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Robust Microbial Recovery After Filtration of Disinfectants Using Microsart® @filter PVDF

Dr. Nils Jäger[1], Anna-Lena Weißenborn[1], Juliane Großmann[2]

1. Lab Essentials Application Development, Sartorius Lab Instruments, Göttingen, Germany

2. Product Management, Lab Essentials Microbiology, Sartorius Lab Instruments, Göttingen, Germany

Correspondence

E-Mail: nils.jaeger@sartorius.com and juliane.grossmann@sartorius.com

Abstract

Disinfectants are used to minimize microorganisms in hygienically critical environments such as pharmaceutical cleanrooms. The rise of microbial resistance against disinfectants poses a serious threat to aseptic processes. Due to high requirements on hygiene in pharmaceutical environments, producers must determine the bioburden of disinfectants during quality control. Microbial examination through membrane filtration encounters difficulties when dealing with antimicrobials. Pharmacopeial guidelines recommend dilution of the sample in suitable diluent or the addition of specific neutralizing agents. Microsart® @filter PVDF shows robust recovery of selected microorganisms after filtration of various disinfectants.

Find out more: www.sartorius.com/en/applications/quality-control-testing/microbiological-quality-control/microbial-enumeration

Introduction

The disinfection of surfaces and objects plays a crucial role in the medical and biopharmaceutical sector. Various types of disinfectants, possessing sporicidal or antimicrobial properties, are applied for this purpose. Disinfectants designated for use in pharmaceutical environments are frequently subjected to sterilization processes, such as gamma irradiation or filtration through 0.2 micron filters. Apart from persistent bacterial and fungal spores, there have been reports of increasing resistance among microorganisms to biocides, including antiseptics, disinfectants and preservatives. This resistance poses a significant threat to aseptic processes that ensure product safety. Consequently, the detection and quantification of microorganisms in disinfectants is a mandatory component of quality control in the manufacturing of these products[1]. Membrane filtration has been established as the regulatory-preferred method for microbial enumeration. However, disinfectants pose a challenge to membrane filtration as their solvents can affect materials that come into contact with the filtered sample and their antimicrobial properties must be effectively neutralized. Besides dilution, the addition of neutralizing agents may be used to neutralize the activity of antimicrobial agents (see Table 1).

Table 1: *Substances with antimicrobial properties and the respective neutralizing agents recommended by USP <61>[2], Ph. Eur. 2.6.12.[3] and JP 4.05[4].*

Antimicrobial substance	Neutralizing agent
Glutaraldehyde, Mercurial	Sodium hydrogen sulfite (Sodium bisulfite)
Phenolics, Alcohol, Aldehydes, Sorbate	Dilution
Aldehydes	Glycine
Quaternary Ammonium Compounds (QACs), Parabens, Bis-biguanides	Lecithin
QACs, Iodine, Parabens	Polysorbate
Mercurials	Thioglycollate
Mercurials, Halogens, Aldehydes	Thiosulfate
EDTA (edetate)	Mg^{2+} or Ca^{2+} ions

The chemical resistant and low binding Microsart® @filter PVDF (polyvinylidene fluoride) are used as disposable devices for microbial enumeration of aqueous and non-aqueous solutions such as disinfectants. The scope of this study was to investigate the recovery of selected microorganisms on Microsart® @filter PVDF after the filtration of various disinfectants and neutralization of their antimicrobial properties.

