

**Final Report submitted to Virox Technologies Inc.
Mississauga, Ontario**

**ASSESSMENT OF THE GERMICIDAL ACTIVITY OF
ACCELERATED HYDROGEN PEROXIDE PLUS (AHP PLUS)**

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July 2002

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A. INTRODUCTION

This study on the germicidal activity of Virox AHP Plus product incorporated testing against six different types of vegetative bacteria, one species of *Mycobacterium*, one type of filamentous fungus and the Sabin strain of poliovirus type 1. The evaluation was conducted using a quantitative carrier test developed in our laboratory, which is now a standard of ASTM International (ASTM, 2000). The sanitizing action of the product was evaluated using a suspension test method.

B. OBJECTIVE

The main objective of this study was to determine the bactericidal, mycobactericidal, fungicidal and virucidal activities of the product at full strength using a quantitative carrier test method and its effectiveness as a sanitizer using a suspension test method.

C. MATERIALS AND METHODS

The Product:

Three separate lots of the product were provided for testing in this study. Upon arrival in our laboratory, the bottles were stored at room temperature in a place with restricted access. The product was tested at full strength.

Carriers:

The inside bottom surface of glass vials (Galaxy Co., Newfield, New Jersey) was used as the carrier for all tests except those against the virus.

Soil Load:

For inoculation of the carriers, all test organisms were first suspended in bovine serum (Gibco BRL Life Technologies Cat. No. 16000-044, NY, USA), at a final concentration of 5%.

Neutralizer, Microbial Diluent and Filter Rinse:

Lethen Broth (with 0.1% sodium thiosulfate pentahydrate) was used as the neutralizer and to rinse the membrane filters and the filter holder unit. A 1% sodium thiosulfate pentahydrate in LB was used as neutralizer for testing with *Pseudomonas aeruginosa*. Normal saline was used to make dilutions of the bacterial suspensions and as the final rinse of the carrier vials and the filter holder unit to aid in rinsing off the froth created by the Lethen broth.

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) and the Sabin vaccine Strain of poliovirus type 1 (ATCC VR-192), already available in our laboratory, were used in this study. A seed culture of *Acinetobacter baumannii* was received through the courtesy of the Microbiology Lab at the Ottawa Hospital. Vancomycin Resistant *Enterococcus* (VRE) and Methicillin Resistant *Staphylococcus aureus* (MRSA) were obtained from Dr. Frank Chan of the Children's Hospital of Eastern Ontario, Ottawa. They were cultured as follows:

a) *Staphylococcus aureus* (ATCC 6538), *Salmonella choleraesuis* (ATCC

10708), *Acinetobacter baumannii*, *MRSA* and *VRE*: Stock suspensions of five of the six vegetative bacteria were prepared by culturing them in tryptic soy broth (TSB) for 24 hours at 37°C. *Pseudomonas aeruginosa* (ATCC 15442) was grown in 1:1000 TSB for 72 hours at 37°C.

b) *Mycobacterium terrae* (ATCC 15755): The mycobacterium was grown in Middlebrook 7H9 broth with ADC enrichment and glycerol, in vented plug seal capped tissue culture flasks. The test suspension was prepared from stocks grown for 21 days. The cell suspension was washed 3 times by centrifugation at 2,500 rpm for 15 minutes and re-suspended in sterile distilled water. The final stock suspension was prepared by re-suspending the bacterial pellets in sterile bijoux bottles containing glass beads to approximately 10^8 cells/mL. The stock suspension was stored at 4°C.

c) *Trichophyton mentagrophytes* (ATCC 9533): A stock suspension of the conidia was obtained by inoculating the center of a Mycobiotic Agar plate 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.

d) The Sabin vaccine strain of poliovirus type 1 (ATCC VR-192): A stock of the virus was prepared by infecting monolayer of Vero cells in 75 cm² flasks. The virus was allowed to adsorb to cells for 60 minutes at 37°C and the infected monolayer kept in minimal essential medium, without any antibiotics and serum, until approximately 75% of the monolayer has been affected by the virus cytopathic effect. The culture was then 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 pool.

