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Broad-spectrum microbicidal activity, toxicologic assessment, and materials compatibility of a new generation of accelerated hydrogen peroxide-based environmental surface disinfectant

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Background: Concerns on human and environmental safety and label claims of many microbicides point to the need for safer, faster acting, and broad-spectrum substitutes. ACCEL TB, a 0.5% accelerated H_2O_2 (AHP)-based disinfectant described here, is a potential candidate.

Methods: ACCEL TB was tested for its broad-spectrum microbicidal activity, safety and materials compatibility using internationally accepted protocols. Activity against bacteria (*Staphylococcus aureus*, *Escherichia coli*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, vancomycin-resistant *Enterococcus faecalis*, methicillin-resistant *Staphylococcus aureus*, and *Salmonella choleraesuis*) was tested with the AOAC use-dilution method and the first tier of a quantitative carrier test (QCT-1). Mycobactericidal activity was tested against *Mycobacterium bovis* and *Mycobacterium terrae* using a quantitative suspension test (QST) and QCT-1, respectively. Fungicidal activity (*Trichophyton mentagrophytes*) was determined with the AOAC test and QCT-1. Activity against several enveloped and nonenveloped viruses was evaluated using the American Society for Testing and Materials (ASTM) method No. E-1053. Sanitizing action was tested against 7 types of vegetative bacteria with method No. DIS/TSS-10. All microbicidal tests contained an added soil load; in all AOAC tests, it was 5% fetal bovine serum, and, in QCT-1, a mixture of 3 types of proteins in phosphate buffer was used instead. The methods to test for acute oral, dermal, inhalation toxicities, and dermal and eye irritation as well as skin sensitization complied with the requirements of the Organization for Economic Cooperation and Development and the US Environmental Protection Agency (OPPTS 870). Standard methods were also used to test compatibility with metals and plastics.

Results: At 20°C, the full-strength product was bactericidal and virucidal in 1 minute and mycobactericidal and fungicidal in 5 minutes. It was nonirritating to skin and eyes. The acute oral LD_{50} (lethal dose 50%) was >5000 mg/kg. It was compatible with 12 types of plastic and 3 out of 4 metals.

Conclusion: The tested formulation showed a high safety and materials compatibility profile in addition to being a fast acting, intermediate-level disinfectant. (Am J Infect Control 2006;34:251-7.)

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Chemical disinfectants are widely used in infection control.[1] Our reliance on them is increasing further in preventive strategies because of rampant antibiotic resistance and mounting threats from emerging and reemerging pathogens. This, in turn, is forcing a closer look at label claims of such products as well as human health and environmental safety of the chemicals in them.[2] Therefore, safer and better substitutes are urgently needed. This report describes our findings on one such formulation.

METHODS

Formulation tested

This study tested ACCEL TB, a new generation of a patented (US. patent No. 6,346,279), 0.5% accelerated

H_2O_2 (AHP)-based, ready-to-use formulation (pH 3.0) designed for the disinfection of hard environmental surfaces. It contains very low levels of certain food-grade anionic and nonionic surfactants, which act in synergy with hydrogen peroxide to produce the desired microbicidal activity. The formulation is in the process of registration as an intermediate-level disinfectant in Canada and the United States.

Microbicidal activity

Internationally accepted methods were used to assess the microbicidal activity of the formulation against 21 different types of organism representing vegetative bacteria, mycobacteria, fungi, and viruses. The details on these organisms are given in Table 1.

Bactericidal activity

The use-dilution method (UDM) of AOAC International[3] and the first tier of the quantitative carrier test (QCT-1) of ASTM International[4] were used to test activity against vegetative bacteria. *Escherichia coli*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, vancomycin-resistant *Enterococcus faecalis*, methicillin-resistant *Staphylococcus aureus*, and *Salmonella choleraesuis* were tested using UDM, and *Staphylococcus aureus* was tested using both UDM and QCT-1 methods. In UDM, stainless steel penicylinders were the carriers, and the performance criterion was no more than 1 culture-positive tube out of 60. In QCT-1, flat-bottomed glass vials were the carriers[4]; the test organism (10 μL) was placed in each vial, dried, and covered with 1 mL of the test substance for the specified contact time at 20°C ± 1°C. At the end of the contact time, 9 mL neutralizer (Letheen broth with 0.1 % sodium thiosulfate) was added to each vial and the test inoculum resuspended with the help of a magnetic stir bar.

