

SPORICIDAL ACTIVITY OF CHEMICAL STERILANTS USED IN HOSPITALS

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ABSTRACT

OBJECTIVE: The current study was designed to assess the sporicidal activity of chemical sterilants using the Association of Official Analytical Chemists (AOAC) sporicidal test. Chemical sterilants are used most commonly in the healthcare setting to disinfect medical instruments such as endoscopes. This study was undertaken to evaluate the efficacy of several newer formulations of glutaraldehydes and hydrogen peroxide as chemical sterilants.

DESIGN: Using the AOAC test the following agents were tested: two 2% alkaline glutaraldehydes (2% Alk-Glut), 2% acid glutaraldehyde (2% Acid Glut), 2% glutaraldehyde-7.05% phenol-1.20% sodium phenate, 10% glutaraldehyde-0.5% phenylphenol-0.1% tertiary amylphenol, and 6% hydrogen peroxide (H_2O_2). Growth in >1 of 60 seeded penicylinders is considered by the AOAC to indicate failure of sporicidal activity.

RESULTS: Test results of the six disinfectants against *Bacillus subtilis* using the manufacturers' specified use-dilution and exposure time were: 0/60 with 2% Alk-Glut (product 1) at 10 hours, 0/60 with 2% Alk-Glut (product 2) at 8 hours, 0/60 with 2% Acid Glut at 10 hours, 2/60 with 2% glutaraldehyde-7.05% phenol-1.20% sodium phenate at 6.75 hours, 0/60 with a 1:5 dilution (2.0% Glut) of 10% glutaraldehyde-0.5% phenyl-

phenol-0.1% amylphenol at 6 hours, 59/60 with a 1:20 dilution (0.5% Glut) of 10% glutaraldehyde-0.5% phenylphenol-0.1% tertiary amylphenol at 12 hours and 0/60 with 6% H_2O_2 at 6 hours. Test results against *Clostridium sporogenes* were: 2/60 with 2% Alk-Glut (product 1) at 10 hours, 1/60 with 2% Alk-Glut (product 2) at 8 hours, 1/60 with 2% Acid Glut at 10 hours, 2/60 with undiluted 2% glutaraldehyde-7.05% phenol-1.20% sodium phenate at 6.75 hours, 6/60 with a 1:5 dilution (2% Glut) of 10% glutaraldehyde-0.5% phenylphenol-0.1% amylphenol at 6 hours, 60/60 with a 1:20 dilution (0.5% Glut) of 10% glutaraldehyde-0.5% phenylphenol-0.1% amylphenol at 12 hours, and 0/60 with 6% H_2O_2 at 6 hours. Dilutions of 2% glutaraldehyde-7.05% phenol-1.20% sodium phenate yielded the following results against *C. sporogenes*: 59/60 with a 1:8 dilution (0.25% Glut) at 6.75 hours, 60/60 with a 1:16 dilution (0.125% Glut) at 6.75 hours, 11/90 with a 1:8 (0.25% Glut) dilution at 12 hours, and 60/60 with a 1:16 dilution (0.125% Glut) at 12 hours.

CONCLUSIONS: Both 2% acid and alkaline glutaraldehydes are effective chemical sterilants. Diluting glutaraldehyde-based sterilants below 2% glutaraldehyde resulted in failure to kill spores of *B. subtilis* and *C. sporogenes* (*Infect Control Hosp Epidemiol* 1993;14:713-718).

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INTRODUCTION

Pathogenic spore-forming bacteria include *Clostridium difficile*, *Bacillus* species, and *Clostridium* species. These organisms have been noted to cause serious infections in hospitalized patients.¹ Bacterial spores are highly resistant to inactivation by physical and chemical agents. Chemical sterilants are used in the healthcare setting to disinfect medical instruments such as endoscopes in a procedure referred to as high-level disinfection and, rarely, to achieve chemical sterilization. Chemical sterilants are comparatively few in number, with glutaraldehyde most commonly used. Glutaraldehyde is an excellent antimicrobial agent; however, at alkaline pH glutaraldehydes are less stable and rapidly lose their activity. These problems of instability and shortened use-life have prompted the development of novel formulations of glutaraldehydes to overcome these limitations. Some of these formulations are based on acid rather than alkaline glutaraldehyde, thereby benefiting from the stability inherent in such solutions.^{2,3} An enhanced activity is claimed for a combination of glutaraldehyde with sodium phenate and phenol disinfectants.⁴ In 1989, a glutaraldehyde with two phenolics was marketed with sporicidal claims at a reduced glutaraldehyde concentration.