D. THE TEST METHODOLOGY

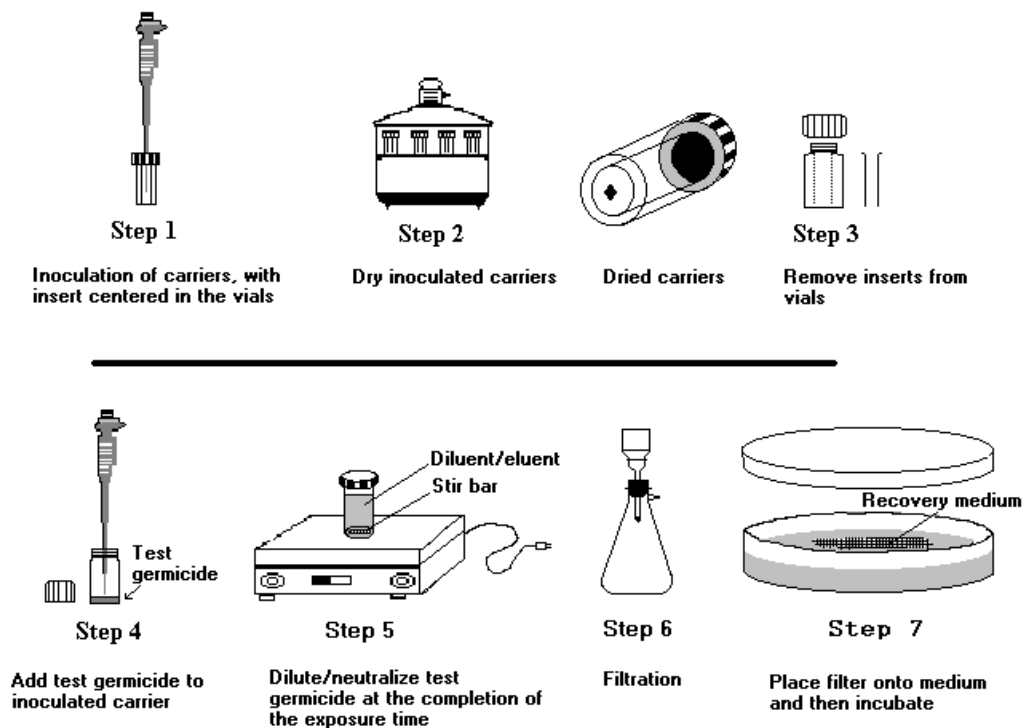
QUANTITATIVE CARRIER TEST:

The quantitative carrier tests used in this evaluation have been designed to: (a) permit the determination of the exact number of colony forming units (CFU) or plaque forming units (PFU) placed on each carrier and the CFU/PFU remaining after the drying of the inoculum, (b) avoid wash-off of any 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) in the case bactericidal tests, capture all the bacterial cells of the test organism on a membrane filter before and after exposure to the test product, (f) removal of any residual germicidal activity by a thorough rinsing of the membrane filter, (g) allow a ratio of 1:100 between the volume of the test microbial inoculum and the volume of the product being evaluated, (h) incorporation of glass inserts to eliminate any false-positive results due to the generation of micro-aerosols in the carriers and (i) give a precise determination of log₁₀ reduction in CFU/PFU of the test organism after exposure to the product under test. This new test method, therefore, eliminates the deficiencies associated with the AOAC Use-Dilution Test (AOAC, 1990) while meeting the Canadian General Standards Board's requirements for germicide test methodology (CGSB, 1997). As stated above, it is now an accepted standard of ASTM (E2111).

The Method for Testing Bactericidal Activity: The general equipment and procedure for testing are given in Figure 1 and Flow Chart 1, respectively.

