Comparative evaluation of the sporicidal activity of new low-temperature sterilization technologies: Ethylene oxide, 2 plasma sterilization systems, and liquid peracetic acid

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Objective: This study was undertaken to evaluate the efficacy of 4 new low-temperature sterilization technologies: ethylene oxide with hydrochlorofluorocarbons, a liquid peracetic acid immersion system (Steris System 1 Processor), and 2 plasma sterilization processes that use vaporized hydrogen peroxide (Sterrad 100 and the Sterrad 100S). The Sterrad 100S system potentially improves sterilizer efficacy by using 2 cycles of a diffusion stage and a plasma stage per sterilization cycle.
Methods: Flat stainless steel carriers were inoculated with approximately 10^6 *Bacillus stearothermophilus* spores. These carriers were aseptically placed in the middle of 40 cm long stainless steel lumens (hollow tubes). Two types of lumen were used: (1) a lumen test unit with a removable 5 cm center piece (1.2 cm diameter) of stainless steel sealed to the narrower steel tubing by hard rubber septums and (2) a straight lumen. Three different diameters of the lumen test unit (1, 2, and 3 mm) and a single diameter of the straight lumen (3 mm) were studied. At least 40 replicates were performed for each type of lumen and sterilization method. After inoculation, the test unit was evaluated in 1 of the low-temperature sterilization technologies. After sterilization, the carriers were cultured in trypticase soy broth for 14 days at 55°C and assessed for growth of *B stearothermophilus* spores.
Results: Our results demonstrated that ethylene oxide with hydrochlorofluorocarbons, the Sterrad 100S, and the Sterrad 100S half cycle were highly effective in killing approximately 10^6 *B stearothermophilus* spores present in the center of narrow-lumen stainless steel tubes. As the lumen diameter decreased with the lumen test unit, the Sterrad 100 demonstrated reduced ability to kill *B stearothermophilus* spores present on the carrier. At the smallest diameter tested (1 mm), the Sterrad 100 system failed 74% of the time. The Steris System 1 was not effective in completely eliminating the 10^6 inoculum under test conditions.
Conclusion: The Sterrad 100S was significantly superior to the Sterrad 100 system and equivalent to ethylene oxide with hydrochlorofluorocarbons. Introduction of this new Sterrad 100S system should improve the margin of safety and reduce processing costs by its use of a shorter cycle time. The Steris System 1 is limited by diffusion of the chemical sterilant into the interior of the lumen test unit. (AJIC Am J Infect Control 1998;26:393-8)

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Low-temperature sterilization is required for temperature- and moisture-sensitive critical medical devices and supplies. Ethylene oxide has been the most widely used low-temperature sterilization process. Health care facilities are currently considering alternative processes to ethylene oxide for several reasons: some states (eg, California and New York) require ethylene oxide emission reductions of 90% to 99.9%; there are

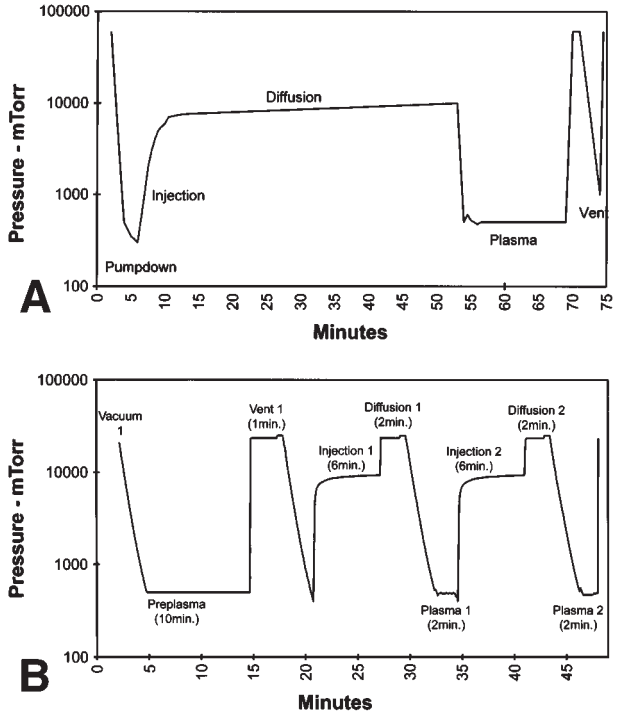

Fig 1. Characteristic cycle curves for the Sterrad 100 **(A)** and Sterrad 100S **(B)**.

