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Evaluation of a Low-temperature Sterilization Process Against Mature Biofilms in Endoscope Model

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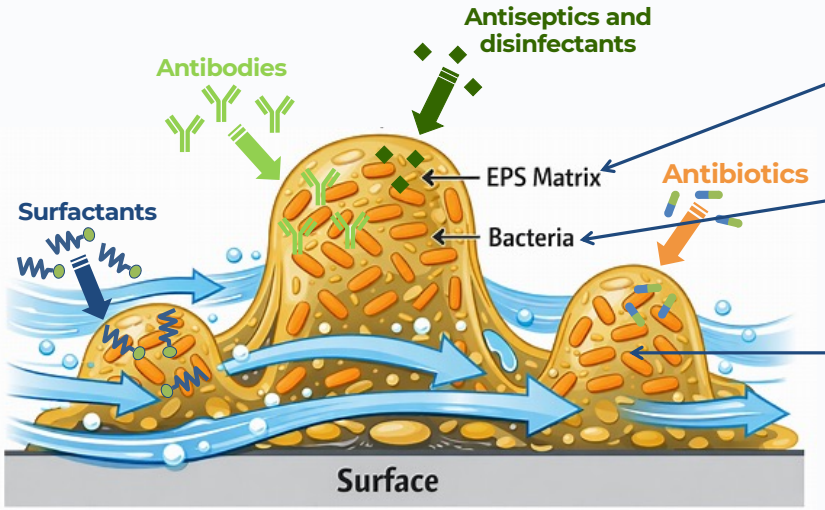
Why biofilm changes the reprocessing challenge



Physical and Chemical Barrier

Reduced metabolic activity:
limit the effect of molecules acting on metabolic processes and reduce membrane permissiveness

High cell density & quorum sensing:
Development of collective defense responses

Biofilm = matrix-enclosed microbial populations adherent to each other and/or to surfaces

Bacteria within biofilm are less susceptible to antimicrobial agent.

Sources: Costerton, J. W., Lewandowski, Z., Caldwell, D. E., Korber, D. R., & Lappin-Scott, H. M. (1995). Microbial biofilms. Microbiological Reviews, 59(3), 239–279
Lebeaux D, Ghigo J-M, Beloin C. Biofilm-related infections: Bridging the gap between clinical management and fundamental aspects of recalcitrance toward antibiotics. Microbiol Mol Biol Rev 2014;78:510e43.
M Exner, G J Tuschewitzki, J Scharnagel. Influence of biofilms by chemical disinfectants and mechanical cleaning. Zentralbl Bakteriol Mikrobiol Hyg B Umwelthyg Krankenhaushyg Arbeitshyg Prav Med. 1987 Apr;183(5-6):549-63.

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Reduced susceptibility of Microbial biofilm

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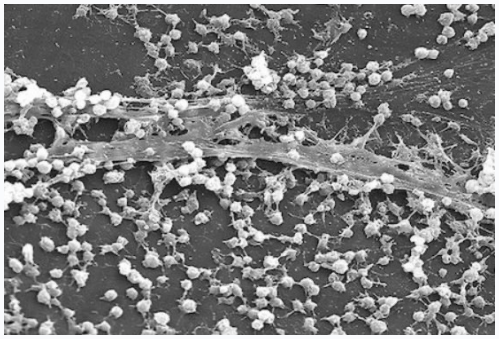
Chemicals	Minimum bactericidal concentration(1)	Disinfection parameters to kill the biofilm cells(2)	Biofilm removal efficacy
Aldehydes	0.007%	1h / 3% or 10 min / 10%	0.007%
P.A.A.	0.0004%	1h / 1%	0.0004%
H_2O_2	0.3%	1h / 3%	0.3%
Chlorine	0.0001%	1h / 0.001%	0.0001%



Staphylococcus aureus biofilm on a catheter.

(1): 5 log reduction in 5 minutes at 20°C against vegetative *P. aeruginosa*

(2) : 10^6 UFC/cm^2

Bacteria within biofilm are 10 to 1000 times less susceptible to disinfection than the same bacteria in suspension.

Sources: M Exner, G J Tuschewitzki, J Scharnagel. Influence of biofilms by chemical disinfectants and mechanical cleaning. Zentralbl Bakteriol Mikrobiol Hyg B Umwelthyg Krankenhaushyg Arbeitshyg Prav Med. 1987 Apr;183(5-6):549-63.

