EMERGING TECHNOLOGIES

A new method to sterilize multichannel flexible colonoscopes

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ABSTRACT

Background: Flexible gastrointestinal (GI) endoscopes have been associated with patient-to-patient transfer of multidrug-resistant bacteria that are not inactivated by high-level disinfection. This has resulted in calls to reprocess GI endoscopes by sterilization. However, traditional low-temperature sterilization methods are not cleared by the United States FDA to terminally sterilize complex multichannel endoscopes.

Aim: Demonstrate that the STERIZONE® VP4 Sterilizer (VP4 Sterilizer) can sterilize a multichannel colonoscope using a new gravity-based inoculation method.

Methods: In accordance with US, EU and Canadian requirements, a direct-inoculation method was developed to demonstrate that the VP4 Sterilizer can sterilize a multichannel colonoscope under both half-cycle and simulated-use conditions.

Findings: Half-cycle and simulated-use testing demonstrated that the VP4 Sterilizer can sterilize a multichannel colonoscope with a sterility assurance level of SAL-6. Validation of the inoculation method using surrogate lumens, confirmed that the center of each lumen contained $>10^6$ test organisms. Furthermore, both high and low-level recovery was achieved for each lumen within a multichannel colonoscope.

Conclusion: Flexible colonoscopes can be terminally sterilized using the VP4 Sterilizer. It is the first vapor-based sterilization technology that is FDA cleared to sterilize a four-channel flexible colonoscope.

KEY WORDS
colonoscope, sterilization

INTRODUCTION

In accordance with the Spaulding Classification scheme, flexible GI endoscopes including colonoscopes and gastroscopes have been traditionally classified as semi-critical devices, meaning that they should be sterilized before use, or if this is not possible, reprocessed using high-level disinfection (HLD) (1). Because flexible endoscopes are temperature sensitive, HLD has been the preferred reprocessing method, reflecting the inadequacy of available low-temperature sterilization technologies. Recently however, both regulatory agencies and the medical community have recognized that GI endoscopes should be reclassified from semi-critical to critical devices, which requires reprocessing by sterilization and not HLD (2).

The desire to sterilize GI endoscopes is in large part caused by recent publicity involving patient-to-patient transfer of multidrug-resistant organisms (MDROs) attributed to endoscopes, particularly duodenoscopes (3). Although some infectious outbreaks have been caused by breaches of reprocessing (4), others have occurred even when endoscopes have been reprocessed according to manufacturer's instructions-for-use (IFU) (3). In particular, Ofstead *et al*, found that viable microbes were identified on GI endoscopes reprocessed using cleaning and disinfection methods provided by the device manufacturer (5).

To address this problem, some device manufacturers have begun to validate the use of ethylene oxide (EtO) as a method for sterilizing GI scopes. However, EtO requires lengthy aeration times and is associated with occupational health and environmental risks. Also, EtO sterilizers are limited in the US to sterilization of devices with a maximum of two lumens (6), which by definition excludes modern GI endoscopes. Furthermore, in studies published by Alfa *et al* involving inoculation and sterilization of flexible surrogate lumens, data shows that EtO efficacy is compromised when inoculum is mixed with inorganic contaminants (7), which are intended to reflect "simulated-use" conditions commonly found in a clinical setting.

Liquid chemical sterilization using peracetic acid is indicated for reprocessing reusable critical and semi-critical heat-sensitive medical devices including flexible endoscopes (8). As reported by McDonnell *et al* (9), half-cycle testing using a peracetic-acid system and commercial duodenoscopes, demonstrated a sterility assurance level of SAL^{-6}. However, reprocessed scopes must be used at point-of-care, since the method does not allow for *terminal* sterilization, which facilitates sterile storage.

