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Concise Communication

Evaluation of a new technology for terminal sterilization of flexible endoscopes using hydrogen peroxide gas plasma

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Abstract

In laboratory testing, a novel hydrogen peroxide gas plasma endoscope sterilizer consistently reduced vegetative organisms, but not bacterial spores, to undetectable levels in the presence of high organism load (≥6.5 $\log_{10}$) and organic material and salts. These findings highlight the importance of meticulous cleaning of endoscopes prior to sterilization.

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Background

Contaminated endoscopes have been linked to infections more than any other medical device.[1] Evidence of contamination despite high-level disinfection has compelled the Food and Drug Administration (FDA) and professional organizations to promote a transition from high-level disinfection to sterilization.[1,2] The transition to sterilization would ensure the process is monitored with physical, chemical, and biological monitors,[1] and since the endoscopes would likely be terminally packaged, they could be safety stored.

The aim of this study was to evaluate the effectiveness of a new hydrogen peroxide gas plasma endoscope sterilizer that recently received FDA 510K clearance. Meticulous cleaning is essential prior to use of low-temperature sterilization technologies because endoscopes may be contaminated with 7–10 $\log_{10}$ colony-forming units (CFUs) of microbes after use,[1,2] and organic material and salts can affect efficacy.[3–5] Here, we tested the hypothesis that the effectiveness of the sterilizer would be reduced in the presence of organic material and/or salt that might be present in the setting of suboptimal manual cleaning.

Materials and methods

Description of the device and chemical and biological indicators

The hydrogen peroxide gas plasma sterilizer (Steroscope, Ideate Medical, St. Louis, MS) is designed for terminal sterilization of flexible endoscopes with as many as 8 internal channels after manual cleaning and drying with filtered forced air for ≥10 minutes. The endoscope is placed inside a container that interfaces with the sterilizer and subsequently provides a sterile storage container. A detailed description of the technology including methods for monitoring with chemical and biological indicators is provided in the supplementary material. Figure 1 shows a picture of the sterilizer and the storage container.

Test organisms and soil

Spore-forming test organisms included *Bacillus atrophaeus* American Type Culture Collection (ATCC) 9372, *Clostridioides difficile* ATCC 43598, and *Clostridium sporogenes* ATCC 19404. Spores were prepared as previously described.[6] The concentration of spores was adjusted such that ~6 $\log_{10}$ CFU, or a higher concentration of 7 to 9.7 $\log_{10}$ CFU, was recovered from the controls. Vegetative test organisms included a clinical carbapenem-resistant *Escherichia coli* (CRE) strain, vancomycin-resistant *Enterococcus faecium* (VRE) strain C68, and *Candida auris* Antibiotic Resistance Bank (AR)-0385 (clade IV; South America origin). For vegetative organisms, soil was included for all tests and the inoculum was adjusted such that ~8 to 9 $\log_{10}$ CFU was recovered from controls.

Test soils included 5% fetal calf serum, Roswell Park Memorial Institute Medium (RPMI) 1640 (Thermo Fischer Scientific, Waltham, MA) with ~0.65% salt and 10% fetal calf serum (Gibco, Gaithersburg, MD), and Artificial Test Soil (ATS)-2015 (Healthmark Industries Company Inc., Fraser, MI). The ATS-2015 soil is intended to simulate a "worst case" challenge.[7]

Duodenoscope used for testing

The duodenoscope used for testing was an OLYMPUS EXERA TJF-160F VIDEO DUODENOSCOPE (AIZU OLYMPUS CO., LTD, Aizuwakamatsu-Shi Fukushima, Japan). This duodenoscope

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Figure 1. Picture of the sterilizer technology and sterilization container.

has a 4.2 mm inner diameter instrument channel with a working length of 124 cm.

Efficacy of the sterilizer against vegetative organisms and spores

Three simulated use methods were used, including inoculation onto steel wires that were placed inside the elevator channel, inoculation directly into the elevator recess and the instrument channel as described by Molloy-Simard et al.,[8] and inoculation into the instrument channel followed by use of a brush to spread the inoculum throughout the lumen. The supplementary material provides details on the 3 test methods. All tests were completed 3 times except the brush method which was completed 1 to 5 times.

Microbiology

The steel wires were mixed using a vortex mixer for 30 seconds in 10 mL trypticase soy broth. Aliquots were serially diluted and plated on selective media; broth enrichment was also performed by incubating the tubes containing the wires at 37°C for 72 hours. Log_{10} reductions were calculated in comparison to untreated control wires.

For the other test methods, the flush-brush-flush method was used to sample the elevator mechanism and the instrument channel.[9] The sampling buffers were filtered with a sterile 0.45 µm membrane (MicroFunnel, Pall Corporation, Ann Arbor, MI), and the filters were incubated after overlaying on selective agar. Log_{10} reductions were calculated in comparison to recovery from inoculated endoscopes not subjected to sterilization.

The selective media for *B. atrophaeus*, *C. sporogenes*, *C. difficile*, *E. faecium*, *E. coli*, and *C. auris* were trypticase soy agar (TSA) (Thermo Fisher Scientific, Waltham, MA), TSA with 5% sheep's blood, *C. difficile* brucella agar, enterococcosel agar (Thermo Fisher Scientific), MacConkey agar (Thermo Fisher Scientific), and Sabouraud dextrose agar (Becton Dickinson, Sparks, MD), respectively.

