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Original Article

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

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Abstract

Objective: To compare the microbicidal activity of low-temperature sterilization technologies (vaporized hydrogen peroxide [VHP], ethylene oxide [ETO], and hydrogen peroxide gas plasma [HPGP]) to steam sterilization in the presence of salt and serum to simulate inadequate precleaning.

Methods: Test carriers were inoculated with *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus*, vancomycin-resistant *Enterococcus, Mycobacterium terrae, Bacillus atrophaeus* spores, *Geobacillus stearothermophilus* spores, or *Clostridiodes difficile* spores in the presence of salt and serum and then subjected to 4 sterilization technologies: steam, ETO, VHP and HPGP.

Results: Steam, ETO, and HPGP sterilization techniques were capable of inactivating the test organisms on stainless steel carriers with a failure rate of 0% (0 of 220), 1.9% (6 of 310), and 1.9% (5 of 270), respectively. The failure rate for VHP was 76.3% (206 of 270).

Conclusion: Steam sterilization is the most effective and had the largest margin of safety, followed by ETO and HPGP, but VHP showed much less efficacy.

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Each year in the United States, ~53,000,000 outpatient surgical procedures and 46,000,000 inpatient surgical procedures are performed.[1] Each of these procedures involves contact by a surgical instrument with a patient's sterile tissue. A major risk of all such procedures is the introduction of infection.[2] Failure to properly sterilize surgical instruments may lead to transmission via these instruments.[2,3]

Most medical and surgical devices used in healthcare facilities are made of materials that are heat stable and thus are sterilized by heat, primarily steam sterilization (SS).[2] However, since 1950, more medical devices and instruments have been made of materials (eg, plastics) that require low-temperature sterilization (LTS). Ethylene oxide gas (ETO) has been used since the 1950s for heat- and moisture-sensitive medical devices. Within the past 30 years, a number of LTS systems (eg, hydrogen peroxide gas plasma [HPGP], vaporized hydrogen peroxide [VHP], hydrogen peroxide plus ozone) have been developed and are being used to sterilize surgical and medical devices.[2,4]

In this study, we compared the microbicidal activity of LTS technologies (ie, VHP, ETO, HPGP) to SS in the presence of salt and serum (Table 1).[5] The addition of salt and serum simulates inadequate cleaning of instruments prior to sterilization.[5] To our knowledge, no studies in the peer-reviewed literature have evaluated the microbicidal activity of vaporized hydrogen peroxide, and none has compared 4 of the 5 sterilization technologies currently available in the United States.[2,4–6]

Methods

Brushed stainless steel discs (1 cm in diameter, 0.7 mm in thickness) were used as carriers (Muzeen and Blythe, Winnipeg, Canada). The test organisms (*Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* antibiotic-sensitive clinical isolate, *Staphylococcus aureus* ATCC 6538, and vancomycin-resistant *Enterococcus* [VRE] ATCC 51299) were grown overnight on trypticase soy agar with 5% sheep blood and then used to make a 0.5 McFarland in RPMI 1640 media (~0.65% salt, RPMI media with ATCC modification, Thermo Fisher Scientific, Waltham, MA) containing 10% fetal calf serum (FCS, Gibco, Gaithersburg, MD).[5] *Mycobacterium terrae* ATCC 15755 (10^{11} CFU/mL) was taken from frozen stock and made into a fine homogenous suspension using a tissue grinder. Serial dilutions were then made to produce a 10^8 CFU/mL suspension using RPMI media containing 10% FCS. *Bacillus atrophaeus* (Thermo Fisher Scientific) and *Geobacillus stearothermophilus* spore (Thermo Fisher Scientific) inoculums were made with commercially prepared spore suspensions of ~10^6 spores/0.1 mL. In making these inoculums, 900 µL of the spore suspensions were mixed with 900 µL RPMI 1640 media and 200 µL 10% FCS, with a final salt concentration of 0.29%. The *Clostridioides difficile* spores from a frozen suspension were used to make the inoculum containing 10% FCS