Additionally, the effectiveness of first-generation vaporized hydrogen peroxide (H_2O_2) sterilizers in sterilizing multi-lumen devices has been evaluated and found inadequate to reprocess a modern GI endoscope. Claim language varies by sterilizer

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manufacturer, but at best is limited to only dual-channel flexible scopes with the longest lumen ≥ 1 mm in Inner Diameter (ID) and ≤ 1000 mm in length, which is well short of the requirements for a modern colonoscope (10).

The process for validating sterilization claims for new device designs is dictated by both international standards (11) and regulatory guidance, such as provided by FDA (12). Specifically, ISO 14937 requires that a sterilizer manufacturer demonstrate that test devices, inoculated with at least 10^6 CFU of a highly resistant organism, can be sterilized under half-cycle conditions. Furthermore, the inoculation must provide the greatest challenge to sterilant penetration, which for vapor-based processes, is in the middle of a lumen.

In addition, FDA requires that test devices must pass simulated-use testing, wherein the microbe suspension is mixed with organic and inorganic soils and inoculated onto devices. For a successful simulated-use validation, testing is to be performed in triplicate with no growth observed following sterilization.

Because of the urgent need for a viable method to terminally sterilize complex GI endoscopes, the effectiveness of a new low-temperature dual-sterilant method was evaluated for reprocessing a flexible video colonoscope. This in turn was completed by use of a new validated test method for direct inoculation of long-lumen multichannel flexible endoscopes.

METHODS

Sterilizer

The STERIZONE® VP4 Sterilizer (VP4 Sterilizer) (TSO3, Inc., Quebec Canada) was used in this study. A detailed description of the device has been previously published (13). The device uses dual sterilants (vaporized H_2O_2 and ozone), in a multiphase process. The device is intended for use in terminal sterilization of cleaned, rinsed, and dried metal and non-metal reusable medical devices. The VP4 Sterilizer uses only a single sterilization cycle irrespective of load configuration, with a maximum load limit of 34 kg (75 pounds).

Test organism

The most resistant microorganism to either hydrogen peroxide or ozone sterilants is *Geobacillus stearothermophilus* spores (14).

Spore suspensions of *G. stearothermophilus* ATCC 7953 (Lot AR-469; population $2{,}2 \times 10^8$ colony forming unit (CFU)/mL) were purchased from iuvo BioScience (Rush, NY). The spore suspension populations were verified and adjusted to achieve a final concentration of $1{,}0\text{-}2{,}5 \times 10^6$ CFU/10 µL, which was used for validation of high-level recovery, as well as half-cycle and simulated-use testing (the latter in combination with 400 ppm AOAC hard water and 5% fetal bovine serum).

The spore suspension was further diluted to 10-100 CFU/10 µL for validation of low-level recovery.

Lumen devices or surrogates

For the purpose of validating expanded sterilization claims, a Pentax Video Colonoscope Model EC-3890Li (Pentax Medical, Tokyo, Japan) was used. The manufacturer identifies seven discrete lumens, consisting of four "channels" (Instrument, Air, Water, and Forward Water Jet, extending from the distal end of the device to the handle) and three "tubes" or umbilical lumens (Suction, Air Feeding, and Water Feeding, extending from the handle to the suction source, air pump, and water bottle, respectively; see Figure 1). Channel dimensions, which are the basis for FDA labeling claims for the VP4 Sterilizer, are ≥ 1,45 mm ID and ≤ 3 500 mm in length, and/or ≥ 1,2 mm ID and ≤ 1 955 mm in length. Tube dimensions, are all ≥ 2,4 mm ID and ≤ 1 580 mm in length. Validation studies were completed on all channels and tubes (seven in total) as defined by Dufresne (15), since all lumens can become contaminated, although the device is commonly referred to as a "four-channel" endoscope (consisting of air, water, suction, and instrument channels).

Development and validation of the inoculation method as well as high-level recovery method was completed by use of surrogate fluoropolymers tubing such as polytetrafluoroethylene (PTFE) or perfluoroalkoxy alkanes (PFA) tubing, which are part of the same group of fluoropolymers tubing used for commercial flexible endoscopes. Tubing diameter and length was selected to correspond to the dimensions found in the Pentax colonoscope. Thus, surrogate PTFE tubing, ranging between 1 mm ID × 3 500 mm length, and 4 mm ID × 1 840 mm length, were selected based on worst-case lumen dimensions (smallest ID and longest length).