potential toxic hazards to staff and patients because ethylene oxide is a probable carcinogen and is flammable; and recent Environmental Protection Agency regulations under the authority of the Clean Air Act banned production of chlorofluorocarbons, which were used as a stabilizing agent in combination with ethylene oxide.[1,2]

Alternative technologies to ethylene oxide with chlorofluorocarbons are being developed at an accelerated pace. These include 100% ethylene oxide, ethylene oxide with a different stabilizing gas such as carbon dioxide or hydrochlorofluorocarbons, vaporized hydrogen peroxide, gas plasmas, ozone, peracetic acid, and chlorine dioxide.[1,2] Some of these new technologies have been cleared by the Food and Drug Administration (FDA) and are commercially available, including peracetic acid plasma sterilization, hydrogen peroxide plasma sterilization, and peracetic acid immersion. These new processes should be compared against the characteristics of an ideal low-temperature (<60°C) sterilant, which include high efficacy, rapid activity, strong penetrability, material compatibility, nontoxicity, adaptability, ability to withstand an organic load, monitoring capability, and cost-effectiveness.[1,3]

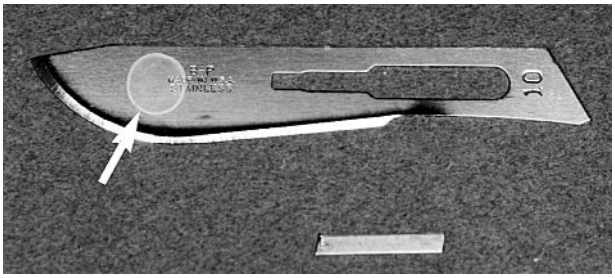

Fig 2. Number 10 Bard-Parker stainless steel surgical blade after being inoculated with a 10 µl suspension of spores *(arrow)*. This carrier was placed into the center of the LTUs (see Fig 3). In addition, a smaller stainless steel carrier cut from a number 10 Bard-Parker surgical blade is shown. This carrier was placed into the center of the straight LTUs (see Fig 3, A).

It is apparent that all currently developed sterilization processes have limitations. Understanding these limitations is crucial in the proper application of these new sterilization technologies within medical facilities. This study was undertaken to evaluate the efficacy of 4 new low-temperature technologies: ethylene oxide with hydrochlorofluorocarbons (EtO-HCFC), liquid peracetic acid (Steris System 1 Processor; Steris Corporation, Mentor, Ohio), and 2 plasma sterilization processes that use vaporized hydrogen peroxide, the Sterrad 100 and the Sterrad 100S (Advanced Sterilization Products, Irvine, Calif.). The Sterrad 100S system potentially improves sterilizer efficacy by using 2 cycles of a diffusion stage and a plasma stage per sterilization cycle.

METHODS

Sterilizers

The ethylene oxide sterilizer (2057 Eagle Series ethylene oxide sterilizer/aerator; AMSCO, Erie, Pa.) was a standard ethylene oxide sterilizer with hydrochlorofluorocarbon carrier gases (27% chlorodifluoromethane, 63% chlorotetrafluoroethane) in 10%:90% ratio (Oxyfume 2002; Allied Signal, Morristown, N.J.). This sterilizer was operated at 54°C with 2-hour exposure to ethylene oxide followed by overnight aeration at 55°C for 12 to 14 hours.