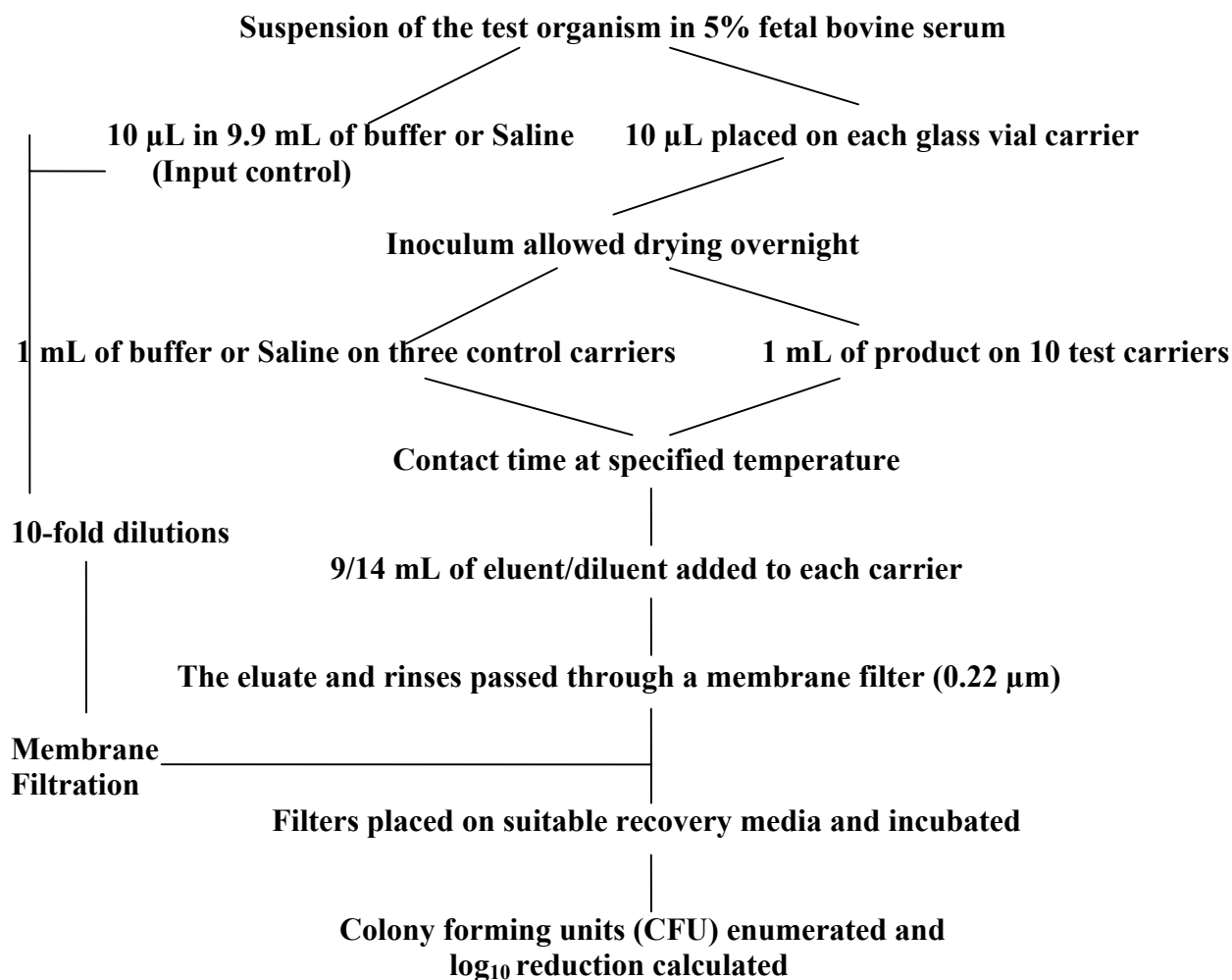
FIGURE 1.

GENERAL STEPS FOR THE QUANTITATIVE CARRIER TEST



FLOW CHART 1

THE BASIC QUANTITATIVE CARRIER METHOD FOR TESTING THE BACTERICIDAL ACTIVITIES OF LIQUID CHEMICAL GERMICIDES



The test involved drying a microbial suspension on a hard surface carrier and covering the dried inoculum with the use-dilution of the disinfectant for the specified contact time at room temperature. At the end of the contact time, an eluent/rinse was used to recover the reaction mixture from the carrier and the eluate was passed through a membrane filter (0.22µm pore diameter) to capture the test organism. The filters were then placed on plates of suitable recovery agar medium and incubated to allow viable organisms to form visible colonies. The numbers of colony forming units (CFU) were recorded and the level of inactivation of the test organism was calculated.