The Environmental Protection Agency (EPA) does not independently test chemical sterilants prior to their registration but has recently undertaken post-registration testing of chemical sterilants to ensure that, once registered, commercially available disinfectants satisfy their registered label claims. Because of concerns regarding the validity of manufacturer's sporicidal label claims, we evaluated the activity of chemical sterilants that are commonly used as high-level disinfectants.

METHODS

The Association of Official Analytical Chemists (AOAC) sporicidal test was used to assess the sporicidal activity of liquid chemical germicides registered by the EPA as "sterilants/disinfectants." In brief, the AOAC sporicidal test was conducted as follows.⁵ *Bacillus subtilis* (ATCC No. 19659) was grown in soil extract nutrient broth, and *Clostridium sporogenes* (ATCC No. 3584) was grown in soil extract-egg-meat medium (Difco Laboratories, Detroit, MI, or an equivalent laboratory-prepared medium [Gayle Mulberry, HillTop BioLabs]) at 37°C for 72 hours. Laboratory-prepared egg-meat media was used in all comparative trials (Tables 1 and 2) because the Difco egg-meat media was unavailable for an extended period of time. The *B subtilis* (after maceration to break the pellicle) and *C sporogenes* cultures were then filtered in a sterile

manner through a funnel containing glass wool into sterile glass tubes. Porcelain penicylinders (ie, carriers) were inoculated by being immersed for 10 to 15 minutes in the glass tubes containing the filtered *C sporogenes* or *B subtilis*. After removal, draining on filter paper and vacuum drying for 24 hours at room temperature, the inoculated penicylinders were placed into the disinfectant solutions and exposed per manufacturer's label claim unless otherwise specified. After exposure to the disinfectant each penicylinder was removed and placed into a 10-mL tube containing thioglycollate broth, and then removed and transferred to a second 10-mL tube containing thioglycollate broth. Both tubes then were capped tightly and incubated at 37°C for 21 days and examined for growth. If no growth was observed after 21 days, the tubes were heat-shocked for 20 minutes at 80°C and reincubated 72 hours at 37°C.

The presence of *B subtilis* and *C sporogenes* spores was monitored by exposing inoculated penicylinders to 2.5 N hydrochloric acid. Test spores resisted hydrochloric acid for two minutes or longer.

The number of organisms per penicylinder (microbial load) was determined as follows: an inoculated penicylinder was placed into a tube containing Tween 80-saline, exposed to ultrasound for 10 minutes, and vortexed for two minutes at setting 5. The number of colony forming units (CFU)/mL was quantitated by a spread plate method.

All products were tested using 30 inoculated penicylinders. The number of *B subtilis* and *C sporogenes* spores on inoculated penicylinders was on average $6.8 \cdot 10^4$ and $8.5 \cdot 10^5$, respectively. The number of spores on inoculated penicylinders did not vary significantly during a test day. All disinfectants were purchased for the study and used within their specified use-life. Sterile distilled water (USP, Travenol Laboratories, Deerfield, IL) was used for disinfectant dilution and all disinfectants were used according to the manufacturers' instructions unless otherwise specified. The active ingredients before dilution, activated shelf life, use-dilution, and sporicidal label claims were: 2% alkaline glutaraldehyde, 14 d, undiluted, and 10 hours at 25°C; 2% alkaline glutaraldehyde, 14 d, undiluted, and 8 hours at 20°C; 2% acid glutaraldehyde, 2 years, undiluted, and 10 hours at 21°C; 10% glutaraldehyde with 0.5% orthophenylphenol and 0.1% paratertiary amyphenol, 30 d, 1:5 dilution for 6 hours at 20°C or 1:20 dilution for 12 hours at 20°C; 2% glutaraldehyde with 7.05% phenol and 1.20% sodium phenate, 30 d, undiluted, and 6.75 hours at 20°C. Because the latter product is recommended to be used at a 1:16 dilution as a high-level disinfectant and as this is the concentration used for endoscope disinfection,⁷ this concentration was tested. Addition-

