

Combined application of simulated reuse and quantitative carrier tests to assess high-level disinfection: Experiments with an accelerated hydrogen peroxide-based formulation

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Background: Heat-sensitive medical devices require chemical disinfection between patients, and certain formulations for this purpose can be reused for several days. Because dilution, evaporation, and breakdown or neutralization of active ingredients can occur during reuse, it is vital to ensure that the solution retains its broad-spectrum germicidal activity even at the end of the recommended reuse period.

Objective: The purpose of this study was to combine the US Environmental Protection Agency's and the Food and Drug Administration's recommended simulated reuse method with recently developed quantitative carrier tests (QCT) to assess the broad-spectrum germicidal activity of a 7% solution of accelerated hydrogen peroxide (pH 2.9) stressed for 14 days.

Materials and methods: On alternate days baths with 3 lots of the test formulation were stressed by the addition of bacteria (*Salmonella choleraesuis*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*) on glass beads and spores (*Bacillus subtilis* and *Clostridium sporogenes*) on metallic penicylinders. In addition, one set of respiratory therapy equipment was subjected to 3 daily cycles of disinfection in each bath. The pH and H₂O₂ levels in the test samples were measured, and they were also subjected to QCTs for their sporicidal, bactericidal, virucidal, fungicidal, and mycobactericidal activities.

Results: After 14 days of reuse, the pH of the test solutions remained essentially unchanged. Although the level of H₂O₂ dropped from a high of 7.66% to as low as 6.40%, all lots showed the required level of broad-spectrum germicidal activity after 14 days of stress.

Conclusions: The stress test and QCT were successfully combined in demonstrating the broad-spectrum germicidal activity of a high-level disinfectant subjected to 14 days of simulated reuse. (Am J Infect Control 2002;30:449-57.)

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The heat-sensitive nature of many medical devices requires that they be chemically disinfected through manual or automated systems between uses.¹ The working solutions of many germicides are meant for use only once, but others are formulated for reuse in manual systems for 14 to 30 days. During such reuse, the germicidal activity of the solution can be compromised as a result of its incremental dilution, evaporation, or breakdown of its active ingredients, as well as the accumulation of soil from the devices

Time	Procedure applied
9:00 am	Remove from the test solution the 200 spore-laden penicylinders added to it on the previous afternoon
9:10 am	Prepare and dry the glass bead carriers for bioburden with vegetative bacteria
10:00 am	Measure level of H ₂ O ₂ in test solution using test strip
10:30 am	Measure pH of test solution
11:00 am	Add to the test solution 880 glass beads with the vegetative bacteria
11:30 am	Remove the glass beads from the test solution
12:00 noon	Add to the test solution the respiratory therapy equipment (1 st cycle)
12:30 pm	Remove respiratory therapy equipment from the test solution
1:00 pm	Add to the test solution the respiratory therapy equipment (2 nd cycle)
1:15 pm	Prepare and dry spore carriers for next day of challenge
1:30 pm	Remove respiratory therapy equipment from the test solution
2:00 pm	Add to the test solution the respiratory therapy equipment (3 rd cycle)
2:30 pm	Remove respiratory therapy equipment from the test solution
3:45 pm	Add 200 penicylinders with dried spores to test solution & leave overnight

Fig 1. Flow chart of the daily cycle of stress of the test solution starting on the second day of the reuse period.

being processed.² A reduction in the germicidal activity of such formulations during reuse could increase the risk of spreading disease.³ Therefore, it is crucial to ensure that products sold for reuse remain active against major classes of pathogens at least for the period claimed on their label.

Standardized and internationally accepted protocols remain unavailable to stress high-level disinfectants during reuse. The only method available for this purpose was developed and endorsed by the US Environmental Protection Agency (EPA)⁴ and the US Food and Drug Administration (FDA).⁵ This protocol challenges the in-use solution with repeated stress in the form of germ-loaded carriers as well as by soaking pieces of respiratory therapy equipment on a prescribed schedule during the recommended period of reuse. Samples of the test solution from the reuse bath are collected on a regular basis and evaluated for their germicidal activity to demonstrate that the stressed solution remains a high-level

disinfectant even at the end of the recommended reuse period. However, the carrier test protocols commonly used for this purpose are now recognized as deficient in design, and their results often difficult to reproduce.^{6,7}

Formulations derived from accelerated hydrogen peroxide (AHP) have demonstrated relatively rapid activity against all classes of human pathogens.⁸ In this study, Virox STF, a 7% AHP solution with broad-spectrum antimicrobial activity, was evaluated for 14-day reuse in the manual disinfection of medical devices. The product is already registered with Health Canada as a high-level disinfectant.

