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Major Article

Elevating the standard of endoscope processing: Terminal sterilization of duodenoscopes using a hydrogen peroxide–ozone sterilizer

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Background: The health care community is increasingly aware of the processing challenges and infection risks associated with duodenoscopes owing to published reports of outbreaks and regulatory recalls. Studies have demonstrated that the current practices are inadequate for consistently producing patient-ready endoscopes. Alternatively, terminal sterilization would offer a greater margin of safety and potentially reduce the risk of patient infection. The purpose of this study was to evaluate the efficacy of a hydrogen peroxide–ozone sterilizer with regulatory clearance for terminal sterilization of duodenoscopes.

Methods and Results: Validation studies were performed under laboratory simulated-use and clinical in-use conditions. The overkill method study demonstrated a reduction of at least 6-log of *Geobacillus stearothermophilus* spores at half-cycle, providing a sterility assurance level of 10^{-6}. In addition, the sterilizer achieved a 6-log reduction of *G stearothermophilus* in the presence of inorganic and organic soils in a simulated-use study. The clinical in-use study confirmed that the sterilizer achieved sterilization of patient-soiled duodenoscopes under actual use conditions.

Conclusions: Simulated-use and clinical in-use studies demonstrated the efficacy of a hydrogen peroxide–ozone sterilizer for terminal sterilization of duodenoscopes. This offers health care facilities a viable alternative for duodenoscope processing to enhance patient safety as part of a comprehensive infection control strategy.

BACKGROUND

While endoscopes play a vital role in the effective delivery of health care and offer patients many benefits, the risks associated with iatrogenic transmission via endoscopes continue to be a significant health care concern. “Failure to consistently and effectively reprocess flexible endoscopes” is listed second on the Top 10 Health Technology Hazards for 2018 published by the Emergency Care Research Institute.[1] Endoscope processing has consistently been listed as a significant hazard for the last decade because endoscopes are associated with more cases of device-mediated patient infections than any other medical instrument.[2] Their design complexity presents a substantial challenge to achieve consistent and effective processing. Duodenoscopes present a particular challenge because they contain multiple long and narrow lumens and are side-viewing endoscopes consisting of a recessed elevator used to direct endoscopic accessories.[3-7] This processing challenge is evidenced by documented cases of duodenoscope contamination[8] and by related patient infections,[9-15] even after adherence to the manufacturer’s processing instructions,[16-19] which include high-level disinfection (HLD). Several outbreaks of patient infections with carbapenem-resistant Enterobacteriaceae (CRE) were linked to contaminated duodenoscopes in the United States and around the world.[17,19,20] CRE are highly transmissible, difficult to treat

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owing to their resistance to antimicrobial treatments, and result in a high mortality rate.[21,22] Investigations from institutions in which outbreaks occurred demonstrated that a single contaminated endoscope can infect several patients with CRE over long periods of time, despite adherence to recommended processing practices.[19] Furthermore, a study from 2015 estimated that one-third of institutions do not use surveillance methods to identify possible duodenoscope-related bacterial transmissions,[23] which reduces the number of declared infections.

Improving safety for endoscopy patients requires a multifactorial approach, including an effective personnel training program, comprehensive quality control systems, validated methods for ensuring adequate processing, and designing processes with margins of safety that address the level of risk associated with the use of these devices. The Food and Drug Administration (FDA) and Centers for Disease Control and Prevention (CDC) have recommended a list of supplemental duodenoscope processing measures to further reduce the risk of infection and increase the safety of these medical devices.[24] These supplemental methods included microbiological culturing, repeat HLD, HLD followed by liquid chemical sterilization, and HLD followed by ethylene oxide (EO) sterilization. However, microbiological culturing has received mixed reviews, since it requires complex staff training, is expensive, and requires aseptic facilities.[25-29] Repeated HLD has not been shown to consistently provide a patient-ready endoscope.[13,30-34] The use of HLD combined with EO sterilization, which is time-consuming,[6] also does not guarantee that the duodenoscope is microorganism free.[31,35] A study by Snyder et al showed no significant difference between duodenoscopes processed by HDL, double HLD, or HLD combined with EO sterilization, with positive bacterial growth being detected following all three processing methods.[31]

Terminal sterilization of duodenoscopes has been identified as a leading solution for improving duodenoscope processing outcomes since it offers a significantly greater margin of safety than HLD.[36-38] According to the Spaulding classification scheme, flexible gastrointestinal endoscopes are classified as semi-critical devices, in which case sterilization is recommended, or if this is not possible, processing using HLD at a minimum.[39-41] Duodenoscopes are designed as semi-critical but are used as critical devices in a significant number of procedures.[6,42] To accommodate this reality, some members of the health care community have proposed to reclassify duodenoscopes as critical[36] by modifying the Spaulding definition of critical items.[38-40,43] Nevertheless, there are challenges associated with transitioning from the current practice to terminal sterilization.