The Sterrad 100 system, which is FDA cleared and in clinical practice, uses a single diffusion stage (hydrogen peroxide vapor) and a plasma stage per sterilization cycle (Fig 1, *A*). In the initial stage of sterilization, the 3.5 ft^3 sterilization chamber is evacuated to a vacuum of approximately 0.5 mm Hg. A small amount of 58% hydrogen perox-

Table 1. Comparative evaluation of the sporicidal activities of new low-temperature sterilization methods

Sterilization method	Test unit type and size			
	LTU, 3 mm	LTU, 2 mm	LTU, 1 mm	SL, 3 mm
10%/90% EtO-HCFC	0/50*	0/40	0/40	0/50
Sterrad 100	2/40	3/40	37/50	0/40
Sterrad 100S	0/50	0/40	0/40	0/40
Sterrad 100S, half cycle	0/40	0/40	0/40	2/40
Steris System Processor	37/40	Not tested	Not tested	Not tested

SL, Straight lumen.
*Number of positive carriers per replicates tested.

ide (1.8 mL), contained in a disposable cassette of 10 premeasured charges, is delivered to the sterilizer (>6 mg/L hydrogen peroxide) through an automated delivery system and allowed to diffuse as a vapor for a fixed period. Then the vapor is excited by a radiofrequency energy of 13.6 MHz, creating a secondary, low-temperature plasma (approximately 104°F) that forms around the products being sterilized. Microbicidal free radicals (eg, hydroxyl and hydroperoxyl) are generated in the plasma. These products recombine at the end of the cycle, leaving water and oxygen as byproducts and thus eliminating the need for aeration.[2]

The Sterrad 100S, which has not yet been FDA cleared, potentially improves sterilizer efficacy by using 2 cycles of a diffusion stage and a plasma stage per sterilization cycle (Fig 1, *B*). This revision, which is achieved by a software modification, reduces total processing time from 73 minutes to 52 minutes. The manufacturer believes that the enhanced activity obtained with the Sterrad 100S is due in part to the pressure changes that occur during the injection and diffusion phases of the process and to the fact that the process consists of 2 equal and consecutive half cycles, each with a separate injection of hydrogen peroxide. Before our initial use of the Sterrad 100S, the machine was evaluated by an Advanced Sterilization Products employee and found to be in proper working order.

The Steris System 1 Processor is a microprocessor-controlled low-temperature sterilization method. The sterilant, 35% peracetic acid, and an anticorrosive agent are supplied in a single-dose container. The container is punctured by the user and the lid of the sterilizer is closed. The concentrated peracetic acid is diluted to 0.2% (0.22 μm) with filtered water at a temperature of approximately 50°C. The diluted peracetic acid is circulated within the chamber of the machine and pumped through the channels of the endoscope for 12 minutes, decontaminating exterior surfaces, lumens, and accessories. Interchangeable trays are available to permit the processing of as many as 3 rigid endoscopes or 1 flexible endoscope. Connectors are available for most types of flexible endoscopes for the irrigation of all channels by forced flow. Rigid endoscopes are placed within a lidded container, and the sterilant fills the lumens by immersion. The peracetic acid is discarded through the sewer, and the instrument is rinsed 4 times with filtered water. Clean filtered air is passed through the chamber of the machine and endoscope channels to remove excess water.[4]

Before our initial use of the Steris System 1, the machine was evaluated by a Steris employee and found to be in proper working order. A diagnostic cycle was conducted daily. After an acceptable diagnostic cycle, test cycles were conducted. During each test cycle, temperature, water pressure, and operating parameters were evaluated and the results were documented on a printout at the end of each cycle. This test is based partially on a conductivity measurement that depends on the presence of buffer and is not a direct indicator of the presence of peracetic acid.[4] The lumen test units (LTUs, described later) were placed in the processor's lidded instrument tray, as recommended in the operator's manual. A spore strip containing at least 10^5 spores of *Bacillus stearothermophilus* (Steris Corporation) was used with each run as a biologic indicator. The spore strip was removed from its glassine envelope and held by a clip provided by the manufacturer. The clip was placed in a designated spot inside the machine. After each test cycle, personnel aseptically transferred the spore strip to trypticase soy broth (Steris Corporation) with phenol red and incubated at 55° to 56°C. In all tests the expected results were observed; no color change (red) for sterilizer runs (negative for growth) and color change (yellow) and turbidity (growth) for control runs. A chemical monitoring strip (Steris