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Table 1. Sterilization Technologies, Cycles and Indicators Used to Assess the Microbicidal Activity of Low-Temperature Sterilization Technologies to Steam Sterilization

Sterilization Unit	Manufacturer, Name and Model	Cycle(s) Used	Chemical Indicator Used	Biological Indicator Used
Steam sterilizer	Steris, Amsco Century V-120 Prevacuum	132°C vacuum-assisted steam sterilization cycle, 4 min	3M Comply SteriGage	3M Attest Rapid 1296 Readout Biological Indicator Steam Pack
100% ethylene oxide (ETO)	Steris Amsco Eagle 3017	High-temperature at 130°F	3M Comply ETO	3M Attest Rapid ETO Test Pack 1298
VHP-V-PRO maX	Steris Amsco V-PRO maX	Non-lumen cycle, 28 min	Steris Verify HPV chemical indicator	Verify V24 self-contained biological indicator
Sterrad NX	ASP Sterrad NX	Non-lumen instruments, 28 min	ASP Sterrad chemical indicator strip	ASP Sterrad Cyclesure 24

and 0.52% salt. The presence of viable *C. difficile* spores was verified before use with a malachite green spore stain, and each suspension was tested according to the Association of Official Analytical Chemists hydrochloric acid protocol.[7]

A 10 µL inoculum of each test suspension containing from 4×10^4 to 3×10^6 test organisms was inoculated in the center of 42 sterile stainless-steel carriers and not dispersed. The carriers were allowed to dry for 1–2 hours in a biological safety cabinet (Labgard, Class II, Type A2, Plymouth, MN). The 42 carriers were divided into 4 sets of 10 carriers, 1 set of 10 carriers for each of 4 sterilizers tested, and 2 control carriers for calculating the viable inoculum per carrier the day of the experiment and 24 hours later (ie, after the ETO cycle). After drying, each set of 10 carriers was labeled with the organism and the sterilizer to be tested and placed on a sterile petri plate. The bottom half of the petri plate was placed in the sterilization pouch according to the manufacturer's guideline for the sterilizer tested, being cautious not to disturb the carriers. Appropriate chemical (each load) and biological indicators (ie, at least daily for SS and LTS) were used to monitor the sterilization process. With 2 exceptions (*C. difficile* spores and steam; *M. terrae* and steam), each organism and sterilizer were tested in triplicate at a minimum, and each replicate contained 10 carriers.

There were no sterilizer indicator failures during the test runs. For example, all chemical indicators demonstrated the appropriate color change indicating the "process" was completed and the biological indicators were negative. The carriers were brought to Central Sterile Processing and were processed in the test sterilizers in an empty load. The sterilizers tested are shown in Table 1. Preventive maintenance was performed on the sterilizers as prescribed by the respective manufacturer.

Once processed through the sterilizer, each carrier was aseptically transferred to 10 mL trypticase soy broth in the biological safety cabinet. The tubes were examined daily, and positive tubes were subcultured to verify the test organism. All tubes were incubated for 7 days at 37°C except the *M. terrae* and *C. difficile*. The *C. difficile* carriers were placed in thioglycollate broth medium, and *M. terrae* carriers were placed in 7H9 broth supplemented with oleic acid, albumin, dextrose, and catalase to enhance growth. Quantitations for *M. terrae* were performed using 7H11 agar and were taped to prevent desiccation, and all *Mycobacterium* broth and plates were incubated at 37°C for 21 days. The *C. difficile* quantitations were plated to sheep blood agar, and all plates and broths were incubated anaerobically using the Pack-Anaero Anaerobic Gas Generating System (Mitsubishi Gas Chemical) at 37°C for 48–72 hours.

To determine whether the presence of salt or serum or both interfered with the VHP, 30 replicates were processed with the stainless-steel carriers in the presence of salt or serum or both using *S. aureus* and *G. stearothermophilus* spores.

We used the Fisher Exact test (2-sided) to compare the inactivation frequency between the different methods of sterilization.