The primary modality currently employed for terminal sterilization of duodenoscopes is EO. In some cases, it has been part of the strategy used to successfully control CRE outbreaks, [18] although previous research has demonstrated that EO sterilization is not always effective in the presence of organic and inorganic soils when tested in surrogate devices.[44,45] While EO has been validated by duodenoscope manufacturers, it has not been cleared by the FDA for sterilization of multichannel gastrointestinal endoscopes.[45,46] The toxicity of EO adds to the requirements for occupational health and safety and necessitates an aeration time of 12 hours or longer to ensure the absence of toxic residues on the endoscope.[6,46] EO sterilization systems may not be accessible to all health care facilities and are costly,[24,36] since they require control of the room temperature and humidity, EO concentration monitoring equipment, and specific facility design requirements, including a nonrecirculating or dedicated ventilation system.[47] When EO sterilization cannot be performed locally, additional logistics and costs associated with transport and longer turnaround times are required.[6] EO sterilization turnaround times are typically 17 to 48 hours, which require a costly increase in duodenoscope inventory.[6,31,34] For these reasons, EO sterilization is not considered a long-term solution,[36,46] highlighting the need for new low-temperature sterilization technologies specifically validated for duodenoscopes.[27,36,37,46]

Recently, multiple hospitals in the United States and Canada implemented hydrogen peroxide–ozone terminal sterilization instead of HLD as an alternate solution to prevent or eradicate patient infections linked to inadequately processed duodenoscopes.[48,49] Since terminal sterilization has been identified as a key solution for improving the safety of reusable flexible endoscopes and members of the health care community expressed the need for terminal sterilization of duodenoscopes, the current study was performed to evaluate the microbicidal efficacy of a hydrogen peroxide–ozone terminal sterilization technology for duodenoscopes with an overkill method, a simulated-use method, and a clinical in-use validation. The tests reported here were performed both under simulated worst-case laboratory conditions and in-use clinical conditions.

METHODS

Sterilizer and duodenoscope description

The STERIZONE VP4 Sterilizer (TSO3 Inc., Quebec City, Canada) was used in this study to evaluate sterilization of duodenoscopes. This sterilizer is intended for use in terminal sterilization of cleaned, rinsed, and dried metal and nonmetal reusable medical devices in health care facilities. The sterilizer uses dual sterilants (vaporized hydrogen peroxide and ozone) in a multiphase process. A detailed description of the sterilizer has been previously published.[50] This hydrogen peroxide–ozone sterilizer was approved by the FDA (K172191), Health Canada (Health Canada License No. 37796, October 23, 2015) and the European Union (CE Mark granted on February 16, 2015) to include sterilization of multichannel flexible duodenoscopes, colonoscopes, and gastroscopes with lumen dimensions listed in the sterilizer's indications for use.

The duodenoscope model used for this study was the Olympus EVIS EXERA II Duodenovideoscope TJF-Q180V (AIZU OLYMPUS CO., LTD, Aizuwakamatsu-Shi Fukushima, Japan), the most widely used duodenoscope in the United States.[51] This duodenoscope is composed of 3 channels (instrument/suction, air, and water), which will be separated into 7 channel segments for the purpose of this study. The term "channel segment" is defined here as a lumen with 2 openings. Its center (halfway point) is the most difficult location to reach for the sterilant, thus the most challenging location to sterilize.

This duodenoscope model has an elevator mechanism, and the wire actuating the elevator mechanism is in a sealed channel. A recall was conducted on this endoscope model (FDA#Z-2807-2015[52]), which led to a newly designed special brush (MAJ-1888) for the mechanism to facilitate cleaning of the device. Another recall was conducted to change the elevator mechanism to comply with the design proposed in the duodenoscope 510(k) (FDA#Z-0757-2016[53]). All devices tested in this study were duodenoscopes with the redesigned sealed elevator mechanism.