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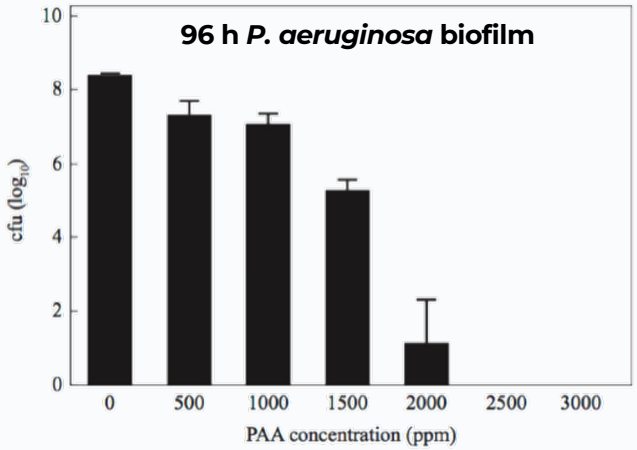
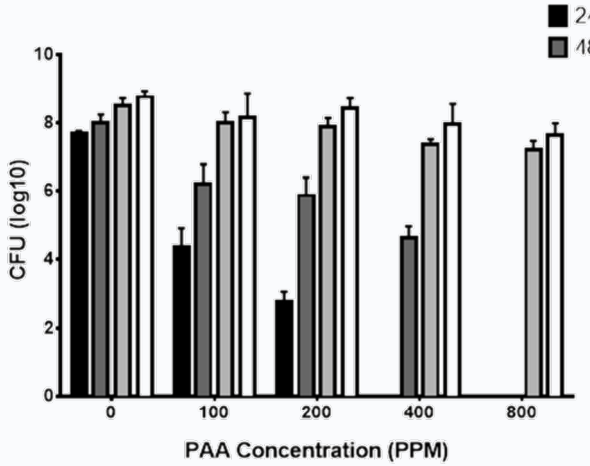
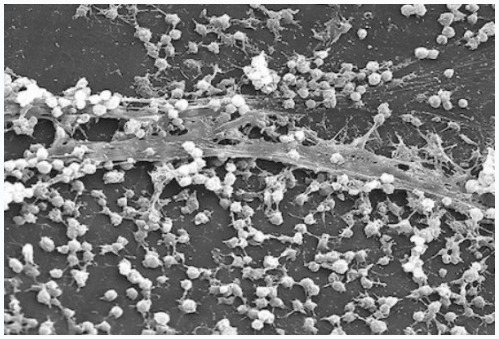
Reduce susceptibility of Microbial biofilm







Staphylococcus aureus biofilm on a catheter.

96-hour *Pseudomonas aeruginosa* biofilm survives 5 min treatment with 2000 ppm of peracetic acid (working condition used in some endoscope washer-disinfectors).

➔Failure of flexible endoscopes treatment might occur when biofilms build up in endoscopes lumens.

Sources: Akinbobola AB, Sherry L, Mckay WG, Ramage G, Williams C. Tolerance of Pseudomonas aeruginosa in in-vitro biofilms to high-level peracetic acid disinfection. J Hosp Infect. 2017 Oct;97(2):162-168.

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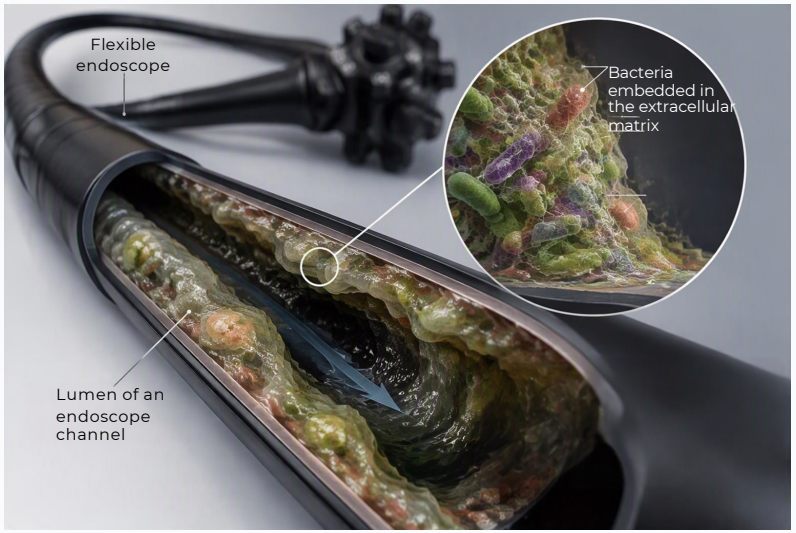
Why biofilms matter for Endoscope Reprocessing



Key facts

- Retained soil, moisture and device damage may favour biofilm development and persistence.
- Biofilm-associated cells are more tolerant than planktonic cells to disinfectant.
- Residual biofilm may contribute to persistent contamination and transmission risk.

Key numbers

5.4%
Duodenoscope
contaminated with high-concern microorganims in US
(FDA postmarket surveillance, 2019 data)

13%
Patient-ready Endoscope
contaminated above the acceptable threshold in 2021 on 18,288 samples
27.9% considering high risk endoscopes
(Pineau, 2023)

5.0%
Endoscope after enhanced reprocessing
contaminated above the acceptable threshold on 9,084 samples
(Bomman et al. 2022)

Can sterilization processes address this challenge?

Sources: S. Haugen. FDA Update on Infections Associated xith Reprocessed Duodenoscopes (May 16, 2019) HICPAC
L. Pineau. Endoscope reprocessing: Retrospective analysis of 90,311 samples. Endosc Int Open 2023; 11: E247–E257
S. Bomman, M. Ashat, N. Nagra, M. Jayaraj, S. Chandra, R. A. Kozarek, A. Ross, R. Krishnamoorthi. Contamination Rates in Duodenoscopes Reprocessed Using Enhanced Surveillance and Reprocessing Techniques: A Systematic Review and Meta-Analysis. Clin Endosc. 2022 Jan 3;55(1):33–40.