Results

Steam sterilization killed all the test organisms (*P. aeruginosa*, *E. coli*, *vancomycin-resistant Enterococcus*, *S. aureus*, *B. atropheaus* spores, *G. stearothermophilus* spores, *C. difficile* spores, and *M. terrae*) inoculated on the stainless-steel carriers in the presence of salt and serum with no failures (0 of 220 replicates) (Table 2). Similarly, the ETO and the HPGP sterilizers were capable of inactivating the test organisms on stainless-steel carriers with a failure rate of 1.9% for both (ie, 6 of 310 for ETO and 5 of 270 for HPGP) (Table 2). Although steam had no failures compared to both ETO and HPGP, which demonstrated some failures for vegetative bacteria, there was no significant difference comparing the failure rate of steam to either ETO ($P > .05$) or HPGP ($P > .05$). The VHP system failed to inactivate all the test organisms in 76.3% of the tests (206 of 270) (Table 2). On vegetative bacteria (*P. aeruginosa*, *E. coli*, *VRE*, *S. aureus*, and *M. terrae*), VHP had a failure rate of 71.7% (129 of 180), and with the spores (*B. atropheaus* spores, *G. stearothermophilus* spores, and *C. difficile* spores), VHP had a failure rate of 85.6% (77 of 90). The failure rate of VHP was significantly higher than the other technologies evaluated for both vegetative bacteria ($P < .00001$) and spores ($P < .00001$).

When the impact of salt and serum were independently assessed using VHP technology, it was found that salt, not serum, had the most significant effect on sterilization failure (Table 3). When the *S. aureus* and *G. stearothermophilus* spores were tested with 10% FCS only, complete inactivation occurred. The RPMI salts (alone or combined with FCS) significantly interfered with the sterilization process for *S. aureus* (ie, 41 of 60, or 68% failure) and *G. stearothermophilus* spores (ie, 60 of 60, or 100% failure).

Discussion

Sterilization technologies are essential for instrument reprocessing in healthcare facilities. Cleaning, or the removal of visible soil (eg, organic and inorganic material) and microbial contaminants from objects and surfaces, precedes sterilization. Cleaning should consistently and reliably remove and/or reduce the organic and inorganic materials before sterilization to avoid interfering with the effectiveness of sterilization and to ensure a sterility assurance level (SAL) of 10^{-6}.[2–4,8] The criticality of cleaning was reconfirmed by

Table 2. Comparative Evaluation of the Microbicidal Activities of Sterilization Technologies in the Presence of Salt and Serum[a]

Organism	Mean Inoculating Suspension/mL	Mean Carrier Quantitation (Day of Run)	Mean Carrier Quantitation (24 h ETO)	% Failure (Carriers Positive/Carriers Tested)			
				Steam	ETO	HPGP	VHP
Vegetative cells							
Pseudomonas aeruginosa	8.1×10^8	2.0×10^6	3.5×10^4	0 (0/30)	0 (0/50)	0 (0/40)	13 (5/40)
Escherichia coli	1.1×10^9	3.4×10^6	5.1×10^5	0 (0/30)	4 (2/50)[b]	3 (1/40)[b]	75 (30/40)
Vanomycin-resistant enterococci	5.9×10^8	2.8×10^6	2.8×10^6	0 (0/30)	8 (4/50)[b]	10 (4/40)[b]	93 (37/40)
Staphylococcus aureus	4.8×10^8	2.3×10^6	2.5×10^6	0 (0/30)	0 (0/40)	0 (0/30)	93 (28/30)
Mycobacterium terrae	1.4×10^9	5.2×10^4	3.2×10^5	0 (0/20)	0 (0/30)	0 (0/30)	97 (29/30)
Vegetative cells, total				0 (0/140)	3 (6/220)	3 (5/180)	72 (129/180)
Bacillus atropheaus spores	1.5×10^7	1.2×10^5	1.3×10^5	0 (0/30)	0 (0/30)	0 (0/30)	83 (25/30)
Geobacillus stearothermophilus spores	5.4×10^6	5.1×10^4	6.0×10^4	0 (0/30)	0 (0/30)	0 (0/30)	73 (22/30)
Clostridiodes difficile spores	1.3×10^7	4.4×10^4	4.2×10^4	0 (0/20)	0 (0/30)	0 (0/30)	100 (30/30)
Spore total				0 (0/80)	0 (0/90)	0 (0/90)	86 (77/90)
Overall total				0 (0/220)	2 (6/310)	2 (5/270)	76 (206/270)