Inoculation and recovery method using surrogate lumens

PTFE lumens (three samples per dimension) were used to develop the inoculation method for each lumen found in the colonoscope, as well as to validate that a minimum of 10^6 spores were deposited in the center of the lumen, as required by FDA.

Each lumen was temporarily placed on a vertical wall such that the middle of the lumen was at the lowest height. A minimum volume of sterile diluent solution (between 40-400 µL, depending on the lumen dimension) was added to 10 µL of inoculum (with and without hard water and serum) in order that the collective volume would flow to the middle of the test lumen. A micropipette with a low retention tip was used to introduce the diluted inoculum into the lumen orifice. Minimal visible droplets were observed on the sides of the tube confirming that the inoculum was deposited in the middle of the lumen. The objective was to use the smallest diluent necessary in order to minimize drying time and to ensure that inoculum was visibly collected in the center of the test lumen. The inoculated tubes were left to dry.

After overnight drying of surrogate lumens, verification of the spore count deposited in the middle of the tube was performed by cutting the middle part of the PTFE tube (about 10 % of its total length) and separating it from the remainder of the tubing. This portion of the tubing underwent recovery with a 100 mL buffer solution. A pour plate method using Trypticase Soy Agar (TSA) was performed to evaluate the population. The plates were incubated at 55-60°C for a minimum of 48 hours. The acceptance criteria for a successful high-level validation required recovery of $> 10^6$ spores.

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Inoculation of the Pentax Colonoscope for half-cycle test and simulated use test

The channels and tubes of each colonoscope were inoculated with 1,0-2,5 × 10^6 CFU/10 µL using a direct inoculation method based on gravity. A volume of 10 µL of inoculum was diluted with 40-400 µL of sterile diluent solution, which was introduced into each lumen orifice separately using a gel loading micropipette. For simulated-use, the inoculum was mixed with hard water and serum as described previously (*Test Organism* Section).

The endoscope was inoculated in two groups: Group 1 included only the Forward Water Jet Channel and Group 2 included all other channels and tubes (six lumens in Group 2). The Forward Water Jet Channel had to be inoculated separately due to its considerable length, extending from the distal end of the scope to the umbilical (Figure 1).

