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White Paper

Validation of design features and a method to reduce the risk of false positives during bioburden filtration testing

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Introduction

Bioburden testing of fluids typically requires filtration through a membrane, using a manifold or a pump. Managing the contamination risk that may create false positive results is a significant concern in the Quality Control laboratory. This risk can originate from the filtration equipment, via backflow, or contaminated residual on the pump head.

These concerns were considered in the design of the new Milliflex Oasis® pump and Milliflex Oasis® filtration device.

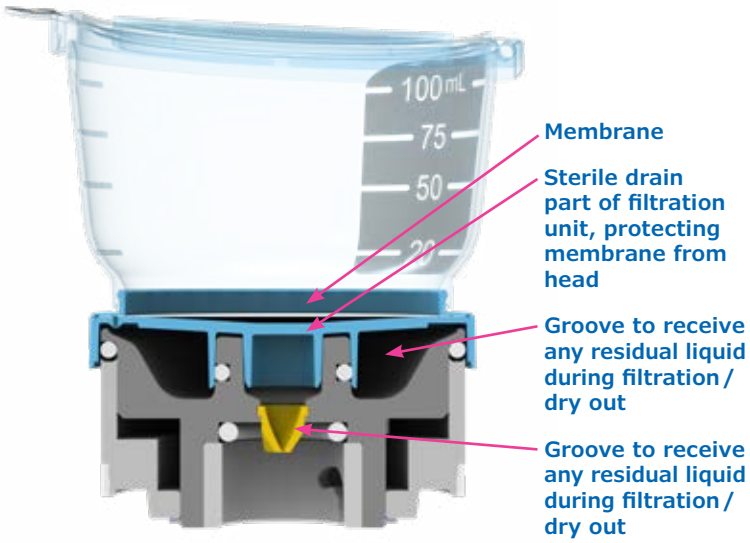

Figure 1: The Milliflex Oasis® pump head with a filtration device: pump head (gray), filtration device (blue), and the check-valve seal (yellow).

System design includes:

- Removable pump head including features presented in **Figure 1**. The stainless steel head can be easily decontaminated without autoclaving, using surface disinfectants.
- Drain, as part of the filtration unit.
- Pump housing with smooth surfaces, material is compatible with a wide range of disinfectants.
- Accessory allowing cleaning and decontamination of the pump's fluid path (**Figure 2**).

Figure 2: Decontamination accessory installed on each head of a Milliflex Oasis® pump. 50 mL of the disinfectant is kept in contact with the fluid path for 15 minutes and rinsed with 250 mL of sterile water.

Methods

Contamination study: 3 Milliflex Oasis® pumps (6 pump heads) were tested, each of which was treated with a different disinfectant, as shown in **Table 1.**

Pump	Disinfectant for pump housing and head	Disinfectant for pump internal decontamination
Pump 1	Wiped with 10% bleach (Clorox) followed by a Prosat (70% IPA) wipe to remove residue	10% bleach (Clorox), rinsing with sterile water
Pump 2	Wiped with quaternary ammonium (Vesta-Syde SQ) from STERIS	Same quaternary ammonium, rinsing with sterile water
Pump 3	Wiped with 6% hydrogen peroxide WFI from STERIS	Same 6% hydrogen peroxide, rinsing with sterile water

Table 1 – List of the disinfectants used to treat each pump

Pumps were first decontaminated using the accessory (**Figure 2**), and then heads were disinfected by wiping after seal removal.

For 18 weekdays, each pump was subjected to the following protocol:

- Wipe the pump's outer surface with the respective disinfectant.
- Filter 100 mL of a Fluid A solution contaminated with 112 to 26000 CFUs using a funnel without membrane in order to contaminate the fluid path. Table 2 presents the strains used and head tested. The right and left heads of each pump are tested separately. Two alternating analysts perform the testing. The right and left pump heads are tested by both analysts.
- Immediately after, place a Milliflex Oasis® filtration unit with a 0.45 µm nitrocellulose membrane on the head. Filter 100 mL of sterile water, transfer to Tryptic Soy Agar (TSA) media and enumerate after three days of incubation at 30–35 °C.

Day	Organism (ATCC #)	Pump	Head
Day 1	*Staphylococcus aureus* (6538)	1, 2, 3	Right
Day 2	*Bacillus subtilis* (6633)	1, 2, 3	Left
Day 3	*Candida albicans* (10231)	1, 2, 3	Right
Day 4	*Aspergillus brasiliensis* (16404)	1, 2, 3	Left
Day 5	*Staphylococcus epidermidis* (12228)	1, 2, 3	Right
Day 6	*Pseudomonas aeruginosa* (9027)	1, 2, 3	Left
Day 7	*Kocuria rhizophila* (9341)	1, 2, 3	Right
Day 8	*Ralstonia pickettii* (27511)	1, 2, 3	Left
Day 9	*Stenotrophomonas maltophilia* (13637)	1, 2, 3	Right
Day 10	*Staphylococcus aureus* (6538)	1, 2, 3	Left
Day 11	*Bacillus subtilis* (6633)	1, 2, 3	Right
Day 12	*Candida albicans* (10231)	1, 2, 3	Left
Day 13	*Aspergillus brasiliensis* (16404)	1, 2, 3	Right
Day 14	*Staphylococcus epidermidis* (12228)	1, 2, 3	Left
Day 15	*Pseudomonas aeruginosa* (9027)	1, 2, 3	Right
Day 16	*Kocuria rhizophila* (9341)	1, 2, 3	Left
Day 17	*Ralstonia pickettii* (27511)	1, 2, 3	Right
Day 18	*Stenotrophomonas maltophilia* (13637)	1, 2, 3	Left

Table 2 – List of strains and pump head positions tested

On days 5, 10, and 15, prior to contamination, the pump fluid path's microbiological load was evaluated. 100 mL of sterile water was filtered on each pump head, this 200 mL flow aseptically collected from each pump drain. Serial dilutions in PBS were plated on TSA, and the remaining volume split and filtered on two 0.45 µm nitrocellulose membranes, which were then transferred to TSA media. Membranes and media were incubated for three days at 30–35 °C.