Figure 1 represents a schematic of the tested duodenoscope model. Number identifications for channel segments and other sites were added to the figure to support what is described in the following sections.

Overkill (half-cycle) test method

The tested duodenoscope model was first evaluated in the hydrogen peroxide–ozone sterilizer with an overkill method performed in triplicate using 2 separate duodenoscopes. The overkill method (or half-cycle method) consists of inoculating the medical devices with more than 1.0×10^6 colony-forming units (CFU) of the most resistant organism identified for this sterilization process and by showing no growth after the half-cycle point of the sterilization cycle (cycle

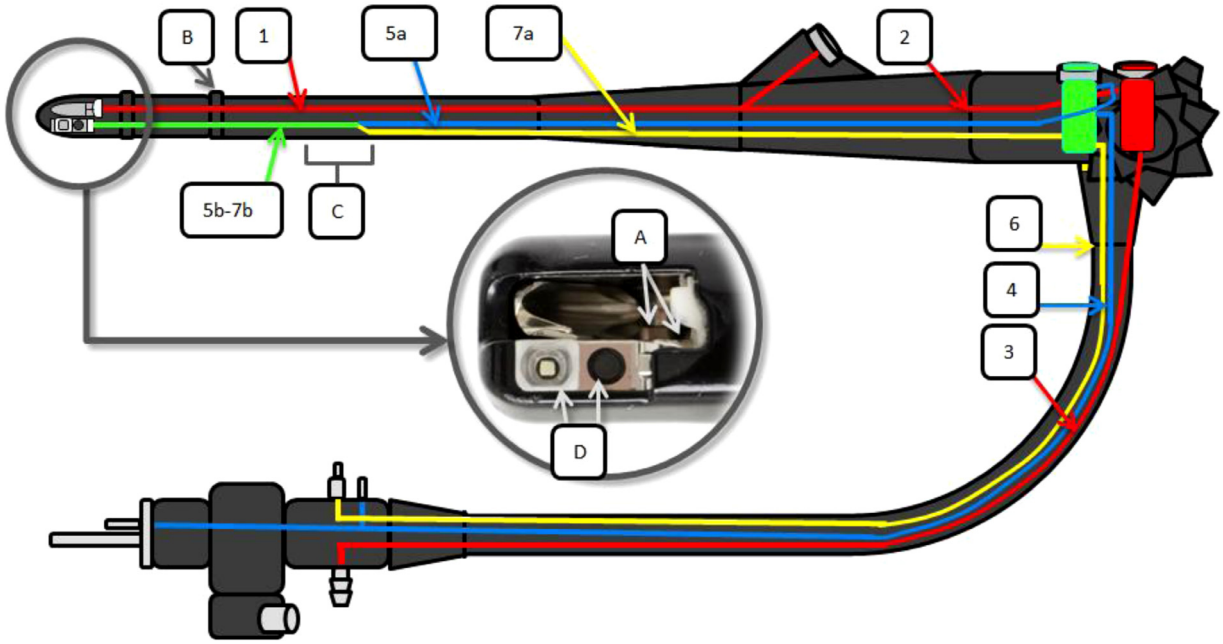

Fig. 1. Schematic duodenoscope channel segments (numbers): (1) Instrument (distal end to instrument channel inlet). (2) Instrument (instrument channel inlet to suction cylinder). (3) Suction. (4) Air (umbilical), (5a/5b) Air (distal end to the air/water cylinder). (6) Water (umbilical). (7a/7b) Water (distal end to the air/water cylinder). Other sites (letters): (A) Elevator mechanism. (B) Bending section adhesive. (C) Insertion tube between the 10 cm-40 cm mark. (D) Adhesives around the light guide and camera objective lenses.

performed included 1 phase of sterilant exposure instead of 2 phases in a complete cycle). This results in a sterility assurance level of 10^{-6},[54] meaning that there is a 1 in 1,000,000 chance of an organism surviving the sterilization process. Although sterilizing with a half-cycle would never be performed in clinical use, this overkill approach recommended by the FDA results in a significant margin of safety, which is not required when validating endoscope processing using HLD.

