Original

Evaluation of Low-Temperature Sterilization using Hydrogen Peroxide Gas Containing Peracetic Acid

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Received 27 March, 2020/Accepted 21 May, 2020

In low-temperature sterilization for the medical field, hydrogen peroxide sterilization is widely used for its safety. However, its low penetrability and residual amount of sterilant are major concerns. Recently, the combination of hydrogen peroxide and peracetic acid has been found to enforce sporicidal effect, with low concentration in hydrogen peroxide. The application of this finding in medical sterilization is still very limited. To elucidate the combination effect, we compare peracetic acid containing hydrogen peroxide gas sterilizer and conventional hydrogen peroxide gas (plasma) sterilizers. The sterilant penetrability was examined in hollow load process challenge devices with inner diameters of 1 and 2 mm and lengths of 1, 2, and 3 m. As a result, peracetic acid containing hydrogen peroxide gas sterilizer demonstrated total inactivation with all diameters and lengths and achieved the highest sterilant penetrability in this study. The amount of residual sterilant on the surface of the sterilized object was 4.2 µg/cm², which corresponds to half amount of those of conventional hydrogen peroxide gas sterilizers. These results suggest that the addition of peracetic acid to hydrogen peroxide gas sterilizer can enhance sterilization efficiency and safety.

Key words : Low-temperature sterilization / Peracetic acid / Sterilant penetrability / Hollow load process challenge device / Residual sterilant.

INTRODUCTION

Low-temperature sterilization methods, including ethylene oxide gas (EOG) sterilization, low-temperature steam formaldehyde sterilization, and hydrogen peroxide gas (plasma) sterilization, are widely used to sterilize materials such as non-heat resistant plastic and rubber products (Dai et al., 2016; Yoshino et al., 2015). The hydrogen peroxide gas (plasma) sterilization method has been widely used in the medical field (Japanese Society of Medical Instrumentation, 2014), because it uses a sterilant with lower toxicity than those in EOG sterilization or low-temperature steam formaldehyde sterilization methods (Bathina et al., 1998). However, the hydrogen peroxide gas (plasma) sterilization method has a limited compatibility range due to the low

penetrability of the hydrogen peroxide (Kanemitsu et al., 2005). In recent years, the combination of peracetic acid with hydrogen peroxide induce synergistic effect for sporicidal activity (Anouar et al, 1993; Leggett et al., 2016). However, application of this mechanism for sterilization in medical field is very limited.

In the hydrogen peroxide gas (plasma) sterilization method, hydrogen peroxide is decomposed into water and oxygen after completion of the sterilization process, and it is considered safe for humans and the environment; however, there are reported cases that hydrogen peroxide gas causes mucosal irritation symptoms in the eye, pharynx and respiratory tract (Yoshida and Kobayashi, 2012a). In these cases, decomposition of hydrogen peroxide is considered to be insufficient and residual hydrogen peroxide exist on sterilized material (Yoshida and Kobayashi, 2012b). The half-life of hydrogen peroxide is one day (JETOC, 1999) and it takes effort to decompose hydrogen peroxide sufficiently after

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TABLE 1. Operation parameters of each sterilizer

Model	hydrogen peroxide gas plasma sterilizer	hydrogen peroxide gas sterilizer	peracetic acid containing hydrogen peroxide gas sterilizer
Sterilization condition			
Sterilization temperature (Catalog value)	47-55 °C	55 °C	50-55 °C
Cycle time	24-60 min	28-50 min	30-47 min
Sterilant concentration	Conc.cycle: H ₂ O ₂ (80-90%) Non-conc.cycle: H ₂ O ₂ (58%)	Conc.cycle: H ₂ O ₂ (80-90%) Non-conc.cycle: H ₂ O ₂ (59%)	Non-conc.cycle: H ₂ O ₂ (8%) Non-conc.cycle: PAA(Less than 10%)
Number of sterilant injections	2	4	2
Plasma	Yes (Sterilization process)	No	Yes (Ventilation process)
Number of ventilations	1	5 to 10	4

The operation parameters of three models of low temperature gas sterilizers were shown. Each parameter was described based on the catalog.

sterilization step. Peracetic acid is known as an unstable chemical compound and decomposition of it proceed effectively after sterilization step because its half time is 22 minutes (JETOC, 2008). Therefore, partial replacement of hydrogen peroxide by peracetic acid could decrease the amount of residual sterilant.