The sporicidal, mycobactericidal, fungicidal, and bactericidal activities of samples of the stressed solutions were assessed with a quantitative carrier test (QCT). This method, which uses flat-bottomed glass vials as carriers, is now a standard of ASTM International,^{9,10} an organization that publishes

consensus-based standards for testing the germicidal activity of chemicals. The virucidal activity of the test samples was determined with the use of metal disks as carriers,⁷ and this protocol has also been recently accepted as a standard of ASTM.¹¹

OBJECTIVE

The objective of this study was to determine if the EPA and FDA stress protocol could be applied in combination with recently developed QCTs to assess the germicidal activity of an AHP-based, high-level disinfectant under simulated reuse for 14 days.

MATERIALS AND METHOD

Test formulation

Three separate lots of the test formulation (Virox STF; Virox Technologies, Mississauga, ON, Canada) were received in 20-L white plastic pails for use in this study. The solutions, derived from a 7% solution of stabilized and AHP, were clear with a pale yellow color and had a slight acidic odor. Upon receipt, the containers were stored in the laboratory at $22 \pm 2^\circ\text{C}$ in an area with controlled access. The product is already registered as a high-level disinfectant in Canada.

Test organisms

Standard strains of *Pseudomonas aeruginosa* (ATCC 15442), *Staphylococcus aureus* (ATCC 6538), *Salmonella choleraesuis* (ATCC 10708), *Mycobacterium terrae* (ATCC 15755), *Trichophyton mentagrophytes* (ATCC 9533), *Bacillus subtilis* (ATCC 19659), *Clostridium sporogenes* (ATCC 7955), and the Sabin strain of poliovirus 1 (ATCC VR-192) were used in this study.

Stock suspensions of *P. aeruginosa*, *S. aureus*, and *S. choleraesuis* were prepared by culturing them in tryptic soy broth (TSB; Difco Labs, Detroit, MI) for 24 hours at 37°C . *B. subtilis* spores were grown aerobically in a 1:10 dilution of Columbia broth (Difco) with manganese for 72 hours at 37°C . *C. sporogenes* spores were grown anaerobically in undiluted Columbia broth for 5 days at 30°C . To yield a concentration of 10^9 spores/mL, the spore suspensions were centrifuged for 10 minutes at 5000 500x g, washed, and resuspended in sterile, distilled, deionized water.

M. terrae was grown in Middlebrook 7H9 broth (Difco) with ADC (albumin, dextrose, catalase) enrichment and glycerol in vented plug seal capped

tissue culture flasks. The test suspension was prepared from stocks grown for 21 days at 37°C . The cell suspension was washed 3 times by centrifugation at 5000 xg for 15 minutes and resuspended in sterile distilled water. The final stock suspension was prepared by resuspending the bacterial pellets in sterile bijoux bottles containing glass beads to about 10^8 cells/mL. The stock solution was stored at 4°C .

A stock suspension of the conidia of *T. mentagrophytes* was obtained by inoculating the center of a Mycobiotic Agar plate (Difco) and incubating it at 28°C for 10 days. Mycelial mats were harvested from the agar surface, homogenized with sterile glass beads in normal saline, and filtered through sterile cotton gauze to remove the hyphae.

Test suspensions of the poliovirus were prepared by infecting monolayers of Vero cells in 75 cm^2 plastic cell culture flasks. The virus was allowed to adsorb to the cells for 60 minutes at 37°C , and the infected monolayer kept in Eagle's minimum essential medium, without any antibiotics and serum, until approximately 75% of the monolayer showed virus-induced cytopathology. The culture was then frozen (-20°C) and thawed 3 times and centrifuged at 1000 xg for 10 min to remove cell debris. The supernatant was used as the virus pool.