The contents of the vial were then filtered through a membrane filter (Millipore Corp: 47 mm diameter; 0.2 pm pore size). The vial was rinsed several times with a total of no less than 100 mL saline, the rinses also passed through the same filter, and the filters were then placed on the agar surface of an appropriate recovery medium. The plates were held at the required temperature and for the desired length of time for the organism to grow. The colonies on the plates were counted and $\log_{10}$ reductions in the viability titer of the test organism calculated. When the number of colony forming units (CFU) was expected to be high, the eluate was first subjected to 10-fold dilutions, and each sample was passed through a separate membrane filter, and the filters were placed on recovery media. The product performance criterion for QCT-1 was >6 $\log_{10}$ in the viability as compared with the control carriers.

In UDM, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Salmonella choleraesuis* were tested using 3 lots of the test solution; one of the lots was 60 days old. For the rest of the bacteria, only 2 lots were tested, and, in QCT-1, 1 lot was tested for *Staphylococcus aureus*.

Mycobactericidal activity

The quantitative suspension test (QST) of Ascenzi et al[5] was used to test activity against *Mycobacterium bovis*-BCG, and QCT-1 was used with *Mycobacterium terrae* as the challenge organism. Two and 3 lots of the test solution were tested for QST and QCT-1, respectively.

Fungicidal activity

In the AOAC fungicidal activity test,[3] a suspension method, each tube with 5 mL test substance (equilibrated to 20°C ± 1°C in a water bath), received 0.5 mL *Trichophyton mentagrophytes* conidial suspension. The tube was agitated gently after adding the suspension and replaced into the water bath. At the end of the required contact time, a loopful was transferred to an appropriately labeled subculture tube containing 10 mL recovery broth. Secondary subcultures were performed >30 minutes after initial subculture by transferring one loopful from each subculture tube into a similarly labeled secondary subculture tube to rule out fungistatic action. The inoculated and control tubes were incubated for 44 to 76 hours at 25°C to 30°C. Subculture tubes were incubated for 10 days at 25°C to 30°C. The tubes were visually examined for growth. The acceptance criterion for fungicidal activity was no growth in all subculture tubes.

The QCT-1 method for fungicidal tests was similar to that described above for bactericidal and mycobactericidal tests, and the performance criterion was also a >5 $\log_{10}$ reduction. Two and 3 lots of the test solution were tested using the AOAC and the QCT-1, respectively.

Virucidal activity

ASTM standard number E-1053[6] was used to test for virucidal activity. Films of test virus were prepared by spreading 0.2 mL suspension uniformly over the inside bottom surface of 3 separate 100 × 15 mm glass Petri dishes. The plates were held at 20°C ± 1°C (relative humidity = 42 %) until visibly dry (approximately 20 minutes). Each dried virus film was exposed to 2 mL test substance (control plates received an equivalent volume of a buffer) at 20°C for the desired contact time. The inoculated surface of the plates was scraped with a cell scraper to resuspend the contents of the inoculum, and the virus-disinfectant mixture was immediately passed through a Sephadex gel column following