In this study, we tested peracetic acid containing hydrogen peroxide gas sterilizer to elucidate the effect of adding peracetic acid to hydrogen peroxide on the sterilization efficiency and safety. The sterilizer is examined in terms of the penetrability of the sterilant and the amount of residual sterilant in comparison with conventional hydrogen peroxide gas (plasma) sterilizers.

MATERIALS AND METHODS

Sterilization system

The three models of sterilizer examined included hydrogen peroxide gas plasma sterilizer (STERRAD® 100NX, Advanced Sterilization Products, Ethicon, Inc., Irvine, CA, USA), hydrogen peroxide gas sterilizer (ES-1400, Canon Lifecare Solutions Inc., Tokyo, Japan) and peracetic acid containing hydrogen peroxide gas sterilizer (STERIACE 100, Saraya, Osaka, Japan). The operation parameters of each sterilizer are listed in Table 1. Each sterilizer has several operation modes; hydrogen peroxide gas plasma sterilizer has Standard cycle (concentrated: hydrogen peroxide concentration 90%), Flex cycle (concentrated: hydrogen peroxide

concentration 80%), Duo cycle (non-concentrated: hydrogen peroxide concentration 58%) and Express cycle (non-concentrated: hydrogen peroxide concentration 58%). hydrogen peroxide gas sterilizer has Standard mode (concentrated: hydrogen peroxide concentration 80%), Soft mode (non-concentrated: hydrogen peroxide concentration 59%) and Short mode (non-concentrated: hydrogen peroxide concentration 59%). Peracetic acid containing hydrogen peroxide sterilizer has Standard cycle (non-concentrated: hydrogen peroxide concentration 8%, peracetic acid <10%) and Quick cycle (non-concentrated: hydrogen peroxide concentration 8%, peracetic acid <10%). The sterilant used for peracetic acid containing hydrogen peroxide sterilizer contains acetic acid, and the concentration of the acetic acid has not been announced.

Evaluation of sterilization temperature

The temperature inside the sterilization chamber was measured using a data logger (Temperature Data Logger HiTemp 140, Madge Tech, Warner, NH, USA) installed in the sterilization rack of each device in the vicinity of the door, center, back, top, and bottom of the rack, as previously described (Noda et al., 2016). The spatial distribution of the temperature in the sterilization chamber was then measured to identify hot and cold spots.

FIG. 1. Equipment for the penetration test of sterilants.

(a) Biological indicators (BIs) from three manufacturers were used. BI (A): Johnson & Johnson; BI (B): Fukuzawa; and BI (C): Saraya. (b) Each BI was inserted into a syringe. (c) A Teflon or stainless-steel tube was connected to the adaptor of a syringe containing a BI, and the syringe with the attached tube was used as a process challenge device (PCD). The PCD was placed in a cold spot in the sterilizer.

Evaluation of sterilant penetrability

To evaluate the sterilant penetrability in each sterilizer, a disposable syringe (10 ml) was installed on one end of a Teflon™ or stainless-steel tube. Tubes were prepared with inner diameters of 1 and 2 mm and lengths of 1, 2, and 3 m, and the tip of the disposable syringe was inserted into one end of the tube, as previously described (Tsurushima et al., 2013). A hollow load process challenge device (PCD) was prepared by inserting a biological indicator (BI) into the prepared syringes. Three types of BI were used [BI (A): Johnson & Johnson; BI (B): Fukuzawa BI (C): Saraya], all of which contained $\geq 10^6$ *Geobacillus stearothermophilus* ATCC 7953 spores.

These were placed in cold spots in each sterilizer and sterilized using a half-cycle method to compare the penetrability of the three-sterilant (Fig. 1). For the test, the hydrogen peroxide gas plasma sterilizer was operated in the standard cycle, the hydrogen peroxide gas sterilizer in the standard mode, and the peracetic acid containing hydrogen peroxide gas sterilizer in the standard mode, and the test was performed three times for each sterilizer.

