A Product based on accelerated and stabilized hydrogen peroxide:

Evidence for broad-spectrum germicidal activity

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Abstract

Introduction: Chemical germicides are important in infection control. However, there are mounting concerns on the human and environmental safety of many germicidal products in use today. Whereas it may be difficult to find formulations which are effective while being totally safe, this paper describes the broad-spectrum germicidal activity of Virox, a product based on accelerated and stabilized hydrogen peroxide; it contains ingredients which are considered safe for humans and benign to the environment.

Objective: The objective was to test the sporicidal, mycobactericidal, fungicidal, bactericidal and virucidal activities of Virox to determine its potential as a broad-spectrum germicide.

Materials & Methods: Three separate lots of the product were tested. The quantitative carrier test used met the requirements of the Canadian General Standards Board to assess germicides for use on environmental surfaces and medical devices. Standard strains of *Bacillus subtilis, Clostridium sporogenes, Mycobacterium terrae, Trichophyton mentagrophytes, Poliovirus type 1 (Sabin), Staphylococcus aureus, Salmonella choleraesuis and Pseudomonas aeruginosa* were used. The soil load was 5% bovine serum and standard hard water (200 ppm calcium carbonate) was the product diluent. Depending on type of activity assessed and the temperature, the contact time ranged from one minute to six hours.

Results: At 20°C, the undiluted product was sporicidal in six hours, mycobactericidal in 20 minutes and fungicidal in five minutes. When diluted 1:16 in hard water, it proved to be bactericidal in one, three and 10 min at 45, 20 and 4°C, respectively; it was virucidal in five minutes at 20°C.

Conclusions: All three lots of the product proved to be sporicidal, mycobactericidal and fungicidal at full strength, and virucidal and bactericidal at a 1:16 dilution. The product is therefore suitable for use as a high-level disinfectant and is safer than many other broad-spectrum germicides.

Key Words: Accelerated and stabilized hydrogen peroxide, infection control, virucide, bactericide, sporicide, fungicide, mycobactericide.

Introduction

Chemical germicides play a crucial role in infection control in many settings and reliance on them is increasing even further with a revival of interest in environmental control to combat antibiotic resistant as well as opportunistic pathogens. However, there is a concomitant heightening of concerns on the human and environmental safety of many germicidal chemicals. This, in turn is focusing greater attention on the search for formulations, which are not only good broad-spectrum germicides, but are also safer for humans and benign for the environment. While the objective of finding highly effective and fast-acting germicidal chemicals which are, at the same time, totally harmless for humans and the environment may be unattainable at the moment, this paper describes the tests on a formulation which meets with nearly all of the requirements in this regard.

The quantitative carrier test used in this study has been developed in our laboratory (1). It has already been used for evaluating the sporicidal, mycobactericidal, virucidal, fungicidal and bactericidal activities of a variety of formulations. The test incorporates all the essential requirements of the Canadian General Standards Board's standard entitled *Assessment of Efficacy of Antimicrobial Agents for Use on Environmental Surfaces and Medical Devices* (2). It also fully conforms to the American Society of Testing and Materials' (ASTM) requirements for evaluating the virucidal activity of liquid chemical germicides to be used on non-porous environmental surfaces (3).

Objective

The objective of this study was to test the sporicidal, mycobactericidal, fungicidal, bactericidal and virucidal activities of Virox to determine its potential as a broad-spectrum germicide.

Materials and methods

The Product: The product, marketed under the trade name Virox, is a colourless and odourless solution, with a pH of 1.3. It contains hydrogen peroxide at a final concentration of 7%, food grade acids and detergents; a combination of these ingredients also makes it non-corrosive. All its components meet the requirements for food contact surfaces. Its germicidal action is believed to be based on its activity as a strong oxidizing agent. Three separate lots of the product were tested in this study. Once received by us, the bottles of the product were stored at room temperature in a place with restricted access. For testing its bactericidal and virucidal activities, a 1:16 dilution of the product was prepared by adding one part of it to 15 parts of water with a standardized level of hardness (see below). The dilution was prepared immediately prior to each test and the diluted product was used only once. In certain cases, the product was tested with an anti-foam to determine if this could, in any way, interfere with its germicidal activity.