Sterilization

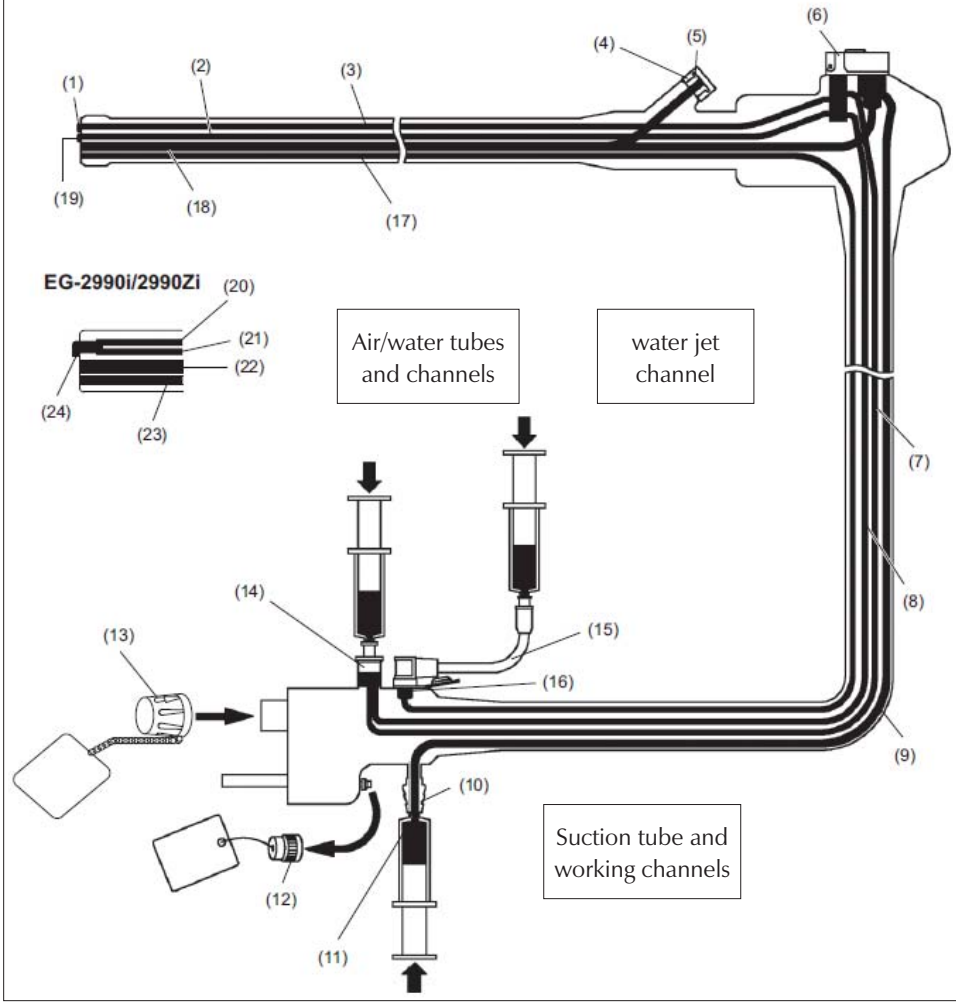
The endoscope was placed in a stainless steel basket and packaged in a full length SteriTite® Container (Case Medical Inc, South Hackensack, NJ). The container was placed on the lower shelf of the sterilizer loading rack.

The load conditions used for the half-cycle and simulated-use validation testing were selected to represent the worst case conditions for sterile efficacy testing. The recommended load temperature to be processed in the STERIZONE® VP4 Sterilizer is 20°C to 26°C. Thus the validation loads were pre-conditioned at 26°C prior to being processed in the sterilizer. The pre-conditioning temperature of 26°C was chosen, due to the fact that this load condition requires the shortest sterilant exposure time and results in the lowest mass of sterilant, and therefore represent the most challenging condition for achieving sterilization efficacy.

For the half-cycle test, the load was exposed to the first phase of the process only. For simulated-use, the load was as exposed to the complete Cycle (two sterilization pulses and full aeration).

Tests were performed in triplicate for each inoculation group under worst-case conditions. Prior to each test, the colonoscope was reprocessed in accordance with the manufacturer's instructions before initiation of the next test, which included cleaning, drying, and storage.

FIGURE 1: Component legend for PENTAX Colonoscope. Inoculated channels and tubes are identified in green (Numbers 2, 3, 7, 8, 9, 17, & 18 – seven lumens in total). Three recovery syringes, with corresponding channels and tubes, are also identified.



(1) Water nozzle
(2) Air channel
(3) Water channel
(4) Inlet seal
(5) Instrument channel inlet
(6) Cleaning adaptor (OF-B153)
(7) Water feeding tube
(8) Air feeding tube
(9) Suction nipple
(10) Suction tube
(11) Syringe
(12) Ventilation cap
(13) PVE soaking cap
(14) Cleaning adapter (OF-G17)
(15) Irrigation tube
(16) OE-C12 without check valve
(17) Forward water jet channel
(18) Instrument channel
(19) Air nozzle

Recovery

Recovery of viable spores was achieved by using a 60 mL syringe and the cleaning connector provided by Pentax, following the cleaning method described in the scope-reprocessing manual.

