

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

**ASSESSMENT OF THE SANITIZING ACTION OF  
A FORMULATION BASED ON 7%  
ACCELERATED HYDROGEN PEROXIDE (AHP)**

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August 2001

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## A. INTRODUCTION

The sanitizing action of a 7% hydrogen peroxide formulation based on Accelerated Hydrogen Peroxide (AHP) technology was evaluated using a suspension test method.

## B. 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: *Escherichia coli*, *Listeria monocytogenes*, *Campylobacter jejuni*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella choleraesuis* using a suspension test method.

## C. MATERIALS AND METHODS

### The Product:

Three 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 a dilution of 1:128 in hard water with 200 parts per million (ppm) as calcium carbonate (CaCO<sub>3</sub>).

**Soil Load:** No soil load was used in this testing.

### 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. 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:

To dilute the product for testing, water with a standard hardness of 200 ppm as CaCO<sub>3</sub> was used as the diluent. The hard water was prepared according to the formula of AOAC International (1990).

### Test Organisms:

The organisms used and their specific strains are given below:

1. *Escherichia coli* O157:H7 (MEA Isolate)
2. *E coli* (ATCC 25404)
3. *Staphylococcus aureus* (ATCC 6538)
4. *Campylobacter jejuni* (ATCC 33560)
5. *Listeria monocytogenes* (ATCC 19112)
6. *Pseudomonas aeruginosa* (ATCC 15442)
7. *Salmonella choleraesuis* (ATCC 10708)

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  $10 \times 10^7$  organism/mL

#### **D. 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 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.

##### **Recovery Media and Detection of Viable Organisms:**

For tests using *Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella choleraesuis*, the filters were placed on TSA plates, incubated at 37°C, monitored, and CFU were recorded at 24-hour intervals for a total of 5 days. For *L. monocytogenes*, the filters were placed on TSA plates, incubated at 37°C, monitored, and CFU were recorded at 48 hours, and every 24-hour interval thereafter for a total of 5 days. For testing using *Campylobacter jejuni*, the filters were placed on modified Skirrow agar plates, incubated at 37°C in a micro-aerophilic atmosphere required for the growth of the *Campylobacter*, monitored, and CFU recorded at 48-hour intervals for a total of 4 days.

##### **Controls:**

Controls were tested by adding 100 µL of bacterial suspension to 9.9 mL Lethen broth instead of the disinfectant.

##### **Neutralization Verification:**

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 for 24 hours at 37°C 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.

#### **E. PRODUCT PERFORMANCE CRITERIA**

The number of test repeats in the bactericidal tests was 6. The test also included three control repeats. The results are reported as  $\log_{10}$  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  $\log_{10}$  under the conditions of this test.

## F. RESULTS

The activity of AHP against *E. coli* (ATCC 25404): Table 1 summarizes the results of the suspension test. 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.

**Table 1. The activity of a 1:128 dilution of 7% Accelerated Hydrogen Peroxide (AHP) Against *E. coli* (ATCC 25404)**

Date of Expt.	Dilution	Lot Number	# Of Carriers	Contact Time	CFU/control Carrier	CFU/test Carrier	Log <sub>10</sub> Reduction
14/06/01	1:128	2664	6	30 sec	$1.01 \times 10^6$	0	6.00
14/06/01	1:128	2753	6	30 sec	$1.01 \times 10^6$	0	6.00
14/06/01	1:128	2754	6	30 sec	$1.01 \times 10^6$	0	5.92

Table 2 summarizes the activity of AHP against *E. coli* O157:H7 (MEA). The product was able to bring about a  $>6 \log_{10}$  reduction in the viability titre of the test organism in a contact time of 30 seconds at room temperature.

**Table 2. The activity of a 1:128 dilution of 7% Accelerated Hydrogen Peroxide (AHP) Against *E. coli* O157:H7**

Date of Expt.	Dilution	Lot Number	# Of Carriers	Contact Time	CFU/control Carrier	CFU/test Carrier	Log <sub>10</sub> Reduction
18/06/01	1:128	2664	6	30 sec	$1.15 \times 10^6$	1	6.06
18/06/01	1:128	2753	6	30 sec	$1.15 \times 10^6$	0	6.06
18/06/01	1:128	2754	6	30 sec	$1.15 \times 10^6$	0	6.06

Table 3 summarizes the activity of AHP against *S. aureus*. The product was able to bring about a  $> 6 \log_{10}$  reduction in the viability titre of *S. aureus* in a contact time of 30 seconds at room temperature.

**Table 3. The activity of a 1:128 dilution of 7% Accelerated Hydrogen Peroxide (AHP) against *S. aureus***

Date of Expt.	Dilution	Lot Number	# Of Carriers	Contact Time	CFU/control Carrier	CFU/test Carrier	Log <sub>10</sub> Reduction
19/06/01	1:128	2664	6	30 sec	$1.63 \times 10^6$	2	6.05
19/06/01	1:128	2753	6	30 sec	$1.63 \times 10^6$	2	6.06
19/06/01	1:128	2754	6	30 sec	$1.63 \times 10^6$	0	6.21

Table 4 summarizes the activity of AHP against *C. jejuni*. The product was able to bring about a  $>6 \log_{10}$  reduction in the viability titre of *C. jejuni* in a contact time of 30 seconds at room temperature.

