A new peroxide-based flexible endoscope-compatible high-level disinfectant

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Semicritical medical devices such as flexible endoscopes require high-level disinfection between each use, and glutaraldehyde is often used for this purpose because of its favorable materials compatibility. However, workplace safety and the relatively slow microbicidal activity of such formulations remain a concern. Although recently introduced substitutes based on 0.55% *ortho*-phthaldehyde (OPA), 7% to 14% hydrogen peroxide, and 0.1% to 0.3% peracids are considered less toxic than glutaraldehyde, OPA can be a potential respiratory sensitizer, and the materials compatibility profile of peroxide/peracids at effective concentrations remains an issue. This study describes a high-level disinfectant/sterilant based on 2% accelerated hydrogen peroxide (AHP). It is a blend of stabilized hydrogen peroxide with safe inerts, which act in synergy, and has a 14-day reuse, 5-minute high-level disinfection, and 6-hour sterilization claim at room temperature. Extensive testing of this formulation using nationally and internationally accepted protocols has found it to be a fast-acting and broad-spectrum microbicide in addition to being biodegradable, virtually nontoxic, and free from volatile organic compounds and alkyl phenol ethoxylates. In addition, materials compatibility testing has proven it to be compatible with flexible endoscopes. Therefore, this new chemistry represents a significant advancement in the design of safer and faster acting, high-level disinfectants. (Am J Infect Control 2006;34:571-7.)

Medical devices are divided into different categories based on the risk of infection involved in their use. Spaulding[1] in 1968 proposed such a classification as critical, semicritical, and noncritical instruments. Spaulding believed that instruments and equipment should be cleaned and reprocessed according to the level of risk associated with their intended use.

In this classification, critical instruments were those that come into contact with bloodstream or sterile areas of the body, such as cardiac catheters, implants, or surgical instruments. These items are required to be reprocessed by sterilization. Semicritical devices are those that only come into contact with mucous membranes of the body and do not contact the sterile part of the body. Examples of these items would be flexible endoscopes, aspirator tubes, bronchoscopes, laryngoscopes, and respiratory therapy equipment. These instruments must be high-level disinfected between uses. Noncritical medical instruments touch intact (unbroken) skin but not the mucous membranes, such as blood pressure cuffs, stethoscopes, and bedpans. These instruments are required to be either low-level disinfected or just cleaned and sanitized in most cases. Semicritical medical devices, such as flexible endoscopes, are heat sensitive and need to be chemically high-level disinfected either manually or in a machine.[2] There has always been a challenge in creating a balance between materials compatibility, toxicity, and microbicidal activity of disinfectants. Generally, broad-spectrum and fast-acting active ingredients are corrosive and/or toxic. For example, chlorine is an effective and rapid microbicide; however, it is not suitable for use on flexible endoscopes because of its high corrosivity. On the other hand, quaternary ammonium compounds have fair material compatibility, but they are not effective against mycobacteria, spores, or nonenveloped viruses[3-5] and, consequently, cannot be used for this application.

Glutaraldehyde is the most commonly used high-level disinfectant for reprocessing flexible endoscopes because of its favorable materials compatibility. However, it is a toxic and irritant chemical, a moderate sensitizer of human skin,[6-9] and a protein fixative.[10] It is classified as a primary dermal irritant, and dermal application to the skin of rabbits caused moderate irritation.[11] Glutaraldehyde causes occupational asthma and rhinitis upon exposure.[12,13] Using a semiquantitative approach, glutataraldehyde was found to be one of the most active mutogenic carbonyl-containing compounds.[14] Glutaraldehyde was also found to be mutagenic, independent of S9 activation.[15] Glutaraldehyde can be absorbed by rubber or plastic parts and can induce cytotoxic reactions.[16]

Some microorganisms have shown resistance against glutaraldehyde. Carson et al[17] showed that TM

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strains of *Mycobacterium chelonei* survived 60 minutes of exposure to 2% glutaraldehye. In addition, Urayama et al[18] showed that *M chelonei* was still detected in endoscopes after a 45-minute exposure to glutaraldehyde. Griffiths et al[19] indicated that a clinical isolate of *M chelonei* was very resistant with little reduction in viable count after 60-minute exposure to 2% glutaraldehyde. Pierce et al[20] suggested that 2% glutaraldehyde failed to disinfect ultrasonic nebulizers heavily contaminated with *Pseudomonas* species. Furthermore, Davison et al[21] showed that 2 isolates of *Salmonella entertitidis*, a major source of infection in poultry, were resistant to glutaraldehyde according to the Association of Official Analytical Chemists use-dilution analysis.