Spore suspension solutions of 1.0×10^6 to 2.5×10^6 CFU/10 μL of *Geobacillus stearothermophilus* ATCC 7953 were prepared from stock solutions (Iuvo BioScience, Rush, NY, USA). *G stearothermophilus* is a spore-forming aerobic thermophilic bacteria that requires 55°C for growth conditions (ATCC 7953). This microorganism was selected because it is the most resistant to hydrogen peroxide and ozone (sterilants of the tested sterilizer), thus the one recommended for sterility testing of hydrogen peroxide and ozone sterilizers. [55]

The inoculation sites tested as part of this overkill validation included the endoscope channels and elevator mechanism (Fig 1: sites 1 to 7, A). The elevator mechanism was inoculated using 10 μL of the prepared spore solution. The spore solution was deposited using a micropipette tip in the hinge and in the recessed space associated with the hinge identified in Figure 1, site A. Then, the mechanism was manipulated (up-down) at least 3 times to allow spreading of the inoculum and penetration in the hinge.

The channels were also inoculated with 10 μL of inoculum (1.0×10^6 to 2.5×10^6 CFU/10 μL) but with added water to dilute the inoculum. For the inoculum to reach the center of each channel segment (the most challenging location to sterilize), sterile water was added so the inoculum could reach the center of the channel by gravity. The needed volume of added sterile water differed depending on the length and diameter of each channel but ranged from 90 μL-390 μL. The controls performed to validate that the inoculum reached the center of the channels and the specifications on the inoculation method are explained in detail in a previous publication.[56]

After the inoculated duodenoscope was dried overnight, it was packaged in a compatible sterilization container (Steritite Full size sealed containers SC06FG, Case Medical Inc., South Hackensack, NJ) and placed individually in a load. Only 1 gastrointestinal endoscope can be sterilized in the sterilizer according to the sterilizer's instructions for use. No washing step was performed prior to sterilization. The recommended load temperature for processing in the tested sterilizer is 20°C-26°C. The loads were conditioned at 26°C ± 1°C before processing. To do so, they were stored in a controlled 26°C temperature room for a minimum of 2 hours with calibrated RTD thermometers (Omega Engineering Inc, Norwalk, CT) placed on the duodenoscope, loading cart, and container to monitor that the required temperature had been reached. The preconditioning temperature of 26°C was chosen owing to the fact that this load condition results in the shortest sterilant exposure time and in the lowest mass of sterilant for the tested sterilizer, therefore representing the most challenging condition for achieving sterility.[50] The loads were then exposed to a half-cycle of the sterilization process.

After sterilization, the containers were opened in a laminar flow hood under aseptic conditions. The elevator mechanism was sampled using a flush-brush-flush method with phosphate sampling buffer (Salts: Fisher Chemical, Fair Lawn, NJ / Tween 80: Fisher Chemical, Fair Lawn, NJ). The channels were sampled by flushing a determined amount of phosphate sampling buffer with the Olympus cleaning connectors (MB-358, MH-946). When a syringe is plugged into the MH-946 connector, the sampling buffer flushes more than 1 channel segment at a time. Specifically, the suction and instrument channels are flushed together (Fig 1, 1,2,3), and the air and water channels are flushed together (Fig 1, 4,5,6,7). The selected sampling buffer volume per group of channels was approximately 10 times the combined internal volume of the channel segments being sampled.

All sampling buffers were filtered using a sterile 0.45 μm membrane (MicroFunnel, Pall Corporation, Ann Arbor, MI). Filters were incubated on agar media (Standard method agar, Benton, Dickinson and Co, Sparks, MD) at 55°C-60°C for 7 days to allow the growth of *G stearothermophilus* bacteria.

Simulated-use test method

The sterilization of the duodenoscope model was also evaluated in the hydrogen peroxide–ozone sterilizer with a simulated-use method performed in triplicate. Three separate duodenoscopes were used in this testing. When performing a simulated-use validation, the most resistant microorganism to the sterilization process is mixed with organic and inorganic soils and inoculated on devices. These simulated soils, including organic and inorganic compounds, mimic clinical use. The inoculated medical devices are not washed (thus simulating a worst-case scenario) and exposed to a complete cycle of the sterilization process.[57] The intended use of the tested sterilizer stipulates that medical devices must be cleaned as per the manufacturer's instructions for use before sterilization. Nevertheless, the cleaning step is omitted in a simulated-use method to perform the test under worst-case conditions.