Three luer-lock connectors are available on the colonoscope, with two of the three connectors associated with more than one channel, and the Forward Water Jet having its own connector (Figure 1). Thus, recovery buffer was passed through more than one channel/tube (with the exception of the Forward Water Jet) using syringes filled with recovery buffer.

The amount of recovery buffer used per channel/tube or group of lumens was 100x the combined internal volume for each lumen or group of lumens. Recovered buffer solution was filtered using a 0,45 μm filter and placed on a TSA plate. Plates were incubated at 55°-60°C for a minimum of 48 hours.

Controls: High level recovery

For high level recovery, each lumen of the Pentax endoscope was tested individually. Each channel was inoculated as described for the half-cycle and simulated use tests. After drying overnight, recovery was performed. A pour plate method using TSA was performed to evaluate the population after heat shock (95-100°C for 15 min) (16). The plates were incubated at 55-60°C for a minimum of 48 hours. A successful high-level validation required recovery of $> 10^6$ spores.

Controls: Low level recovery

In order to confirm low-level recovery, the standard spore suspension was diluted to 10-100 CFU/10 μL. Each channel was inoculated as described for the half-cycle test, but using 10-100 CFU/10 μL spore suspension. After drying overnight, recovery was performed. Recovered buffer solution was filtered using a 0,45 μm filter and placed on a TSA plate. Plates were incubated at 55°-60°C for a minimum of 48 hours. The recovery percentage was calculated using the count of the inoculating spore suspension as 100%. A successful low-level validation required recovery of a minimum of 25% spores.

TABLE 1: Half-cycle and simulated-use validation results

Channel description	Half-cycle Results (# positive lumens/# lumens tested)	Simulated-use Results (# positive lumens/# lumens tested)
Instrument Channel	0/3	0/3
Suction Tube	0/3	0/3
Air Channel	0/3	0/3
Air Feeding Tube	0/3	0/3
Water Channel	0/3	0/3
Water Feeding Tube	0/3	0/3
Forward Water Jet Channel	0/3	0/3

TABLE 2: Recovered population from the middle of the test surrogate (PTFE) lumens

Tubes description	Middle section length	Spore alone		Spore mixed with 5% serum and 400 ppm hard water	
		Recovered population	Percentage	Recovered Population	Percentage
1 mm × 3 500 mm	35 cm	$1,27\times10^6$	79 ± 7%	$1,09\times10^6$	79 ± 7%
		$1,29\times10^6$		$1,24\times10^6$	
		$1,47\times10^6$		$0,83\times10^6$	
2 mm × 1 580 mm	16 cm	$1,27\times10^6$	78 ± 3%	$1,12\times10^6$	79 ± 8%
		$1,34\times10^6$		$1,02\times10^6$	
		$1,38\times10^6$		$1,21\times10^6$	
3 mm × 1 580 mm	16 cm	$1,39\times10^6$	90 ± 8%	$1,27\times10^6$	80 ± 6%
		$1,64\times10^6$		$1,34\times10^6$	
		$1,38\times10^6$		$1,38\times10^6$	
4 mm × 1 840 mm	18 cm	$1,52\times10^6$	92 ± 5%	$1,15\times10^6$	79 ± 8%
		$1,66\times10^6$		$1,01\times10^6$	
		$1,53\times10^6$		$1,03\times10^6$	

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RESULTS

Half-cycle and simulated-use testing of video colonoscope

No viable microorganisms were recovered from any of the inoculated challenges subsquent to exposure to either half-cycle or simulated-use testing conditions (Table 1), despite the fact that six inoculated lumens (within Group 2) were sterilized simultaneously.

Controls – verification of inoculum in the center of test lumens

All lumens were inoculated with a spore suspension of 1,71 × 10^6 CFU/10μL (spore alone) or between 1,02 and 1,53 × 10^6 CFU/10μL when spores were mixed with 5% serum and 400 ppm hard water. High-level recovery using PTFE lumens confirmed that a population of at least 10^6 spores was recovered from the middle of all test lumens, irrespective of ID or length. This was true if the suspension was used either alone (78-92% recovery) or if combined with serum and hard water (74-80% recovery – See Table 2).