Glutaraldehyde reuse commercial formulations have generally 20 to 45 minutes contact time for mycobacteria inactivation and 6 to 10 hours for sporicidal activity.[22] However, Mbithi et al[23] stated that 2% glutaraldehyde may become ineffective against nonenveloped viruses and mycobacteria in much less than 14 days in reuse baths meant for the disinfection of endoscopes. Furthermore, glutaraldehyde has poor cleaning activity and has a strong odor.

Ortho-phthaldehyde (OPA) is an aromatic aldehyde, which is currently in wide use. It is compatible with flexible endoscopes. It is less toxic than glutaraldehyde. OPA is also faster acting than glutaraldehyde against mycobacteria but is a much slower sporicidal.[22] Although OPA is less toxic than glutaraldehyde, it still has some inhalation and irritation concerns. William and Sokol[24] described 9 episodes of anaphylaxis following cystoscopy caused by OPA.

Rideout et al[25] showed that OPA has the same predictors of respiratory sensitization as glutaraldehyde as well as an aromatic group. Joshi and Rosenfeld[26] explained the 2 cases of OPA-induced allergic reactions in patients undergoing surveillance cystoscopy.

Peracetic acid is also used as a high-level disinfectant/sterilant. Peracids have broad-spectrum antimicrobial activity and are friendly to the environment.[27] However, peracid solutions have poor stability,[28] are corrosive to many materials, smell pungent, and are potent tumor promoters and are weak carcinogens.[29]

Commercial hydrogen peroxide is another broad-spectrum active antimicrobial that is used in this area. Commercial hydrogen peroxide solutions typically have poor stability. Commercial hydrogen peroxide is a very slow active antimicrobial,[28] and useful concentrations for high-level disinfection are corrosive to many medical instruments such as flexible endoscopes.

The objective of this paper is to report on a newly developed, high-level disinfectant/chemisterilant that addresses the concerns regarding the abovementioned chemicals. This new product is based on accelerated hydrogen peroxide (AHP) technology. AHP is a synergistic blend of commonly used, safe ingredients that, when combined with low levels of hydrogen peroxide, dramatically increase its germicidal potency. AHP contains only those ingredients on the Generally Regarded as Safe listing published by the Food and Drug Administration (FDA), which represents unsurpassed health, safety, and environmental friendly profiles.

MATERIALS AND METHODS

Formulation tested

The product tested in this study, Accel HLD 5 (Virox Technologies, Ontario, Canada), is a newly developed, AHP-based, high-level disinfectant and chemisterilant. Accel HLD 5 is a blend of 2% hydrogen peroxide, anionic surfactants, nonionic surfactants, and stabilizers. It is a clear, slightly yellowish liquid, odorless and has a pH of 2.5 to 3.0. It is free from volatile organic compounds and alkyl phenol ethoxylates. The formulation is registered for use in Canada and will soon be registered in the United States as well. Accel HLD 5 was tested for its antimicrobial activity, stability, toxicity, dermal and eye irritancy, biodegradability, and materials compatibility using well-recognized protocols.

Antimicrobial tests

Three lots of the test solution were stressed for 14 days using procedures that meet with the requirements of the US FDA and Health Canada. The stressing was carried out according to the procedures described by Sattar et al.[2]

Soil load

To increase the level of stress to the disinfectant solution, fetal bovine serum (FBS) at a final concentration of 2% was added to each container with the test product. The objective of this was to simulate loading with organic material. FBS is universally accepted as a soil load in testing microbicidal activity of liquid chemical disinfectants.[30] It was noninhibitory for all the organisms used in this study. The addition of contaminated carriers as a bioburden and the soaking of several items of respiratory equipment over the 14-day stress cycle further simulated the challenge the product may face under reuse.