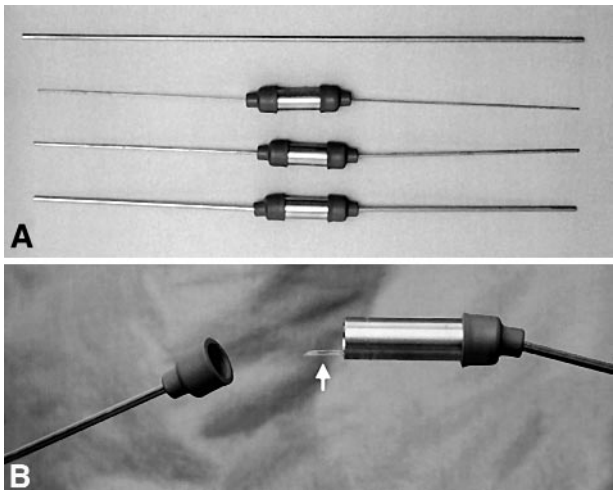

Fig 3. A, LTUs of varying diameters (diameters 1 mm, 2 mm, 3 mm), including straight LTU made of stainless steel (diameter 3 mm, top). **B**, Test objects were placed into the LTUs by aseptically unsealing the hard rubber septums connecting the stainless steel tubing.

Corporation) was also used with each run as a qualitative chemical indicator to detect the presence of peracetic acid at concentrations of at least 1500 ppm. A clip was used to anchor the strip and permit easy retrieval. In all tests the color change was complete (violet to white).

Test pathogens

The sporicidal activity of the sterilization processes were assessed with *B stearothermophilus* (lot number PB49T) prepared by Dr. Irving J. Pflug (University of Minnesota, Minneapolis).

Carrier inoculation and quantitation

A use-dilution spore suspension was prepared by making a 1:10 dilution of a stock spore suspension (approximately 10^9 colony-forming units [cfu]) in 0.1% Tween 80 in sterile water. Quantitation of the use dilution was performed in triplicate by vortex mixing this suspension for 2 minutes, transferring a 10 μL sample to a tube containing 9.99 ml sterile water (1:1000 dilution), and heat shocking at 100°C for 15 minutes. After heat shocking, these dilutions were placed in an ice-water bath and allowed to cool. Then serial dilutions (×3) of each sample were made and placed in triplicate into trypticase soy agar with the pour-plate method. Plates were incubated at 55° to 60°C for 48 hours. Stock and use-dilution spore suspensions were stored at 4°C until use.

Once the use-dilution spore suspension was confirmed to have approximately 10^8 cfu/ml, the carriers were inoculated. The carriers were number 10 Bard-Parker stainless steel surgical blades (Becton Dickinson Acute Care, Franklin Lakes, NJ) or rectangles (1 mm by 10 mm) cut from the Bard-Parker blades (Fig 2). Before inoculation of these carriers, the use-dilution spore suspension was prepared by sonic treatment for 5 minutes, chilling in an ice-water bath for 2 minutes, vortex mixing for 2 minutes, and chilling again in an ice-water bath for 2 minutes. These steps were repeated 2 more times. After achievement of a homogeneous suspension, carriers were aseptically placed in a sterile petri dish and inoculated with 10 μL of this suspension in a biological safety cabinet. To ensure that the spores remained in suspension during the inoculation, the suspension was vortex mixed for approximately 30 seconds after every 40 inoculations. Carriers were allowed to air dry overnight and were stored in an airtight container 7 days before use.

Carriers were quantitated in triplicate before use and on a weekly basis by placing the carrier in a test tube containing 10 mL sterile water and sterile glass beads. These tubes were sonically treated for 5 minutes, chilled in an ice-water bath for 2 minutes, vortex mixed for 2 minutes, and then chilled in an ice-water bath again for 2 minutes. After these steps were repeated twice, the samples were heat shocked for 15 minutes at 100°C and then chilled in an ice-water bath. Serial dilutions (×4) were made for each sample, with the last 2 dilutions of each set being plated in triplicate into trypticase soy agar with the pour-plate method. These plates were incubated at 55° to 60°C for 48 hours and assessed for growth.

