + / catalase –	• No identification	
48	Vent	• *Bacillus Gram* – / oxydase –	• No identification	• Soil and water
	Outlet	• *Bacillus Gram* + / catalase +	• *Curtobacterium pusillum*	
		• *Cocci Gram* + / catalase +	• No identification	
		• *Bacillus Gram* + / catalase +	• No identification	
		• *Bacillus Gram* – / oxydase +	• No identification	
72	Vent	• *Bacillus Gram* – / oxydase –	• No identification	
	Outlet	• *Bacillus Gram* – / oxydase +	• No identification	• Soil and water
		• *Bacillus Gram* + / catalase +	• No identification	

General Conclusion

The results of this study show that bioburden was not present in the tested Millistak+® Pod filters from three different filter lots post-water flush and prior to use. The recommended flushing protocol (100 L/m^2 at 600 LMH) has been followed. The data showed limited bioburden growth up to 24 hours after the flush of the Millistak+® Pod filters. However, after 48 and 72 hours of storage, a more significant level of bioburden was observed.

Millistak+® Pod filters will support bioburden growth. Identification of the bioburden shows that microorganisms are from environmental and human origin and therefore introduced from external sources. No biorbuden was present after the 0.2 µm filter, and therefore retained any bioburden introduced into the process resulting in a very low risk process.

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