Soil load

To increase the level of stress to the test solution used in this study, fetal bovine serum (FBS; GIBCO, Burlington, Ontario) at a final concentration of 2% was used to simulate loading with organic material. FBS was noninhibitory for all the organisms used in this study, and it is also universally accepted as a soil load in testing the germicidal activity of liquid chemical disinfectants.¹² The addition of germ-laden carriers as bioburden and the soaking of several items of respiratory equipment over the 14-days stress cycle simulated the challenge the product may face under reuse.

Carriers for the bioburden

Two types of carriers were used to challenge the test solution with the bacterial bioburden. Pyrex glass beads of 6 mm in diameter (Catalogue No: 7268-6; Corning, New York) were soaked in separate suspensions of *S. choleraesuis*, *S. aureus*, and *P. aeruginosa*. Stainless steel penicylinders (8.0 mm outer diameter, 6 mm inner diameter, 10 mm length; Catalog No; 07-907-5; Fisher Scientific, Whitby,

Ontario) were soaked separately in suspensions of *B subtilis* and *C sporogenes* spores. Holding them for 45 minutes at 37°C before use dried the inocula on all the carriers. The same carriers were used repeatedly in the testing with decontamination, washing, and autoclave sterilization in between.

Carriers for the germicide test

Flat-bottomed glass vials (Galaxy Co, Newfield, NJ), 20 mL in capacity, were used as carriers in QCTs for bactericidal, sporicidal, mycobactericidal, and fungicidal activities.

PROCEDURE FOR MANUAL REUSE STRESS

The flow chart (Fig 1) lists the timing and the sequence of the main steps in the stress protocol.

Three plastic baths (Rubbermaid, Winchester, Va), each containing undiluted 10 L of one of the test lots, were set up in parallel in the laboratory.

The number of viable organisms on representative carriers used as bioburden was determined by adding 3 inoculated carriers of each type separately to a tube with 10 mL of phosphate buffer (pH 7.2) with 1 % Tween 80 (Bioshop Canada Inc, Burlington, ON). The tubes were vortexed for 1 minute, the eluates diluted as required in phosphate buffer, and appropriate dilutions were individually passed through 47 mm diameter membrane filters (0.22 µm pore diameter). Each filter was placed on the recovery agar medium in a 100 mm diameter plastic culture plate. The plates of tryptic soy agar (Difco) used for all the bacteria except *C sporogenes* were held at 37°C for 24 hours. The plates of fastidious anaerobic agar (Difco) used for the recovery of *C sporogenes* were incubated at 30°C for 48 hours.

Gram stains were performed on colonies isolated from one set of the carrier counts for each challenge organism to confirm their identity by morphology and staining characteristics.

In addition to the stress from the contaminated glass beads and penicylinders, a complete set of inhalation therapy equipment was subjected to 3 cycles of processing in each germicide bath on each one of the 14 days of stress: (1) flexible, clear plastic CF cuffed tracheal tube 10.02 mm OD and 7.5 mm ID (Catalog #5-10115; Kendal Sheridan, Mansfield, MA); (2) flexible tubing 1.83 m in length (Catalog #301016;

Respironics Inc, Murrysville, PA); (3) 2.0-L capacity breathing bag (Catalog #5005; CH Medical Ltd, Exeter, England); (4) face mask (CH Medical Ltd); and (5) plastic, 22-mm bifurcator Y-connector (Intersurgical, Inc, Liverpool, NY). Each germicide soak of the above-listed equipment lasted for 30 minutes. Between germicide treatments, the items were subjected to minimal pretreatment consisting of cleaning with a detergent (Sparkleen; Fisher Scientific, Ottawa, ON) and a rinse in sterile distilled water.

The concentration of H₂O₂ in the test samples was determined by iodometric titration.¹³ High-range peroxide test strips (Serim Research Corp, Elkhart, IN) were also used once a day as an additional qualitative measure to confirm that the H₂O₂ concentration in the baths was above the minimum effective level. A pH meter (Accument; Fisher Scientific) was used daily to determine the pH of the test solution in each bath before the first challenge.