TABLE 1
ACTIVITY OF CHEMICAL STERILANTS AGAINST *CLOSTRIDIUM SPOROGENES* AND *BACILLUS SUBTILIS* SPORES USING THE AOAC SPORICIDAL TEST

Chemical Sterilant (Exposure Time)	Number of Positive Penicylinders per 60 Replicates	
	<i>C sporogenes</i>	<i>B subtilis</i>
2% alkaline glutaraldehyde (10 hours)	2	0
2% alkaline glutaraldehyde (8 hours)	1	0
2% acid glutaraldehyde (10 hours)	1	0
2% glutaraldehyde-7.05% phenol-1.20% sodium phenate (6.75 hours)	2	2
2% glutaraldehyde-0.1% phenylphenol-0.02% amylphenol (6 hours)	6	0
0.5% glutaraldehyde-0.025% phenylphenol-0.005 amylphenol (12 hours)	60	59
6% hydrogen peroxide (6 hours)	0	0

Mean number of *C sporogenes* and *B subtilis* spores per penicylinder was $8.52 \cdot 10^5$ and $6.8 \cdot 10^4$ respectively.

ally, when this testing was conducted the sterilization claim for a 1:20 dilution of 10% glutaraldehyde with 0.5% orthophenylphenol and 0.1% paratertiary amylphenol was 12 hours.

RESULTS

All but one chemical sterilant tested at the manufacturers' recommended concentration and exposure time demonstrated excellent activity against *B subtilis* (Table 1). The one failure occurred with the 1:20 dilution of 10% glutaraldehyde-0.5% phenylphenol-0.1% amylphenol, which demonstrated no measurable activity against *B subtilis*.

Against *C sporogenes* all but two chemical sterilants demonstrated excellent activity (Table 1). Again the 1:20 dilution of glutaraldehyde-phenylphenol-amylphenol demonstrated no measurable activity. Breakthrough was noted with the 1:5 dilution of glutaraldehyde-phenylphenol-amylphenol.

We tested, at a 1:16 dilution and recommended exposure time of 6.75 hours, a glutaraldehyde-phenol-sodium phenate product that appeared to have a sporicidal label claim at this dilution. We also tested this

TABLE 2
ACTIVITY OF A DILUTED GLUTARALDEHYDE-BASED DISINFECTANT AGAINST *CLOSTRIDIUM SPOROGENES* AND *BACILLUS SUBTILIS* SPORES USING THE AOAC SPORICIDAL TEST AT VARIOUS EXPOSURE TIMES

Chemical Sterilant (Exposure Time)	Number of Positive Penicylinders per 60 Replicates	
	<i>C sporogenes</i>	<i>B subtilis</i>
0.25% glutaraldehyde-0.88% phenol-0.15% sodium phenate (6.75 hours)	59/60	28/60
0.25% glutaraldehyde-0.88% phenol-0.15% sodium phenate (12 hours)	11/90	12/90
0.125% glutaraldehyde-0.44% phenol-0.075% sodium phenate (6.75 hours)	60/60	63/90
0.125% glutaraldehyde-0.44% phenol-0.075% sodium phenate (12 hours)	60/60	139/150

recommended and an extended exposure time. At all but the higher concentration and extended exposure time, this product failed to demonstrate sporicidal activity (Table 2). Even at the higher concentration and longer exposure time, breakthrough was noted.

When the Difco egg-meat media was compared with laboratory-prepared egg-meat media, we found a significant difference in the number of positive penicylinders for only one anylate (Table 3); for each, a greater number was observed with the Difco media.

DISCUSSION

High-level disinfection is used for decontaminating equipment that has come into contact with mucous membranes such as endoscopes and anesthesia equipment. Gastrointestinal endoscopes in particular are likely to be contaminated during use with spore-forming bacteria.

Glutaraldehyde-based disinfectants, which are EPA-registered as chemical sterilants, are extensively used in the healthcare industry as high-level disinfectants. Their main advantages include a broad spectrum of antimicrobial activity, activity in the presence of organic matter, rapid inactivation of microorganisms with the exception of spores, relative ease of use, and lack of corrosive action against metals, rubber, and cements.