THE SUSPENSION TEST:

The test was carried out by adding 100 µL of the bacterial suspension with soil load to 900 µL of the test product in a 2 mL capacity cryovial, vortexed to mix and allowed to sit for the required contact time at room temperature. At the end of the contact time, the reaction mixture received 14.0 mL of the neutralizer and vortexed. This mixture was passed through a membrane filter and the vial was rinsed 2x with 10.0 mL of saline. The membrane filtration technique was the same as that in the quantitative carrier test for bactericidal activity.

Recovery Media and Detection of Viable Organisms:

For bactericidal testing using *S. aureus*, *P. aeruginosa*, *choleraesuis*, *A. baumannii*, VRE and MRSA the filters were placed on TSA plates, incubated at 37°C, monitored, and the CFU recorded at 24 hour intervals for a total of 5 days. For mycobactericidal testing using *M. terrae*, the filters were placed on 7H11 agar, incubated at 37°C, monitored, and the 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 and incubated at 28°C, monitored, and the CFU recorded at 4 days, and every 24 hour interval thereafter for a total of 10 days

Controls:

For the quantitative carrier test for bactericidal activity, control carriers were used in the same manner as test carriers except that normal saline was applied to the dried inoculum instead of the test product.

Suspension Test - Controls were tested by adding 100µL of bacterial suspension to 900 µL Lethen broth instead of the disinfectant.

VIRUCIDE TEST:

Stainless steel disks (1 cm in diameter) were used as carriers and each disk placed in each well of a 12-well cell culture plate. Each carrier received 10 µL of the test virus containing 5% serum as soil load. After the inoculum had been allowed to dry, each disk in each well was either exposed to 50 µL the test product or EBSS for the required contact time at room temperature. At the end of the contact time, 950 µL of EBSS was added to both the test and control wells as eluent/neutralizer. A pipette was used to suck the eluent in and out onto the carriers to remove inoculum off the carriers. The eluate was transferred into a sterile labeled dilution vial, vortexed to mix. The control and test eluates were serially diluted and inoculated into cell culture monolayer for virus plaque assays. The plaque forming units (PFU) were determined and log₁₀ reduction calculated.

Plaque Assay For Poliovirus

Confluent monolayers of Vero cells were trypsinized and dispensed into 12-well cell culture plates (Corning cat #08-757-16B) for all plaque assays. The cells were dispensed at a density (approximately 1 x 10⁶ cells/well) to allow for formation of confluent monolayers within 24-48 hour. Each assay included three wells as cell controls and each dilution of the sample tested was inoculated into at least three wells.

The growth medium from each plate was aspirated and 100 µL of the appropriate dilution of the test virus suspension was then dispensed directly onto each monolayer. Each dilution was titrated in triplicate. The plates were incubated for 60 minutes at 37°C in a 5% CO₂ atmosphere

to allow for virus adsorption. Each monolayer was overlaid with 2 mL of an overlay medium containing 2X MEM supplemented with HEPES, L-glutamine, non-essential amino acids (NEAA), and 2% FBS, 26 mM MgCl₂ and Noble Difco Agar. The ratio of the agar and the supplemented medium was 1:1. Once the overlay had solidified, the plates were held for 40 hrs in a 5% CO₂ atmosphere at 37°C.

At the end of the required incubation period for the plaque assay, 2 mL of a 3.7 % solution of formaldehyde in saline was added to each well and the plates were left for three to four hours to fix the cells and inactivate the virus. The fixative and the agar overlay were then removed from each plate and each well received 2 mL of a 0.1 % aqueous solution of crystal violet to stain the cells. Following a contact time of about five minutes, the stain was aspirated, the well washed in tap water and the plates allowed to dry to determine the plaque counts.

Neutralization Verification:

Bactericidal Test:

One part of the use-dilution of the product was mixed with 14 parts of the neutralizer. The test organism was added to the neutralized solution to give an estimated 20-100 CFU. The neutralizer alone was used as the control solution. At the end of a contact time of 5 minutes at 20°C, the mixture was passed through a membrane filter to capture the bacteria. The filters were placed on the appropriate recovery medium. The plates were incubated and the colonies counted.

The time of 5 minutes was selected in these experiments because it is the maximum delay that may occur between the initial dilution of the product in the carrier vial and the last lot of rinse passed through the membrane filter.

