

Final Report submitted to Virox Technologies Inc.  
Mississauga, Ontario

**ASSESSMENT OF THE GERMICIDAL ACTIVITY OF  
7% ACCELERATED HYDROGEN PEROXIDE  
AGAINST *ACINETOBACTER BAUMANNII* USING A  
QUANTITATIVE CARRIER TEST AND A SUSPENSION  
TEST**

**Syed A. Sattar, Ph.D.**  
Professor of Microbiology  
Centre for Research in Environmental Microbiology (CREM)  
Faculty of Medicine, University of Ottawa  
Ottawa, Ontario, Canada  
K1H 8M5

*Phone:* (613) 562-5800 ext. 8314; *Fax:* (613) 562-5452

This study was conducted with the technical assistance of  
**Teresa Burke, M.L.T.**

November, 1999

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## A. OBJECTIVE

The main objective of this study was to determine if the product is bactericidal at a 1:16 dilution.

## B. MATERIALS AND METHODS

### **The Product:**

Three separate lots of 7% Accelerated Hydrogen Peroxide were provided for testing in this study. Upon receipt in our laboratory, they were stored at room temperature in a safe and secure location with controlled access.

### **Carriers:**

The inside bottom surface of glass vials (Galaxy Co., Newfield, New Jersey) was used as the surface for the quantitative carrier tests. The glass vials were also used for the suspension test but it was not critical to have a flat surface as with the carrier test. The vials were reused after their decontamination, cleaning and autoclave sterilization.

### **Soil Load:**

For both the quantitative carrier test and the suspension test, the test organism was first suspended in bovine serum (Gibco BRL Life Tech. Cat. No. 16000-044, N.Y., USA) at a final concentration of 5%.

### **Neutralizer, Microbial Diluent and Filter Rinse:**

Modified Lethen Broth (with 0.1% sodium thiosulphate pentahydrate and Tween 80) was used as the neutralizer. It was also used to make post-test dilutions of the bacterial suspensions and to rinse the filters and the filter holding unit. Phosphate buffer (PB), at pH 7.2, was used as the final rinse of the vials and the unit. (It aided in rinsing the froth created by the Lethen broth).

**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 from AOAC International (1990).

### **Test Organism: *Acinetobacter baumannii***

A seed culture of the bacterium was received through the courtesy of the Microbiology Lab at the Ottawa Hospital. A stock suspension of the bacterium was prepared by culturing it in tryptic soy broth (TSB; Difco) for 18-24 hours at 37°C.

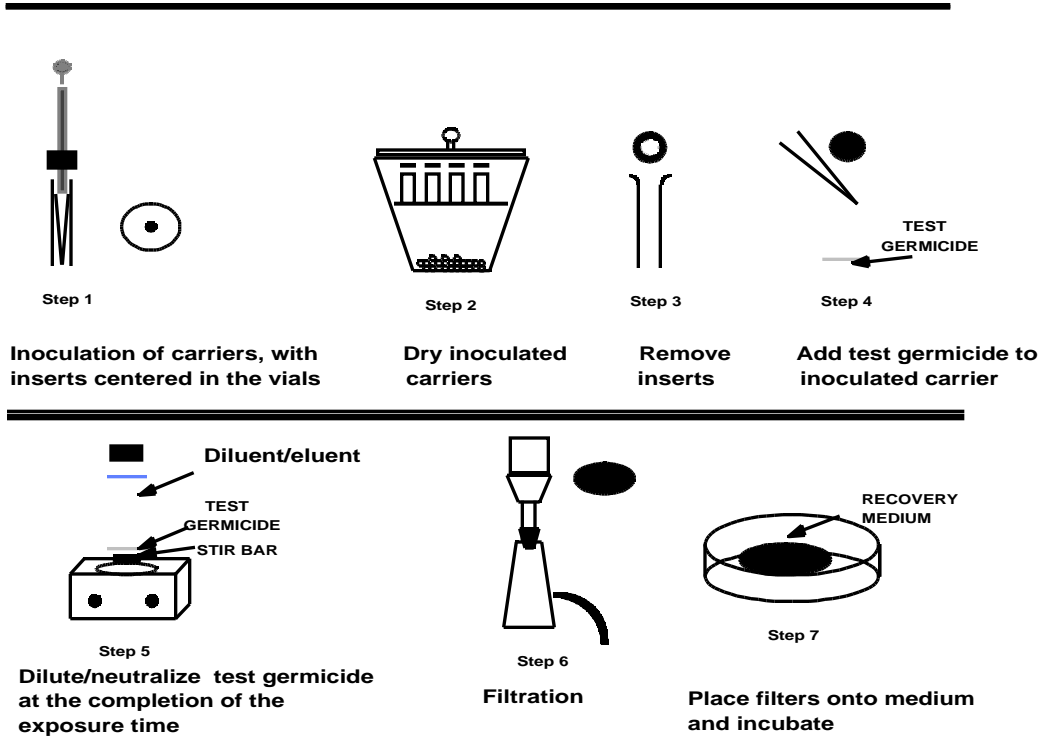
## C. THE QUANTITATIVE CARRIER TEST METHODOLOGY

The quantitative carrier test used has been designed to: (a) permit the determination of the exact number of colony forming units (CFU) placed on each carrier and the CFU remaining 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 the cells of the test organisms 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) incorporation of glass inserts to eliminate any false-positive results due to the generation of micro-aerosols in the carriers and (h) give a precise determination of log<sub>10</sub> reduction in viable cells of the test organism after exposure to the product under test.