Note. ETO, ethylene oxide; HPGP, hydrogen peroxide gas plasma; FCS, fetal calf serum; ND, not done.
[a]To simulate inadequate cleaning, the inoculum for the vegetative bacteria contained 10% FCS and 0.65% salt but 10% FCS and 0.29% salt for the spores *B. atropheaus* and *G. stearothermophilus;* and 10% FCS and 0.52% salt *C. difficile* spores
[b]Runs with ETO and HPGP failure of vegetative bacteria had higher carrier quantitation (day of run) than the mean carrier quantitation for the other runs and that organism (ie, 4.07×10^6 vs 2.54×10^6 for VRE; 8.30×10^6 vs 2.40×10^6 for *E. coli*).

Table 3. Ability of Vaporized Hydrogen Peroxide to Inactivate the Microbial Load on Stainless-Steel Carriers in the Presence of Salt or Serum or Both

Organism	Additive	Inoculating Suspension	Carrier Quantitation	% Failure (Carriers Positive/Carriers Tested)
Staphylococcus aureus	10% FCS	1.33×10^8	6.57×10^5	0 (0/10)
				0 (0/10)
				0 (0/10)
	RPMI medium (salts)	3.67×10^8	1.79×10^6	100 (10/10)
				100 (10/10)
				0 (0/10)
	Both	2.93×10^8	1.52×10^6	100 (10/10)
				100 (10/10)
				10 (1/10)
Geobacillus stearothermophilus spores	10% FCS	5.43×10^5	6.03×10^3	0 (0/10)
				0 (0/10)
				0 (0/10)
	RPMI medium (salts)	2.70×10^5	5.47×10^3	100 (10/10)
				100 (10/10)
				100 (10/10)
	Both	5.77×10^5	6.97×10^3	100 (10/10)
				100 (10/10)
				100 (10/10)

Note. FCS, fetal calf serum.

Alfa et al[5] in a classic study that evaluated various LTS technologies and showed sterilization failure in the presence of salt, serum and lumen test units. Given that organic material and salts are known to influence the sterilization capacity of LTS technologies,[5,8,9] we investigated the impact of inadequate cleaning and salt or crystalline residues on the efficacy of the sterilization technologies used in the United States. One LTS technology cleared in the United States for medical and surgical instruments, a hydrogen peroxide-ozone sterilizer, was not evaluated in this study because it was not available at UNC Health Care. A simulated use and clinical in-use study employing this technology was recently published by the manufacturer.[10]

The literature contains a paucity of information on the comparative microbicidal activity of the sterilization technologies cleared by the Food and Drug Administration (FDA) for sterilizing medical and surgical devices.[5,8–12] For example, no studies in the peer-reviewed literature have compared 4 sterilization methods used commonly in US healthcare facilities, and only a few studies have evaluated 3 sterilization technologies.[5,11,12] To our knowledge, no studies in the peer-reviewed literature have evaluated the microbicidal activity of the FDA-cleared VHP sterilizer (Table 1) used for sterilization of medical and surgical instruments. Our results evaluate the comparative efficacy of 4 sterilization techniques using stainless-steel carriers in the presence of salt and serum. The results illustrate that steam sterilization is the most effective and has the largest margin of safety, followed by both EO and HPGP and lastly, VHP.[13] Steam sterilization is the most robust sterilization process and the least affected by protein, salt, and lubricants.[13] The reason that ETO and HPGP had failures with vegetative bacteria but not spores is not clear, but these failures could be attributed to the lower salt concentration for spores (0.29%) than vegetative bacteria (0.65%) or to higher microbial load (see Table 2). VHP has a significantly narrower margin of safety in killing vegetative bacteria and spores in the presence of a salt and serum challenge. These findings support the findings of Alfa et al[5] for ETO and HPGP on the effect of salt and serum on sterilization efficacy.