Controls: High level recovery

The population of the spore suspension used for high level recovery was 1,71 × 10^6 CFU/10μL (spores alone) or between 1,02 and 1,53 × 10^6 CFU/10μL for spores mixed with 5% serum and 400 ppm hard water. High-level recovery for each inoculated channel and tube within the colonoscope also confirmed a population of at least 10^6 spores. This was confirmed when the suspension was used alone (77-95% recovery by lumen) or with serum and hard water (91-105% recovery by lumen – See Table 3).

Controls: Low level recovery

The population of the spore suspension used for low level recovery was determined to be between 69-90 CFU/10μL; low-level recovery was not done with spores mixed with serum and hard water. Low-level recovery was lower than with high-level recovery, but was judged to be satisfactory, particularly considering the long lengths and complicated access found with the test endoscope (range 29-67 % recovery by lumen – See Table 4).

DISCUSSION

In 2015, the STERIZONE® VP4 Sterilizer was approved by Health Canada and the EU to include sterilization of multichannel flexible GI endoscopes including colonoscopes and gastroscopes. It was subsequently cleared by FDA in June 2016 to include sterilization of flexible endoscopes with lumens ≥ 1,45 mm ID and ≤ 3 500 mm in length (and/or ≥ 1,2 mm ID

TABLE 3: High level recovery for each inoculated channel and tube found in the Pentax video colonoscope

Channel description	Spores alone		Spores mixed with 5% serum and 400 ppm hard water	
	Population recovered (CFU)	Recovery percentage (%) ± SD	Population recovered (CFU)	Recovery percentage (%) ± SD
Instrument Channel	1,27 × 10^6	87 ± 12	1,02 × 10^6	96 ± 8
	1,55 × 10^6		1,11 × 10^6	
	1,66 × 10^6		1,02 × 10^6	
Suction Tube	1,39 × 10^6	90 ± 9	1,05 × 10^6	103 ± 4
	1,54 × 10^6		1,42 × 10^6	
	1,71 × 10^6		1,11 × 10^6	
Air Channel	1,43 × 10^6	84 ± 7	1,11 × 10^6	99 ± 3
	1,32 × 10^6		1,05 × 10^6	
	1,56 × 10^6		1,14 × 10^6	
Air Feeding Tube	1,50 × 10^6	95 ± 6	1,02 × 10^6	91 ± 3
	1,70 × 10^6		1,00 × 10^6	
	1,65 × 10^6		1,01 × 10^6	
Water Channel	1,57 × 10^6	94 ± 2	1,02 × 10^6	99 ± 3
	1,63 × 10^6		1,27 × 10^6	
	1,63 × 10^6		1,13 × 10^6	
Water Feeding Tube	1,61 × 10^6	84 ± 10	1,03 × 10^6	105 ± 9
	1,42 × 10^6		1,18 × 10^6	
	1,27 × 10^6		1,20 × 10^6	
Forward Water Jet Channel	1.29 × 10^6	77 ± 2	1.15 × 10^6	95 ± 17
	1.30 × 10^6		1.14 × 10^6	
	1.35 × 10^6		1.04 × 10^6	

and ≤ 1 955 mm in length). To date, the VP4 Sterilizer is the only vapor-based sterilizer to receive FDA clearance to sterilize a four-channel flexible GI endoscope.

Numerous methods have been published on how to inoculate and recover test organisms from lumens for use in sterilization validation studies. However, in general the methods have been validated for only simple lumen devices, and do not reflect multiple, long lumens as found in a GI endoscope. Furthermore, many of the methods require use of a surrogate lumen and not actual endoscopes, as mandated by FDA.

For example, Okpara-Hofmann *et al* described the use of either stainless steel squares or wire carriers, inoculated with 10^6 bacterial spores, and placed in the middle of an endoscope biopsy channel (17). The longest endoscope evaluated had a biopsy channel of 2,8 mm ID and was only 1 160 mm long. The author's counseled against direct inoculation of the endoscope due to low colony counts in recovery, caused by the spore suspension being lost in "niches and lumens."