The first tier of the quantitative carrier test (QCT-1) used in this evaluation[31] meets the requirements of the Canadian General Standards Board for testing microbicides to be used on environmental surfaces and medical devices[32] and is an accepted standard of the American Society for Testing and Materials (ASTM) International.[33] The method is designed to assess the sporicidal, bactericidal, mycobactericidal, and fungicidal activities of liquid chemicals and uses the inside bottom surface of glass vials as the carrier for the challenge microorganism.

Ten microliters of the test microbial suspension, without any added soil load, was dried in each carrier, and the dried inoculum was then overlaid with 1 mL disinfectant sample to be tested. The carriers are held for the required contact time at 20°C. The inoculum was eluted, and the needed dilutions of the eluate were made and separately passed through membrane filters. The filters were placed on suitable recovery media and incubated, colonies counted, and $\log_{10}$ reductions calculated as described below. Control carriers were used in the same manner as test carriers, except saline solution was applied to the dried inoculum instead of the disinfectant.

Virucidal activity was determined using protocol E1053 of ASTM International.[34] Virus suspension (200 μL) was placed into the middle of a glass Petri dish and spread with a glass rod. The inoculum was left to dry and then exposed to 2 mL test formulation for a contact time of 5 minutes. Earle's balanced salt solution was used for the control samples. At the end of the contact period, 200 μL virus-disinfectant mixture was transferred into 1.8 mL neutralizer to stop the reaction. A 1.2-mL volume of the neutralized samples was layered onto a 5-mL column of Sephadex LH-20. Serial dilutions from the eluates were performed and used for plaque assay.

Product performance criteria

Ten test and 3 control carriers were used in each QCT-1 test. Three glass Petri dishes were used as carriers for each control and test samples in the virucidal activity. The results are reported as $\log_{10}$ reductions in viability in reference to the controls. For a sample to be regarded as bactericidal, sporicidal, or mycobactericidal, it was necessary to get a reduction in the viability titer of the test organism $>6 \log_{10}$ under the conditions of the test; $>5 \log_{10}$ reduction was needed for fungicidal activity and $>4 \log_{10}$ for virucidal activity.[31] The average of the several replicates for each lot was used to calculate colony-forming units per control carrier and colony-forming units per test carrier after exposure to the product.

Stability tests

Three production lots of the test formula were observed for stability for a total period of 12 months since the date of production. Samples were kept in the same packaging form as it is marketed and were maintained at ambient temperature and humidity in a designated storage area. The determining factors in maintaining product efficacy were (1) hydrogen peroxide content no lower than 90% of the nominal concentration, (2) pH lower than pH 2.4 and no higher than 3.0, and (3) homogeneity of the solution (no evidence of clouding, creaming, or sedimentation). A product was considered to remain effective as claimed if these conditions were met at the time of examination. This was done to comply with paragraph C.01.062 in the Food and Drugs Act, wherein the concentration of medicinal active in a drug product cannot lie outside of a band defined by 90% to 110% of the nominal concentration.

Toxicity tests

The acute eye irritation/corrosion test was performed using the OECD 405 test method.[35] A dose of 0.1 mL test solution was instilled in the conjunctival sac of 1 eye of the rabbit. The other eye remained untreated and served as the control. The eye of the rabbit was not washed out during the 24-hour exposure period.

The acute dermal irritation/corrosion test was performed using the OECD 404 test method.[36] A dose of 0.5 mL test article was topically applied by patch application to a chosen intact test site of the skin of the rabbit. The test solution stayed in contact with the skin for a 4-hour period. An untreated control site was concurrently run. Because a corrosive effect was not observed in the initial animal, a confirmatory test was performed in a similar manner on 2 additional animals. The test sites were evaluated immediately (only for the initial animal) and at 1, 24, 48, 72, 96, 120, 144, and 168 hours after the exposure period.

The acute oral toxicity study was performed using the OECD 425 test method.[37] The first animal was dosed at 2000 mg/kg of the test solution. Because the first animal survived, 4 additional animals were dosed at approximately 48-hour intervals. A total of 5 female rats were dosed. All animals received the test article by oral gavage using a feeding cannula. The animals were observed for a 14-day period after dosing. Body weights were recorded before initiation of the treatment, at day 7, and at the end of the study. No effects of toxicity or mortalities were observed postdosing and during the 14-day observation period in any of the animals. All 5 rats gained body weight by day 7 and at the end of the study. At the end of the 14-day observation period, each animal was killed and submitted for gross necropsy.