The findings regarding the robustness of steam sterilization should not be used to suggest that cleaning is unimportant for steam sterilization. In contrast, the data demonstrate how important cleaning is prior to sterilization because salt and organic matter left on instruments can interfere with sterilization. These results, and those of other investigators,[5,9,12,13] highlight the importance of real-time monitoring methods prior to sterilization that are reliable and validated and that assess the effectiveness of cleaning that is predictive of microbial contamination, infection risk, and/or an SAL of 10^{-6}.[14] Current assessment tools (eg, visual, adenosine triphosphate [ATP]) are not predictive of microbial contamination or infection risks. Investigators have demonstrated that visual assessment and ATP lack the sensitivity required to ensure effective decontamination.[15,16]

In the experiments with VHP, salt was the factor that significantly impaired the sterilization outcome because carriers inoculated with organisms (ie, *S. aureus* and *G. stearothermophilus* spores) and 10% FCS alone were sterile (ie, 0 of 60). Several investigators have shown that spores and bacteria occluded inside salt crystals were very resistant to LTS technologies.[5,17,18] Alfa et al[18] found that salt was the principal compounding factor that interfered with ETO sterilization; similarly, salt was the principal component that interfered with VHP. This interference may be due to the crystalline matrix or salt crystals impeding the penetration of the sterilant to the spore.[17,18] A study conducted in 1967 demonstrated that protection of organisms by crystalline material was not limited to LTS technologies but also applied to moist and dry heat.[19] In this evaluation, we considered factors that can interfere with sterilization such as salt, organic matter, and a concentrated inoculum (not dispersed), and we used stainless-steel carriers, which allowed direct exposure of the microbes to the sterilant. Complex medical (eg, lumens [length, diameter], scratches, and crevices) and surgical instruments (eg, lumens [length, diameter], hinged instruments, and robotic instruments) would represent a greater challenge to sterilization. Thus, LTS technology will need to be optimized to achieve an SAL of 10^{-6} (eg, an adapter supplying an additional source of hydrogen peroxide) for complex, lumened instruments such as endoscopes.[14,20] Other factors can affect the sterilization process: different salts, carriers, microbial load, device design (eg, hinges), restrictive flow (eg, sharp bends, blind lumens), construction materials, and type of simulated soil.[11,12] The impact of naturally occurring build-up biofilm on medical and surgical instruments and whehter these materials could be a source of microorganisms that increases the risk of sterilization failure and infection needs to be better understood.[3]

In summary, our findings demonstrate the limitations of sterilization technologies. The results illustrate that the organic and salt challenges used in this investigation had no effect on SS, had minimal effect on ETO and HPGP, but significantly impaired VHP. Clearly, an SAL of 10^{-6} was not consistently achieved under these experimental conditions. These findings reinforce the need for meticulous cleaning and reliable and validated cleaning monitoring methods that are predictive of infection risk.

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Letter to the Editor

Reply to "Comparative evaluation of the microbicidal activity of low-temperature sterilization technologies to steam sterilization"

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To the Editor—This letter is in response to the article by Rutala et al[1] that compared the microbial kill of steam, ethylene oxide (ETO), hydrogen peroxide gas plasma (HPGP), and vaporized hydrogen peroxide (VHP) in the presence of salt and serum in standard sterilization cycles.

Unfortunately, at this time, there are no 'standard' gaseous hydrogen peroxide sterilization processes. The article fails to consider that although both HPGP and VHP processes use gaseous hydrogen peroxide as the sterilant, the processes are distinct and different in the way they operate. Even though 28-minute HPGP and VHP cycles are used, these cycles use significantly different concentrations of sterilant. The HPGP exposure is 25.6 mg/L H_2O_2 for 7 minutes whereas the VHP exposure is 9.1 mg/L H_2O_2 for 12 minutes. The importance of disinfectant concentration is explained in the 2008 CDC Guideline for Disinfection and Sterilization in Healthcare Facilities where it is stated that "The more concentrated the disinfectant, the greater is its efficacy and the shorter the time necessary to achieve microbial kill."[2] For these evaluations with no chamber load, sterilant concentration should have been considered.