Materials and Methods

Table 2: *Hardware, solutions & consumables used in the study.*

Product	Order No.
Microsart® @filter 100 PVDF, 0.45 µm	16D04--10-06--BL 16D04--10-06--TG 16D04--10-06-ACG
2 branch Microsart® Manifold	168M2-MS
Microsart® e. jet pump	166MP-4
BD Trypticase™ Soy Agar	254086
BD buffered sodium chloride–peptone solution	257087

Table 3: *Certified disinfectants tested in this study.*

Chemical entity	Classification	Composition of the undiluted disinfectants
Alcohol	General disinfectant, antiseptic, antiviral	70% isopropyl alcohol, 70% ethanol
Hypochlorous acid	Sporicidal	2000 ppm hypochlorous acid
Hydrogen peroxide	Sporicidal, antiseptic	6% hydrogen peroxide

Pseudomonas aeruginosa ATCC® 9027™ and *Staphylococcus aureus* ATCC® 6538™ were selected as test microorganisms as they are recommended by pharmacopeias covering microbiological examination of nonsterile products and EN 13727-2012[5] for assessing bactericidal activity of chemical disinfectant and antiseptic products for use in medical areas. Spread plates and membrane filtrations with Microsart® @filter PVDF without disinfectants served as controls.

Preparation and filtration of the samples

As recommended by USP <61>, Ph. Eur. 2.6.12. and JP 4.05, the certified disinfectants (Table 3) were diluted 1 to 10 in sterile buffered sodium chloride (NaCl)-peptone solution. Prior to filtration of the samples, membrane filters in the Microsart® @filter unit were pre-wetted by filtration of 50 mL of buffered NaCl-peptone solution. 10 mL of the respective sample was aseptically mixed with 90 mL buffered NaCl-peptone solution, applied to the Microsart® @filter PVDF and subsequently filtered. For effective neutralization of the antimicrobial properties, the membranes were rinsed three times with 100 mL buffered NaCl-peptone solution. During the last rinsing step, <100 colony forming units (CFU) of the respective test micro-organisms were spiked into the buffered NaCl-peptone solution. Finally, the membrane filters were placed on 90 mm Tryptic Soy Agar (TSA) plates and incubated at 35 °C for two days (Table 4). Five replicates were performed.

Table 4: *Schematic workflow for filtration of disinfectants.*

Step	Description
1	Pre-wetting with 50 mL NaCl-peptone solution
2	Filtration of 100 mL of the 1 to 10 diluted disinfectant
3	Rinse 2x with 100 mL NaCl-peptone solution
4	Rinse 1x with 100 mL NaCl-peptone solution spiked with test strain
5	Transfer of the membrane to 90 mm TSA plates
6	Incubation at 35 °C (± 2.5) for two days

Results

The recovery rates of *S. aureus* and *P. aeruginosa* on Microsart® @filter PVDF were above 80% for all tested disinfectants (Figure 1).