Virucidal Test:

To determine if the dilution of the product at the end of the contact time was sufficient to render it ineffective against the test virus, 100 µL of the test virus was added to 900 µL of a 1/100 dilution of the test product. The same amount of virus was also added to 900 µL of EBSS to act as a control. The tubes were allowed to stand for 5 minutes and they were then inoculated onto cell monolayer for virus plaque formation.

Toxicity and Interference with Plaque Formation:

To determine the effect of the diluted test product on the cell monolayer and the plaque forming ability of the test virus, 100 µL of a 1/100 dilution of the test product was placed into six wells of a twelve-well plate while the other six wells received EBSS as control and allowed to incubate for 30 minutes. The cells were observed under the microscope for signs of toxicity of the test product. The cells were then washed once with EBSS and virus diluted to give countable plaques/well, was added to each well. The virus was allowed to adsorb for 60-90 minutes at 37°C. Each cell monolayer was then overlaid and the plates incubated at the appropriate temperature for the development of the virus plaques.

E. PRODUCT PERFORMANCE CRITERIA

The numbers of test carriers in the bactericidal and virucidal test were between 5-10. Each test also included three control carriers. The results are reported as log₁₀ reductions in viability in reference to the control carriers.

For a product to be considered an effective disinfectant it was expected to reduce the viability titre of each bacterial test organism by at least 6 log₁₀ (at least 1 million-fold), the mycobacterium

and the fungus by $\geq 5 \log_{10}$ and the virus by $\geq 3 \log_{10}$ under the conditions of this test. In sanitizer tests, the target was a minimum reduction of $5 \log_{10}$.

F. RESULTS

Activity of the product against *Staphylococcus aureus* (Carrier Test Method): Table 1 summarizes the results of tests against *S. aureus*. All three lots of the product were able to bring about a $>6 \log_{10}$ reduction in the viability titre of *S. aureus* in a contact time of 5 minutes at room temperature indicating bactericidal activity against this organism.

Table 1. The activity of the Product against *Staphylococcus aureus*

Lot Number	Date of Experiment	Contact Time (minutes)	CFU/Control Carrier	Average CFU Test Carrier	Log ₁₀ Reduction
2974	18/04/02	5	1.11×10^7	0	7.74
2976	18/04/02	5	1.11×10^7	0	7.74
2977	18/04/02	5	1.11×10^7	0	7.74

Activity of the product against *Staphylococcus aureus* (Suspension Test Method): Table 2 summarizes the results of the suspension test. All three lots were able to bring about a $6\text{-}\log_{10}$ reduction in the viability titre of *S. aureus* in a contact time of 30 seconds at room temperature indicating bactericidal activity against this organism.

Table 2. The activity of the Product against *Staphylococcus aureus*

Lot Number	Date of Experiment	Contact Time (seconds)	CFU/Control Carrier	Average CFU Test Carrier	Log ₁₀ Reduction
2974	5/06/02	30	9.50×10^5	0	5.97
2976	5/06/02	30	9.50×10^5	0	5.97
2977	5/06/02	30	9.50×10^5	0	5.97

Activity of the product against *Pseudomonas aeruginosa* (Carrier Test Method): Table 3 summarizes the results of tests against *P. aeruginosa*. All three lots of the product were able to bring about a $>6 \log_{10}$ reduction in the viability titre of *P. aeruginosa* in a contact time of 5 minutes at room temperature indicating bactericidal activity against this organism.

Table 3. The activity of the Product against *Pseudomonas aeruginosa*

Lot Number	Date of Experiment	Contact Time (minutes)	CFU/Control Carrier	Average CFU Test Carrier	Log ₁₀ Reduction
2974	16/05/02	5	2.04×10^6	0	6.31
2976	16/05/02	5	2.04×10^6	0	6.31
2977	16/05/02	5	2.04×10^6	0	6.31

Activity of the product against *Pseudomonas aeruginosa* (Suspension Test Method): Table 4 summarizes the results of the suspension test. All three lots were able to bring about a $>7 \log_{10}$ reduction in the viability titre of *P. aeruginosa* in a contact time of 30 seconds at room temperature.