Efficacy test methods

The sporicidal activities of the sterilization systems were assessed by aseptically placing inoculated carriers into 40 cm long stainless steel–lumen tubes of varying diameters (1 mm, 2 mm, and 3 mm; supplied by Advanced Sterilization Products; Fig 3). Two types of lumen tubes were used, an LTU with a removable 5 cm center piece (1.2 cm diameter) of stainless steel sealed to the narrower steel tubing by hard rubber septums and a straight lumen. After inoculation, the Bard-Parker carriers were placed in the middle of the lumened tubes (LTUs) or the rectangles were placed in the middle of the straight-lumen tubes. The test units were then tested in 4 runs of 10 test units/run with one of the 4 low-temperatures sterilization processes according to manufacturer's recommendations. No other medical devices or supplies were placed in the sterilizer when

the Sterrad 100, Sterrad 100S, and Steris System 1 were tested. Additional medical devices or supplies were placed in the ethylene oxide sterilizer during test runs.

After sterilization the carriers were aseptically removed and placed in tubes containing 10 mL trypticase soy broth. These tubes were incubated at 55° to 60°C for 14 days and observed for growth. After 14 days they were heat shocked and reincubated an additional 7 days to ensure that no latent spores were present. Positive culture results were confirmed as representing the test organism with a biochemical identification strip for *Bacillus* species, (API 50 CH; bioMérieux Vitek, Inc., Hazelwood, Mo), along with its corresponding medium (API 50 CHB Medium). Organism identification was confirmed on all positive carriers when sets of 10 replicates had fewer than 5 positive carriers. When sets had more than 5 positive carriers, organism identification was confirmed at a 1:5 ratio.

RESULTS

Overall EtO-HCFC sterilization, the Sterrad 100S, and Sterrad 100S half cycle were highly effective in killing approximately 10^6 *B stearothermophilus* spores present in the center of narrow-lumen stainless steel tubes (Table 1). As the lumen diameter decreased, the Sterrad 100 demonstrated a reduced ability to kill *B stearothermophilus* spores present on the carrier. At the smallest diameter tested, 1 mm, the Sterrad 100 failed 74% of the time. The Sterrad 100S was significantly superior to the Sterrad 100 and equivalent to EtO-HCFC. Even when a half cycle was tested, the Sterrad 100S demonstrated excellent sporicidal activity that was not statistically different from the activity demonstrated by the full-cycle Sterrad 100S and EtO-HCFC. The Steris System 1 Processor was not effective in completely eliminating the inoculum of approximately 10^6 *B stearothermophilus* spores under our test conditions.

DISCUSSION

Medical devices that have contact with sterile body tissues or have sterile body fluid flow through them are considered critical items. These items should be sterile when used because any microbial contamination could result in disease transmission. Such items include surgical instruments, biopsy forceps, and implants.[5] If these items are heat resistant, the recommended sterilization process is steam sterilization, which carries the largest margin of safety. However, reprocessing heat- and moisture-sensitive items requires use of low-temperature sterilization technology. Regardless of the sterilization method used, thorough manual cleaning must precede sterilization to remove organic debris and salts. This investigation was undertaken to evaluate the microbicidal potencies of alternative low-temperature sterilization technologies.

Sterilization processes must be cleared by the FDA. Clearance requires that the process eliminate 10^6 cfu of an organism highly resistant to the process. Typically the organism most resistant to the sterilization process is used. For ethylene oxide sterilization technologies, the recommended test organism is *Bacillus subtilis* and for the hydrogen peroxide plasma process and peracetic acid immersion process it is *B stearothermophilus*. Our studies were conducted with 10^6 cfu of an organism highly resistant to all tested processes, *B stearothermophilus*. The FDA requires that sterilizer microbicidal performance be tested under simulated use conditions.[6] These include, in addition to the use of 10^6 cfu of the most resistant test organism, a design configuration (such as lumens) that provides the greatest barrier for penetration of the sterilant. We chose to use 40 cm long stainless steel tubes with diameters as small as 1 mm because most of the laparoscopes and arthroscopes used in health care have shorter lengths or larger diameters than did our test units. The FDA also requires that the test organism be prepared with an inorganic and organic load.[6] Our experimental design did not incorporate either a salt (inorganic) or protein residue (organic). The inorganic or organic load on used medical devices after cleaning is unknown. Investigators have shown that plasma and ethylene oxide sterilizers effected a 6 $\log_{10}$ reduction of the bacterial inoculum on penicylinders[7]; however, inorganic and organic residues protect the microorganisms from the lethal effect of gas or plasma sterilants. Studies have demonstrated that spores of *Bacillus* species occluded in salt crystals are more resistant to inactivation by ethylene oxide[9] than are spores associated with organic matter.[10,11] It is unlikely that cleaned medical instruments would ordinarily have microorganisms protected in crystals.