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Advantages and limitations of sterilization processes

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Endoscope sterilization challenges | Long / narrow lumen | Material constraints | Residual moisture and potential organic residues

Method	Penetration potential	Advantage	Key limits	Conclusion
Steam	Excellent lumen access if air is removed	Robust and residue free	Incompatible with thermolabile endoscopes	**Not adapted**
Ethylene oxide	High gas diffusivity in complex geometry	Broad material compatibility	Long cycles; Toxic residues	**Strongest diffusion**
VH_2O_2 / plasma	Cycle and lumen specific	Fast cycle; low-temperature; low residues	Restriction for long/narrow lumens	**Strict IFU limits for long channels**
LTSF	Vacuum and steam assisted distribution	Low-temperature; potential for complex lumens	Air removal, condensation, adsorption and formaldehyde residues requiring controls	**Validate worst case**
Radiation	Not dependent on gas or liquid flow through the lumen	Terminal sterilization; No chemical residues	Industrial process; Polymer ageing and electronic compatibility	**Industrial use only**

Sources: ISO 11135:2014 + Amd 1:2018 (EtO); ISO 22441:2022 (VH_2O_2); ISO 25424:2018 (LTSF); CDC, Guideline for Disinfection and Sterilization in Healthcare Facilities (2008, updates); FDA, Sterilization for Medical Devices.

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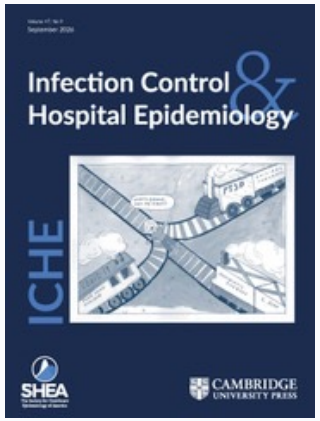
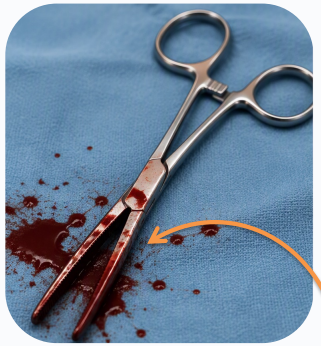


Comparative evaluation of the microbial activity of low-temperature sterilization technologies to steam sterilization

W. Rutala et al., 2020

Does Blood on « Dirty » Instruments Interfere With the Effectiveness of Sterilization Technologies?

W. Rutala et al., 2020



A Comparative Study of Ethylene Oxide Gas, Hydrogen Peroxide Gas Plasma, and Low-Temperature Steam Formaldehyde Sterilization

K. Kanemitsu et al., 2016

Spores + soil

Method	Percentage of no growth	
Steam	Blood + *G. stearothermophilus*	100%
Ethylene oxide	Blood + *B. athrophaeus*	10%
	Blood or serum + *G. stearothermophilus*	100%
Vaporized H_2O_2	Salt and serum + *G. stearothermophilus*	27%
H_2O_2 plasma	Blood + *G. stearothermophilus*	40%
LTSF 37%	Bovine serum + *G. stearothermophilus*	100%
Radiation	Not evaluated	

Sources: Rutala W, Gergen M, Weber DJ. Does Blood on "Dirty" Instruments Interfere With the Effectiveness of Sterilization Technologies? Infection Control & Hospital Epidemiology. 2020;41(S1):s194-s195. doi:10.1017/ice.2020.734
Rutala W, Gergen M, Sickbert-Bennett E, Weber DJ. Comparative Evaluation of the Microbicidal Activity of Low-Temperature Sterilization Technologies to Steam Sterilization. Infection Control & Hospital Epidemiology. 2020;41(S1):s176-s177. doi:10.1017/ice.2020.707
Kanemitsu K, Imasaka T, Ishikawa S, et al. A Comparative Study of Ethylene Oxide Gas, Hydrogen Peroxide Gas Plasma, and Low-Temperature Steam Formaldehyde Sterilization. Infection Control & Hospital Epidemiology. 2005;26(5):486-489. doi:10.1086/502572

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Gastroenterology 2017;153:1018–1025

CLINICAL—BILIARY

Randomized Comparison of 3 High-Level Disinfection and Sterilization Procedures for Duodenoscopes

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[1]Division of Infection Control/Hospital Epidemiology, Beth Israel Deaconess Medical Center, Boston, Massachusetts; [2]Harvard Medical School, Boston, Massachusetts; [3]Division of Gastroenterology, Beth Israel Deaconess Medical Center, Boston, Massachusetts; [4]Department of Pharmacy and Health Systems Sciences, Northeastern University, Boston, Massachusetts; and [5]Division of General Medicine and Primary Care, Beth Israel Deaconess Medical Center, Boston, Massachusetts

See Covering the Cover synopsis on page 875; see editorial on page 892.

Keywords: DISINFECTS Study; Surveillance; Endoscopy; Non-outbreak Setting.