Carriers: 20mL glass vials with a diameter of 24 mm (catalog #5260-G; Galaxy Co, Newfield, NJ) were used as carriers for sporicidal, bactericidal, mycobactericidal and fungicidal tests. Each vial contained a custom-made glass insert (Galaxy) to avoid any false positives due to the deposition of micro aerosols with viable test organisms deposited on the inside surface of the vial. An adjustable Eppendorf pipette (catalog #2244000-4; Brinkman Instruments, Mississauga, Ontario) with disposable tips (catalog #2249040-1.

Brinkman Instruments) was used to carefully place the test inoculum (10 µL) at the centre of the inside bottom surface of each vial. The inoculum was allowed to dry at room temperature for at least one hour. The glass inserts were then removed and the vials capped and placed at room temperature till ready to be used.

For virucidal testing, stainless steel disks (1 cm in diameter) were used (4). The disks were punched out from a sheet of #4 polished stainless steel purchased locally. The vials and the disks were reused after their decontamination, cleaning and autoclave sterilization.

Soil Load: For inoculation of the carriers, all test organisms were first suspended in bovine serum (Cansera, Rexdale, ON) at a final concentration of 5%.

Standard Hard Water: When the product was to be tested after dilution, water with a standard hardness of 200 parts per million (ppm), as calcium carbonate was used as the diluent. The hard water was prepared according to the formula of AOAC International (5).

Microbial Diluents and Filter Rinse: Phosphate buffer at pH 7.2, was used to make dilutions of the spores and the vegetative bacterial cells and to rinse the membrane filters in tests for sporicidal and bactericidal activities. The diluent and filter rinse used in mycobactericidal and fungicidal test was sterile normal saline (0.85% sodium chloride). Earle's balanced salt solution (EBSS; GIBCO) was used to prepare the dilutions of the virus prior to infectivity assays.

Test Organisms: The organisms used and their specific strains are given below:

1. *Bacillus subtilis* (ATCC 19659): The spores were grown in a 1:10 dilution of Columbia broth (Difco), with manganese, for 72 hours at 37°C. For 10^{10} spores/ml, the suspension was centrifuged, washed and resuspended in sterile dis tilled deionized water.

2. *Clostridium sporogenes* (ATCC 7955): *C. sporogenes* spores were grown anaerobically in undiluted Columbia broth for five days at 30°C. For 10^{9} spores/ml, the spore suspension was centrifuged, washed and resuspended in sterile distilled deionized water.

3. *Pseudomonas aeruginosa* (ATCC 15442), *Staphylococcus aureus* (ATCC 6538) and *Salmonella choleraesuis* (ATCC 10708): Stocks of the three vegetative bacteria were prepared by culturing them in tryptic soy broth (TSB; Difco) for 24 hours at 37°C. The broth culture with 5% serum was used as the inoculum for the carriers.

4. *Mycobacterium terrae* (ATCC 15755): *M. terrae* was grown in 7H9 broth (Difco) containing glycerol but no antibiotics. The suspension was centrifuged at 1,000 x g for 15 minutes and the pellet resuspended in 1-2 ml of deionized distilled water to give approximately $2.5x10^{9}$ cells/ml. Bovine serum at a final concentration of 5% was added to the suspension before it was used for the inoculum of the carriers.

5. *Trichophyton mentagrophytes* (ATCC 9533): Stock suspensions of the conidia were obtained by inoculating the center of a Mycobiotic Agar plate and incubating it at 28°C for nine days. Mycelial mats were harvested from the agar surface, homogenized with sterile glass beads in norma l saline and filtered through sterile cotton to remove the hyphae. The conidial suspension also contained bovine serum at a final concentration of 5%.