The delineation of the gaseous hydrogen peroxide processes like HPGP and VHP, with the subsequent comparisons of efficacy minus any consideration of sterilant concentration, seems to imply that there is a benefit from plasma within the sterilization process. This contention contradicts the current understanding of the purpose of a gas plasma in HPGP systems, in which it is known that the plasma step has little to no contribution to sterilizer efficacy. In the only research ever published to evaluate the impact of plasma in a HPGP process, the plasma phase appeared to be nonsporicidal.[3]

The detoxifying (residual sterilant removing) effect of the plasma would have no impact on gaseous hydrogen peroxide microbial lethality; thus, the ~3-fold sterilant concentration difference (25.6 vs 9.1 mg/L H_2O_2 for the HPGP and VHP systems, respectively) is clearly responsible for the observed efficacy differences in HPGP and VHP processes. Higher concentration is not always beneficial. Beyond efficacy, hospitals also consider the gentleness of the sterilization process to include the potential impact of higher sterilant concentrations and higher sterilant dose on device material compatibility (especially devices susceptible to reaction with the highly oxidizing hydrogen peroxide sterilant) or device biocompatibility as well as the potential impact of plasma on medical device surfaces.

Both the HPGP and VHP sterilization cycles have been cleared by the Food and Drug Administration (FDA), so both have demonstrated the ability to achieve a sterility assurance level (SAL) of 10E-6 for their claimed processes. The CDC disinfection guidelines[2] specify that even salts dissolved within surrogate body fluids dissolve with 60 seconds of nonflowing water; therefore, showing that, from a use perspective, the protective nature of salt has little clinical relevance. Although salt has been shown historically by many investigators to potentially impede hospital sterilization of medical devices, the emphasis of these results should be to highlight the need for thorough cleaning methodologies.

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Reply to Randal W. Eveland regarding comparative evaluation of the microbicidal activity of low-temperature sterilization technologies to steam sterilization

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To the Editor—We thank Dr Randal Eveland, Steris Corporation, for his letter regarding our paper that compared the microbicidal activity of low-temperature sterilization technologies (ie, vaporized hydrogen peroxide [VHP], ethylene oxide [ETO], and hydrogen peroxide gas plasma [HPGP]) to steam sterilization in the presence of salt and serum to simulate inadequate precleaning.[1] As noted in our paper, the literature contains a paucity of information on the comparative microbicidal activity of the sterilization technologies cleared by the Food and Drug Administration (FDA) for sterilizing medical and surgical devices. We believe that the data from this study will help clinicians in infection prevention assess the robustness of healthcare sterilization technologies and the risk of infection to patients when an uncleaned instrument is unintentionally brought into the operating room or used on a patient.

We agree with Dr Eveland there are differences in concentration and duration of the VHP and HPGP cycles. Our experiments compared the microbicidal activity of FDA-cleared, low-temperature sterilization technologies to steam sterilization in the presence of salt and serum. The addition of salt and serum simulated inadequate cleaning of instruments prior to sterilization. We evaluated the "robustness" of sterilization technology that is used by hospitals throughout the United States. Robustness is defined as the ability to withstand and overcome adverse conditions or rigorous testing.

Concerning plasma in the HPGP technology, our intention was not to define the components of the cycle that created the robustness (eg, higher concentrations of hydrogen peroxide, plasma); it was solely to define whether FDA-cleared sterilization technologies had the same robustness or ability to inactivate microorganisms in the presence of organic matter and salt. Our results demonstrated that some sterilization technologies were more "forgiving" or safe when cleaning is not complete. Because protein (organic matter) remains on cleaned surgical instruments,[2] we must investigate at what point the presence of protein overwhelms the ability of the sterilizer to inactivate contaminating microorganisms. Alternatively, we should consider using the most robust sterilization technologies that inactivate microorganisms in the presence of organic matter and salt when possible.

