

A COMPARATIVE STUDY OF ETHYLENE OXIDE GAS, HYDROGEN PEROXIDE GAS PLASMA, AND LOW-TEMPERATURE STEAM FORMALDEHYDE STERILIZATION

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ABSTRACT

OBJECTIVE: To compare the efficacies of ethylene oxide gas (EOG), hydrogen peroxide gas plasma (PLASMA), and low-temperature steam formaldehyde (LTSF) sterilization methods.

METHODS: The efficacies of EOG, PLASMA, and LTSF sterilization were tested using metal and plastic plates, common medical instruments, and three process challenge devices with narrow lumens. All items were contaminated with *Bacillus stearothermophilus* spores or used a standard biological indicator.

RESULTS: EOG and LTSF demonstrated effective killing of *B. stearothermophilus* spores, with or without serum, on plates, on instruments, and in process challenge devices. PLAS-

MA failed to adequately sterilize materials on multiple trials in several experiments, including two of three plates, two of three instruments, and all process challenge devices.

CONCLUSIONS: Our results suggest that PLASMA sterilization may be unsuccessful under certain conditions, particularly when used for items with complex shapes and narrow lumens. Alternatively, LTSF sterilization demonstrates excellent efficacy and is comparable to EOG sterilization. LTSF could potentially act as a substitute if EOG becomes unavailable due to environmental concerns (*Infect Control Hosp Epidemiol* 2005;26:486-489).

Autoclaves are often used to sterilize medical instruments composed of heat-resistant material.¹ However, ethylene oxide gas (EOG), hydrogen peroxide gas plasma (PLASMA), and low-temperature steam formaldehyde (LTSF) sterilization methods are employed when materials are not heat resistant.² Understanding the strengths and limitations of various methods is essential to obtain optimal results. Ideally, a device has a smooth surface, has a simple structure, and can be washed easily prior to sterilization. However, many instruments have complex structures and may be difficult to wash. Sometimes sterilization is performed without the complete removal of adherent material such as blood or sputum. Primary washing significantly impacts the efficacy of sterilization^{3,5} and is a vital factor when items contain bacteria or spores.

Few published studies have directly compared low-temperature sterilization methods.^{6,7} We sought to compare the efficacies of EOG, PLASMA, and LTSF sterilization methods for structurally simple and complex devices contaminated with bacterial spores.

METHODS

Sterilization Systems

The low-temperature sterilization systems examined included EOG (ΣIIER-012W, Sakura Seiki, Tokyo, Japan),

PLASMA (STERRAD 100, Johnson & Johnson, Tokyo, Japan), and LTSF (GEF 449, Getinge AB, Getinge, Sweden). The LTSF system used a 37% formaldehyde solution (Wako Pure Chemical Industries, Osaka, Japan). The programs for each system are detailed in Table 1.

Biological Indicators and Bacterial Spores

Biological indicators were as follows: for the EOG system, a filter paper containing 4.0×10^6 spores of *Bacillus subtilis* var *niger* (American Type Culture Collection 9372, Simicon, Munich, Germany); and for the LTSF system, a filter paper containing 1.4×10^6 spores of *B. stearothermophilus* (American Type Culture Collection 7953, Simicon). Because there is not a standard biological indicator for PLASMA sterilization, we used the same filter paper, containing 1.4×10^6 spores of *B. stearothermophilus*, that was used for the LTSF system. We used the original spore suspension of *B. stearothermophilus* (American Type Culture Collection 7953), containing 1.2×10^8 spores/mL of 99% ethanol, and prepared three spore suspensions containing 0.6×10^9 colony-forming units/mL suspended in 6 g/mL of bovine serum albumin (BSA), 0.6 g/mL of BSA, and 0.9% saline. Preparation was as follows: 1 mL of the original suspension was centrifuged (15,000 rpm for 5 minutes) and the ethanol supernatant was

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TABLE 1
LOW-TEMPERATURE STERILIZATION SYSTEM PROGRAMS

System	Temperature (°C)	Duration of	
		Sterilization (min)	Total Program Time (min)
EOG	45	240	1,010
PLASMA	45	19*	75
LTSF	80	10	160

EOG = ethylene oxide gas; PLASMA = hydrogen peroxide gas plasma; LTSF = low-temperature steam formaldehyde.

*Total time of PLASMA condition.

discarded. After that, the spore sediment was suspended in 200 µL of bovine serum (6 g/100 mL), 1/10 of bovine serum (0.6 g/100 mL), or 0.9% saline.