6. The Sabin Strain of Poliovirus Type 1 (ATCC VR-192): The virus stocks were prepared in Vero cells in 75 cm^2 flasks. After virus adsorption for 60 min at 37°C, the infected monolayers were kept in Eagle's minimum essential medium (EMEM; GIBCO), without any serum, until nearly 75% of the monolayer had been affected by virus cytopathology. The cultures were the n frozen (-20°C) and thawed three times and the suspension was centrifuged at 1,000 x g for 10 minutes to remove cellular debris. The supernatant was used as the virus inoculum after bovine serum was added to it to a final concentration of 5%.

The quantitative carrier test methodology

The procedure for testing sporicidal, mycobactericidal, fungicidal and bactericidal activities: The general procedure used in this study is given in Flow Chart 1. Each glass vial received 10 µL of a suspension of the test organism. Using a 10 µL Eppendorf pipette, the inoculum was placed at the centre of the inside bottom surface of the vial. The inoculum was allowed to dry and each vial received 1 ml of the test

germicide to completely cover the dried inoculum. At the end of the contact time, 9 ml of phosphate buffered or normal saline was added to each vial and the test inoculum resuspended with the help of a magnetic stirrer 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 of phosphate buffer or normal saline and the rinses also passed through the same filter. The filter was then placed on the agar surface of the medium suitable for the recovery of the test organism. 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 titre of the test organism calculated.

When the number of colony forming units was expected to be high, the eluate from the vial was first subjected to a series of 10-fold dilutions and each diluted sample was passed through a separate membrane filter and the filters placed on recovery media.

The method has been designed to: (a) determine the exact number of viable units of the test organism placed on each carrier and those remaining viable after the drying of the inoculum, (b) avoid wash-off of any cells of the test organism, (c) allow complete recovery of the inoculum from the carrier surface, (d) arrest the test product's activity by dilution immediately at the end of the contact time, (e) capture all of the test organisms after exposure to the test product, (f) remove any residual germicidal activity by a thorough rinsing of the filter for all tests except for virus; ensure that any residual germicide did not affect the cell cultures or virus quantitation in the virucidal tests, (g) eliminate any false-positive results due to the generation of micro-aerosols in the carriers and (h) give a precise determination of log10 reduction in viable cells of the test organism after exposure to the test product.

This new test method, therefore, eliminates the deficiencies associated with the AOAC Use-Dilution Test (5) while meeting the Canadian General Standards Board's requirements for germicide test methodology (2).

Controls: Control carriers were treated in the same manner as test carriers except that phosphate buffer (pH 7.2), known to be harmless to the test organisms, was applied to the dried inoculum instead of the disinfectant in all tests except those for virucidal activity. In tests for virucidal activity, Earle's balanced salt solution (EBSS) was used on the control carriers.

Detection of Viable Organism: For tests with *B. subtilis, P. aeruginosa, S. aureus and S. choleraesuis,* the filters were placed on TSA plates, incubated at 37°C. For *C sporogenes*, the filters were placed on fastidious anaerobic agar (FAA), incubated at 30°C. The culture plates for the sporicidal and bactericidal tests were observed every 24 hours and the final count for CFU were recorded at the end of the fifth day. For tests using *M. terrae*, the filters were placed on 7H11 agar, incubated at 37°C and CFU recorded at weekly intervals for a total of four weeks. For fungicidal testing with *T. mentagrophytes*, the filters were put on mycobiotic agar held at 28°C and CFU recorded at four days, and every 24 hours thereafter for a total of 10 days.