TESTING FOR GERMICIDAL ACTIVITY

The QCT used in this evaluation was developed in our research laboratory to address the recognized deficiencies in the AOAC International Use-Dilution Test.¹⁴ The study method not only meets the requirements of the Canadian General Standards Board¹² for testing germicides to be used on environmental surfaces and medical devices, but it is also now an accepted standard of ASTM International,⁹ an organization that sets standards for germicide test methods. The details of the procedure have been reported previously.^{7,8} It is designed to assess the sporicidal, bactericidal, mycobactericidal, and fungicidal activities of liquid chemicals. Briefly, it uses the inside bottom surface of flat-bottomed glass vials as the carrier for the challenge microorganism. Ten µL of the test suspension is placed in each carrier, and the inoculum is dried. The dried inoculum is then overlaid with 1 mL of the test germicide, and the carriers are held for the required contact time at the desired temperature. The inoculum is then eluted, and the needed dilutions of the eluates are separately passed through membrane filters. The filters are placed on suitable recovery media and incubated; the colonies are counted and the log₁₀ reductions are calculated.

Recovery media and detection of viable organisms

For the sporicidal testing with *B subtilis*, the filters were placed on trypticase soy agar plates, incubated

at 37°C, monitored, and the number of colony forming units (CFU) recorded at 24-hour intervals for a total of 5 days. For *C sporogenes*, the filters were placed on fastidious anaerobic agar, incubated at 30°C, monitored, and the number of CFU recorded at 48 hours, and every 24-hour interval thereafter for a total of 5 days. For mycobactericidal testing with *M terrae*, the filters were placed on 7H11 agar (Difco), incubated at 37°C, monitored, and the number of CFU recorded at weekly intervals for a total of 4 weeks. For fungicidal testing with *T mentagrophytes*, the filters were placed on Sabouraud's dextrose agar (Difco) and incubated at 28°C, monitored, and the number of CFU recorded at 4 days and every 24-hour interval thereafter for a total of 10 days.

Controls

Control carriers were used in the same manner as test carriers except phosphate buffer was applied to the dried inoculum instead of the disinfectant for the sporicidal tests, and sterile saline was applied to the dried inoculum instead of the disinfectant for the bactericidal, mycobactericidal, and fungicidal tests.

Neutralizer, microbial diluent, and filter rinse

Lethen Broth (with 0.1% sodium thiosulphate pentahydrate) was used as the neutralizer. Phosphate buffer, pH 7.2, was used as the diluent as well as the rinse for filters and filter holders in the sporicidal test; this was replaced with normal saline (0.85% NaCl) in the mycobactericidal and fungicidal tests.

Test for virucidal activity

Disks (0.75 mm thick and 1 cm in diameter) of brushed stainless steel (magnetized) were used as carriers, and each disk placed in each well of a plastic cell culture 12-well plate (Corning) received 10 µL of the test virus. After the inoculum had dried, each disk was either exposed to 50 µL the test product or Earle's balanced salt solution (EBSS) for 5 min at 20°C. At the end of the contact time, 950 µL of EBSS was added to both the test and control wells as eluent or neutralizer. To ensure the efficient recovery of any viable organisms, the disk was gently scraped with the pipette tip by repeatedly pipetting the eluent over its surface. The eluate was transferred into a sterile labeled dilution vial and vortexed to mix. The control and test eluates were serially diluted and inoculated into cell culture monolayers for virus plaque assays.

The plaque forming units were determined, and log₁₀ reduction was calculated.

Plaque assay for the virus

Monolayers of Vero cells in 12-well cell culture plates were used for the plaque assays. Each assay included 3 wells as cell controls, and each dilution of the sample tested was inoculated into at least 3 wells. The growth medium was removed, and each well received 100 µL of the sample to be tested. The plates were held for 60 minutes at 37°C in a 5% CO₂ atmosphere to allow for virus adsorption. Each monolayer was then overlaid with 2 mL of an overlay containing 2 × Eagle's minimum essential medium supplemented with N-2-hydroxyethylpiperazine N'-2-ethanesulfonic acid (HEPES), L-glutamine, nonessential amino acids, and 2% FBS, 26 mmol/L MgCl₂ and 0.6% agar (Difco). Once the overlay had solidified, the plates were held for 40 hours in a 5% CO₂ atmosphere at 37°C. The cells were fixed in 3.7% formaldehyde in normal saline and stained with a 0.1% solution of crystal violet.

