

**Final Report submitted to Virox Technologies Inc.
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**ASSESSMENT OF THE MICROBICIDAL ACTIVITY OF
AN ACCELERATED HYDROGEN PEROXIDE BASED-
FORMULATION (AHP-5) AGAINST *ACINETOBACTER
BAUMANNII* USING A SUSPENSION TEST METHOD**

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OBJECTIVE

The main objective of this study was to determine the effectiveness of AHP-5 as a sanitizer 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.

Neutralizer, Microbial Diluent and Filter Rinse:

Lethen Broth (with 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:

Water with 200 ppm as calcium carbonate (CaCO_3) was used as the diluent for the product.

Test Organism:

A seed culture of *Acinetobacter baumannii* 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 24 hours at 37°C.

METHODOLOGY FOR THE SUSPENSION TEST:

The test was carried out by adding 100 μL of the bacterial suspension with no 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 20°C. At the end of the contact time, the reaction mixture received 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 in the same manner as the test by adding 100 μL of bacterial suspension to 900 μL saline instead of the test formulation.

Recovery Media and Detection of Viable Organisms:

The controls and test samples were passed through 47 mm diameter membrane filters (Millipore; 0.22 μm pore diameter). The filters were then placed on plates with trypticase soy agar (TSA), incubated at 37°C, and the colony forming units (CFU) recorded at 24 hour intervals for a total of 5 days.

Neutralization Verification:

Bactericidal Test:

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 20°C, the mixture was passed through a membrane filter to capture the bacteria. The filters were placed on TSA plates 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 numbers of repeats in the test were 6. The results are reported as log₁₀ reductions in viability in reference to the control carriers.

For a product to be considered effective in the sanitizer test, the target was a minimum reduction of 3 log₁₀.

RESULTS

Activity of the product against *Acinetobacter baumannii*: Table 1 summarizes the result of the suspension test. The product was able to bring about a >5log₁₀ reduction in the viability titre of *A. baumannii* in a contact time of 30 seconds at room temperature indicating bactericidal activity against this organism.

Table1: The activity of a 1:128 dilution of AHP-5 against *Acinetobacter baumannii*

Lot Number	Date of Experiment	Contact Time	CFU/Control Carrier	Average CFU Test Carrier	Log ₁₀ Reduction
3783	30/07/04	30 seconds	7.03 X 10 ⁵	0	5.85

Neutralization to arrest microbicidal activity: Table 2 summarizes the result of the neutralization test. 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 2: Neutralization Verification of AHP-5

Test Organism	Product dilution used in testing	Number of colonies on plates after exposure to a 10-fold dilution of the test solution in the neutralizer	Number of colonies on plates after exposure to the neutralizer
<i>Acinetobacter baumannii</i>	1:128	170/183	200/196

CONCLUDING REMARKS

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