Testing for Virucidal Activity: The virucidal test method (4) was also designed to be fully quantifiable in a closed system and to include all the necessary controls. Stainless steel disks (1cm in diameter) were used as carriers and each disk received 10 µL of the test virus in 5% serum. After the inoculum had been allowed to dry, it was either exposed to EBSS or the test product for the required contact time at the specified temperature. Each disk was then placed aseptically in a vial containing 990 µL of an eluent/diluent and vortexed to recover the inoculum. The control and test eluates were inoculated into monolayers of Vero cells for virus plaque assays. The plaque forming units (PFU) were determined after three days of incubation and log10 reductions in virus titer calculated.

In line with the requirements for the CGSB standard (2), additional controls were included in all virucidal tests to ensure that (a) the non-virucidal and non-cytotoxic levels of the test product did not interfere with the ability of the virus to form plaques or (b) the cell cultures to detect virus and (c) the dilution of the virus-

product mixture at the end of the contact time was sufficient to arrest the virucidal activity of the test product.

Product performance criteria

The number of test carriers in each sporicidal, mycobactericidal, fungicidal and bactericidal test was 10. Each of these tests also included three control carriers. In virucidal tests, there were five test and three control carriers. The results are reported as $\log_{10}$ reductions in viability in reference to the control carriers.

For a product to be considered sporicidal, bactericidal or mycobactericidal, it was expected to reduce the viability titer of each test organisms by a minimum of 6 $\log_{10}$ (at least one million-fold) under the conditions of this test. A minimum of 5 $\log_{10}$ was expected for fungicidal activity and a minimum reduction of 3 $\log_{10}$ in PFU for virucidal activity (2).

Results

Sporicidal activity of full-strength Virox: As shown in Table 1, all three lots of the product could bring about a >7 $\log_{10}$ and a >8 $\log_{10}$ reduction in the titre of *C. sporogenes* and *B. subtilis* spores, respectively, in a contact time of six hours at 20±°C, indicating sporicidal activity against these organisms in our test protocol,

The activity of full-strength Virox against *Mycobacterium terrae*: The product proved to be mycobactericidal by reducing the titer of *M. terrae* by >6 $\log_{10}$, as summarized in Table 2.

The activity of full-strength Virox against the conidia of *T. mentagrophytes*: As shown in Table 3, all three lots of the product could bring about a >5 $\log_{10}$ reduction in the viability of the fungal conidia in a contact time of five minutes at 20±°C, indicating fungicidal activity against this organism.

Activity of diluted Virox against the vegetative bacteria: In addition to the broad-spectrum germicidal activity shown by the undiluted product, a 1:16 dilution of all three lots of the product in hard water was able to reduce the viability titre of the three bacteria by at least 6 $\log_{10}$ in each test. The results of these tests conducted at 4°C, 20°C and 45°C, are summarized in Table 4-6. The presence of an anti-foam did not interfere with its bactericidal activity and it could bring about a >6 $\log_{10}$ in the viability of the bacteria in one minute at 45°C (Table 7).

Activity of diluted Virox against the virus: Two lots of the product could bring about an almost 5 $\log_{10}$, and one lot nearly a 4 $\log_{10}$ reduction in the infectivity titre of the virus in a contact time of five minutes at 20°C. Such performance meets the criterion for virucidal activity specified in the CGSB standard.

A further 1:100 dilution of the diluted product tested for virucidal activity did not interfere with plaque formation by the Poliovirus because the number of plaques on the monolayers with and without prior exposure to it was almost the same (Table 9). These findings show that the absence of Poliovirus plaques was not due to any interference due to residual amounts of the products in the dilutions of virus-germicide mixtures plaque assayed. Therefore, the virucidal activity recorded in this study is not due to any false-positive findings.

As can be seen from the data summarized in Table 10, a 1:100 dilution of the virus-product mixture was sufficient to arrest its virucidal activity. This made it unnecessary to use any chemicals or gel filtration to neutralize/remove any residual virucidal activity at the end of the contact time.

