

Final Report submitted to Virox Technologies, Inc.  
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**EVALUATION OF THE MYCOBACTERICIDAL AND  
FUNGICIDAL ACTIVITIES OF ACCEL-TB**

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## A. OBJECTIVE OF THE STUDY

The main objective of this study was to evaluate the mycobactericidal and fungicidal activities of three lots of ACCEL-TB a 0.5% accelerated hydrogen peroxide (AHP)-based formulation. The quantitative carrier test used to assess the mycobactericidal activities of samples of the solutions incorporates all-important elements specified in the Canadian General Standards Board's document number CAN/CGSB-2.161-M97 entitled *Assessment of Efficacy of Antimicrobial Agents for use on Environmental Surfaces and Medical Devices*. The carrier test method is a standard (E-2111) of ASTM International (ASTM 2000).

## B. MATERIALS AND METHODS

### Disinfectants:

Three lots of the product, as supplied by the sponsor, were tested in this study. Once received here, the product was stored at room temperature in a place where only authorized personnel had access.

### 2. Soil Load:

For inoculation of the carriers, the test organisms were first suspended in a tripartite soil load: 25 $\mu$ L of bovine serum albumin, 100 $\mu$ L of mucin and 35 $\mu$ L of Tryptone were added to 340 $\mu$ L of the mycobacterial or fungal suspension. The soil load mixture contains a level of protein roughly equal to that in 5% bovine serum.

### 3. The Challenge Organisms:

A standard strain of *Mycobacterium terrae* (ATCC 15755) and *Trichophyton mentagrophytes* (ATCC 9533), already available in our laboratory, were used in this study. They were cultured as follows:

The mycobacterium was grown in Middlebrook 7H9 broth with ADC enrichment and glycerol, in vented plug seal capped cell 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 with sterile water. The stock solution was stored at 4°C.

A stock suspension of the conidia was obtained, by inoculating the center of a Sabouraud dextrose agar plate and incubating it at 28°C for 10 days. Mycelia mats were harvested from the agar surface homogenized with sterile glass beads in normal saline and filtered through sterile cotton to remove the hyphae.)