BACKGROUND AND AIMS: Duodenoscopes have been implicated in the transmission of multidrug-resistant organisms (MDRO). We compared the frequency of duodenoscope contamination with MDRO or any other bacteria after disinfection or sterilization by 3 different methods. **METHODS:** We performed a single-center prospective randomized study in which duodenoscopes were randomly reprocessed by standard high-level disinfection (sHLD), double high-level disinfection (dHLD), or standard high-level disinfection followed by ethylene oxide gas sterilization (HLD/ETO). Samples were collected from the elevator mechanism and working channel of each duodenoscope and cultured before use. The primary outcome was the proportion of duodenoscopes with an elevator mechanism or working channel culture showing 1 or more MDRO; secondary outcomes included the frequency of duodenoscope contamination with more than 0 and 10 or more colony-forming units (CFU) of aerobic bacterial growth on either sampling location. **RESULTS:** After 3 months of enrollment, the study was closed because of the futility; we did not observe sufficient events to evaluate the primary outcome. Among 541 duodenoscope culture events, 516 were included in the final analysis. No duodenoscope culture in any group was positive for MDRO. Bacterial growth of more than 0 CFU was noted in 16.1% duodenoscopes in the sHLD group, 16.0% in the dHLD group, and 22.5% in the HLD/ETO group ($P = .21$). Bacterial growth or 10 or more CFU was noted in 2.3% of duodenoscopes in the sHLD group, 4.1% in the dHLD group, and 4.2% in the HLD/ETO group ($P = .36$). MRDOs were cultured from 3.2% of pre-procedure rectal swabs and 2.5% of duodenal aspirates. **CONCLUSIONS:** In a comparison of duodenoscopes reprocessed by sHLD, dHLD, or HLD/ETO, we found no significant differences between groups for MDRO or bacteria contamination. Enhanced disinfection methods (dHLD or HLD/ETO) did not provide additional protection against contamination. However, insufficient events occurred to assess our primary study end-point. ClinicalTrials.gov no: NCT02611648.

Recent outbreaks of infections because of carbapenem-resistant Enterobacteriaceae and other multidrug-resistant organisms (MDRO) have been attributed to contaminated duodenoscopes, despite reported compliance with manufacturer recommendations for reprocessing.[1–6] The reasons for duodenoscope contamination when reprocessing is conducted appropriately are not fully understood, and may relate to biofilm formation within the duodenoscope.

An advisory panel convened by the US Food and Drug Administration (FDA) agreed that current standards for duodenoscope reprocessing did not provide a reasonable level of safety and effectiveness.[7] Experts have suggested the adoption of additional measures like double reprocessing cycles and ethylene oxide sterilization to supplement current standards.[8–10] These additional measures are expensive, time consuming, and not readily available in all institutions. Furthermore, the effectiveness of these additional measures has never been systematically studied in a non-outbreak setting. Studies comparing different methods of duodenoscope reprocessing are therefore needed if we are to identify the optimal method of duodenoscope reprocessing.

In the DISINFECTS trial (Duodenoscope Infection Surveillance IN Functioning automated Endoscope reprocessors in Conjunction with eThlyene oxide Sterilization),

Abbreviations used in this paper: CFU, colony-forming units; dHLD, double high-level disinfection; FDA, US Food and Drug Administration; HLD, high-level disinfection; HLD/ETO, standard high-level disinfection followed by ethylene oxide gas sterilization; MDRGN, multidrug-resistant Gram-negative pathogens; MDRO, multidrug-resistant organisms; MRSA, methicillin-resistant Staphylococcus aureus; sHLD, standard high-level disinfection; VRE, vancomycin-resistant enterococci.

Most current article

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http://dx.doi.org/10.1053/j.gastro.2017.06.052



516 samples from duodenoscopes

	Cleaning Simple HLD	Cleaning Double HLD	Cleaning Simple HLD Ethylene oxide
> 0 CFU	16.1%	16.0%	22.5%
≥ 10 CFU	2.3%	4.1%	4.2%

Sources: Snyder G, Wright S, Smithey A, Bartley A, King A, Sawhney M ...Randomized Comparison of 3 High-Level Disinfection and Sterilization Procedures for Duodenoscopes Gastroenterology, 2017; 153, 1018-1025

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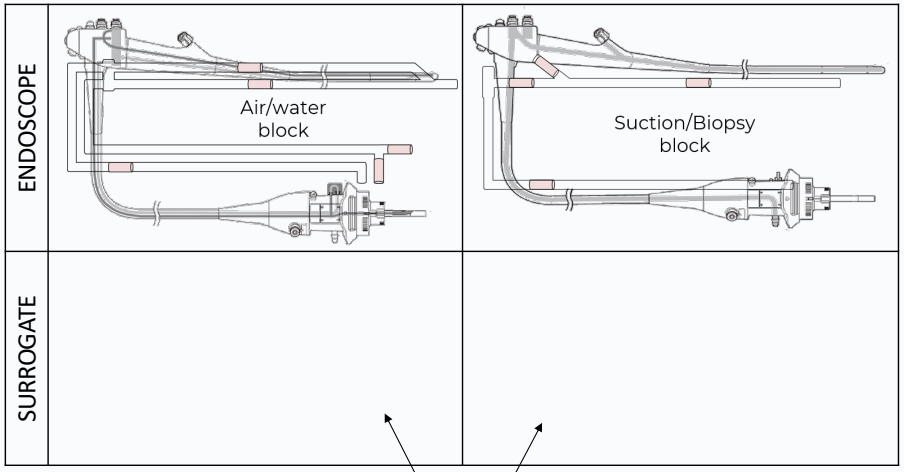
LTSF 2%