Discussion and concluding remarks

Germicides used for infection control usually have unknown targets and should therefore be capable of activity against a broad-spectrum of pathogens. In view of this, a fully quantitative hard surface carrier protocol was used to determine the activity of the test product against all major classes of human pathogens. All three lots of Virox proved to be sporicidal, mycobactericidal, fungicidal, virucidal and bactericidal The

procedure, as reported here, met the testing requirements of a Canadian national standard (2) except that it used the flat bottom surface of a glass vial instead of penicylinders for testing the product's sporicidal, mycobactericidal fungicidal and bactericidal activities. The testing of its virucidal activity fully conforms to the requirements specified in the CGSB standard (2) as well as the recently revised ASTM standard (3).

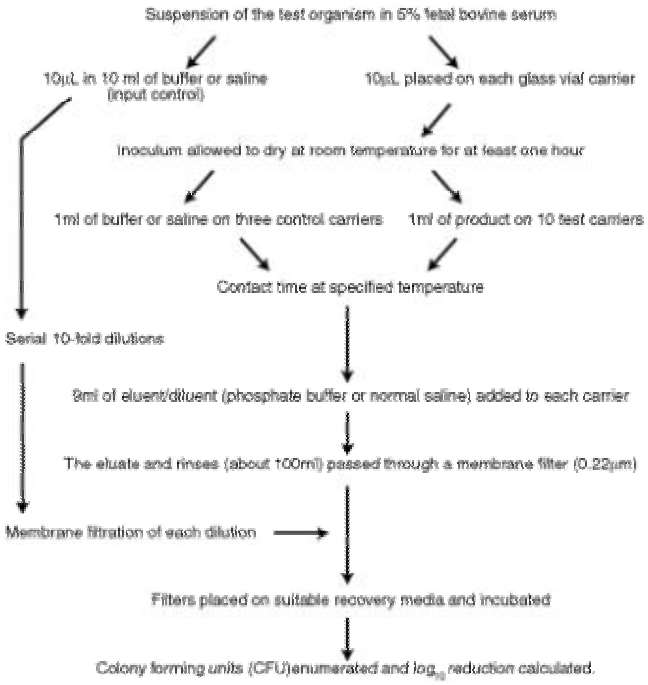
Hydrogen peroxide has a long history as a germicide but its activity in unformulated solution is quite weak and slow (6, 7). In view of this, great care must be exercised in choosing only those hydrogen peroxide-based products that have been properly formulated to accelerate, and enhance, its germicidal activity. The product evaluated here represents a new generation of hydrogen peroxide-based germicides in which the potency and performance of the active ingredient have been enhanced and accelerated through the use of appropriate acids and detergents. This provides a product with superior cleaning power as well as increased germicidal potency. Such products have numerous potential applications where cleanup and disinfection are crucial. Furthermore. since hydrogen peroxide does not leave any toxic residues, it also has the potential to replace more toxic formulations.

Addition of antifoam to germicide formulations is often desirable when surface wetting of narrow lumens, process lines or other difficult-to-treat surfaces is involved. Low-foaming or non-foaming germicides are also needed for the machine decontamination of medical devices. The results reported here for antifoam addition suggest no detrimental effect on product performance with their inclusion.

The product met all the performance criteria set in this study. It, therefore, appears to have the potential as a high-level disinfectant in the undiluted form and as a general purpose disinfectant when diluted 1:16. The diluted product proved to be bactericidal in one minute at 45°C and this combination of time and temperature could be very useful in the decontamination of food processing equipment and facilities; its bactericidal activity in three minutes at 20°C is also superior to many other environmental surface disinfectants which may require three times as long to achieve the same result. The diluted product also proved to be virucidal in five minutes at 20°C, while many other products in this category generally show poor activity against viruses. It should also be noted here that, in Canada, a product with activity against a Poliovirus is regarded as a general virucide. This is because non-enveloped viruses such as polio viruses are generally more resistant to many types of chemical germicides (8, 9) and a formulation that can inactivate a prototypical non-enveloped virus is most likely to work against a wide variety of viral pathogens.