**Table 4. The activity of a 1:128 dilution of 7% Accelerated Hydrogen Peroxide (AHP) against *C. jejuni***

Date of Expt.	Dilution	Lot Number	# Of Carriers	Contact Time	CFU/control Carrier	CFU/test Carrier	Log <sub>10</sub> Reduction
1/08/01	1:128	2664	6	30 sec	1.65 X 10 <sup>6</sup>	0	6.22
1/08/01	1:128	2753	6	30 sec	1.65 X 10 <sup>6</sup>	0	6.22
1/08/01	1:128	2754	6	30 sec	1.65 X 10 <sup>6</sup>	0	6.22

Table 5 summarizes the activity of AHP against *L. monocytogenes*. The product was able to bring about a >5 log<sub>10</sub> reduction in the viability titre of *L. monocytogenes* in a contact time of 30 seconds at room temperature.

**Table 5. The activity of a 1:128 dilution of 7% Accelerated Hydrogen Peroxide (AHP) against *L. monocytogenes***

Date of Expt.	Dilution	Lot Number	# Of Carriers	Contact Time	CFU/control Carrier	CFU/test Carrier	Log <sub>10</sub> Reduction
20/06/01	1:128	2664	6	30 sec	2.43 X 10 <sup>5</sup>	0	5.39
20/06/01	1:128	2753	6	30 sec	2.43 X 10 <sup>5</sup>	1	5.39
20/06/01	1:128	2754	6	30 sec	2.43 X 10 <sup>5</sup>	1	5.39

Table 6 summarizes the activity of AHP against *P. aeruginosa*. The product was able to bring about a >5 log<sub>10</sub> reduction in the viability titre of *P. aeruginosa* in a contact time of 30 seconds at room temperature.

**Table 6. The activity of a 1:128 dilution of 7% Accelerated Hydrogen Peroxide (AHP) Against *P. aeruginosa***

Date of Expt.	Dilution	Lot Number	# Of Carriers	Contact Time	CFU/control Carrier	CFU/test Carrier	Log <sub>10</sub> Reduction
21/06/01	1:128	2664	6	30 sec	2.30 X 10 <sup>6</sup>	1	5.36
21/06/01	1:128	2753	6	30 sec	2.30 X 10 <sup>6</sup>	1	5.36
21/06/01	1:128	2754	6	30 sec	2.30 X 10 <sup>6</sup>	1	5.36

Table 7 summarizes the activity of AHP against *S. choleraesuis*. The product was able to bring about a > 6 log<sub>10</sub> reduction in the viability titre of *S. choleraesuis* in a contact time of 30 seconds at room temperature.

**Table 7. The activity of a 1:128 dilution of 7% Accelerated Hydrogen Peroxide (AHP) against *S. choleraesuis***

Date of Expt.	Dilution	Lot Number	# Of Carrier	Contact Time	CFU/control Carrier	CFU/test Carrier	Log <sub>10</sub> Reduction
21/06/01	1:128	2664	6	30 sec	1.36 X 10 <sup>6</sup>	1	6.13
21/06/01	1:128	2753	6	30 sec	1.36 X 10 <sup>6</sup>	1	6.13
21/06/01	1:128	2754	6	30 sec	1.36 X 10 <sup>6</sup>	0	6.13

**Neutralization Verification results of all three lots of the product:** Table 8 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 test formulation in the neutralizer was sufficient to arrest its germicidal activity.

**Table 8. Neutralization to arrest the germicidal activity of the test formulation.**

<b>Test Organism</b>	<b>Product dilution used in testing</b>	<b>Number of colonies on plates after exposure to a 15-fold dilution of the test solution in the neutralizer</b>	<b>Number of colonies on plates after exposure to the neutralizer</b>
<i>Staphylococcus aureus</i>	1:128	13/13	10/14
<i>Pseudomonas aeruginosa</i>	1:128	8/13	7/11
<i>Salmonella choleraesuis</i>	1:128	10/7	12/11
<i>Listeria monocytogenes</i>	1:128	20/26	21/20
<i>Campylobacter jejuni</i>	1:128	27/24	25/26
<i>E. coli</i>	1:128	6/7	11/4

#### **G. CONCLUDING REMARKS**

All three lots of the test formulation, when diluted 128-fold, were able to reduce the viability titres of *L. monocytogenes*, *C. jejuni*, *E. coli* O157:H7, *E. coli* (ATCC 25404), *S. aureus*, *P. aeruginosa* and *S. choleraesuis* a by a  $>5 \text{ Log}_{10}$  with a contact time of 30 seconds at room temperature in a suspension test.