Product performance criteria

In each QCT, 10 test carriers and 3 control carriers were used. The results are reported as log₁₀ reductions in viability in reference to the control carriers. For a product to be considered bactericidal, sporicidal, or mycobactericidal, it was expected to reduce the viability titre of all the test organisms by a minimum of 6 log₁₀ (at least 1 million-fold) under the conditions of this test. A minimum of 5-log₁₀ reduction was expected for fungicidal activity and no less than a 3-log₁₀ reduction for virucidal activity.

RESULTS

Bioburden

As specified in the EPA protocol, each bath with the test germicide received enough serum to give a final serum concentration of 2%. As shown in Table 1, every day each bath also received 880 glass beads coated with each of the 3 types of vegetative bacteria and 200 penicylinders contaminated separately with 1 of the 2 types of spores. On the first day, each bath received the carriers contaminated with *S aureus* and *B subtilis*. The carriers used on the following day contained *P aeruginosa* and *C sporogenes*, and those for the third day were contaminated with *S choleraesuis* and *B subtilis*. The cycle was repeated for the remaining days of stress. This represented a daily input of a total of 1080 bacteria or spore-contaminated carriers

Table 1. Recovery of the bacterial bioburden from the carriers and the estimated level of CFU added to each germicide bath per day

Carrier type	Organism	CFU recovered after drying	Carriers added to each germicide bath	Total CFU added to each germicide bath	Total CFU/mL of each bath
Glass beads	<i>P aeruginosa</i>	1.76×10^5	880	1.55×10^8	1.55×10^4
	<i>S aureus</i>	7.2×10^4	880	6.33×10^7	6.33×10^3
	<i>S choleraesuis</i>	5.7×10^4	880	5.01×10^7	5.01×10^3
Penicylinders	<i>C sporogenes</i>	1.03×10^5	200	2.06×10^7	2.06×10^3
	<i>B subtilis</i>	8.47×10^5	200	1.69×10^8	1.69×10^4

Table 2. Hydrogen peroxide concentration and pH of the test solutions during stress

Lot number	Days of stress	pH	% H ₂ O ₂
2484	7	2.93	7.5
2485	7	2.91	7.6
2486	7	2.89	7.6
2484	14	2.98	6.9
2485	14	2.90	6.9
2486	14	2.89	6.8

per bath per day. The level of contamination on each glass bead and penicylinder with the dried inoculum is shown in Table 1. On average, each test solution received 8.95×10^5 and 9.48×10^5 CFU/mL of the bacteria and the spores, respectively, resulting in a cumulative bacterial bioburden of 1.84×10^4 CFU/mL of test solution per day. This challenge slightly exceeded the EPA's recommended level of 10^4 CFU/mL.

pH and H₂O₂ concentration

The pH of the test solutions at the start of the experiment ranged from 2.84 to 2.88, and, as can be seen from Table 2, it remained essentially unchanged even after 14 days of reuse.

The H₂O₂ levels in the 3 test solutions at the start of the experiment ranged from 7.49% to 7.66%. As shown in Table 2, these levels dropped to 6.40% to 6.43% after 14 days of reuse. The test strips showed the germicide concentrations to be in the acceptable levels.

Sporicidal activity

Table 3 summarizes the results of testing against the spores of *B subtilis* and *C sporogenes*. All of the samples tested after 7 and 14 days of stress were able to

reduce their viability titre $> 7 \log_{10}$ in a contact time of 6 hours at 20°C, indicating high sporicidal activity even at the end of the reuse period.

Mycobactericidal activity

As summarized in Table 4, the test solutions collected after 7 days of stress were able to reduce the viability titre of *M terrae* by $> 6.5 \log_{10}$ after a contact time of 25 minutes at 20°C. For those obtained after 14 days of stress, a contact time of 35 minutes was needed for the same level of mycobactericidal activity.

Fungicidal activity

The test solutions were able to reduce the viability titre of the fungal conidia by $> 5 \log_{10}$, even after 14 days of stress in a contact time of 20 minutes at 20°C (Table 5).

Bactericidal activity

The test solutions retained strong bactericidal activity, even after 14 days of stress (Table 6). They were able to inactivate all 3 types of vegetative bacteria by $> 6 \log_{10}$ in a contact time of 5 minutes at 20°C.