EtO-HCFC, the Sterrad 100S, and the Sterrad 100S half cycle were highly effective in killing approximately 10^6 *B stearothermophilus* spores or more present in the center of narrow stainless steel–lumen tubes. No failures were detected with either EtO-HCFC or the Sterrad 100S. Two failures out of 160 replicates were noted with the Sterrad 100S run at a half cycle after inoculation with a mean of 1.35×10^6 spores. Because killing has been demonstrated to be linear with time, this suggests that a full cycle run is capable of reliably killing more

than 10^{12} ($12 \log_{10}$) bacterial spores.[12] As the lumen diameter decreased with the LTUs, the Sterrad 100 demonstrated reduced ability to kill *B stearothermophilus* spores present on the carrier. At the smallest diameter tested, 1 mm, the Sterrad 100 system failed 74% of the time. However, it should be noted that the Sterrad 100 is FDA cleared for use with medical devices with lumen diameters 3 mm or larger and not longer than 40 cm.

The Steris System 1 is commonly used in the United States for processing flexible endoscopes, and 2 clinical trials have demonstrated favorable results during clinical use.[13,14] In an experimental study, endoscopes contaminated with more than 10^6 *Pseudomonas aeruginosa* or more than 10^4 *B subtilis* spores showed no growth. However, 10% (2/24) of spore strips containing 10^6 *B subtilis* spores showed a small number of survivors, fewer than 10 per strip.[4] In our experiments, we believe that the failure to eliminate high levels of spores from the center of our test unit was related to the inability of the liquid peracetic acid to completely diffuse into the center of the 3 mm diameter test unit. This may be caused by an air lock or air bubbles formed in the lumen, impeding the flow of the sterilant through the long and narrow lumen and limiting complete access to the *Bacillus* spores. This restricted diffusion environment that exists with the test units (which simulate rigid endoscopes) would not exist with flexible endoscopes processed in the Steris System 1 because they are connected to channel irrigators to ensure that the sterilant has direct contact with contaminated surfaces.[11] On the basis of published studies and ongoing work in our laboratory, we believe that the failure of the Steris System 1 to completely eliminate extremely high numbers of a highly resistant bacteria *(B stearothermophilus)* does not represent a clinical hazard because rigid endoscopes after proper cleaning are contaminated only with low numbers of more susceptible bacteria.[15] Additionally, in a preliminary set of experiments with at least 10^5 *P aeruginosa* or *Staphylococcus aureus* with the same methods, we found complete elimination of these bacteria in all runs (10 replicates/test organism). Further studies should be undertaken to assess the ability of the Steris System 1 to remove clinically relevant levels of vegetative bacteria (including mycobacteria) from rigid endoscopes.

The Sterrad 100S was significantly superior to the Sterrad 100 system and equivalent to EtO-HCFC. Introduction of this new system should improve the margin of safety and reduce processing costs by its use of a shorter cycle time. Although the Steris System 1 Processor failed under our test conditions, the clinical relevance of this finding is unclear. Further studies should be undertaken to assess the ability of the Steris System 1 Processor to eliminate vegetative bacteria (including mycobacteria) from the lumens of rigid endoscopes.

The Steris System 1 Processor was kindly loaned by Steris Corporation, Mentor, Ohio. The Sterrad 100S was kindly loaned by Advanced Sterilization Products, Irvine, California.

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