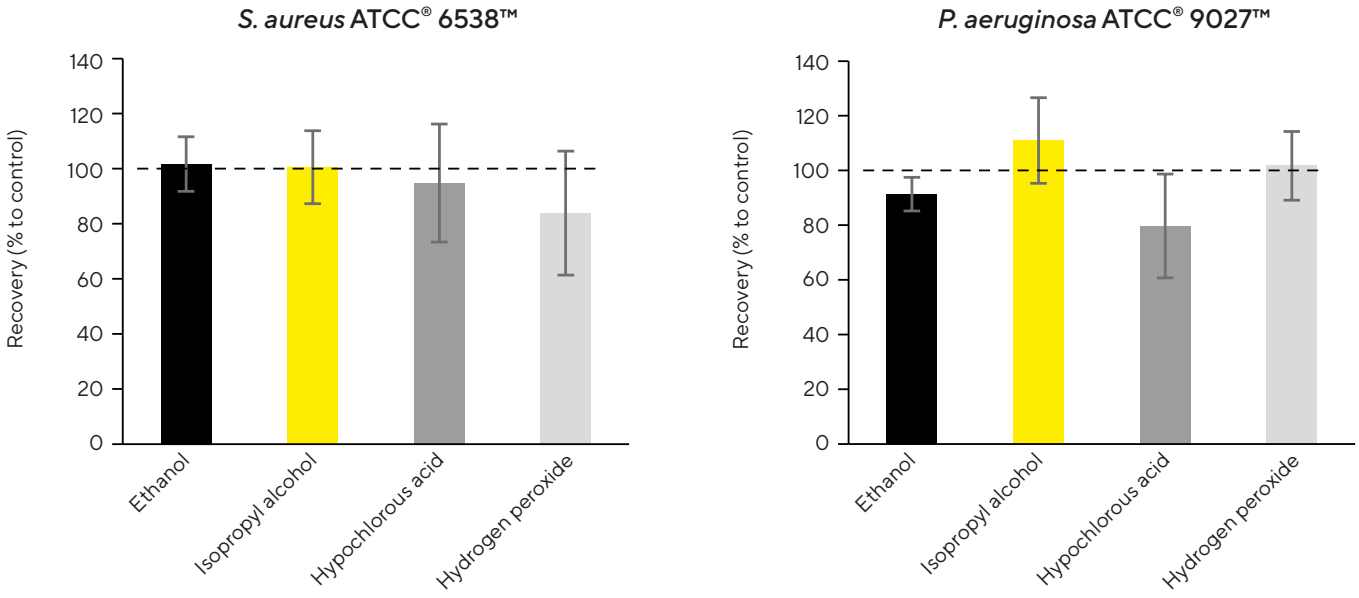

Figure 1: *Recovery rates of S. aureus and P. aeruginosa after filtration of different 1 to 10 diluted disinfectants on Microsart® @filter PVDF and incubation on TSA. The recovery rate is shown in percentage with the reference (Microsart® @filter PVDF without disinfectant) set as 100%.*

The morphology and size of *P. aeruginosa* and *S. aureus* colonies on Microsart® @filter PVDF were similar to controls (Figure 2).

In summary, these results indicate the effective neutralization of antimicrobial properties by dilution and rinsing with buffered NaCl-peptone solution.

***S. aureus* ATCC® 6538™**

***P. aeruginosa* ATCC® 9027™**

Figure 2: *Exemplary images of S. aureus and P. aeruginosa recovered on Microsart® @filter PVDF and incubated on TSA after filtration of different 1 to 10 diluted disinfectants.*

Conclusion

Microbiological testing of disinfectants intented for the use in hygienic critical environments is a mandatory component of microbiological quality control. Due to their low affinity and chemical resistance, Microsart® @filter PVDF are ideally suited for this application. The suitability is confirmed by the findings of this study, in which robust recoveries of *P. aeruginosa* and *S. aureus* were obtained after filtration of various samples with antimicrobial properties in accordance with pharmacopeial guidelines. Only by dilution and rinsing with buffered NaCl-peptone solution, microbial recoveries above 80% were achieved. To obtain higher recovery rates, the use of sodium thiosulfate as a neutralizer for hypochlorous acid or hydrogen peroxide is a pharmacopeia-compliant option. The results highlight the low binding properties of the Microsoft® @filter PVDF membrane as well as the effective neutralization of the antimicrobial properties of disinfectants by buffered NaCl-peptone solution.

1. EU GMP Annex 1: Manufacture of Sterile Medicinal Products (2022)
2. US Pharmacopoeia (USP) <61> - Microbiological Examination of Nonsterile Products: Microbial Enumeration Test
3. European Pharmacopoeia (Ph. Eur.) 2.6.12. - Microbiological Examination of Non-sterile Products: Microbial Enumeration Test
4. Japanese Pharmacopoeia (JP) 4.05 – Microbiological Examination of Non-sterile Products
5. EN 13727-2012 Chemical Disinfectants and Antiseptics - Quantitative Suspension Test for the Evaluation of Bactericidal Activity in the Medical Area - Test Method and Requirements

Germany
Sartorius Lab Instruments GmbH & Co. KG
Otto-Brenner-Strasse 20
37079 Goettingen
Phone +49 551 308 0

USA
Sartorius Corporation
565 Johnson Avenue
Bohemia, NY 11716
Phone +1 631 254 4249
Toll-free +1 800 635 2906

For further information, visit
www.sartorius.com

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