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**ASSESSMENT OF THE MICROBICIDAL  
ACTIVITY OF VIROX AHP BROAD SPECTRUM  
CLEANER AND NO RINSE SANITIZER  
(AHP-BSC)**

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## TABLE OF CONTENTS

	Page No.
OBJECTIVE	3
MATERIALS AND METHODS	3
The Product	3
Soil Load	3
Neutralizer, Microbial Diluent and Filter Rinse	3
Standard Hard Water	3
Test Organisms	3
1. <i>C. jejuni</i> (ATCC 33560)	3
2. <i>L. monocytogenes</i> (ATCC 19112)	3
3. <i>P. aeruginosa</i> (ATCC 15442)	3
4. <i>S. choleraesuis</i> (ATCC 10708)	3
5. <i>E. coli</i> (ATCC 10708)	3
THE TEST METHODOLOGY	3
Suspension Test	3
Controls	4
Recovery Media and Detection of Viable Organisms	4
Neutralization Verification	4
PRODUCT PERFORMANCE CRITERIA	4
RESULTS	4
Activity of AHP-BSC against <i>C. jejuni</i>	4
Activity of AHP-BSC against <i>L. monocytogenes</i>	5
Activity of AHP-BSC against <i>P.aeruginosa</i>	5
Activity of AHP-BSC against <i>E. coli</i>	5
Activity of AHP-BSC against <i>S. choleraesuis</i>	5
Neutralization Verification Results to Arrest Activity of AHP-BSC	6
CONCLUDING REMARKS	6

## OBJECTIVE

The objective of this study was to determine the effectiveness of the formulation as a sanitizer at a dilution of 1:128 against the following foodborne bacteria: *Listeria monocytogenes*, *Campylobacter jejuni*, *Escherichia coli*, *Salmonella choleraesuis* and *Pseudomonas aeruginosa* using a suspension test method.

## MATERIALS AND METHODS

### The Product:

One lot of the product was provided for testing in this study. Upon arrival in our laboratory, the bottle was stored at room temperature in a place with restricted access. The product was tested at a dilution of 1:128.

### Soil Load:

No soil load was used in this testing.

### Neutralizer, Microbial Diluent and Filter Rinse:

Lethen Broth (with 1% sodium thiosulphate pent hydrate) was used as the neutralizer and to rinse the membrane filters and the filter holder unit. 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.

### Standard Hard Water:

Water with 200 ppm as calcium carbonate ( $\text{CaCO}_3$ ) was used as the diluent for the product.

### Test Organisms:

The organisms used and their specific strains are given below:

1. *Campylobacter jejuni* (ATCC 33560)
2. *Listeria monocytogenes* (ATCC 19112)
3. *Pseudomonas aeruginosa* (ATCC 15442)
4. *S. choleraesuis* (ATCC 10708)
5. *Escherichia coli* (ATCC 25404)

Stock suspensions of the bacteria except *C. jejuni* were prepared by culturing them in tryptic soy broth (TSB; Difco) for 24 hours at 37°C. The stock suspension of *C. jejuni* was prepared by suspending colonies in sterile saline of the bacteria grown on modified Skirrow agar plates to give an average of  $1.0 \times 10^7$  cfu/mL

## THE TEST METHODOLOGY

### The Suspension Test:

The test was carried out by adding 100 µL of the bacterial suspension, without soil load, to 900 µL of the test formulation 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 was transferred into a vial containing 9.0 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.

### Controls:

Controls were tested by adding 100 µL of bacterial suspension to 900 µL of saline instead of the disinfectant.

### Recovery Media and Detection of Viable Organisms:

The control suspensions and the test samples were passed through 47 mm diameter membrane filters (Millipore; 0.22 µm pore diameter). The filters for all the bacteria except *Campylobacter jejuni* were placed on TSA plates, incubated at 37°C, and the colony forming units (CFU) recorded at 24 hour intervals for a total of 5 days. For testing with *Campylobacter jejuni*, the filters were placed on modified Skirrow agar plates, incubated at 37°C in a micro-aerophilic atmosphere, monitored, and CFU recorded at 48-hour intervals for a total of 4 days.

### Neutralization Verification:

One part of the use-dilution of the product was mixed with 9 parts of the neutralizer. The test organism was added to the neutralized solution. The neutralizer alone was used as the control solution. At the end of a contact time of 5 minutes at room temperature, 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 vial and the last lot of rinse passed through the membrane filter.