Evaluation of residual sterilant

The amount of residual sterilant remaining on the surface of the object to be sterilized was measured using a reflection-type photometer (RQflex Plus, Merck, Darmstadt, Germany). The amounts of hydrogen peroxide and peracetic acid was evaluated by Reflectoquant® Peroxide Test and Reflectoquant® Peracetic Acid Test strips (Reflectoquant, Sigma-Aldrich, St. Louis, MO, USA), respectively. Silicone rubber, which is porous and easily retains sterilant, was used as the object to be sterilized in the evaluation. Silicone rubber wrapped in a sterilized bag was placed in the upper-middle of the sterilization chamber and sterilized. After sterilization, the sterilized bag was opened, the silicone rubber was immersed in recovery solution (purified water), and the residual components were recovered for 10 min. Subsequently, each test strip for hydrogen peroxide and peracetic acid was immersed in the recovered solution for 2 s, and the amount of residual sterilant per unit area was measured with a reflection photometer.

TABLE 2. Measurement of sterilization temperature.

Model Measurment	hydrogen peroxide gas plasma sterilization	hydrogen peroxide gas sterilization	peracetic acid containing hydrogen peroxide gas sterilization
Cold spot	Bottom front	Bottom front	Bottom front
Hot spot	Upper center	Upper back	Upper back
Hot spot (Max temperature, Cycle)	65.4 °C Standard cycle	58.4 °C Soft mode	54.5 °C Standard cycle

The temperature inside chamber were measured by data loggers. They were installed at 9 points in the upper and lower sterilization rack and were used to determine the cold spot and hot spot in each sterilizer.

RESULTS

Evaluation of sterilization temperature

The observed cold spots in all three sterilizers were near the lower door. Considering hot spots, that in the hydrogen peroxide gas plasma sterilizer was in the upper middle of the chamber, whereas those in the hydrogen peroxide gas sterilizer and peracetic acid containing hydrogen peroxide gas sterilizer were in the upper back. The sterilization temperature at the hot spot of each sterilizer was measured. The highest temperature at the hot spot among the three models was 65.4 °C in the hydrogen peroxide gas plasma sterilizer standard cycle. The peak temperature of the hydrogen peroxide gas sterilizer was 58.4 °C in soft mode, and that of the peracetic acid containing hydrogen peroxide gas sterilizer was 54.5 °C in the standard cycle (Table 2).

Evaluation of sterilant penetrability

Three types of integrated media BI were used based on the recommendation for each sterilizer to achieve a fair comparison of the three sterilizers. The sensitivity of the BIs was predicted to differ because of differences in the structure of the vial and spore application methods of the BIs. The results were judged as positive if there was failure in even one trial to kill spores in the BI, and negative if all three trials were successful in killing spores (Table 3).

For the evaluation using the Teflon tube, BI (A) showed a positive reaction in all tubes in the hydrogen peroxide gas plasma sterilizer and hydrogen peroxide gas sterilizer, whereas BI (B) showed a negative reaction in all cases. For BI (C), the hydrogen peroxide gas plasma sterilizer showed a positive reaction in all

tubes except the $\phi 2$ mm $\times$ 1 m tube, and the hydrogen peroxide gas sterilizer showed a positive reaction with the $\phi 1$ mm $\times$ 3 m tube. The peracetic acid containing hydrogen peroxide gas sterilizer showed negative reactions with all BI and tube combinations.

In evaluation with a stainless-steel tube, in the hydrogen peroxide gas plasma sterilizer and hydrogen peroxide gas sterilizer, BI (A) showed positive reactions in all tubes and BI (B) showed negative reactions in all tubes. For BI (C), the hydrogen peroxide gas plasma sterilizer was positive with the $\phi 1$ mm $\times$ 2 m, $\phi 1$ mm $\times$ 3 m, and $\phi 2$ mm $\times$ 3 m tubes, and the hydrogen peroxide gas sterilizer was positive with the $\phi 1$ mm $\times$ 2 m and $\phi 1$ mm $\times$ 3 m tubes. The peracetic acid containing hydrogen peroxide gas sterilizer showed negative reactions for all BI and tube combinations.