Diab-Elschahawi *et al* also described use of an inoculated wire carrier placed in the midpoint of a surrogate stainless steel lumen measuring 0,7 mm × 500 mm[18]. Although the carrier was significantly longer than used by Okpara-Hofmann, the carrier was not qualified for use in long flexible lumens.

TABLE 4: Low level recovery for each inoculated channel and tube found in the Pentax video colonoscope

Channel description	Inoculum (CFU) population	Recovery (CFU) percentage	Recovery percentage (%)
Instrument Channel	76	45	67 ± 14
	78	45	
	78	65	
Suction Tube	69	42	63 ± 13
	69	35	
	69	53	
Air Channel	76	44	52 ± 13
	78	48	
	78	29	
Air Feeding Tube	90	42	47 ± 6
	90	48	
	80	33	
Water Channel	69	20	54 ± 28
	69	34	
	69	58	
Water Feeding Tube	88	19	29 ± 7
	88	30	
	88	28	
Forward Water Jet Channel	84	42	39 ± 10
	84	26	
	76	28	

Dufresne *et al* tested surrogate lumens made of stainless steel tubing with diameters ranging between 0,5-4,0 mm, and lengths ranging between 450-700 mm (19). For the smallest diameter lumens, tubing was directly inoculated with spore suspension. For all other tubing, the microbial challenge was created by placing an inoculated wire inside the channel, which was longer than the lumen to be sterilized.

Finally, McDonnell *et al* reported the direct inoculation of a four-channel duodenoscope by flushing 0,5 mL spore suspension (with a titer of 10^8 CFU/mL) through the port and through each channel of the device (9). Satisfactory high and low-level validation was reported. Nonetheless, the method would not satisfy FDA requirements for validation of a vapor-based sterilization process, which requires confirmation that the inoculum is deposited into the middle of each channel.

Due to the complexity of modern GI endoscopes, and FDA's specific requirements for the location of inoculum and validation of spore recovery, neither carriers nor conventional direct inoculation methods are satisfactory. In particular, carriers are difficult to insert into endoscope lumens due to valves and other restrictions, which are not found in surrogate lumens. In addition, spores may be lost due to the interaction of the carrier with lumen walls during insertion. Therefore, a new validated test method was required for direct inoculation of long-lumen multichannel flexible endoscopes. The gravity-based inoculation method described herein satisfied FDA requirements for targeted inoculation and recovery efficacy.

Application of the direct inoculation method confirmed that the VP4 Sterilizer achieves a six log spore reduction in each of seven colonoscope lumens under half-cycle and simulated-use conditions. This represents the first sterilization validation of a modern multichannel GI endoscope using a vapor-based sterilant.

The development of sterilization methods for long-lumen devices is an important advancement. It is reported that more than 10 million GI endoscopic procedures are performed every year in the US, which equates to a significant risk of patient-to-patient transfer of MDROs (2). However, sterilization does not necessarily compensate for inadequate or timely cleaning of the endoscope immediately following a procedure. Thus, successful reprocessing of a complex endoscope must be viewed in the context of thorough bedside cleaning, manual cleaning, automated endoscope reprocessing, and terminal sterilization.

CONCLUSIONS

A new gravity based inoculation method using sterile diluent demonstrated that spores were consistently deposited in the center of each test lumen as required by FDA for sterilization validation studies. Furthermore, both high and low-level recovery confirmed that spores could be recovered from inoculated lumens. Application of the method to half-cycle and simulated-use testing with a multichannel colonoscope was confirmed, verifying that complex GI scopes can be terminally sterilized using the STERIZONE® VP4 Sterilizer. The FDA's clearance of this device for the terminal sterilization of multichannel video colonoscopes is a milestone in reducing risk for patients using these critical medical devices.

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