Spores suspension solutions of 1.0×10^6 to 2.5×10^6 CFU/10 μL of *G stearothermophilus* ATCC 7953 mixed with fetal bovine serum (for final concentration of 5%) and hard water (final concentration of 400 ppm) were prepared (Microorganism: Iuvo BioScience, Rush, NY/Fetal bovine serum: BenchMark Gemini Bio-product, West Sacramento, CA/Hard water salts [AOAC 960.09E]: Fisher Chemical, Fair Lawn, NJ, and Avantor Performance Materials Inc [J.T. Baker], Central Valley, PA).

The duodenoscopes were then inoculated, conditioned, sterilized with a full cycle and samples were incubated, as described previously in the overkill validation method. In addition, 3 sites targeting materials susceptible to potential degradation from processing[5] were tested as part of the simulated-use validation. These additional sites included the bending section adhesive, the insertion tube surface, and the adhesives around the light source and camera objective lenses (see Fig 1, sites B, C, and D). These sites were tested on endoscopes that were cycled (manually cleaned and sterilized) at least 15 times each in the hydrogen peroxide–ozone sterilizer prior to testing. The additional sites were inoculated, processed in the sterilizer, and then sampled by swabbing (Puritan HydraFlock swab, Guilford, ME).

Clinical in-use test method

A clinical in-use study was performed to evaluate the efficacy of processing clinically soiled duodenoscopes following the standard processing steps performed in a health care facility but with the terminal step being sterilization in the hydrogen peroxide–ozone sterilizer instead of HLD. Hospital-owned duodenoscopes were used during clinical procedures and assessed for their sterility after processing in the hydrogen peroxide–ozone sterilizer. Five separate duodenoscopes were used in the clinical study. The tested duodenoscopes were not in new condition. Signs of wear, including ridges on the insertion tube and cracks in the bending section, were visually observed. All tested duodenoscopes were taken from surgeries during which patients were not on an antimicrobial regimen at the time of the endoscopic retrograde cholangiopancreatography procedure, since antimicrobials could reduce the bioburden level on the endoscope post procedure and bias the results. Endoscopes from 11 surgical procedures were tested: endoscopes 1-3 to establish precleaning contamination levels, endoscopes 4-8 to establish post–manual cleaning contamination levels, and endoscopes 9-11 for poststerilization assessment. Precleaning and postcleaning controls were taken to evaluate the contamination levels of the duodenoscopes, by sampling each channel group (channel segments 1-2-3 and 4-5-6-7 sampled together as explained in the overkill section), the elevator mechanism, and the insertion tube surface of the endoscope. Precleaning controls were sampled after the clinical procedure, but prior to the operating room bedside cleaning. They were performed in triplicate. Manual cleaning of the tested devices was performed by the hospital sterile processing department personnel according to their internal processing procedure, which complies with the endoscope manufacturer's instructions for cleaning, but no automated endoscope reprocessor or HLD step was performed prior to terminal sterilization. Postcleaning controls were sampled after the manual cleaning performed by the hospital personnel. They were performed in quintuplicate.

The test endoscopes were packaged in compatible containers (Steritite Full-size sealed containers SC06FG, Case Medical Inc, South Hackensack, NJ) and processed according to the sterilizer's instructions for use. Following sterilization, the duodenoscopes were transported and sampled under aseptic conditions with the same sampling methods as described in the previous sections. The duodenoscopes were tested for contamination in the channels, elevator mechanism, and insertion tube surface (Fig 1, sites 1-7, A, C). All sampling buffers were filtered using a sterile 0.45 μm membrane (MicroFunnel, Pall Corporation, Ann Arbor, MI) and membranes were plated. Each sample was tested and incubated using appropriate media to culture for a large spectrum of microorganisms: aerobic bacteria (blood agar, 37°C, 7 days), anaerobic bacteria (blood agar, 37°C, 7 days), yeasts and molds (Sabouraud Dextrose agar, 20°C-25°C, 7 days) (all agar plates from Hardy Diagnostic, Santa Maria, CA). Anaerobic agar plates were placed in anaerobic jars with corresponding reagents and anaerobic indicators (BBL GasPak system jar, BD GasPak EZ, and BBL indicator strip: Becton, Dickinson and Company, Sparks, MD) for the duration of the incubation. All plates were incubated for 7 days since it was demonstrated that microbial cultures from gastrointestinal endoscopes can require more than 2 days to efficiently recover microorganisms and demonstrate contamination.[58]

The surgical procedure, the manual cleaning of the endoscopes, and the precleaning and postcleaning recovery control sampling were performed at Centre Hospitalier de l'Université Laval (Québec City, Canada). Packaging and sterilization of the tested duodenovideoscopes were performed at Hotel-Dieu de Québec Hospital (Québec City, Canada). Both hospital centers are affiliates of the Centre Hospitalier Universitaire hospital group and routinely exchange sterilization services. The device sampling post-sterilization was performed at TSO3 Inc facilities (Québec City, Canada).