The evidence available suggests that Virox and other products based on accelerated hydrogen peroxide technology, apart from being good germicides, are safer for humans and benign to the environment. Therefore, they show considerable promise as disinfectants for use in healthcare facilities, daycare centers, nursing homes, medical, dental and veterinary clinics, food processing establishments, commercial facilities as well as domestic settings.

Flow Chart 1



The basic quantitative carrier method for testing the sporicidal, mycobactericidal, fungicidal and bateriacidal activities of liquid chemical germicides.

Table 1

The sporicidal activity of full-strength Virox at 20±°C in a contact time of six hours.

Product Lot Number	CFU/Control carrier	CFU/test carrier	Log_{10} Reduction
Bacillus subtilis spores			
007	$1.96x10^8$	0	>8
008	$1.96x10^8$	0	>8
009	$1.56x10^8$	0	>8
Clostridium sporogenes spores			
007	$3.12x10^7$	0	>7
008	$3.12x10^7$	0	>7
009	$1.36x10^7$	0	>7

Table 2

The activity of full-strength Virox against Mycobacterium terrae at 20±1°C in a contact time of 20 minutes.

Product Lot Number	CFU/Control carrier	CFU/test carrier	Log_{10} Reduction
007	$2.53x10^6$	0	>6

008	$1.78x10^6$	0	>6
009	$1.86x10^6$	0	>6

Table 3

The activity of full-strength Virox against the conidia of Trichophyton mentagrophytes at 20±°C in a contact time of five minutes.

Product Lot Number	CFU/Control carrier	CFU/test carrier	Log_{10} Reduction
007	$5.40x10^5$	0	>5
008	$4.00x10^5$	0	>5
009	$4.00x10^5$	0	>5

Table 4

The activity of a 1:16 dilution of Virox against *Pseudomonas aeruginosa.*

Product Lot Number	Contact Temperature	Contact Line	CFU/Control carrier	CFU/test carrier	Log_{10} Reduction
007	20°C	3 min	$1.96x10^6$	0	>6
008			$1.96x10^6$	0	>6
009			$1.25x10^6$	0	>6
007	4°C	10 min	$1.79x10^6$	0	>6
008			$1.79x10^6$	0	>6
009			$1.25x10^6$	0	>6
007	45°C	1 min	$1.45x10^6$	0	>6
008			$1.45x10^6$	0	>6
009			$2.27x10^6$	0	>6

Table 5

The activity of a 1:16 dilution of Virox against Staphylococcus aureus.

Product Lot Number	Contact Temperature	Contact Line	CFU/Control carrier	CFU/test carrier	Log_{10} Reduction
007	20°C	3 min	$1.66x10^6$	0	>6
008			$1.77x10^6$	0	>6
009			$1.77x10^6$	0	>6
007	4°C	10 min	$1.68x10^6$	0	>6
008			$1.68x10^6$	0	>6
009			$1.68x10^6$	0	>6
007	45°C	1 min	$1.79x10^6$	0	>6
008			$1.79x10^6$	0	>6
009			$2.40x10^6$	0	>6

Table 6

The activity of a 1:16 dilution of Virox against Salmonella cholerasuis.

Product Lot Number	Contact Temperature	Contact Line	CFU/Control carrier	CFU/test carrier	Log_{10} Reduction
007	20°C	3 min	$3.86x10^6$	0	>6
008			$3.86x10^6$	0	>6
009			$2.38x10^6$	0	>6
007	4°C	10 min	$1.65x10^6$	0	>6
008			$1.65x10^6$	0	>6
009			$2.38x10^6$	0	>6
007	45°C	1 min	$1.16x10^6$	0	>6
008			$1.16x10^6$	0	>6
009			$1.11x10^6$	0	>6

Table 7

The activity of a 1:16 dilution of Virox with an anti-foam against three types of vegetative bacteria after a contact time of one minute at 45°C*.