Table 1. Particulars on the organisms tested in this study*

Organism (ATCC No.)	Medium/host cells for growth	Medium/host cells for recovery
Staphylococcus aureus (6538)	Synthetic broth	Neutralizer: Letheen broth with 1.0% sodium thiosulfate (primary), Letheen broth with 0.07% lecithin and 0.5% Tween 80 (secondary) Agar plate media: Trypticase soy agar (TSA) with 5% sheep blood
Pseudomonas aeruginosa (15442)	Nutrient broth	Neutralizer: Letheen broth with 1.0% sodium thiosulfate (primary), Letheen broth with 0.07% lecithin and 0.5% Tween 80 (secondary) Agar plate media: Trypticase soy agar (TSA) with 5% sheep blood
Salmonella choleraesuis (10708)	Synthetic broth	Neutralizer: Letheen broth with 1.0% sodium thiosulfate (primary), Letheen broth with 0.07% lecithin and 0.5% Tween 80 (secondary) Agar plate media: Trypticase soy agar (TSA) with 5% sheep blood
Methicillin-resistant *S aureus* (MRSA) (33592)	Synthetic broth	Neutralizer: Letheen broth with 1.0% sodium thiosulfate (primary), Letheen broth with 0.07% lecithin and 0.5% Tween 80 (secondary) Agar plate media: Trypticase soy agar (TSA) with 5% sheep blood
Vancomycin-resistant *Enterococcus faecalis* (VRE) (51575)	Fluid thioglycolate medium	TSA with 5% sheep blood
Escherichia coli O157:H7 (35150)	Synthetic broth supplemented with 1.5% dextrose	Neutralizer: Letheen broth with 1.0% sodium thiosulfate (primary), Letheen broth with 0.07% lecithin and 0.5% Tween 80 (secondary) Agar plate media: Trypticase soy agar (TSA) with 5% sheep blood
Acinetobacter baumannii (19606)	Nutrient broth	Neutralizer: Letheen broth with 1.0% sodium thiosulfate (primary), Letheen broth with 0.07% lecithin and 0.5% Tween 80 (secondary) Agar plate media: Trypticase soy agar (TSA) with 5% sheep blood
Klebsiella pneumoniae (4352)	Nutrient broth	Neutralizer: Letheen broth with 1% sodium thiosulfate and 0.05% catalase
Mycobacterium bovis (BCG) (OT 451C150)	7H9 broth with Tween 80	Neutralizer: Letheen broth with 1% sodium thiosulfate
Mycobacterium terrae (15755)	7H9 broth with ADC enrichment and glycerol	7H11 agar neutralizer: Letheen broth with 0.1% sodium thiosulfate
Trichophyton mentagrophytes (9533)	Potato dextrose agar	Neutralizer: Letheen broth with 1.0% sodium thiosulfate (primary), Letheen broth with 0.07% lecithin and 0.5% Tween 80 (secondary)
Poliovirus type 1- Brunhilde (VR-1000)	Vero cells	Removal of microbicide residual: Sephadex filtration Media: Eagle's minimal essential media + 1% serum + 10 μg/mL gentamicin + 100 units/mL penicillin + 2.5 μg/mL amphotericin B
HIV-1, strain HTLV-IIIB	MT-2 cells (human $CD4^+$ lymphocytes)	Removal of microbicide residual: Sephadex filtration Media: RPMI 1640 supplemented with 15% heat-inactivated FBS + 10 μg/mL gentamicin + 100 units/mL penicillin + 2.5 μg/mL amphotericin B
Feline calicivirus strain F9-a surrogate for noroviruses (VR-782)	CRFK cells	Removal of microbicide residual: Sephadex filtration Media: E-MEM + 5% heat-inactivated FBS + 10 μg/mL gentamicin + 100 units/mL penicillin + 2.5 μg/mL amphotericin B
Human coronavirus 229E-also a surrogate for the SARS virus (VR-740)	MRC-5 (human embryonic lung) cells	Removal of microbicide residual: Sephadex filtration Media: E-MEM + 2% FBS + 10 μg/mL gentamicin + 100 units/mL penicillin + 2.5 μg/mL amphotericin B
Herpes virus types 1 (VR-733)	Rabbit kidney (RK) cells	Removal of microbicide residual: Sephadex filtration Media: E-MEM + 5% FBS+ 10 μg/mL gentamicin + 100 units/mL penicillin + 2.5 μg/mL amphotericin B
Herpes virus types 2 (VR-734)	Rabbit kidney (RK) cells	Removal of microbicide residual: Sephadex filtration Media: E-MEM + 5% FBS+ 10 μg/mL gentamicin + 100 units/mL penicillin + 2.5 μg/mL amphotericin B
Human rhinovirus type 37 (VR-1147)	MRC-5 cells	Removal of microbicide residual: Sephadex filtration Media: E-MEM + 10% FBS+ 10 μg/mL gentamicin + 100 units/mL penicillin + 2.5 μg/mL amphotericin B
Human rotavirus Wa strain (2018)	MA-104 cells	Removal of microbicide residual: Sephadex filtration Media: E-MEM + 0.5 μg/mL trypsin, 2.0 mmol/L L-glutamine + 10 μg/mL gentamicin + 100 units/mL penicillin + 2.5 μg/mL amphotericin B
Human influenza virus A - PR-8 strain (VR-544)	Rhesus monkey kidney (RMK) cells	Removal of microbicide residual: Sephadex filtration Media: E-MEM + 1% FBS + 10 μg/mL gentamicin + 100 units/mL penicillin + 2.5 μg/mL amphotericin B
Bovine viral diarrhea virus-surrogate for hepatitis C virus (VR-1422)	Cultures of bovine turbinate (BT) cells	Removal of microbicide residual: Sephadex filtration Media: E-MEM + 2% FBS+ 10 μg/mL gentamicin + 100 units/mL penicillin + 2.5 μg/mL amphotericin B