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

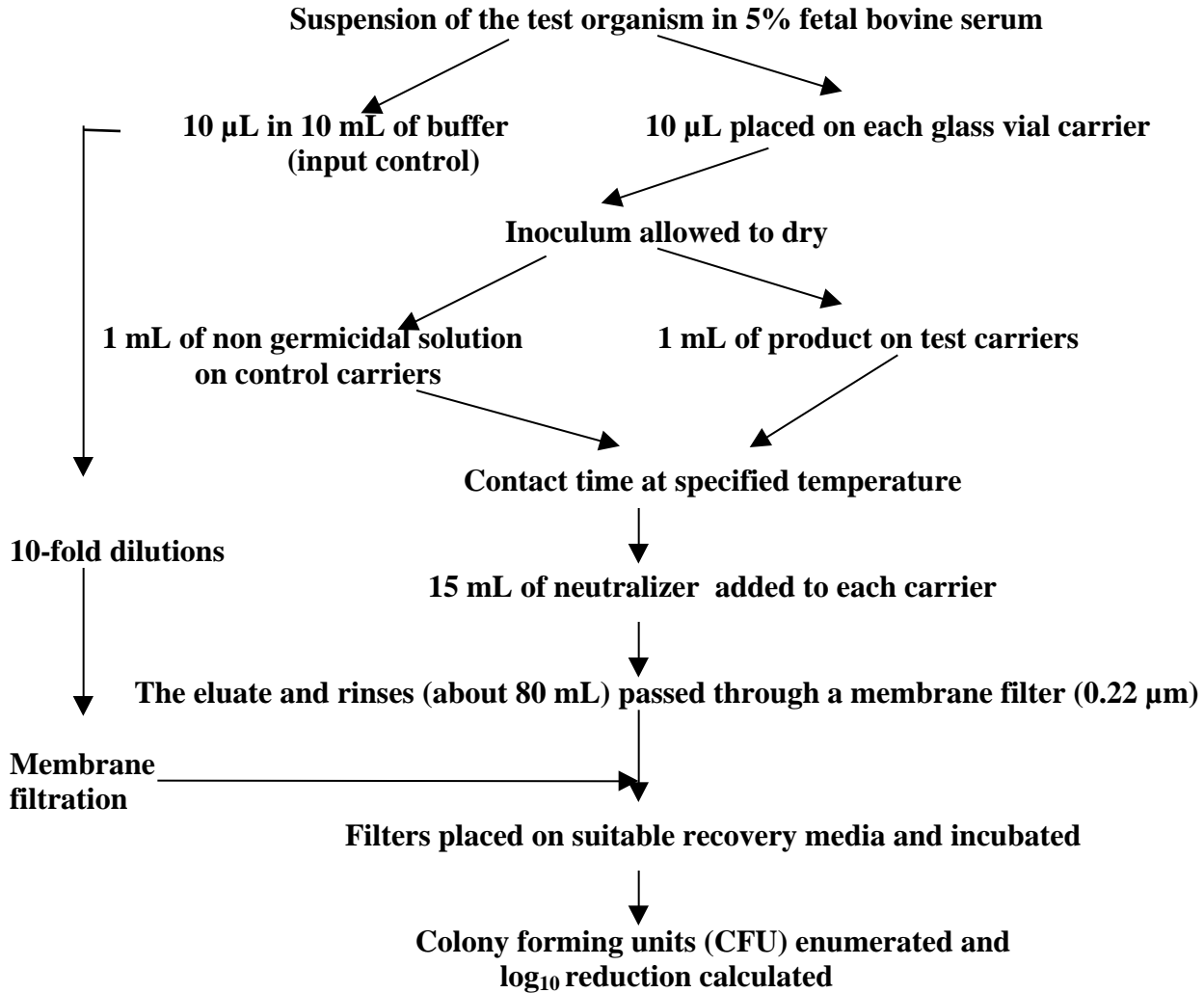
Figure 1.