Table 4. The activity of the Product against *Pseudomonas aeruginosa*

Lot Number	Date of Experiment	Contact Time (seconds)	CFU/Control Carrier	Average CFU Test Carrier	Log ₁₀ Reduction
2974	18/06/02	30	2.30 x 10 ⁷	0	7.36
2976	18/06/02	30	2.30 x 10 ⁷	0	7.36
2977	18/06/02	30	2.30 x 10 ⁷	0	7.36

Activity of the product against *Salmonella choleraesuis* (Carrier Test Method): Table 5 summarizes the results of *S. choleraesuis* testing. All three lots of the product were able to bring about a >6log₁₀ reduction in the viability titre of *S. choleraesuis* in a contact time of 5 minutes at room temperature indicating bactericidal activity against this organism.

Table 5. The activity of the Product against *Salmonella choleraesuis*

Lot Number	Date of Experiment	Contact Time (minutes)	CFU/Control Carrier	Average CFU Test Carrier	Log ₁₀ Reduction
2974	9/05/02	5	2.26 X 10 ⁶	0	6.34
2976	9/05/02	5	1.17 X 10 ⁶	0	6.07
2977	9/05/02	5	1.17 X 10 ⁶	0	6.07

Activity of the product against *Salmonella choleraesuis* (Suspension Test Method): Table 6 summarizes the results of the suspension test. All three lots were able to bring about a >6log₁₀ reduction in the viability titre of *S. choleraesuis* in a contact time of 30 seconds at room temperature.

Table 6. The activity of the Product against *Salmonella choleraesuis*

Lot Number	Date of Experiment	Contact Time (seconds)	CFU/Control Carrier	Average CFU Test Carrier	Log ₁₀ Reduction
2974	24/06/02	30	1.30 X 10 ⁶	0	6.11
2976	24/06/02	30	1.30 X 10 ⁶	0	6.11
2977	24/06/02	30	1.30 X 10 ⁶	0	6.11

Activity of the product against Methicillin Resistant *Staphylococcus aureus* (Suspension Test Method): Table 7 summarizes the results of the suspension test. All three lots were able to bring about a >6-log₁₀ reduction in the viability titre of MRSA in a contact time of 30 seconds at room temperature indicating bactericidal activity against this organism.

Table 7. The activity of the Product against Methicillin Resistant *S. aureus*

Lot Number	Date of Experiment	Contact Time (seconds)	CFU/Control Carrier	Average CFU Test Carrier	Log ₁₀ Reduction
2974	27/06/02	30	1.70 X 10 ⁶	0	6.23
2976	27/06/02	30	1.70 X 10 ⁶	0	6.23
2977	27/06/02	30	1.70 X 10 ⁶	0	6.23

Activity of the product against Vancomycin Resistant *Enterococcus* (Suspension Test Method): Table 8 summarizes the results of the suspension test. All three lots were able to bring

about a $>6\text{-log}_{10}$ reduction in the viability titre of VRE *in* a contact time of 30 seconds at room temperature indicating bactericidal activity against this organism.

Table 8. The activity of the Product against Vancomycin Resistant *Enterococcus*

Lot Number	Date of Experiment	Contact Time (seconds)	CFU/Control Carrier	Average CFU Test Carrier	Log ₁₀ Reduction
2974	3/07/02	30	5.7×10^6	2	6.54
2976	3/07/02	30	5.7×10^6	2	6.62
2977	3/07/02	30	5.7×10^6	2	6.47

Activity of the product against *Acinetobacter baumannii* (Carrier Test Method): Table 9 summarizes the results of the suspension test. All three lots were able to bring about a $>6\text{-log}_{10}$ reduction in the viability titre of *A. baumannii* in a contact time of 5 minutes at room temperature indicating bactericidal activity against this organism.

Table 9. The activity of the Product against *Acinetobacter baumannii*

Lot Number	Date of Experiment	Contact Time (minutes)	CFU/Control Carrier	Average CFU Test Carrier	Log ₁₀ Reduction
2974	7/06/02	5	1.02×10^6	0	6.00
2976	26/06/02	5	1.71×10^6	0	6.23
2977	26/06/02	5	1.71×10^6	0	6.23

Activity of the product against *Mycobacterium terrae* (Carrier Test Method): Table 10 summarizes the results of the Carrier test. All three lots were able to bring about a $>5\text{-log}_{10}$ reduction in the viability titre of *M. terrae* in a contact time of 5 minutes at room temperature indicating bactericidal activity against this organism.