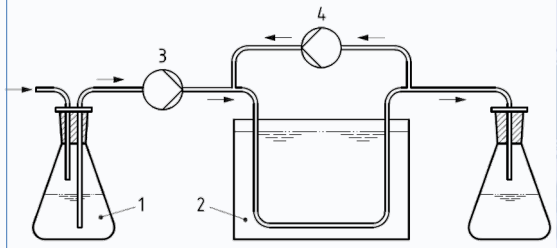
Endoscope surrogate



Architecture challenges:

- **Long channels:** up to 2900 mm
- **Narrow lumen:** 2 mm internal diameter
- **Multiple pathways**

ISO 15883-5:2021 biofilm



1: Growth medium
2: Water bath at 30°C
3: Supply pump
4: Agitation pump

Bioburden challenges:

- **High cellular density: > 8 log(CFU/cm²)**
- **Complex extracellular matrix constituting one of the most robust test soil proposed by ISO 15883-5.**

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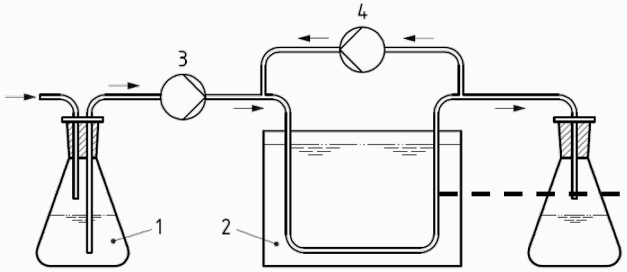
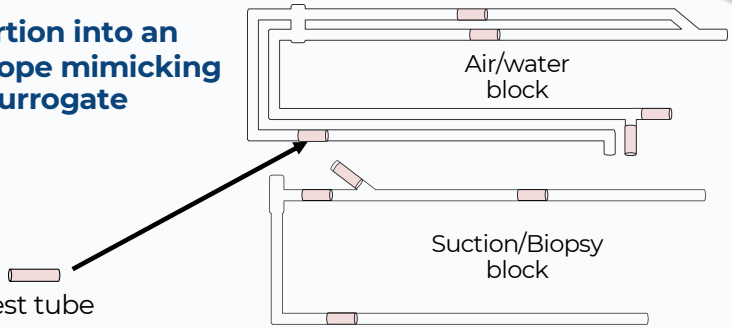
1 *P. aeruginosa* Biofilm formation according to ISO 15883-5 (96 hours of growth)

1: Growth medium
2: Water bath at 30°C
3: Supply pump
4: Agitation pump



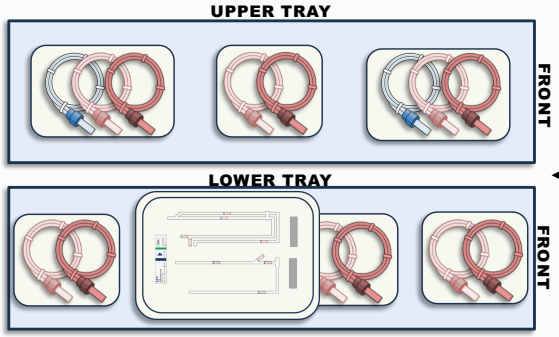
2 Insertion into an endoscope mimicking surrogate

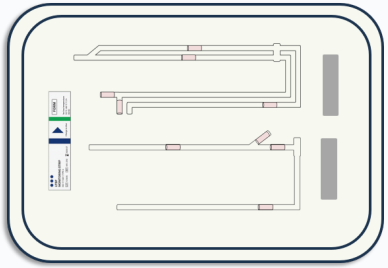


3 Submission to a 60°C LTSF sterilization cycle



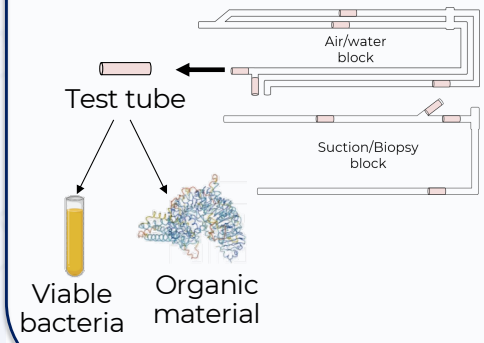
60°C standard cycle





Double packed in sterilization pouch

4 Aseptic collection and sampling



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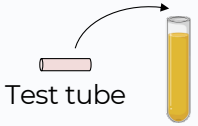
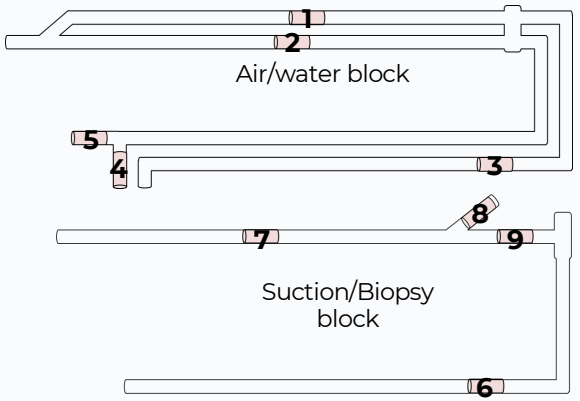
Sterilization efficacy:



Cycle control:
14/14 BI with no growth – SAL of -6 according to ISO 24424

	Growth after 7 days of incubation at 36±1°C		
Test tube Position	**Assay 1**	**Assay 2**	**Assay 3**
Positive control (log(CFU/cm²))	**8.3**	**8.2**	**8.2**



The LTSF sterilization cycle resulted in no detectable growth under the test conditions (*i.e.* total inactivation of the biofilm)

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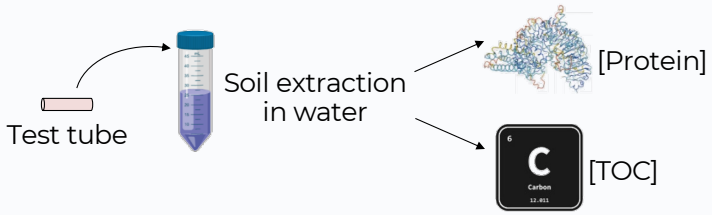
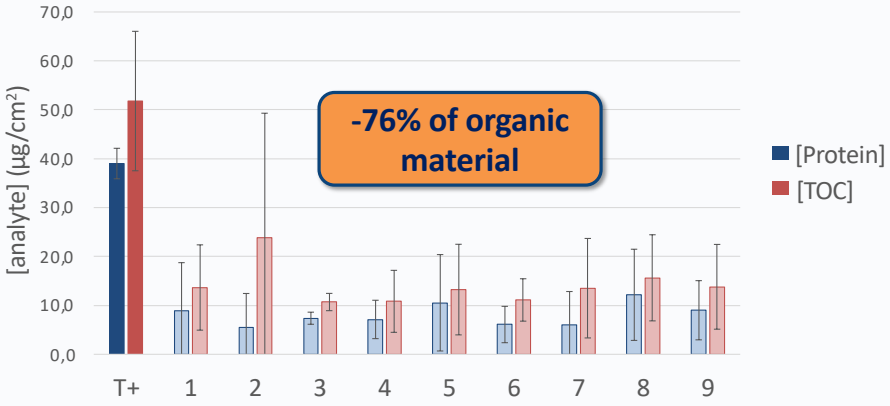
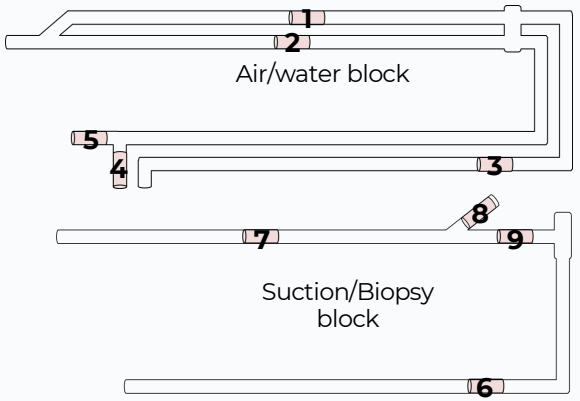
Evaluation of the LTSF Technology Against Mature Biofilm

Evaluation of the level of soil:





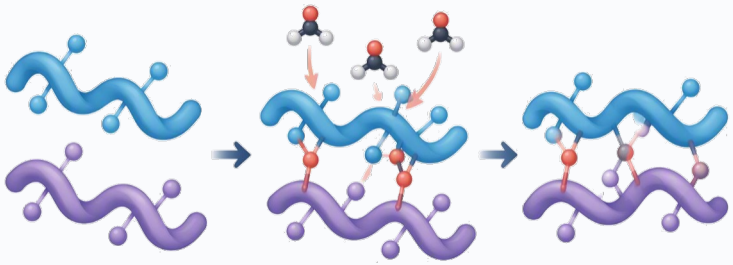


The LTSF sterilization cycle was associated with a decrease of the amount of extractable organic material.

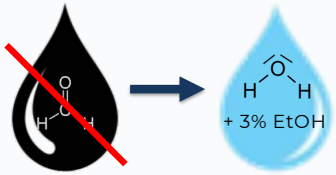
Evaluation of the LTSF Technology Against Mature Biofilm

True soil removal? OR Reduced recovery efficiency?

The formaldehyde is a fixing agent







The LTSF sterilization cycle was associated with a decrease of the amount of extractable organic material.

1. Even in absence of formaldehyde a reduction of protein and TOC is observed.
   → True removal efficacy against biofilm.
2. No reduction of the organic material is observed.
   → Reduced recovery efficiency?
   → True removal efficacy formaldehyde dependant?

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Key take-home messages

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1 A stringent biofilm model

A 96-hour *P. aeruginosa* biofilm reached > 8 log(CFU/cm^2) with resilient organic soil in an endoscope mimicking surrogate with 2 mm lumens and channels up to 2900 mm.

2 LTSF achieved complete inactivation throughout complex lumens

With the standard 60°C LTSF cycle, no growth was detected on 27/27 test pieces after 7 days of incubation in nutrient medium.

3 LTSF may have a cleaning efficacy

Additional controls are required shall be made to evaluate if the LTSF cycle has a genuine removal activity.

LTSF can penetrate a complex endoscope model and completely inactivate a mature *P. aeruginosa* biofilm in the presence of moist organic soil.

What's next?