Sampling methods validation

The sampling methods for all test sites were validated by direct inoculation of the sites with 10 CFU-100 CFU of the *G stearothermophilus* spore solution to demonstrate low-level recovery. The tested sites were inoculated, left to dry overnight, and sampled as explained in previous sections. All sites were tested in triplicate. The recovery results obtained ranged from 63%-82%, thus showing that all sampling methods are acceptable based on ANSI/AAMI/ISO 11737-1:2018.[59]

RESULTS

Overkill and simulated-use validation

No growth was observed for the sampling of the elevator mechanism and channels following exposure to a half-cycle of the sterilizer using an overkill method validation. No growth was also observed for the sampling of the elevator mechanism, channels, and possible degradation sites following exposure to a complete cycle of the sterilizer using a simulated-use method validation. The results are summarized in Table 1.

Clinical in-use validation

Endoscopes from 11 surgical procedures were tested as part of the clinical in-use validation. Table 2 presents the contamination levels

Table 1
Poststerilization sampling results for tested duodenoscopes using overkill and simulated-use methods

Sampled site	Validation method	Microorganism contamination type	Results of replicate No. (CFU)		
			1	2	3
All channels combined	Overkill	$1.0 - 2.5 \times 10^6$ CFU of *G stearothermophilus*	-	-	-
Elevator mechanism			-	-	-
All channels combined	Simulated use	$1.0 - 2.5 \times 10^6$ CFU of *G stearothermophilus*	-	-	-
Elevator mechanism		mixed with fetal bovine serum and hard water	-	-	-
Flexible section adhesive			-	-	-
Insertion tube			-	-	-
Light source and camera lens adhesives			-	-	-

-: No growth (sterile).
CFU, Colony-forming unit.

Table 2
Contamination levels of clinically-used duodenoscopes before sterilization

Sampled site	Microorganism contamination type	Results of surgical procedure test No. (CFU)							
		Precleaning samples			Postcleaning samples				
		1	2	3	4	5	6	7	8
Instrument/suction channel group	Aerobic	+	+++	+++	++	+	++	+	+
	Anaerobic	+++	+++	+++	++	++	+++	+++	+++
	Yeasts and molds	+	++	+++	++	-	+	+	+
Air/water channel group	Aerobic	++	++	+++	+	+	-	+	+
	Anaerobic	++	++	+++	+	++	+	++	++
	Yeasts and molds	++	+	++	+	-	-	+	+
Elevator mechanism	Aerobic	++	+++	+++	+++	++	+++	++	++
	Anaerobic	+++	++	+++	+++	++	+++	++	+++
	Yeasts and molds	++	+	++	+++	+	+	+	+
Insertion tube surface	Aerobic	++	+++	++	+	-	-	+	-
	Anaerobic	++	+++	++	-	-	+	-	-
	Yeasts and molds	+	+	-	+	-	-	-	-

-: No growth (sterile); +: < 10 CFU; ++: 10 to 200 CFU; +++: > 200 CFU.
CFU, Colony-forming unit.

from surgical procedures 1-3 (precleaning contamination levels) and 4-8 (post–manual cleaning contamination levels).

Table 3 presents the results from surgical procedures 9-11 in which the duodenoscopes were sampled post sterilization. All tested sites showed no growth for aerobic bacteria, anaerobic bacteria, and yeasts and molds.

Discussion

Terminal sterilization has been proposed as one potential solution for improving the safety of reusable flexible endoscopes, specifically duodenoscopes. For decades, the standard practice has been HLD of duodenoscopes, resulting in limited development of new technologies targeted for terminal sterilization of these complex devices. As duodenoscopes are heat labile, only 2 low-temperature modalities are currently available for terminal sterilization, including EO and hydrogen peroxide–ozone sterilization.