Materials

The initial experiments used plates made of stainless steel, copper, and low-density polyethylene, each measuring $1 \times 4 \text{ cm}^2$. Ten microliters of the three bacteria spore suspensions (6 g/mL of BSA, 0.6 g/mL of BSA, and 0.9% saline) was spread onto each plate's surface and allowed to dry. After placement into a test tube to prevent contamination, plates were sterilized by EOG, PLASMA, or LTSF. Each condition was repeated three times. Afterward, on a clean bench equipped with horizontal laminar filtrated air-flow, the plates were placed in a sterilized pouch containing 10 mL of tryptone soy broth-soybean casein digest medium (TSB) (CM129, Oxoid, Hampshire, England) in 0.05% Tween 80 (Kanto Chemical Co., Tokyo, Japan). Each plate was washed thoroughly in broth by rubbing it in the pouch. After washing, the TSB was transferred to a sterile tube and incubated at 90°C for 60 minutes and then placed in a water

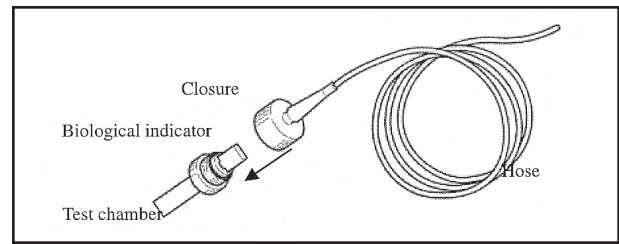


FIGURE. The process challenge device.

bath at $56 \pm 2^\circ\text{C}$ for 7 days. If turbidity could be observed with the naked eye, the test result was considered positive. Bacteria were confirmed as gram-positive bacilli by Gram stain.

Three commonly used medical tools, Haemostatic forceps (straight 140 mm, Shanghai, China), a dissector (MH-370, Olympus, Tokyo, Japan), and an airway tube (100/303 Portex, Smith Medical Co., Ltd., London, England), were contaminated with *B. stearotherophilus* spores. Because the tool surfaces were too smooth to allow adherence of serum or saline, the spores were diluted in 99% ethanol. Thirty microliters of the resulting suspension (containing 3.6×10^6 spores) was applied to the irregular surface of the tool (eg, the serrated tips of the forceps) using a micropipette and allowed to dry. Next, each item was packed in a sterilization bag: HM304 (Hogi Medical, Tokyo, Japan) for EOS and LTSF and a STERRAD bag for PLASMA (Johnson & Johnson). After sterilization, the packages were opened and cultured in the same manner as described for the steel, copper, and polyethylene plates.

The process challenge device (Figure), Helix PCD (Getinge AB), was composed of two portions: a chamber used to place the biological indicator and a hose made of

TABLE 2
EFFICACIES OF LOW-TEMPERATURE METHODS TO STERILIZE MATERIALS CONTAMINATED WITH *BACILLUS STEAROTHERMOPHILUS* SPORES

Material	Spore Suspension	Sterilization System									
		EOG			PLASMA			LTSF			
		1	2	3	1	2	3	1	2	3	
Stainless steel plate	6 g/mL of serum	-	-	-	-	-	-	-	-	-	-
	0.6 g/mL of serum	-	-	-	-	-	-	-	-	-	-
	0.9% saline	-	-	-	-	+	-	-	-	-	-
Copper plate	6 g/mL of serum	-	-	-	-	-	-	-	-	-	-
	0.6 g/mL of serum	-	-	-	-	-	-	-	-	-	-
	0.9% saline	-	-	-	-	-	-	-	-	-	-
Polyethylene plate	6 g/mL of serum	-	-	-	-	-	-	-	-	-	-
	0.6 g/mL of serum	-	-	-	-	-	-	-	-	-	-
	0.9% saline	-	-	-	-	-	+	-	-	-	-

EOG = ethylene oxide gas; PLASMA = hydrogen peroxide gas plasma; LTSF = low-temperature steam formaldehyde.

TABLE 3

EFFICACIES OF LOW-TEMPERATURE METHODS TO STERILIZE MEDICAL INSTRUMENTS CONTAMINATED WITH *BACILLUS STEAROTHERMOPHILUS* SPORES

Instrument	Sterilization System								
	EOG			PLASMA			LTSF		
	1	2	3	1	2	3	1	2	3
Forceps	-	-	-	-	+	-	-	-	-
Dissector	-	-	-	-	+	-	-	-	-
Airway tube	-	-	-	-	-	-	-	-	-

EOG = ethylene oxide gas; PLASMA = hydrogen peroxide gas plasma; LTSF = low-temperature steam formaldehyde.

TABLE 4

GROWTH FROM BIOLOGICAL INDICATORS AFTER STERILIZATION OF PROCESS CHALLENGE DEVICES

Process Challenge Device	Positive Control	Sterilization System								
		EOG			PLASMA			LTSF		
		1	2	3	1	2	3	1	2	3
Helix PCD* (ID, 2 mm; hose, 1.5 m)	+	-	-	-	+	-	+	-	-	-
Modified process challenge device (ID, 0.96 mm; hose, 1.5 m)	+	-	-	-	+	+	+	-	-	-
Modified process challenge device (ID, 0.96 mm; hose, 3.0 m)	+	-	-	-	+	+	+	-	-	-

ID = internal diameter; EOG = ethylene oxide gas; PLASMA = hydrogen peroxide gas plasma; LTSF = low-temperature steam formaldehyde.