Biodegradability test

Accel HLD 5 was tested for its inherent biodegradability using the OECD 302B test method.[38]

Flexible endoscope compatibility test

The Olympus flexible gastroscope, model GIF-Q160, was tested for its compatibility with the test solution. The scope was rinsed with deionized water and dried.

Table 1. Sporicidal activity of the 14 day stressed formulation after a contact of 6 hours at 20°C

Lot No.	Date of experiment	CFU per control carrier	CFU per test carrier	Log_{10} reduction
Bacillus subtilis (ATCC 19659)				
3575	04/07/04	2.79×10^6	0	6.45
3576	04/07/04	2.79×10^6	0	6.45
3577	04/07/04	2.79×10^6	0	6.45
Clostridium sporogenes (ATCC 7955)				
3575	05/26/04	3.02×10^6	0	6.48
3576	05/26/04	3.02×10^6	0	6.48
3577	05/26/04	3.02×10^6	0	6.48

Table 2. Bactericidal activity of the 14 day stressed formulation after a contact of 5 minutes

Lot No.	Date of experiment	CFU per control carrier	CFU per test carrier	Log_{10} reduction
Staphylococcus aureus (ATCC 6538)				
3575	08/12/04	5.48×10^6	0	6.73
3576	08/12/04	5.48×10^6	0	6.73
3577	08/12/04	5.48×10^6	0	6.73
Pseudomonas aeruginosa (ATCC 15442)				
3575	08/24/04	1.49×10^7	0	7.17
3576	08/24/04	1.49×10^7	0	7.17
3577	08/24/04	1.49×10^7	0	7.17
Salmonella choleraesuis (ATCC 10708)				
3575	08/25/04	8.28×10^6	0	6.91
3576	08/25/04	8.28×10^6	0	6.91
3577	08/25/04	8.28×10^6	0	6.91

Table 3. Mycobactericidal activity of the 14 day stressed formulation after a contact of 5 minutes

Lot No.	CFU/control carrier	CFU/test carrier	Log_{10} reduction
Mycobacterium terrae (ATCC 15755)			
3575	3.07×10^6	0	6.49
3576	3.07×10^6	0	6.49
3577	3.07×10^6	0	6.49

Table 4. Fungicidal activity of the 14 day stressed formulation after a contact time of 5 minutes

Lot No.	Date of experiment	CFU/control carrier	CFU/test carrier	Log_{10} reduction
Trichophyton mentagrophytes (ATCC 9533)				
3575	04/16/04	1.32×10^6	0	6.12
3576	04/16/04	1.32×10^6	0	6.12
3577	04/16/04	1.32×10^6	0	6.12

Table 5. Virucidal activity of the stressed formulation after a contact time of 5 minutes

Lot No.	Date of the experiment	PFU/control	PFU/test	Log_{10} reduction
Poliovirus type 1, Sabin (ATCC VR-192)				
3575	04/09/09	1.35×10^4	0	4.13
3576	04/09/09	1.35×10^4	0	4.13
3577	04/09/09	1.35×10^4	0	4.13

Each part of the scope was photographed to compare before and after exposure. The scope was soaked for 1000 cycles of 5-minute high-level disinfection contact time (84 hours). Every 24 hours, the scope was visually observed for any damage. Type of rinsing water does not affect the material compatibility. However, it is important that the rinsing water be free from microorganisms to avoid recontamination. Therefore, submicron-filtered tap water, which is mostly used in health care settings can be used instead of distilled (DI) water.

RESULTS

Sporicidal activity

Table 1 gives the results of the sporicidal tests. All 3 lots of the product showed sporicidal activity against *B subtilis* and *C sporogenes*, with a reduction in the viability titer of >6 log_{10} in a contact time of 6 hours at 20°C.

Bactericidal activity

Table 2 shows the results of bactericidal activity. The stressed disinfectant displayed bactericidal activity against the 3 vegetative bacteria. A reduction in the viability titer of >6 log_{10} in a contact time of 5 minutes was obtained.