While EO sterilization of medical devices has been available since the 1950s, duodenoscopes do not fall within the indications for use of commercially available EO sterilizers.[46] However, duodenoscope manufacturers list EO sterilization as a validated processing modality in the device labeling. Despite this, adoption of EO sterilization of duodenoscopes has been limited owing to environmental and safety issues, long turnaround times, and lack of accessibility.[6,46,47] In

Table 3
Clinical conditions study poststerilization sampling results of tested duodenoscopes

Sampled site	Microorganism contamination type	Results of surgical procedure test No. (CFU)		
		9	10	11
Lumens (all channels combined)	Aerobic	-	-	-
	Anaerobic	-	-	-
	Yeasts and molds	-	-	-
Elevator mechanism	Aerobic	-	-	-
	Anaerobic	-	-	-
	Yeasts and molds	-	-	-
Insertion tube surface	Aerobic	-	-	-
	Anaerobic	-	-	-
	Yeasts and molds	-	-	-

-: No growth (sterile).
CFU, Colony-forming unit.

contrast, hydrogen peroxide–ozone sterilization was introduced in 2014 and recently gained regulatory clearance for terminal sterilization of duodenoscopes. This sterilization modality has shorter turnaround times but is not currently listed as a validated processing method in duodenoscope manufacturers' instructions for use. In general, both sterilization modalities are harsher on materials[36,41] and will reduce duodenoscope use life relative to HLD processes.

This study was performed to evaluate the microbicidal efficacy of a hydrogen peroxide–ozone terminal sterilization technology for duodenoscopes with an overkill method, a simulated-use method, and a clinical in-use validation. All the validation methods used are those recommended by the FDA in its *Guidance on Premarket Notification 510(k) Submissions for Sterilizers Intended for Use in Health Care Facilities.*[57]

The first set of laboratory tests was performed using the overkill method in which the device was sterilized with only one-half of the normal sterilization cycle. Although sterilizing with a half-cycle would never be performed in clinical use, this overkill approach recommended by the FDA provides a significant margin of safety not present when processing endoscopes using HLD. In this testing, no microorganisms were recovered from all duodenoscopes, demonstrating a reduction of at least 6-log of *G stearothermophilus* at half-cycle, providing a sterility assurance level of 10^{-6}.

The second set of performed laboratory tests used a simulated-use approach, in which soils were added to the inoculum mimicking clinical organic and inorganic compounds. For all trials, the test devices were processed multiple times before the sterility tests were performed. The intended use of the tested hydrogen peroxide–ozone sterilizer stipulates that medical devices must be cleaned as per the manufacturer's instructions for use before sterilization. However, for the purposes of this study, the cleaning step was omitted to perform the test under worst-case conditions. In all trials, no microorganisms were recovered from the tested duodenoscopes after sterilization, including from sites composed of materials known to degrade from repetitive processing. Although the study designs differ, these results contrast other low-temperature sterilization methods that were reported by Alfa et al[44,45], in which ethylene oxide failed to achieve sterility of narrow flexible lumens in the presence of serum and salt.

Both the overkill and the simulated-use methods demonstrated that the tested hydrogen peroxide–ozone sterilizer achieves sterilization of contaminated duodenoscopes under worst-case conditions not typically encountered in clinical application.

The last part of this study was an evaluation of the sterile efficacy of clinically-soiled duodenoscopes with all the processing steps typically performed in a health care facility. The tested sterilizer and duodenoscopes were hospital owned. The duodenoscopes had 1-6 years of clinical use with a history of being processed with HLD and were functional. However, damage was visually observed on the bending section adhesives and insertion tubes, which represented actual use conditions.

Contamination was present on all tested duodenoscopes after the procedure and immediately prior to sterilization. As expected, a reduction of the bioburden was shown by the precleaning controls versus the postcleaning controls on the duodenoscope surface and air/water channels. However, a measurable reduction of the bioburden was not observed for the elevator mechanism and instrument/suction channels of the duodenoscope. This may have resulted from the sampling design, in which the sampling fluid was flushed through the instrument/suction channel and contacted the elevator prior to collection. Consequently, it is possible that the apparent instrument/suction channel contamination arises from the elevator mechanism rather than the channel itself. The elevator mechanism of the endoscope showed consistent growth on all postcleaning tests, even after the recommended manual cleaning with an enzymatic detergent and brushing with the newly designed brush. This supports previous conclusions regarding the challenge of effectively cleaning duodenoscopes.[3–7] After terminal sterilization, the duodenoscopes were aseptically sampled and cultured for the detection of a large spectrum of microorganisms. Despite the known cleaning challenges and the existing wear of the duodenoscopes, no bacterial growth was observed on any of the processed duodenoscopes after terminal sterilization in the tested hydrogen peroxide–ozone sterilizer under clinical conditions.