Table 10. The activity of the Product against *Mycobacterium terrae*

Lot Number	Date of Experiment	Contact Time (minutes)	CFU/Control Carrier	Average CFU Test Carrier	Log ₁₀ Reduction
2974	25/04/02	5	2.0×10^5	0	5.30
2976	25/04/02	5	2.0×10^5	0	5.30
2977	25/04/02	5	2.0×10^5	0	5.30

Activity of the product against *Trichophyton mentagrophytes* (Carrier Test Method): Table 11 summarizes the results of the carrier test. All three lots were able to bring about a $>5\text{-log}_{10}$ reduction in the viability titre of *T. mentagrophytes* in a contact time of 5 minutes at room temperature indicating bactericidal activity against this organism.

Table 11. The activity of the Product against *T. mentagrophytes*

Lot Number	Date of Experiment	Contact Time (minutes)	CFU/Control Carrier	Average CFU Test Carrier	Log ₁₀ Reduction
2974	24/05/02	5	1.13×10^5	0	5.05
2976	24/05/02	5	1.13×10^5	0	5.05
2977	24/05/02	5	1.13×10^5	0	5.05

Activity of the Product against *Polio Sabin 1*: As seen in Table 12, The Product was able to bring about a $>3 \log_{10}$ reduction in the viability titre of the Poliovirus in a contact time of 5 minutes at $20 \pm 1^\circ\text{C}$, indicating virucidal activity against this organism.

Table 12. The activity of the Product against *Poliovirus type 1 (Sabin)*

Lot Number	Date of Experiment	Contact Time (minutes)	PFU/Control Carrier	Average PFU Test Carrier	Log ₁₀ Reduction
2974	22/04/02	5	1.28×10^4	0	4.10
2976	22/04/02	5	1.28×10^4	0	4.10
2977	29/04/02	5	8.00×10^4	0	4.70

Neutralization Verification results of all three lots of the product: Table 13 summarizes the results of the neutralization test of the product. The absence of any significant difference in the number of colonies of the test organism in the test and control was taken to mean that a 1:15 dilution of the Product in the neutralizer was sufficient to arrest its germicidal activity.

Table 13. 15-fold dilution of test solution in neutralizer to arrest the germicidal activity.

Test Organism	Product dilution used in testing	Number of colonies on plates after exposure to a 15-fold dilution of the test solution in the neutralizer	Number of colonies on plates after exposure to the neutralizer
<i>Staphylococcus aureus</i>	Full strength	98/107	91/99
<i>Pseudomonas aeruginosa</i>	Full strength	107/124	94/107
<i>Salmonella choleraesuis</i>	Full strength	60	41/45
<i>Acinetobacter baumannii</i>	Full strength	21/31	16/23
VRE	Full strength	40/37	29/25
<i>T. mentagrophytes</i>	Full strength	44/45	47/48
<i>M. terrae</i>	Full strength	75/58	59/58

Cytotoxicity of the Test Product: At a dilution of 1:100 in EBSS, the product showed no apparent toxicity for the cell line used in this study.

Interference with Plaque Formation: Pre-exposure of the cell monolayers to a 1:100 dilution of the test product in EBSS did not interfere with the plaque formation by the virus tested in the study. Any interference by the residual amounts of the product would have resulted in significantly lower numbers of plaques in the monolayers pre-treated with its dilution when compared to the number of plaques in the control monolayers.

Dilution of the Product to Arrest its Virucidal Activity: Adding the viruses to a 1:100 dilution of the product in EBSS did not result in any loss in their infectivity, which indicates that the dilution of the test product in EBSS at the end of the contact time was sufficient to arrest its virucidal activity.

G. CONCLUDING REMARKS

All three lots of the formulation tested were able to meet the product performance criteria under the conditions of the testing carried out in this study.

H. REFERENCES

ASTM International (2000); Document # E-2111-00, ASTM International, West Conshohocken, PA.

ASTM International (2002); Document # E-2197-02, ASTM International, West Conshohocken, PA.

AOAC International (1998); Official Methods of Analysis of the AOAC. AOAC, Washington, D.C.

Canadian General Standards Board (1997); Assessment of Efficacy of Antimicrobial Agents for Use on Environmental Surfaces and Medical Devices. Document: #CAN/CGSB-2.161-M97. CGSB, Ottawa, Canada.