Regarding the comparison of HPGP to VHP and materials compatibility, there are other factors involved in materials compatibility than the hydrogen peroxide concentration alone. Although the theoretical concentration of hydrogen peroxide for HPGP is higher than for VHP (ie, 25.6 vs 9.1 mg/L hydrogen peroxide for the HPGP and VHP, respectively), the plasma process quickly removes the hydrogen peroxide from the load by dissociating unreacted hydrogen peroxide into oxygen and water, eliminating the need for aeration.[3] The VHP sterilizer passes hydrogen peroxide through a catalytic converter where it is reduced to water and oxygen. The HPGP system has 3 potential advantages. First, because the plasma quickly removes the residual hydrogen peroxide, rather than a gradual release with VHP, there may be improved material compatibility and biocompatibility. However, we have not been able to find any data on the internet or in the peer-reviewed literature that demonstrated that VHP is more or less materials compatible or biocompatible than HPGP. Second, regarding environmental hydrogen peroxide levels, for both sterilizers there were no notable emissions from the sterilizers during the cycle. However, other investigators measured significant hydrogen peroxide emissions when the VHP chamber door was open compared

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to the HPGP (eg, hydrogen peroxide ranging from 5 to 17 ppm for VHP vs ≤0.3 ppm for HPGP).[3] This demonstrated that HPGP emits less hydrogen peroxide into the breathing zone of the operator who opens the sterilizer door to remove the load. Third, the higher concentration of hydrogen peroxide in the HPGP sterilizer is a potential safety factor, which resulted in the significantly higher margin of safety for HPGP vs VHP (ie, failure rate of HPGP of 1.9% vs 76.3% for VHP).

As it pertains to FDA clearance and the test methodology, there are 2 issues. First, there are limitations to the effectiveness of sterilization technologies even though they are FDA-cleared and have been demonstrated to achieve a sterility assurance level of 10^{-6} under the test conditions. In our study, we assessed the margin of safety or robustness associated with the sterilization technologies currently used in healthcare facilities. Steam sterilization, which is most common and used for sterilization of instruments that are heat resistant, is the most effective and robust sterilization technology. Salt was the principal component that interfered with VHP, which is likely due to the salt crystals impeding the penetration of the sterilant to the microbe. Second, many salts (eg, most sodium, potassium and ammonium salts) are soluble in water and dissolve in water and are removed from surgical instruments when immersed in water[4]; however, some salts are insoluble or have a low solubility in water. Some salts, such as calcium carbonate, can occlude microbial exposure and dramatically affect the time required for inactivation. For example, the time required for inactivation of 8.0×10^3 *Bacillus subtilis* spores by ethylene oxide (1,200 mg/L) at 54°C with unoccluded spores is 30 seconds, but for spores occluded in calcium carbonate, the inactivation time is >2 weeks.[5] Additionally, to understand the dynamics of a cleaning process and the potential effect of soils on a sterilization process, the different ingredients in the soil (ie, organic to inorganic ratio) need to be examined independently.[4]

Lastly, as demonstrated by this study, not all sterilization technologies used in healthcare to sterilize surgical instruments are equal and have the same robustness. Although FDA-cleared sterilization technologies theoretically kill a very large number of microorganisms on instruments (eg, 12 logs or a trillion), healthcare personnel can unintentionally impede the effectiveness of sterilization technology by improper cleaning of the instruments prior to sterilization. Cleaning, or the removal of visible soil and microbial contaminants from objects, precedes sterilization. If instruments are not properly cleaned prior to sterilization and are then placed in a low-temperature sterilization technology such as VHP, there is a possibility of failure. However, the robustness of some sterilization technology, such as steam sterilization, makes it exceedingly unlikely that a steam-sterilized instrument will be the source of infection. Most medical and surgical devices used in healthcare facilities are made of materials that are heat stable and thus are sterilized by heat, primarily steam sterilization. The data demonstrate how important cleaning is prior to sterilization because salt and organic matter left on instruments can interfere with low-temperature sterilization. These findings reinforce the need for meticulous cleaning and for reliable and validated cleaning monitoring methods that are predictive of microbial contamination and infection risk.

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