Results

Chemical and biological indicators

For all test runs, the 3M Attest Vaporized Hydrogen Peroxide Type 4 Tri-Metric Chemical Indicators indicated that exposure time, temperature, and hydrogen peroxide concentrations were achieved. All the biological indicators (N = 21 total during 7 sterilizer cycles) passed indicating no surviving *G. stearothermophilus* spores.

Efficacy of the sterilizer against vegetative organisms

The inoculum recovered from the controls ranged from 7.9 to 9.1 log_{10} CFU. For each method and for all 3 organisms, no organisms were recovered after the sterilization cycle was completed. The soil included with the test runs included 5% fetal calf serum (N = 3 experiments for each organism), ATS-2015 (N = 4 experiments), and RPMI (N = 3 experiments).

Efficacy of the sterilizer against bacterial spores

Table 1 shows the recovery of spores from control and test duodenoscopes for the 3 different test methods and 3 types of soil. No spores were recovered from wires in the absence of soil. In the presence of soil, no spores were recovered after sterilization when the inoculum resulted in recovery of $\leq$6.2 log_{10} CFU spores from the control endoscopes, but low levels of spores were frequently recovered when $\geq$6.5 log_{10} CFU spores were recovered from the control endoscopes.

Discussion

To obtain FDA clearance, the manufacturers of the Steroscope device were required to provide evidence that a half-cycle eliminated 6 log_{10} CFU of *G. stearothermophilus* spores with no soil and a full cycle eliminated 6 log_{10} CFU of spores in simulated-use testing with soil.[10] In our testing, the sterilizer eliminated vegetative organisms under "worst case" simulated-use conditions including high organism load and presence of organic material and salts. The technology consistently eliminated 6.0 to 6.2 log_{10} CFU of spores in accordance with the FDA requirement that no survivors are recovered after challenge with 6 log_{10} of spores with soil.[10] However, the failure to eliminate 6.5 and 6.8 log_{10} CFU of *B. atrophaeus* spores in 2 of the tests could be interpreted as failure to achieve sterilization. The device did not eliminate higher numbers of spores (ie 7.1 to 9.7 log_{10} CFU recovered from control endoscopes) in the presence of soil.

Our results suggest that the sterilizer is a promising technology that could provide one means to move toward a transition from high-level disinfection to sterilization of endoscopes. However, our results also highlight the importance of meticulous cleaning prior to use of the sterilizer. Previous reports have similarly demonstrated that FDA-cleared low-temperature sterilization technologies sometimes fail in the presence of salt, serum, blood, and

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Table 1. Recovery of spores (mean $\log_{10}$ CFU [95% CI]) of *Bacillus atrophaeus*, *Clostridium sporogenes*, and *Clostridioides difficile* from control and test endoscopes using 3 different test methods and 3 types of soil

	Inoculated wires		Inoculation of elevator recess and instrument channel		Brush inoculation of entire instrument channel	
	Control	Test	Control	Test	Control	Test
No soil						
B. atrophaeus	8.1 (8.1–8.1)	0	–	–	–	–
C. sporogenes	8.2 (7.9–8.5)	0	–	–	–	–
C. difficile	6.0 (5.5–6.5)	0	–	–	–	–
5% fetal calf serum (FCS)*						
B. atrophaeus	8.3 (7.8–8.7)	.5 (–.1–1.0)	8.6 (8.1–9.1)	1.7 (1.4–2.0)	6.8 (6.1–7.5)	2.2 (1.0–3.4)
C. sporogenes	6.0 (5.8–6.2)	0	9.7 (9.5–9.9)	1.3 (1.0–1.6)	6.2 (5.9–6.5)	0
C. difficile	6.0 (5.6–6.4)	0	7.1 (6.9–7.3)	1.5 (1.0–2.0)	6.2 (6.2–6.2)	0
Roswell Park Memorial Institute Medium (RPMI)						
B. atrophaeus	6.5 (5.9–7.3)	1.9 (1.6–2.2)	–	–	–	–
C. sporogenes	–	–	–	–	–	–
C. difficile	6.0 (5.7–6.3)	0	–	–	–	–
Artificial Test Soil (ATS)-2015						
B. atrophaeus	–	–	–	–	7.1 (5.3–8.9)	1.2 (–1.2–3.6)
C. sporogenes	–	–	–	–	7.3 (6.3–8.3)	2.1 (–1.9–3.9)
C. difficile	–	–	–	–	7.0 (5.1–8.8)	1.5 (–.3–3.3)

CFU, colony-forming unit.
N = 3 for all test methods except the brush throughout the instrument channel group (N = 5, *B. atrophaeus* with 5% FCS; N = 1, *C. difficile* with 5% FCS; N = 2 for other test conditions).
*Inoculation of elevator recess and instrument channel with ~6 $\log_{10}$ CFU of the test spores in 5% FCS (N = 3 for each organism) resulted in complete reduction with no spores recovered (data not shown).

lubricating fluid (e.g., a >70% failure rate was observed for an FDA-cleared technology when tested with salt and serum).[1–5]

Our study has some limitations. Given the labor-intensive nature of the study methods, a limited number of tests were performed. The study involved simulations of contamination with only one type of duodenoscope which did not have a disposable end cap that allows for easier cleaning of the elevator mechanism. Additional studies are needed in clinical settings with a wide range of in-use endoscopes.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/ice.2024.236

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