Virucidal activity

As shown in Table 7, the test solutions were able to bring about an almost $5 \log_{10}$ reduction in the viability titre of the poliovirus in a contact time of 5 minutes at 20°C.

DISCUSSION

The focus of this study was to apply the EPA and FDA simulated reuse stress protocol in combination with recently developed QCTs to assess the broad-spectrum germicidal activity of the stressed product. This is the first study where the findings of such

Table 3. Sporidicidal activity of the stressed solutions with a contact time of 6 hours at 20°C*

Lot number	Days of stress	CFU/Control Carrier × 10 ⁷		CFU/test carrier		Log ₁₀ reduction	
		<i>B subtilis</i>	<i>C sporogenes</i>	<i>B subtilis</i>	<i>C sporogenes</i>	<i>B subtilis</i>	<i>C sporogenes</i>
2484	7	1.89	12.8	0	0	7.28	8.09
2485	7	2.46	12.8	0	0	7.39	8.09
2486	7	2.46	12.8	0	0	7.39	8.09
2484	14	2.18	6.34	0	0	7.34	7.80
2485	14	2.18	6.34	0	0	7.34	7.80
2486	14	2.18	6.34	0	0	7.34	7.80

*Counts were calculated from 10 test and 3 control carriers in each test.

Table 4. Activity of the stressed formulation against *Mycobacterium terrae* at 20°C*

Lot number	Days of stress	Contact time (min)	CFU/control carrier × 10 ⁶	CFU/test carrier	Log ₁₀ reduction
2484	7	25	2.73	0	6.43
2485	7	25	2.73	0	6.43
2486	7	25	2.73	0	6.43
2484	14	35	4.70	1	6.67
2485	14	35	4.70	0	6.67
2486	14	35	4.70	0	6.67

*Counts were calculated from 10 test and 3 control carriers in each test.

Table 5. Activity of the stressed formulation against the conidia of *T mentagrophytes* with a contact time of 20 minutes at 20°C*

Lot number	Days of stress	CFU/control carrier × 10 ⁵	CFU/test carrier	Log ₁₀ reduction
2484	7	8.23	0	5.91
2485	7	8.23	0	5.91
2486	7	8.23	0	5.91
2484	14	47.0	0	6.67
2485	14	47.0	8	6.48
2486	14	47.0	1	6.59

*Counts were calculated from 10 test and 3 control carriers in each test.

Table 6. Bactericidal activity of the test solutions collected after 14 days of stress and tested with a contact time of 5 minutes at 20°C*

Lot number	Average CFU/control carrier			CFU/test carrier			Log ₁₀ reduction		
	<i>S choleraesuis</i> × 10 ⁷	<i>S aureus</i> × 10 ⁶	<i>P aeruginosa</i> × 10 ⁶	<i>S choleraesuis</i>	<i>S aureus</i>	<i>P aeruginosa</i>	<i>S choleraesuis</i>	<i>S aureus</i>	<i>P aeruginosa</i>
2484	2.51	8.63	1.65	0	0	0	7.39	6.93	6.22
2485	2.51	8.63	1.65	0	0	0	7.39	6.93	6.22
2486	2.39	8.63	1.65	0	0	0	7.38	6.93	6.22

*Counts were calculated from 10 test and 3 control carriers in each test.

a combination have been reported. The types of stress placed on the test formulation during this investigation included the following: (1) addition of bovine serum to a final concentration of 2% to each

test bath at the start of the study, representing the input of organic and inorganic soil into the baths along with the medical devices being processed; (2) daily input of 880 glass beads with a dried inocu-

Table 7. Virucidal activity of the test solutions collected after 14 days of stress and tested at a contact time of 5 minutes at 20°C*

Lot number	PFU/control carrier × 10 ⁴	PFU/test carrier	Log ₁₀ reduction
2484	9.0	0	4.95
2485	9.0	0	4.95
2486	9.0	0	4.95

*Counts were calculated from 5 test and 3 control disk carriers in each test.

lum of 1 of the 3 types of vegetative bacteria; (3) daily input of 200 penicylinders coated with a dried layer of 1 of the 2 types of bacterial spores; and (4) 3 processing cycles per day of a complete set of respiratory therapy equipment. By the end of the 14-day reuse period, each bath had received a total of 2800 penicylinders and 12,320 glass beads and processed 42 sets of respiratory therapy equipment. This represents a substantial challenge to any high-level disinfectant under reuse.