### PRODUCT PERFORMANCE CRITERIA

The number of repeats in the suspension test was six. The test also included three control repeats. The results are reported as log<sub>10</sub> reductions in viability in reference to the control. For a product to be considered bactericidal it was expected to reduce the viability titre of each test organism by at least 5 log<sub>10</sub> under the conditions of this test.

### RESULTS

**Activity of AHP-BSC against *C. jejuni*:** Table 1 summarizes the activity of AHP-BSC against *C. jejuni*. The product was able to bring about a >5 log<sub>10</sub> reduction in the viability titre of *C. jejuni* in a contact time of 30 seconds at room temperature.

Table 1: The activity of AHP-BSC against *C. jejuni*

Date of Expt.	Dilution	Lot Number	# Of Carriers	Contact Time	CFU/control Carrier	CFU/test Carrier	Log <sub>10</sub> Reduction
04/11/04	1:128	3811	6	30 sec	1.50X 10 <sup>5</sup>	0	5.18

**Activity of AHP-BSC against *L. monocytogenes*:** Table 2 summarizes the activity of AHP-BSC against *L. monocytogenes*. The product was able to bring about a  $>5 \log_{10}$  reduction in the viability titre of *L. monocytogenes* in a contact time of 30 seconds at room temperature.

Table 2: The activity of AHP-BSC against *L. monocytogenes*

Date of Experiment	Dilution	Lot Number	# Of Repeats	Contact Time	CFU/control	CFU/test	Log <sub>10</sub> Reduction
12/11/04	1:128	3811	6	30 sec	$6.30 \times 10^5$	0	5.80

**Activity of AHP-BSC against *P. aeruginosa*:** Table 3 summarizes the activity of AHP-BSC against *P. aeruginosa*. The product was able to bring about a  $>5 \log_{10}$  reduction in the viability titre of *P. aeruginosa* in a contact time of 30 seconds at room temperature.

Table 3: The activity of AHP-BSC against *P. aeruginosa*

Date of Experiment	Dilution	Lot Number	# Of Repeats	Contact Time	CFU/control	CFU/test	Log <sub>10</sub> Reduction
8/11/04	1:128	3811	6	30 sec	$7.90 \times 10^5$	0	5.89

**Activity of AHP-BSC against *E. coli* :** Table 4 summarizes activity of AHP-BSC against *E. coli*. The product was able to bring about a  $>5 \log_{10}$  reduction in the viability titre of *E. coli* in a contact time of 30 seconds at room temperature indicating bactericidal activity against this organism.

Table 4: The activity of AHP-BSC-BSC against *E. coli*

Date of Experiment	Dilution	Lot Number	# Of Repeats	Contact Time	CFU/control	CFU/test	Log <sub>10</sub> Reduction
21/07/04	1:128	3990	6	30 sec	$1.14 \times 10^5$	0	5.14

**Activity of AHP-BSC against *S. choleraesuis*:** Table 5 summarizes the result of the suspension test. The product was able to bring about a  $>5 \log_{10}$  reduction in the viability titre of *S. choleraesuis* in a contact time of 30 seconds at room temperature indicating bactericidal activity against this organism.

Table 5: The activity of AHP-BSC against *S. choleraesuis*

Date of Experiment	Dilution	Lot Number	# Of Repeats	Contact Time	CFU/control	CFU/test	Log <sub>10</sub> Reduction
27/07/01	1:128	3990	6	30 sec	$6.47 \times 10^6$	0	5.81

**Neutralization Verification results of all three lots of the product:** Table 4 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 1:10 dilution of the Product in the neutralizer was sufficient to arrest its germicidal activity.

Table 4: Neutralization Verification of AHP-BSC

<b>Test Organism</b>	<b>Product dilution used in testing</b>	<b>Number of colonies on plates after exposure to a 10-fold dilution of the test solution in the neutralizer</b>	<b>Number of colonies on plates after exposure to the neutralizer</b>
<i>P. aeruginosa</i>	1:128	314/307	317/313
<i>L. monocytogenes</i>	1:128	131/132	195/211
<i>C. jejuni</i>	1:128	14/12	15/6
<i>S. choleraesuis</i>	1:128	216/238	277/274
<i>E. coli</i>	1:128	8/5	1/6

#### CONCLUDING REMARKS

The formulation tested was able to meet the product performance criteria under the conditions of the testing carried out in this study.