Disadvantages include a pungent and irritating odor, a relatively slow mycobactericidal activity com-

TABLE 3

COMPARISON OF EFFECT OF CULTURE MEDIA ON THE ACTIVITY OF GLUTARALDEHYDE-BASED DISINFECTANTS AGAINST *CLOSTRIDIUM SPOROGENES** AT VARIOUS EXPOSURE TIMES

Chemical Sterilant (Exposure Time)	Number of Positive Penicylinders per 30 Replicates	
	Difco Egg-Meat Media	Laboratory-Prepared Egg-Meat Media
2% alkaline glutaraldehyde (20 minutes)	22	8†
2% alkaline glutaraldehyde (1 hour)	3	1
2% alkaline glutaraldehyde (10 hours)	1	6
2% acid glutaraldehyde (20 minutes)	30	30
2% acid glutaraldehyde (1 hour)	30	7†
2% acid glutaraldehyde (10 hours)	0	0
0.5% glutaraldehyde-0.025% phenylphenol-0.005 amyphenol (20 minutes)	30	30
0.5% glutaraldehyde-0.025% phenylphenol-0.005 amyphenol (1 hour)	30	30
0.5% glutaraldehyde-0.025% phenylphenol-0.005 amyphenol (12 hours)	30	30
0.125% glutaraldehyde-0.44% phenol-0.75% sodium phenate (20 minutes)	30	30
0.125% glutaraldehyde-0.44% phenol-0.75% sodium phenate (1 hour)	30	30
0.125% glutaraldehyde-0.44% phenol-0.75% sodium phenate (12 hours)	30	30

* Mean number of *C sporogenes* spores per penicylinder was $1.2 \cdot 10^6$ for Difco egg-meat media and $4.0 \cdot 10^5$ for laboratory-prepared egg-meat media.

† Statistically significant difference ($P < 0.05$) by two-sided Fisher's exact test when comparing number of positive penicylinders per 30 replicates with the two different culture media.

glutaraldehyde vapor.^{2,3} Exposure to levels of 0.3 to 0.4 ppm can cause nose and throat irritation, burning eyes, and headaches. For this reason, the Occupational Safety and Health Administration (OSHA) has imposed a ceiling limit of 0.2 ppm. Because of the new OSHA limit, diluted glutaraldehydes, which would result in lower ambient air levels of glutaraldehyde, have been marketed over the last several years.

Until recently there was no requirement for EPA verification of manufacturer's sporicidal claims for chemical sterilants. In 1991, the EPA entered into an interagency agreement with the Food and Drug Administration (FDA) to test antimicrobial products registered with the EPA as sterilants and determine their effectiveness. To meet current EPA registration requirements as a sporicidal agent, a chemical sterilant must be tested against *C sporogenes* ATCC 3584 and *B subtilis* ATCC 19659 using the AOAC Sporicidal Test. Killing on all of the 120 carriers representing each of two types of surfaces (porcelain penicylinders and silk suture loops) must be demonstrated using at least three product samples representing three different batches, one of which is at least 60 days old (240 carriers per sample; a total of 720 carriers). The EPA's rationale for testing sterilants was that these products

are crucial for infection control since their failure could pose a significant risk to the public and because of increased controversy regarding the validity of product label claims.

In December 1991, a chemical sterilant (1:16 dilution of 2% glutaraldehyde-7.05% phenol-1.20% sodium phenate) failed to pass EPA postregistration testing and the EPA issued a "stop sale, use or removal order" against this product.⁷ One batch of another diluted glutaraldehyde (1:20 dilution of 10% glutaraldehyde-0.5% phenylphenol-0.1 amyphenol) failed postregistration testing and the EPA issued a "stop sale action" and requested a product recall in May 1992.⁸ This latter EPA product recall was challenged in U.S. District Court and resulted in a preliminary finding that the test data were unreliable for reasons including a failure to follow "good laboratory practices" and of product overdilution. The court issued a restraining order prohibiting the EPA from publishing, disseminating, or releasing to the public these test data.^{9,10} In November 1992, the manufacturer of this product temporarily removed it from the marketplace due to inconsistent test data and prohibitive costs involved in being regulated by both the EPA and FDA.