The challenge to the tested formulations was even greater because, in contrast to the 20-L volume recommended for each germicide bath in the EPA protocol, this study uses only 10 L, thus representing a higher dilution of the active ingredients during its reuse.

The glass beads were used as carriers for the vegetative bacteria because purchase of additional stainless steel penicylinders would have meant a substantial expense. The beads also represented a different kind of hard surface with which the germicide would have to cope.

The germicide test protocol to assess the sporicidal, fungicidal, mycobactericidal, and bactericidal activities of the test samples was not only fully quantitative, but also designed to allow the same type of carriers and basic procedure to be used. Such a “harmonized” approach makes for a more meaningful comparison of the various levels of germicidal activity desired in a high-level disinfectant.⁹ Because practical considerations limit the use of glass vials as carriers in virucidal tests, stainless steel disks were used instead as carriers.^{7,10,11}

Because the entire study was conducted in one place, it avoided the need to transport the samples of stressed solutions for germicidal tests to another location. This is also the first study where samples of the germicide under reuse have been subjected to testing against all major classes of pathogens.

All the microorganisms used in this study represented ATCC strains recommended for use in germicidal tests in the United States^{9,11,14} and in Canada.¹² The membrane filtration step used in all the tests except those against the virus allowed for the capture and detection of even low numbers of viable organisms and reduced, as much as possible, the risk of carryover of any disinfectant residue to the recovery medium. The incorporation of a neutralizer additionally reduced this risk.

For the past several decades, glutaraldehyde has remained the leading germicidal ingredient in products marketed as high-level disinfectants. Although nearly 74% (14 of 19) of such products registered with the US FDA are still glutaraldehyde-based (<http://www.fda.gov/cdrh/ode/germlab.html>), formulations containing alternative germicides are gradually being developed and marketed to enhance the workplace and environmental safety of chemical germicides in general, and that of high-level disinfectants in particular. The use of oxidizing agents such as hydrogen peroxide and peracetic acid, alone or in combination, shows considerable promise in this regard, and 4 of the 19 high-level disinfectants currently registered with the FDA represent such formulations.

A detailed evaluation of the materials compatibility of the formulation tested herein was not within the scope of this research. In general, no apparent damage to various components of the inhalation therapy equipment was observed in spite of its repeated exposure to the test product. Because of their chemical nature, items containing halogenated polyethylenes, chemically resistant rubbers, and high-quality stainless steel should be considered compatible for prolonged immersion in the test formulation. Conversely, any strong oxidizing solution, such as the one tested here, may be unsuitable for use on items containing aluminum, anodized metals, brass, carbon steels, copper, and iron. Consequently, users are urged to investigate the presence of any of these components in the items to be disinfected in this solution.

The AHP product tested in this study represents a relatively recent development in the search for alternatives to glutaraldehyde. Its unique combination of ingredients not only accelerates the activity of hydrogen peroxide, but also enhances its materials compatibility. Formulations derived from this technology are registered for marketing in Canada. Label claims are for 14-day reuse in the cold soaking of instruments,

thus decreasing overall processing costs. The product is particularly suited for use on items with high-quality stainless steels or chemically resistant polymers or rubbers. Specific examples include medical and veterinary surgical tools (eg, blades, pliers, or pins), certain rigid ophthalmologic and odontologic instruments (eg, tonometer tips, extraction tools, or drill bits), and respiratory accessories, such as respiratory masks, endotracheal tubes, tubing, and connectors. Nonclinical applications include soaking instruments used in tattoo parlors and podiatry.

The stress test protocol used in this investigation remains a cumbersome and time-consuming procedure. It also requires much planning, coordination, and the involvement of at least 2 skilled and experienced researchers to conduct it properly. Furthermore, the procedure is not yet recognized as a standard by any organization that deals with germicide test methodology. Therefore a need exists to improve or develop a suitable substitute for the method so that it could be proposed as a standard for the purpose. However, the details described here could help in comparing the performance of a given formulation in the existing method when compared with that in any improved or modified method.

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