**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 (0.22 µm 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 procedure for the suspension test involved adding 100 µL of the test organism to 900 µL of the product; swirling gently and allowing to sit for the required contact time after which 9.0 mL of neutralizer was added and vortexed. This mixture was passed through a membrane filter and the carrier vial was rinsed 2x with 10 mL Lethen broth and then 3x with 15 ml of PB. The membrane filtration technique was the same as with the quantitative carrier test.

**Recovery Media and Detection of Viable Organisms:** The control suspensions and eluates tested were passed through 47 mm dia. membrane filters (Millipore; 0.22µm pore diameter). The filters were then placed on the surface of tryptic soy agar (TSA) in 100 mm dia. disposable plastic plates, incubated at 37°C, and the colony forming units (CFU) were recorded at 24 hour intervals for a total of 5 days.

**Controls: Carrier Testing** - Control carriers were used in the same manner as the test carriers except that a non germicidal solution was applied to the dried inoculum instead of the disinfectant.

**Suspension Testing** - control carriers were used in the same manner as the test except Lethen broth was used instead of the disinfectant.

**Neutralization Verification:** One lot of the product was tested against the organism to verify if the product could be neutralized. The procedure was as follows: 10-100 CFU (Colony Forming Units) of the organism were pipetted into 9.9 mL Lethen broth + product (100 µL). After 5 minutes of contact at room temperature the solution was filtered, the filters placed on TSA plates and incubated at 37°C overnight. The resulting colony counts were compared with a control (organism in Lethen broth, no product).

## D. PRODUCT PERFORMANCE CRITERIA

Carrier Test- the number of test carriers in each test was 10.

Suspension Test- the number of test carriers in each test was 6.

Both test methodologies included three control carriers.

- The results are reported as log<sub>10</sub> reductions in viability in reference to the control carriers.

For a product to be considered bactericidal it was expected to reduce the viability titre of the test organism by at least 6 log<sub>10</sub> (at least 1 million-fold) on each test carrier under the conditions of these tests.

## E. RESULTS

### Quantitative Carrier Test:

**Activity of the product against *Acinetobacter baumannii*:** Table 1 summarizes the results of the quantitative carrier testing. All three lots were able to bring about a >6 log<sub>10</sub> reduction in the viability titre of *A. baumannii* in a contact time of 5 minutes at room temperature indicating bactericidal activity against this organism in our test protocol.

**Table 1. Activity of a 1:16 Dilution of the Product Against *A. baumannii* in a Carrier Test**

Lot Number	Date of expt.	Contact Time (minutes)	CFU/control carrier	CFU/test carrier	Log <sub>10</sub> Reduction
11061	10/11/99	5	3.85 x 10 <sup>6</sup>	0	6.59
11118	11/11/99	5	3.05 x 10 <sup>6</sup>	0	6.48
11221	11/11/99	5	3.05 x 10 <sup>6</sup>	0	6.48

## Suspension Test:

**Activity of the product against *Acinetobacter baumannii*:** Table 2 summarizes the results of the suspension test. All three lots were able to bring about a >6 log<sub>10</sub> reduction in the viability titre of *A. baumannii* in a contact time of 30 seconds at room temperature indicating bactericidal activity against this organism in our test protocol.

**Table 2. The Activity of a 1:16 Dilution of the Test Product Against *Acinetobacter baumannii* in a Suspension Test**

Lot Number	Date of expt.	Contact Time (seconds)	CFU/in controls	CFU/in test	Log <sub>10</sub> Reduction
11061	22/11/99	30	7.4 x 10 <sup>6</sup>	0	6.87
11118	16/11/99	30	1.19 x 10 <sup>6</sup>	1	6.95
11221	16/11/99	30	1.19 x 10 <sup>6</sup>	5	6.78

**Neutralization Verification Results of the Product:** Table 3 summarizes the results of the neutralization test. The product was able to be neutralized when tested against the organism in this study.(note: testing was done in duplicate).

**Table 3: Neutralization of the Test Product**

Date of Experiment	Lot Number	Organism	Contact time	CFU in Control	CFU in Tests
22/11/99	11061	<i>Acinetobacter baumannii</i>	5 minutes	26/28	24/30

## F. DISCUSSION AND CONCLUDING REMARKS

For the past 27 years our laboratory has been active in the development of methods for assessing the microbiocidal activity of chemical germicides and in the evaluation of formulations for application on semi-critical medical devices, non-porous environmental surfaces and human skin. The laboratory has a quality control/quality assurance unit as required by the U.S. Environmental Protection Agency. Submissions based on our evaluations of the germicidal activity of disinfectant formulation have been found to meet the registration requirements of Health Canada.

This study used a fully quantitative hard surface carrier test and a suspension test to determine the germicidal activity of the product. All three lots of the product proved to be bactericidal. The quantitative procedure, as reported here, met the testing requirements of a Canadian national standard (CGSB, 1997) except that it used the flat surface of a glass vial instead of penicylinders for testing the product's bactericidal activities.