Mycobactericidal activity

As summarized in Table 3, all 3 lots of the product showed mycobactericidal activity, with a reduction in the viability titer of >6 log_{10} in a contact time of 5 minutes.

Fungicidal activity

As shown in Table 4, all 3 lots of the product also showed fungicidal activity of >6 log_{10} in a contact time of 5 minutes, higher than the product performance criterion of 5 log_{10}.

Virucidal activity

The results for virucidal activity are given in Table 5. All 3 lots of Accel HLD 5 showed virucidal activity, with a reduction in the viability titer of >4 log_{10} in a contact time of 5 minutes.

Table 6. H_2O_2 concentration and pH of the disinfectant during stress

Lot No.	Days of stress	pH	% H_2O_2
3575	7	2.51	1.73
3576	7	2.53	1.73
3577	7	2.55	1.73
3575	14	2.43	1.67
3576	14	2.45	1.67
3577	14	2.45	1.67

Table 7. Stability results for 3 lots of Accel HLD 5

	Lot 1	Lot 2	Lot 3
Initial H_2O_2%	2.04	2.05	2.04
Initial pH	2.55	2.56	2.60
Initial appearance	Clear liquid	Clear liquid	Clear liquid
Final H_2O_2%	1.86	1.89	1.90
Final pH	2.52	2.59	2.56
H_2O_2 loss%	8.8	7.8	6.8
Final appearance	Clear liquid	Clear liquid	Clear liquid

The results show that the product have at least one year of shelf life.

Hydrogen peroxide levels and pH

The hydrogen peroxide concentration and the pH were monitored after 7 and 14 days of stress and did not show any significant change (Table 6).

Stability tests

Table 7 shows the results for the stability test of 3 lots of Accel HLD 5. The results show that the product has at least 1 year of shelf life.

Toxicity tests

Acute eye irritation/corrosion test. Because a corrosive effect was not observed in the initial animal, a confirmatory test was performed in a similar manner on 2 additional animals. Irritancy evaluations were carried out at 1, 24, 48, and 72 hours following test article instillation. Based on these observations, the test solution was found to be mildly irritating to eyes.

Acute dermal irritation/corrosion test. Because a corrosive effect was not observed in the initial animal, a confirmatory test was performed in a similar manner on 2 additional animals. The test sites were evaluated immediately (only for the initial animal) and at 1, 24, 48, 72, 96, 120, 144, and 168 hours after the exposure period. Based on these test results, the solution was classified as a slight irritant.

Acute oral toxicity study. No effects of toxicity or mortalities were observed postdosing and during the 14-day observation period in any of the animals. All 5 rats gained body weight by day 7 and at the end of the study. At the end of the 14-day observation period, each animal was killed and submitted for gross necropsy. No gross pathologic findings were observed in any rat at necropsy. Based on these results, the acute oral lethal dose (LD) 50 in rats of the test solution was found to be in excess of 2000 mg/kg. Therefore, the test article is considered not to present a significant acute toxic risk if swallowed. The Globally Harmonized Classification System for Chemicals and Mixtures classifies compounds in which the estimated LD50 is greater than 2000 mg/kg with no deaths or evidence of toxicity as being category 5 chemicals.[39]

Biodegradability test. The test solution showed 73.5% biodegradation in 28 days. The criterion for this test is more than 20% biodegradation in 28 days, which shows that the test material exceeds the criterion and is therefore inherently biodegradable. This means that the product has the potential to degrade and is not persistent.

Flexible endoscope compatibility test. Table 8 shows the test results.

DISCUSSION

High-level disinfectants are required for reprocessing semicritical and critical medical devices such as flexible endoscopes. However, current products such as those based on glutaraldehyde and OPA have been under increased scrutiny because of their less than ideal toxicity profile. Although OPA has not been in the market for a long time, the inhalation studies suggest that, as an aromatic aldehyde, OPA is considered toxic.