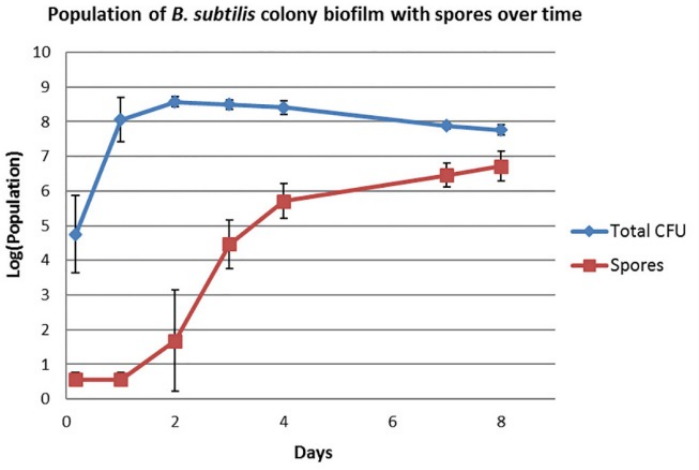
Biofilms and spores: the ultimate challenge?

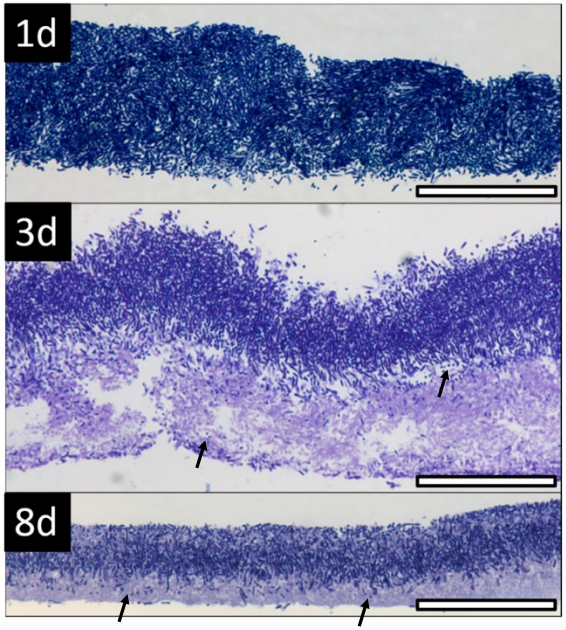
Journal of Microbiological Methods 148 (2018) 181–187
Contents lists available at ScienceDirect
Journal of Microbiological Methods
journal homepage: www.elsevier.com/locate/jmicmeth

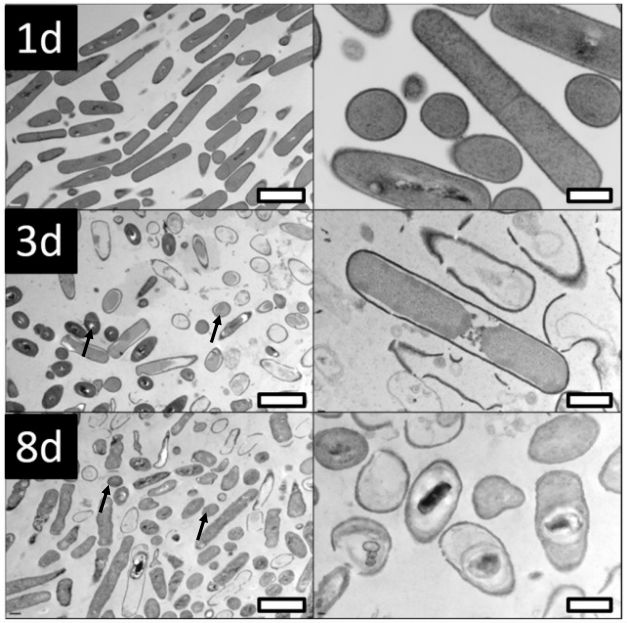
Production and analysis of a *Bacillus subtilis* biofilm comprised of vegetative cells and spores using a modified colony biofilm model

Laura (Kaatz) Wahlen[a,*], Jason R. Mantei[a], James P. DiOrio[a,b], Christopher M. Jones[a], Mark E. Pasmore[a]

[a] Baxter Healthcare Corporation, Round Lake, Illinois, USA
[b] BioPhia Consulting, Lake Forest, Illinois, USA







Sources: Wahlen L, Mantei J, DiOrio J, Jones C, Pasmore M. Production and analysis of a *Bacillus subtilis* biofilm comprised of vegetative cells and spores using a modified colony biofilm model. Journal of Microbiological Methods. 148 (2018): 181-187