*All percentage values given are vol/vol except for sodium thiosulfate, which was wt/vol.

Table 2. Bactericidal activity of ACCEL TB in a contact time of 1 minute at 20°C (AOAC use- dilution test)

Organism tested	CFU/control carrier	Number of positive carriers/number tested		
		Lot No. 3635	Lot No. 3646	Lot No. 3647
Staphylococcus aureus	1.73×10^5	1/60	0/60	0/60
Pseudomonas aeruginosa	1.16×10^5	1/60	0/60	0/60
Salmonella choleraesuis	7.20×10^5	1/60	0/60	0/60
MRSA	2.10×10^6	NT	0/10	0/10
E coli	1.01×10^6	NT	0/10	0/10
VRE	4.60×10^6	NT	0/10	0/10
Acinetobacter baumannii	4.7×10^6	NT	0/10	0/10

NT, Not tested.

ASTM standard E-1482[7] to remove as much cytotoxicity as possible. The filtrate (10^{-1} dilution) was then subjected to a 10-fold dilution series and assayed for infectious virus. The acceptance criterion for this test was no infectivity for the virus-disinfectant mixture at any dilution tested and a 3-log reduction beyond the cytotoxicity level. Two lots of the test solution were tested in all virucidal tests.

Sanitizing activity test

DIS/TSS-10 test method[8] was used with glass squares (2.5 × 2.5 cm) in Petri plates. Each carrier received 20 μL 48-hour-old bacterial culture using a calibrated pipette. The inoculum was spread to within 0.13 inches (0.3 cm) of the edges of the carrier. All plates were then placed at 35°C to 37°C for 20 to 40 minutes in a chamber with 40% ± 2% relative humidity (RH). The carriers with the dried inoculum were placed at room temperature, and each plate with 5 carriers received 5 mL test substance for a contact time of 30 seconds. At the end of the contact time, 20 mL of an appropriate neutralizer solution was used to arrest the activity of the test substance. One-milliliter volumes of the eluates were assayed for viable organisms by incubating the inoculated tubes for 48 hours at 35°C to 37°C. The product performance criterion was a >3 $\log_{10}$ (>99.9%) reduction in numbers of viable organism as compared with the carrier quantitation control. *Staphylococcus aureus* and *Klebsiella pneumoniae* were tested using 3 lots of the test solution, including a 60-day-old sample, and the rest of the bacteria were tested using 2 lots.