Reprocessing medical devices in a rapid manner is desirable and largely contingent on the exposure time of the disinfectant to be mycobactericidal or, in some cases, sporicidal. The antimicrobial activity of aldehydes is rather slow. Glutaraldehyde-based formulations have from 10- to 40-minute tuberculocidal and over 10-hour sporicidal contact times. For OPA, contact times are 12 minutes and 32 hours, respectively.[22] Other high-level disinfectants such as peracid and peroxide have known to have materials compatibility concerns. The balance between user safety, microbicidal activity, and materials compatibility has always been a significant challenge for product formulators. Traditional commercial hydrogen peroxide by itself is one of the oldest known disinfectants. It is environmentally friendly because it decomposes to water and oxygen. It is not toxic at disinfection levels and is naturally generated in many settings. However, its microbial activity is very slow. It is well-known that 7% hydrogen peroxide commercial products have 30-minute tuberculocidal and 6-hour sporicidal contact time.[22] At this concentration, peroxide is corrosive to most items because of its oxidizing nature. It is also difficult to formulate

Table 8. Flexible endoscope compatibility test results

Inspection point	Prestudy condition	Inspection results	Comments
Image quality-videoscope	Image is crisp with uniform brightness. No stains. No defects.	No change	No damage is observed
Insertion tube (polymer coat)	Smooth surface, shiny, clear topcoat.	No change	No change in color shine, and flexibility; no tackiness, blistering, bubbling, buckling
Insertion tube (boot)	Bright White	No change	No yellowing or other color change
Insertion tube (markings)	Smooth	No change	No crazing, no loss of gloss, no swelling of tapered end
Distal end (glue condition)	Smooth, shiny, no chips, no discoloration	Black, shiny, no pitting	No whitening, no roughing of the surface, no chipping of the edges
Distal end (rubber condition)	Smooth	Tight, normal	No crazing/swelling, no loss of gloss
Leak test	Watertight	No leak	No damage was observed
Plastic grip and angulation knobs, visual inspection	Smooth, black, no defects	No change	No damage was observed
Nameplates	Intact, no discoloration	No change	No damage was observed
Lightguide tube (polymer coat)	Smooth, shiny, clear.	No change	No damage was observed
Lightguide tube (boot)	Smooth	No change	No damage was observed
Lightguide connector (body)	Smooth, no defects	No change	No damage was observed
Lightguide connector (electrical connectors, videoscopes only)	No corrosion	Clean	No damage was observed
Lightguide connector (boot)	Smooth Was duplicated from above	No change	No damage was observed
Lightguide connector (labels/markings)	Intact, no discoloration	No change	No damage was observed

stabilized hydrogen peroxide solutions containing other inert ingredients. Commercial peracetic acid solutions containing 1% peroxide and 0.08% peracid have 25-minute tuberculocidal and 6-hour sporicidal contact time.[22] However, Accel HLD 5 (2% hydrogen peroxide solution) has 5-minute tuberculocidal and 6-hour sporicidal contact time. Despite its fast acting germicidal activity, Accel HLD 5 is proven to be a relatively mild solution for end users. It is slightly irritating to skin and mildly irritating to eyes according to accepted standard tests methods, which is the same as 3% topical hydrogen peroxide solutions typically found and used in hospitals.

This study shows that accelerated hydrogen peroxide ''AHP'' technology is now able to address the above concerns. All ingredients used in AHP formulations are on the FDA's Generally Recognized as Safe list and the Environmental Protection Agency's inerts list. They are free from aquatic toxicants including alkyl phenol ethoxylates or nonyl phenol ethoxylates. AHP is also free from volatile organic compounds and is inherently biodegradable. Based on these findings, it is now possible to have a faster acting, high-level disinfectant that is not only safer for end users but also compatible with flexible endoscopes. All antimicrobial test results in this study are based on in vitro testing performed at third party labs. Although in vitro tests in this study simulate real-life situations and are sufficient for registration in countries such as Canada, antimicrobial tests on endoscopes are still required to be performed to register this product with the US FDA.

In summary, the AHP-based, high-level disinfectant tested in this study proved to be a broad-spectrum microbicide, fast acting, and safer to end users and the environment and is considered to be compatible with flexible endoscopes. Accel HLD5, therefore, addresses many of the concerns relating to other types of actives in processing flexible endoscopes and other heat/chemical sensitive medical devices.

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