Test organism	CFU/Control carrier	CFU/test carrier	Log_{10} Reduction
Pseudomonas aeruginosa	$2.27x10^6$	0	>6
Staphylococcus aureus	$1.40x10^6$	0	>6
Salmonella choleraesuis	$1.11x10^6$	0	>6

*Lot#009 was tested in these experiements

Table 8

The activity of a 1:16 dilution of Virox against Poliovirus type 1 (Sabin) at 20°C with a contact time of five minutes.

Product Lot Number	Input control	PFU/control carrier	PFU/test carrier	Log_{10} Reduction
007	$8.3x10^{66}$	$8.7x10^4$	1.34	4.8
008	$8.3x10^6$	$8.7x10^4$	1	4.9
009	$8.3x10^6$	$8.7x10^4$	10	3.94

Table 9

The effect of diluted Virox on the plaque forming ability of the poliovirus.

Plaques on cell monolayer controls	Plaques on cell monolayers pre-exposed to 1/100 dilution of the test product	Plaques on cell monolayers pre-exposed to EBSS
0	7	5
0	5	7
0	6	5
-	7	-
-	5	-
-	4	-
Mean±SD	5.67±1.1	5.83±1.24

Cell monolayers were first exposed to either a 1:100 dilution of the test product or EBSS and incubated for 30 minutes at 37°C. They were then washed with EBSS and inoculated with the virus for plaque assay. Cell control monolayers were treated in the same way but did not receive any virus. - = not done.

Table 10

The effectiveness of dilution/neutralization as a means of arresting the virucidal activity of Virox.

Plaques on cell monolayer controls	Plaques on cell monolayers exposed to one part virus mixed with nine parts of a 1/100 dilution of the test product.	Plaques on cell monolayers exposed to one part virus mixed with nine parts EBSS
0	5	5
0	3	3
0	4	4
-	3	-
-	5	-
-	9	-
Mean°SD	4.83°2.03	4.0°0.82

One part of the virus was mixed with either the diluted test product or EBSS and held at room temperature for five minutes. Plaque assays were then carried out. Cell monolayer controls received EBSS only. -= not done.

References

1. Springthorpe. VS and Sattar, SA. "A new quantitative sporicide test: Concepts, development and current status". Presentation at the Annual Conference of AOAC International, San Diego, California Sept., 1997.

2. Canadian General Standards Board. "Assessment of efficacy of antimicrobial agents for use on environmental surfaces and medical devices". 1997. Document #CAN/CGSB-2.161-M97. CGSB, Ottawa, Canada.

3. American Society for Testing and Materials. "Standard method for efficacy of virucidal agents intended for inanimate environmental surfaces". 1996. Document No E-1053-96. ASTB, Conshohocken, PA.

4. Lloyd-Evans N, Springthorpe, VS and Sattar, SA. "Chemical disinfection of rotavirus-contaminated inanimate surfaces". *J. Hyg*, 1986; 97:163-173.

5. AOAC. "Official methods of analysis". 1990. AOAC International, Washington, DC

6. Best, M, Springthorpe, VS and Sattar, SA. " Feasibility of a comb ined carrier test for disinfectants: studies with a mixture of five types of microorganisms." *Am. J. Infect. Control.* 1994; 22:152-162.

7. Saurina, G., Landman, D. and Quale, JM. "Activity of disinfectants against Vancomycin-resistant Enterococcus faecium". *Infect. Control and Hosp. Epidemiol*. 1997; 18:345-347.

8. Sattar, SA, Springthorpe, VS, Karim, Y and Loro, P. "Chemical disinfection of non-porous inanimate surfaces experimentally contaminated with four human pathogenic viruses". *Epidemiol. Infect*. 1989; 102:493-505.

9. Sattar, SA and Springthorpe. "Environmental spread and germicide control of viruses in hospitals". *Infect Contr. Steriliz. Technol*. 1996.,2(7):30-36.