Soil load

In the AOAC tests, fetal bovine serum (Gibco, Burlington, ON) at a final concentration of 5% was used as a soil load. In QCT-1, a tripartite soil load prepared in phosphate buffer[4,8,9] was used instead. Five hundred microliters test inoculum was prepared by adding to each 340 μL of the microbial suspension 25 μL, 100 μL, and 35 μL of stock solutions of 5% bovine serum albumin (Sigma, St. Louis, MO), 0.4% bovine mucin (Sigma, St. Louis, MO), and 5% tryptone (Ditco, Detroit, MI), respectively; the total protein content of this soil load is roughly equal to that in 5% bovine serum.

Neutralization of microbicidal activity

Letheen broth (Difco) with 1.0% (wt/vol) sodium thiosulfate was used as the neutralizer in the QCT-1 testing. Letheen broth with 0.07% lecithin and 0.5% Tween 80 was used as the secondary neutralizing subculture medium.

Controls

Suitable controls were incorporated to check for sterility of media, reagents and carriers, effectiveness of the microbicide neutralization procedure, and carrier population counts.

Toxicity tests

All toxicity tests were performed at Stillmeadow Laboratory (Sugar Land, TX) using the methods of the Organization for Economic Cooperation and Development (OECD) and the US Environmental Protection Agency (OPPTS 870). One lot of the test solution was used for all toxicity tests.

Materials compatibility

ASTM standards G-1[10] and G-31[11] were used to test the compatibility of the formulation with plastics and metals. The plastics tested were high-density polyethylene (HDPE), low-density polyethylene (LDPE), polytetrafluoroethylene (Teflon), polypropylene (PP), acrylobutadiene styrene (ABS), polyvinyl chloride (PVC), and polysulfone. The metals tested were brass, cold rolled steel, aluminum, and stainless steel.

RESULTS

Bactericidal activity of full-strength ACCEL TB

As shown in Table 2, 7 different species of vegetative bacteria were tested using the UDM, and all lots of the product proved to be bactericidal in 1 minute, at 20°C, in the presence of 5% serum. The number of viable organisms on the carriers was also determined and ranged between 1.0×10^5 and 5.0×10^6 CFU/carrier.

Activity of the test formulation was also assessed against *S aureus* using the QCT-1. Here again, the contact time was 1 minute at 20°C. With a baseline titer of 8.95×10^6 CFU/control carriers, no viable organisms were detected on any of the 6 test carriers, giving a reduction of nearly 7 $\log_{10}$.

Table 3. Fungicidal activity of full-strength ACCEL TB as tested against *Trichophyton mentagrophytes* using QCT-1 with a contact time of 5 minutes at 20°C

Lot number	CFU/control carrier	CFU/ test carrier	Log_{10} reduction
3635reg	8.83×10^5	3	5.47
3636reg	8.83×10^5	2	5.64
3637reg	8.83×10^5	2	5.64

Table 4. Virucidal activity of full-strength ACCEL TB in 1 minute at 20°C (ASTM E-1053)

Virus	Infective units of virus on control carriers (log_{10})	Test substance cytotoxicity (log_{10})	Log_{10} reduction in infectivity
Poliovirus type 1 (Sabin)	4.75	<0.5	>4.25
HIV type 1	5.5	<1.5	>4
Feline calicivirus	6.75	<0.5	>6.25
Human coronavirus 229E	4.5	<0.5	>4
Influenza A virus (PR-8)	5.25	<0.5	>4.75
Human rhinovirus type 37	4.75	<0.5	>4.25
Human rotavirus (Wa)	5.5	<0.5	>5
Herpes virus type 1	5.25	<0.5	>4.75
Herpes virus type 2	4.75	<0.5	>4.25
Bovine viral diarrhea virus (surrogate for hepatitis C virus)	4.5	<0.5	>4.38
Bovine viral diarrhea virus (surrogate for hepatitis C virus)-confirmatory assay	4.75	<0.5	>4.47

Mycobactericidal activity

Two lots (3646 and 3647) of the test formulation were tested against *M bovis* using QST. The level of challenge was approximately 1.3×10^8 CFU/mL. The formulation was able to reduce the viability titer of the test organisms to undetectable levels (>6.8 log_{10} reduction) after a contact time of only 5 minutes at 20°C in the presence of 5% serum.