On day 18, each pump's fluid path was decontaminated using the decontamination accessory. The microbiological load in the fluid path was evaluated by filtration of the 200 mL of rinse. The filtration heads were disinfected by wiping with the respective product, after seal removal.

Organism recovery study: One pump head was decontaminated by wiping after seal removal. Heads were returned to the pump after replacing the seals. On each pump head, recovery of several challenge microorganisms was tested in triplicate by filtration with Milliflex Oasis® units, using spiked Fluid A. Recovery was calculated against dilution plating on the same media. **Table 3** lists the microbes and media included in the study. Negative controls were done by sterile Fluid A filtration at the beginning and end of the study.

Organism (ATCC #)	TSA 30–35 °C / 3 days	SDA 20–25 °C / 3 days
Staphylococcus aureus (6538)	✔	
Bacillus subtilis (6633)	✔	
Candida albicans (10231)	✔	✔
Aspergillus brasiliensis (16404)	✔	✔
Pseudomonas aeruginosa (9027)	✔	
Kocuria rhizophila (9341)	✔	

Table 3 – Recovery microbes and media

Results and Discussion

Contamination study: During the 18 day study, a total of 54 membranes (9 per pump head) were used to filter sterile water after intentional contamination of the pump through the head. All membranes showed no colony forming units after three days of incubation. The design features of the pump head and the filtration unit prevented backflow contamination from the pump head to the membrane even without any autoclaving of the head. Daily surface wiping of the head with a disinfectant successfully managed this risk.

Figure 3 displays the bioburden of the rinse from the three pumps' drains on different days, and after decontamination on the last day. Pump 3 showed no detectable bioburden during the entire study. The microbial flora in the fluid path of pump 3 may have required more than 3 days of incubation for detection. In spite of high levels of bioburden obtained

on pumps 1 and 2, no contamination was observed on the membranes used with these pumps. Other experimental data, not displayed here, suggests that the rinse bioburden may originate from the pump engine's flora and not the tested organisms. Each time following decontamination, bioburden decreased to an undetectable level.

Organism recovery study: recoveries obtained via filtration compared to those via plating are presented in **Table 4.** The acceptable recovery levels show that the pump head decontamination method does not cause inhibition by the tested disinfectants.

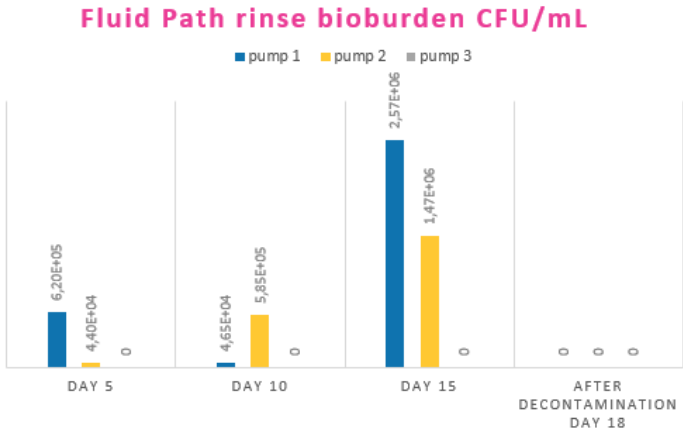

Figure 3: Pump drain rinsing bioburden level in CFU/mL.

Organism (ATCC #)	Recovery % versus plating					
	Pump 1		Pump 2		Pump 3	
	TSA	SDA	TSA	SDA	TSA	SDA
Staphylococcus aureus (6538)	80%	–	80%	–	88%	–
Bacillus subtilis (6633)	104%	–	97%	–	100%	–
Candida albicans (10231)	115%	89%	118%	89%	118%	103%
Aspergillus brasiliensis (16404)	94%	94%	100%	100%	100%	100%
Pseudomonas aeruginosa (9027)	124%	–	105%	–	116%	–
Kocuria rhizophila (9341)	98%	–	101%	–	91%	–
Initial negative control: 0 CFU			**Final negative control: 0 CFU**			

Table 4 – Recovery study results for membrane filtration

Summary

Bioburden testing of water, raw materials, and in-process samples typically requires membrane filtration using a manifold or a pump. Due to the time and resources required to conduct investigations, managing the risk of false positive results is a significant concern in Quality Control laboratories. A study was performed in the BioReliance® Corporation GMP laboratories which demonstrated the ability of the new Milliflex Oasis® to efficiently manage the microbial contamination risk. Three Milliflex Oasis® pumps were used for 18 days and deliberately contaminated with several microorganisms. Wiping the outer surfaces and heads of the pump with a disinfectant every day and decontaminating the fluid path and head once a month managed to avoid false positives when filtering sterile water. No autoclaving of the pump heads was necessary, hence the bioburden test is a user-friendly method without the risk of false positives.

Furthermore, recovery testing showed that the disinfectants used for head and pump decontamination did not inhibit microbial growth with the filtration unit.

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