Evaluation of residual sterilant

The highest concentration of residual hydrogen peroxide was 11.0 $\mu\text{g}/\text{cm}^2$ after the hydrogen peroxide gas sterilizer soft mode. The residual amount for the peracetic acid containing hydrogen peroxide gas sterilizer standard cycle was 4.2 $\mu\text{g}/\text{cm}^2$, which was low in comparison with the values observed for the hydrogen peroxide gas plasma sterilizer and the hydrogen peroxide gas sterilizer. In the peracetic acid containing hydrogen peroxide gas sterilizer, the amount of residual peracetic acid was 0.26 $\mu\text{g}/\text{cm}^2$ (Fig. 2).

DISCUSSION

In this study, we confirmed that the temperature inside chamber of hydrogen peroxide gas plasma sterilizer was higher than those in hydrogen peroxide gas sterilizer and

TABLE 3. Sterilant penetration test results.**Teflon tube**

hydrogen peroxide gas plasma sterilization (standard cycle) <i>n</i> = 3						
Measurements	Ø1 mm×1 m	Ø1 mm×2 m	Ø1 mm×3 m	Ø2 mm×1 m	Ø2 mm×2 m	Ø2 mm×3 m
BI (A)	+	+	+	+	+	+
BI (B)	—	—	—	—	—	—
BI (C)	+	+	+	—	+	+

hydrogen peroxide gas sterilization (standard mode)						
Measurements	Ø1 mm×1 m	Ø1 mm×2 m	Ø1 mm×3 m	Ø2 mm×1 m	Ø2 mm×2 m	Ø2 mm×3 m
BI (A)	+	+	+	+	+	+
BI (B)	—	—	—	—	—	—
BI (C)	—	—	+	—	—	—

peracetic acid containing hydrogen peroxide sterilization (standard cycle)						
Measurements	Ø1 mm×1 m	Ø1 mm×2 m	Ø1 mm×3 m	Ø2 mm×1 m	Ø2 mm×2 m	Ø2 mm×3 m
BI (A)	—	—	—	—	—	—
BI (B)	—	—	—	—	—	—
BI (C)	—	—	—	—	—	—

(—) Negative (+) Positive

Stainless steel tube

hydrogen peroxide gas plasma sterilization (standard cycle) <i>n</i> = 3						
Measurements	Ø1 mm×1 m	Ø1 mm×2 m	Ø1 mm×3 m	Ø2 mm×1 m	Ø2 mm×2 m	Ø2 mm×3 m
BI (A)	+	+	+	+	+	+
BI (B)	—	—	—	—	—	—
BI (C)	—	+	+	—	—	+

hydrogen peroxide gas sterilization (standard mode)						
Measurements	Ø1 mm×1 m	Ø1 mm×2 m	Ø1 mm×3 m	Ø2 mm×1 m	Ø2 mm×2 m	Ø2 mm×3 m
BI (A)	+	+	+	+	+	+
BI (B)	—	—	—	—	—	—
BI (C)	—	+	+	—	—	—

peracetic acid containing hydrogen peroxide sterilization (standard cycle)						
Measurements	Ø1 mm×1 m	Ø1 mm×2 m	Ø1 mm×3 m	Ø2 mm×1 m	Ø2 mm×2 m	Ø2 mm×3 m
BI (A)	—	—	—	—	—	—
BI (B)	—	—	—	—	—	—
BI (C)	—	—	—	—	—	—

(—) Negative (+) Positive

Three types of BIs were inserted in hollow load PCD, which composed by the syringe connecting to stainless steel or Teflon tube. The inner diameters of prepared tubes were 1 mm and 2 mm and the length used were 1 m, 2 m and 3 m. The hollow load PCD was operated by half cycle of each sterilizer's mode and the color change in BIs was confirmed after 24 hours incubation. Each test was performed three times and the results were judged as negative (—) if spores in BIs were killed in all three trials and judged as positive if spores survived in even one trial.

FIG. 2. Measurement of residual sterilant.