Results from this study demonstrate that the tested hydrogen peroxide–ozone sterilizer is effective for terminal sterilization of duodenoscopes, which could be implemented as part of a comprehensive infection control strategy. Terminal sterilization of duodenoscopes offers a higher safety margin than the current practice of HLD, although it is important to emphasize that it does not eliminate the requirement for effective cleaning of the device following the manufacturer's instructions for use. Cleaning of the device prior to sterilization is critical to prevent an accumulation of organic material and the formation of biofilm, both of which could inhibit sterilization and present a risk to patients. Biofilms may contribute to the recovery of viable microorganisms post processing even when current guidelines are followed.[31,60]

The bulk of the literature about the processing of duodenoscopes relates to the currently recommended guidelines of performing HLD. Recent studies show positive bacterial contamination of duodenoscopes after HLD[58,60-62] and double-HLD processing.[13,30-34] And while it has been part of a strategy used to successfully control CRE outbreaks,[18] Snyder et al[31] sampled 173 duodenoscopes after processing with HLD followed by EO sterilization and recovered viable organisms from 22.5% duodenoscopes. It should be noted that the duodenoscopes included in the Snyder et al study predated the duodenoscope elevator design change discussed in the material section. Visrodia et al reported negative cultures for 51 duodenoscopes subjected to combined HLD and EO sterilization,[34] though the details of this study are not currently published. Naryzhny et al detected a rate of positive cultures of 1.2% after EO sterilization, although they were culturing for CRE bacteria only.[35]

The current study demonstrated the absence of detectable microorganisms in duodenoscopes following hydrogen peroxide–ozone sterilization, using culturing methods covering a large spectrum of microorganisms. The data presented here are from a limited sample size, but provide the first set of data to support terminal sterilization using a hydrogen peroxide–ozone sterilizer. Future work should include clinical studies in additional health care facilities. This would not only increase the confidence in the conclusions of this study, but also evaluate the impact of variable processing practices on the efficacy of the sterilization process. Additional proposed studies will evaluate of the sterile efficacy of hydrogen peroxide–ozone sterilization in the presence of biofilms and the formation of biofilm when low-temperature terminal sterilization is routinely used compared with HLD.

CONCLUSIONS

This study showed that the hydrogen peroxide–ozone sterilizer can effectively sterilize duodenoscopes, under laboratory worst-case conditions and in actual clinical use. The overkill method validated the sterility assurance level of 10^{-6}, while the simulated-use method demonstrated that a duodenoscope can be sterilized in the presence of organic and inorganic soils. Furthermore, high-risk materials susceptible to potential degradation with repetitive processing were able to be effectively sterilized. Finally, duodenoscopes that were clinically soiled were sterilized in a health care facility.

Limitations of the study

The conclusions from the clinical in-use study are limited by the small sample size. As clinical endoscopy studies include uncontrolled

variables, such as bioburden load, endoscope age and condition, patient soil composition, and hospital practice, the conclusions of the in-use clinical study would be strengthened by including duodenoscope samples from additional health care facilities.

In addition, the elevator mechanism was sampled using a flush-brush-flush method, while the internal duodenoscope channels were sampled with a flush-only method. The use of a flush-only method for the channels is recognized as a potential limitation compared with use of a flush-brush-flush method. A study by Alfa et al, published in 2017,[63] stated that a flush-only method with the use of a tensioactive agent such as Tween 80 seemed less effective than a flush-brush-flush method and that the potential role of such agents in terms of sample extraction efficiency from endoscope channels needed to be further evaluated. Also, a study by Alfa et al published in 2017[64] stated that the use of a flush-brush-flush method improved recovery of bacteria in biofilms. However, this study does not provide a statistically significant difference between a flush-only and a flush-brush-flush method, explained by Alfa et al, owing to the limited sample size. Both of these studies were published after the testing presented in this article. In contrast, a study by Brandabur et al[61] concluded that among the 4 methods of sampling used in their large-scale 4,032 specimens study (flush-only and flush-brush-flush methods), no significant differences were detected, since the standard deviation was higher within an individual sampling method than between the different sampling methods.

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