The mycobactericidal activity of the test formulation was further confirmed with 3 lots of the formulation using QCT-1 and *M terrae* as the challenge. The level of challenge here was 2.4×10^6 CFU/control carrier. It was able to reduce the viability titer of the test organisms to undetectable levels after a contact time of only 5 minutes at 20°C. This represented a reduction of approximately 6.38 log_{10}. Therefore, the test formulation was able to meet the performance criteria for both methods used to assess mycobactericidal activity.

Table 5. Sanitizing activity of ACCEL TB in 30 seconds at 20°C

Test microorganism	Lot number of formulation	Count/ control carrier	Count/ test carrier	Log_{10} reduction
Staphylococcus aureus	3646	4.1×10^5	<30.2	>4
Staphylococcus aureus	3647	9.6×10^5	<30.2	>4
Staphylococcus aureus	4635	2.6×10^6	<30.2	>4
Klebsiella pneumoniae	3646	1.7×10^7	<30.2	>5
Klebsiella pneumoniae	3647	1.3×10^5	<30.2	>3
Klebsiella pneumoniae	4635	3.7×10^7	<30.2	>5
Salmonella choleraesuis	3646	9.4×10^6	<30.2	>5
Salmonella choleraesuis	3647	9.4×10^6	<30.2	>5
Pseudomonas aeruginosa	3635	2.5×10^5	<30.2	>3
Pseudomonas aeruginosa	3646	2.5×10^5	<30.2	>3
E. coli O157:H7	3647	9.6×10^6	<30.2	>5
E. coli O157:H7	3646	9.6×10^6	<30.2	>5
MRSA	3646	2.0×10^6	<30.2	>4
MRSA	3647	2.0×10^6	<30.2	>4
VRE	3646	1.2×10^5	<30.2	>3
VRE	3647	1.2×10^5	<30.2	>3

Fungicidal activity

Two lots of the test formulation were tested for their fungicidal activity using the AOAC suspension test. The level of challenge was 8.3×10^7 CFU, and both lots were able to reduce the viability titer of the organism to undetectable levels in 5 minutes at 20°C in the presence of 5% serum.

As shown in Table 3, 3 additional lots of the formulation were tested for their fungicidal activity using QCT-1. The level of challenge was 8.83×10^5 CFU/carrier, and all lots of the product were able to reduce the viability titer of the fungal conidia by at least 5.47 log_{10} in 5 minutes at 20°C in the presence of the tripartite soil load.

Virucidal activity

As shown in Table 4, both lots of the product tested proved to be virucidal in 1 minute at 20°C in the presence of 5% serum.

Sanitizing activity

As shown in Table 5, all lots of the product were able to work as a sanitizer in 30 seconds at 20°C in the presence of 5% serum, against specified bacteria.

Toxicity

The findings of toxicity testing (Table 6) showed the tested formulation to fall into EPA toxicity category IV, making it exempt from any precautionary statements on the label.

Table 6. Summary of toxicity tests on one lot of ACCEL TB

Test method	Results
Acute oral toxicity study (UDP) on rats	There was no mortality in the study. LD_{50} >5000 mg/kg
Acute dermal toxicity study in rabbits	There was no mortality in the study. LD_{50} >5050 mg/kg
Acute inhalation toxicity study in rats	There was no mortality in the study. LC_{50} >2.59 mg/L
Acute eye irritation study in rabbits	Nonirritating (the product is assigned to toxicity category IV)
Acute dermal irritation study in rabbits	Slightly irritating (the product is assigned to toxicity category IV)
Skin sensitization (local lymph node assay in mice)	The product is not a sensitizer

Materials compatibility

None of the 12 types of plastics tested were affected by the test formulation after being exposed to the test solution for 2900 cycles of disinfection contact time. Among the metals, only brass showed minor to moderate damage, after 7200 cycles of disinfection contact time, whereas all others remained unaffected.