*Getinge AB, Getinge, Sweden.

polytetrafluoroethylene (internal diameter, 2 mm; length, 1.5 m). We also tested two modified process challenge devices with more complex shapes. In these, a hose with an internal diameter of 0.96 mm and an outer diameter of 1.56 mm was connected to the Helix chamber. The hose was either 1.5 or 3.0 m long. In each experiment, air tightness between the chamber and the hose was confirmed. Each process challenge device was double packed into an HM304 or a STERRAD bag before sterilization. A biological indicator was placed into the process challenge device before sterilization. An unsterilized biological indicator was used as the positive control for each experiment. After sterilization, the biological indicator was removed, placed in a test tube containing 10 mL of TSB, and incubated at 90°C for 60 minutes. The samples of *B. stearothermophilus* were then incubated in a water bath at 56 ± 2°C for 7 days while the samples of *B. subtilis* var *niger* were incubated at 33°C for 7 days in a separate water bath. If turbidity could be observed with the naked eye, the test result was considered positive. Bacteria were confirmed as gram-positive bacilli by Gram stain.

RESULTS

Sterilization of all plates using EOG and LTSF resulted in complete eradication of *B. stearothermophilus* spores in both the presence and the absence of serum. However, two samples subjected to PLASMA sterilization yielded positive cultures (Table 2). Experiments using medical instruments demonstrated similar results with EOG and LTSF, with negative cultures from forceps, dissectors, and airway tubes in all cases. The PLASMA system did not ad-

equately sterilize the forceps and dissector in one of three trials (Table 3).

Similarly, experiments with process challenge devices demonstrated that EOG and LTSF completely eradicated the spores from the Helix PCD and two modified devices. The PLASMA method was unsuccessful in two of three trials with the Helix PCD and in all trials with the modified process challenge devices (Table 4).

DISCUSSION

EOG, PLASMA, and LTSF are low-temperature sterilization systems indicated for materials that are not heat sensitive.⁸ The sporicidal efficacy and safety (in terms of residual formaldehyde) of LTSF sterilization has been previously reported.⁹ Equipment requiring sterilization varies in design, durability, and cost. Selecting an appropriate sterilization technique based on design characteristics of materials is essential. In clinical settings, there is a demand for low-cost, high-performance methods with short handling time and low toxicity.¹⁰⁻¹⁴ However, the optimal means to sterilize specific objects is not always clear.

Although the importance of washing items before sterilization is well documented, thorough washing of instruments of complex design and shape may not be possible. Sometimes washing is performed inappropriately.¹⁵⁻¹⁷ The impact of insufficient washing on the efficacies of EOG, PLASMA, and LTSF is an important issue. Our study was designed to assess efficacy when objects were not washed prior to sterilization.

We tested instruments that were difficult to wash to assess and compare the efficacies of EOG, PLASMA,

and LTSF. Regardless of complexity, both EOG and LTSF resulted in eradication of bacterial spores in all experiments. However, PLASMA methods produced variable results. In particular, adequate sterilization was not achieved when instruments of complex structure were tested, even those used routinely in clinical practice. PLASMA fared even more poorly in experiments with process challenge devices. These results suggest that PLASMA sterilization should be discouraged, particularly when the instrument is not washed or is inadequately washed prior to sterilization. These results are similar to those reported by Gaspar et al.²

In recent years, several nosocomial infections have been reported related to the use of flexible endoscopes.¹⁸⁻²⁰ With this in mind, we sought to compare the efficacies of low-temperature methods using modified process challenge devices, as the hollow portion of some endoscopes is narrower than the Helix PCD. When items that are longer and narrower than the lumen specified on the STERRAD web site are to be sterilized, the manufacturer of the PLASMA system recommends attachment of a cassette that contains a high concentration of hydrogen peroxide into one side of the lumen. Our results demonstrated that PLASMA sterilization is inadequate for hollow items with a narrow internal diameter (confirming the recommendations provided by the manufacturer). The modified 1.5- and 3.0-m process challenge devices were not previously standardized but were created to validate the capabilities of the different sterilizers under challenging conditions. The modified process challenge devices were more difficult to sterilize because of their narrower internal diameters and longer hoses compared with the Helix PCD. EOG and LTSF efficiently sterilized the biological indicator in both modified process challenge devices in all three experiments, whereas the PLASMA sterilizer failed to do so in all three experiments.

Our results demonstrated similar efficacies of EOG and LTSF in sterilization of the modified process challenge devices. Many complex instruments used clinically require sterilization. It is unclear whether the Helix PCD is representative of all such instruments. Thus, there is a need to design and validate a standardized protocol to further examine the efficacy of low-temperature sterilization methods.

This study demonstrated that EOG and LTSF systems are efficacious for simple as well as complex instruments contaminated with bacterial spores and serum. Because of environmental concerns, we expect the Japanese market to

increase the use of LTSF rather than EOG for low-temperature sterilization. Our results suggest that PLASMA methods may be inadequate for sterilizing complex instruments, especially those with long, narrow lumens that cannot be properly washed prior to sterilization.

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