Our data support the EPA actions involving glutaraldehyde diluted below a 2% concentration. As with the EPA, we were unable to reproduce the manufacturer's sporicidal label claims for diluted glutaraldehydes using the standard AOAC sporicidal test. Our data further suggest that 2% glutaraldehyde-based formulations demonstrate excellent sporicidal activity when used at their recommended exposure times. Our findings corroborate those of Powers and Russell¹¹ who demonstrated excellent sporicidal activity with a 2% alkaline glutaraldehyde and 2% glutaraldehyde-7.05% phenol-1.20% sodium phenate. However, poor sporicidal activity was demonstrated using a 1:16 dilution of the latter product.

Acid glutaraldehydes have been reported to be less sporicidal but are more stable than alkaline glutaraldehyde.^{12,13} Our data demonstrated no measurable difference between the 2% alkaline and 2% acid glutaraldehydes at the manufacturer's recommended exposure times for sporicidal activity. However, at short exposure times (20 minutes and one hour) alkaline glutaraldehydes were superior. Our data demonstrate that 2% alkaline glutaraldehyde ensured significant sporicidal activity within one hour. Longer exposure times (eg, 6 to 10 hours) are required to demonstrate complete inactivation (0 positive penicylinders/720 replicates) when using EPA criteria¹⁴ for claiming "sporicidal" activity. An exposure time of 6 to 10 hours generally is impractical in a clinical setting.

Chemical sterilants that do not meet the EPA registration criteria as a liquid chemical sterilant also do not meet current criteria established by the Centers for Disease Control and Prevention (CDC) for high-level disinfection. Thus, a disinfectant that cannot substantiate a sporicidal claim should not be used as a high-level disinfectant.⁷ This CDC recommendation is consistent with the "APIC Guidelines for Selection and Use of Disinfectants," which recommends the use of sporicidal agents such as 2% glutaraldehyde-based formulations for high-level disinfection of semicritical patient care items.²

In our studies, 2% glutaraldehydes demonstrated an occasional positive penicylinder per 60 replicates; however, this is unlikely to represent a public health problem for three reasons. First, the AOAC test is very conservative because spores dried on penicylinders are more difficult to inactivate than spores suspended in a liquid media. Second, a much larger load of spores is employed than would be present on a properly cleaned patient care item. Third, the spores used in this procedure are more resistant than the common microbial contaminants found on patient care items.

Our comparative data were based primarily on

using a laboratory-prepared egg-meat media, as the Difco egg-meat medium was unavailable. Comparing Difco-Ortho egg-meat media and laboratory prepared egg-meat media suggests that the Difco may present a more stringent challenge of chemical sterilants. This may be attributable to better *Clostridium* growth in the Difco media, which may have resulted in a higher spore load per penicylinder.

We conclude that glutaraldehyde preparations that are diluted below 2% glutaraldehyde should be used as chemical sterilants only following independent verification of their label claims. Hydrogen peroxide demonstrated excellent sporicidal activity. In one recent study, hydrogen peroxide was more effective in the high-level disinfection of flexible endoscopes than a 2% glutaraldehyde solution. Hydrogen peroxide has not been used widely for endoscope disinfection because there continue to be concerns that its oxidizing properties may be harmful to some components of the endoscope.¹⁵ The use of hydrogen peroxide as a chemical sterilant warrants further study.

ADDENDUM

In June 1993, the FDA and EPA executed a "Memorandum of Understanding" that gives the FDA primary responsibility for sterilants used on critical and semicritical devices, while disinfectants used on noncritical surfaces will be regulated by the EPA. The FDA intends to promulgate the new rules based on the agreement, but a timetable for completion of this process has not been announced.

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International Conference on the Prevention of Infection

The deadline for abstract submission to the Third International Conference on the Prevention of Infection (CIPI) is January 1, 1994; the meeting will be held April 5-6, 1994 in Nice, France. English is the official language of the conference, which will include original reports, state-of-the-art lectures, and plenary sessions featuring leading infection control and infectious diseases

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