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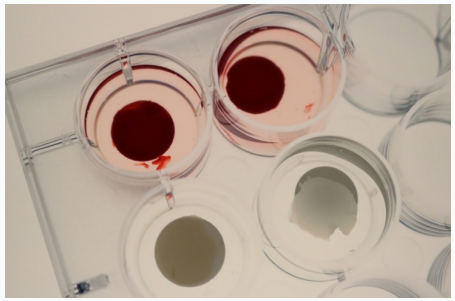
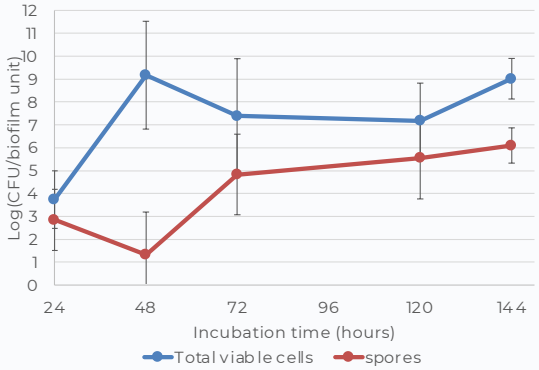
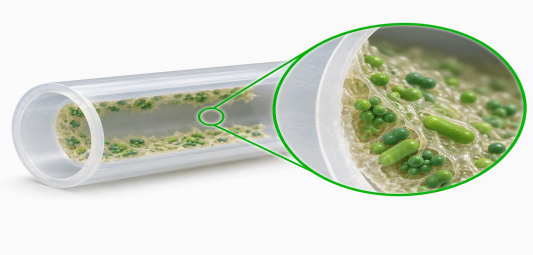
What's next?

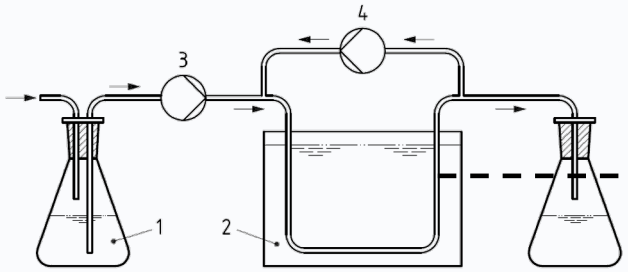
Biofilms and spores: the ultimate challenge?



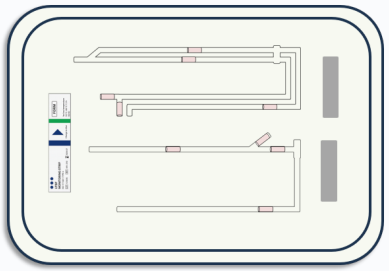
Adaptation of the model from a flat surface to a tube







1: Growth medium
2: Water bath at 30°C
3: Supply pump
4: Agitation pump





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What's next?

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Exploring other sterilization processes

Method	Mature *P. aeruginosa* biofilm inactivation		Inactivation of spores embedded in mature biofilm	
LTSF	27/27 test pieces with no growth	100%		?%
VH_2O_2 / plasma	biofilm-specific efficacy undocumented	?%		?%
Ethylene oxide	No direct evidence identified	?%		?%
Radiation	*S. aureus* biofilm inactivation reported at 4 kGy	?%		?%

Published studies do not currently provide a standardized comparison of sterilization processes using the same mature biofilm and device geometry.

Sources: Chang G, Luo Z, Zhang Y, et al. Effect and Mechanism of Eliminating Staphylococcus aureus by Electron Beam Irradiation and Reducing the Toxicity of Its Metabolites. Applied and Environmental Microbiology. 2023;89(3):e02075-22.

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Thank you

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Study manager
Eurofins Biotech Germande

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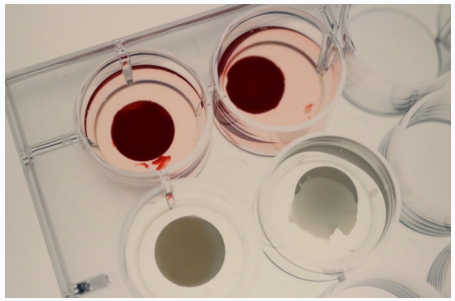
What's next?

Biofilms and spores: the ultimate challenge?



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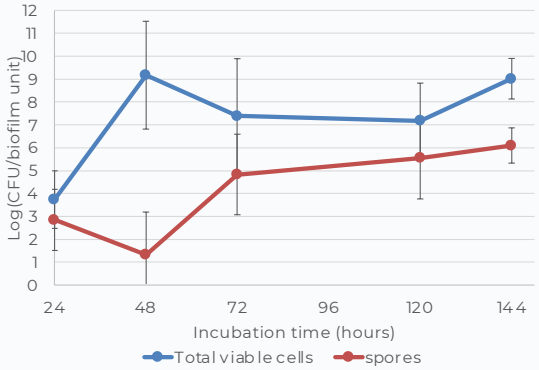

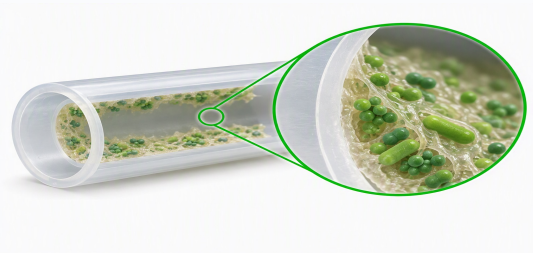

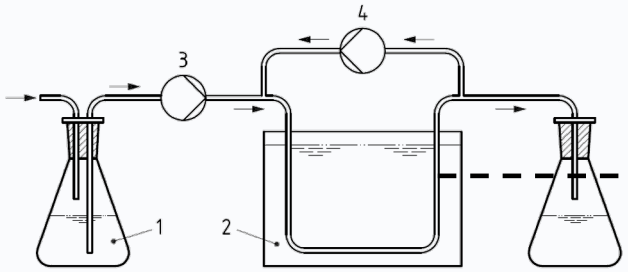

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