Silicone rubber (5×5 cm) was sterilized in each mode and immersed in purified water. The amounts of hydrogen peroxide and peracetic acid in the solution were measured using a reflection photometer. The residual sterilant amount was normalized by the area of the silicone rubber.

peracetic acid-containing hydrogen peroxide gas sterilizer and reached 65.4°C . The utilization of plasma for sterilization is considered to trigger this difference (Mounir, 2005). There are cases where sterilization must be performed at $\leq 60^\circ\text{C}$ (Yoshida and Kobayashi, 2013). It is not desirable for the temperature inside the sterilization chamber of a low-temperature sterilization device to exceed 60°C .

We found that peracetic acid-containing hydrogen peroxide gas sterilizer was a higher sporicidal effect than conventional hydrogen peroxide gas sterilizers. Hydrogen peroxide and peracetic acid are known to show the synergistic effect on sporicidal activity so that they are suggested to play different roles; hydrogen peroxide would compromise the spore coat and peracetic acid would destroy the spore inner membrane (Leggett et al., 2015; Mark et al., 2016).

Another reason for the difference in the sporicidal effect is because the conventional hydrogen peroxide gas (plasma) sterilizers inactivate microorganisms with active species composed mainly of hydrogen peroxide and hydroxyl radical. In contrast, the peracetic acid-containing hydrogen peroxide gas sterilizer is thought to inactivate microorganisms using singlet oxygen and organic radicals generated from peracetic acid, in addition to hydroxyl radical produced from hydrogen

peroxide (Marquis et al., 1995). Because singlet oxygen and organic radicals have a longer life than hydroxyl radical (Gutowski and Kowalczyk, 2013), the peracetic acid-containing hydrogen peroxide gas sterilizer is thought to enable free radicals for sterilization to reach more areas of items for sterilization than is possible in the hydrogen peroxide gas sterilizers.

We also found that hydrogen peroxide sterilizers show lower sporicidal effect in stainless tube than in PTFE tube while the peracetic acid-containing hydrogen peroxide gas sterilizer shows high sporicidal effect in both tubes. This result suggests that hydrogen peroxide is decomposed by transition metal (Ni, Cr and Fe) in stainless steel (Amou et al., 2018), while peracetic acid is not affected.

We observed that the range of residual hydrogen peroxide is from $4.2 \mu\text{g}/\text{cm}^2$ to $11 \mu\text{g}/\text{cm}^2$ and computed from the ISO10993-5 extract protocol, these values are corresponding to the hydrogen peroxide solution ranged from 25.2 mg/L (0.74 mM) to 66.0 mg/L (1.94 mM). These values are lower than the concentrations that inhibit cell growth by around 75% (IC75) of 3T3 fibroblasts (2.2 mM) (Botta et al., 2014). However, the residual sterilant amounts on medical materials are known to be correlated with cytotoxicity potential of hydrogen peroxide gas sterilizers (Ikarashi et al.,

1995). Our findings that residual sterilant in peracetic acid containing hydrogen peroxide gas sterilizer is 1/2th those in conventional hydrogen peroxide gas sterilizers would potentially contribute to reduce the cytotoxicity. The residual peracetic acid of peracetic acid containing hydrogen peroxide gas sterilizer was 0.26 µg/cm², which was calculated the equivalent concentration of 1.56 mg/L peracetic acid solution by the ISO10993-5 extract procedure. Previous study showed that from 150 mg/L to 300 mg/L peracetic acid did not affect L929 fibroblast cell viability (Viola, 2017). Our data suggests that the replacement of hydrogen peroxide by peracetic acid enhances the safety of hydrogen peroxide gas (plasma) sterilizer.

In this study, we found that adding peracetic acid to hydrogen peroxide allows enhancement in the sterilant penetrability and reduction in residual sterilant. These findings could impose the compatibility of hydrogen peroxide gas (plasma) sterilization.

ACKNOWLEDGMENTS

This work was financially supported by the SARAYA Co. Ltd. The sponsors had no role in the study design; collection, analysis, and interpretation of data; writing of the report; or the decision to submit the article for publication.

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