DISCUSSION

Chemical disinfectants constitute the backbone of infection control. Fourteen types of chemicals singly or in various combinations are in over 90% of the products currently used for this purpose,[2] and several of them either have a rate of microbial kill too slow for field use and/or a poor safety profile.[2]

Hydrogen peroxide is among the oldest microbicides known, and it is generated naturally in many settings. However, it is relatively unstable by its very nature and somewhat slow acting when used on its own.[12] Both of these weaknesses have been addressed in that it is now possible to produce highly stabilized forms of H_2O_2 in solution and also speed up its microbicidal action. Furthermore, its corrosivity has been tamed, thus widening its materials compatibility. The AHP technology has combined these developments to enhance the potential of H_2O_2 as a microbicide, resulting in formulations for a variety of applications.[13,14] The stabilizers, surfactants, and other excipients in these formulations have a high safety and biodegradability profile and are free from aquatic toxicants such as nonyl phenol ethoxylates (NPEs) or alkyl phenyl ethoxylates (APEs).[15,16] The findings summarized here further substantiate the desirable attributes of the AHP-based formulation evaluated.

The formulation's microbicidal activity was assessed using relevant AOAC and ASTM protocols, and, in the case of fungicidal and mycobactericidal activities, the findings with the suspension test were reconfirmed with the more stringent QCT-1. As shown in Table 1, the vegetative bacteria tested represented a wide array of nosocomial pathogens. The viruses tested also included important nosocomial pathogens or their accepted surrogates.[17] Although certain of the enveloped viruses tested are not known to spread through contaminated environmental surfaces, they were included to ensure that ACCEL TB, based on a relatively new technology, is successful in dealing with both nonenveloped and enveloped varieties. ASTM protocol No. E-1053 was used for this purpose because it is currently the only protocol accepted by the US Environmental Protection Agency for submission of data for virucidal activity.

All evaluations for microbicidal activity were performed with an added soil load in microbial suspensions. The soil load was either 5% serum at its final concentration or the tripartite mixture of proteins.[4,9,17] Such soil loads not only simulate the presence of body fluids but also add to the level of stringency of the methods for added confidence in the test data.[14]

The initial fungicidal tests were based on AOAC's suspension protocol.[3] Because this was considered a weaker challenge to the test formulation, further testing was carried out using QCT-1 to ascertain properly that ACCEL TB has good fungicidal activity.

QCT-1 used in some of the tests reported here offers several advantages over the existing AOAC methods.[14] It has been designed to (1) permit the determination of the exact number of CFU remaining after the drying of the inoculum; (2) avoid wash off of any of the test organism; (3) allow complete recovery of the inoculum from the carrier surface; (4) arrest the test substance's activity by dilution immediately at the end of the contact time; (5) in the case of bactericidal, mycobactericidal, and fungicidal tests, capture all the cells of the test organisms on a membrane filter before and after exposure to the test product; (6) remove any residual microbicidal activity by a thorough rinsing of the membrane filter; (7) incorporate glass inserts to eliminate any false-positive results because of the generation of microaerosols in the carriers; and (8) give a precise determination of $\log_{10}$ reduction in CFU of the test organism after exposure to the product under test. It therefore, eliminates the deficiencies associated with the AOAC methods. Because the QCT-1 meets the requirements for microbicide testing as in the Canadian General Standards Board,[18] data based on it are acceptable for product registration in Canada.

In summary, the AHP-based environmental surface disinfectant tested in this study proved to be a broad-spectrum and fast-acting microbicide with high safety and materials compatibility profiles. It, therefore,

addresses many of the concerns relating to other types of actives in wide use in infection control today.[2,19-21]

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