## C. MICROBIOLOGICAL TESTS TO ASSESS THE GERMICIDAL ACTIVITIES:

### 1. The Test Methodology

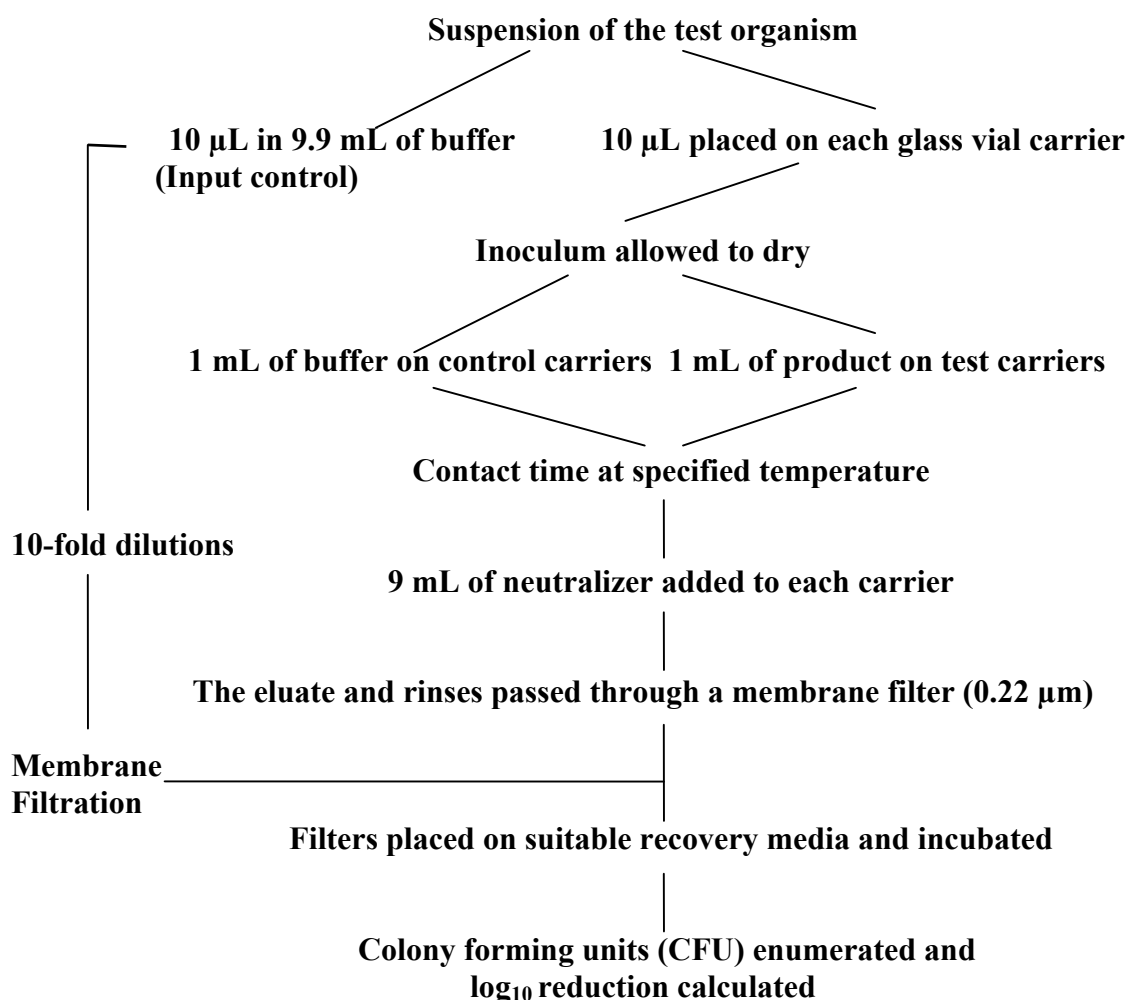
The quantitative carrier tests used in this evaluation have been designed to: (a) permit the determination of the exact number of colony forming units (CFU) placed on each carrier and the CFU remaining after the drying of the inoculum, (b) avoid wash-off of any cells of the test organism, (c) allow complete 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 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  $\log_{10}$  reduction in CFU of the test organism after exposure to the product under test. This new test method, therefore, eliminates the deficiencies associated with the AOAC's test for mycobactericidal activity (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 International.

The Flow Chart outlines the methodology used for the germicide test.

### FLOW CHART\*

#### BASIC METHOD FOR TESTING GERMICIDES USING THE QUANTITATIVE CARRIER TEST



\*This basic procedure was used for testing mycobactericidal activities of the test product(s).

### Recovery Media and Detection of Viable Organisms:

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 dextrose agar and incubated at 28°C, monitored, and CFU recorded at 3 days, and every 24 hour interval thereafter for a total of 10 days.

### Controls:

Control carriers were used in the same manner as test carriers except sterile saline was applied to the dried inoculum instead of the disinfectant.

### Neutralizer, Microbial Diluent and Filter Rinse:

Lethen Broth (with 1% sodium thiosulphate pentahydrate) was used as the neutralizer. It was also used to rinse the membrane filters and the filter holder unit. Normal saline (0.85% NaCl) was used as the diluent and filter rinse. (It worked well in rinsing out the froth created by the Lethen broth).

### Product Performance Criteria

In each test, 10 test carriers and 3 control carriers were used. The results are reported as log<sub>10</sub> reductions in viability in reference to the control carriers. Under the conditions of this test, for the product to be considered mycobactericidal, it was expected to reduce the viability titre of the test organisms by a minimum of 4 log<sub>10</sub> and a 5 log<sub>10</sub> for fungicidal activity.

## D. RESULTS

**Activity of ACCEL-TB against *Mycobacterium terrae*:** Table 1 summarizes the results of testing against *M. terrae*. All three lots of the product were able to bring about a >4 log<sub>10</sub> reduction in the viability titre of *M. terrae* in a contact time of 1 minute at room temperature, indicating mycobactericidal activity against this organism in our test protocol.

**Table 1: The Activity of ACCEL-TB against *Mycobacterium terrae*.**

Lot Number	Date of Experiment	Contact Time	Number of Carriers	CFU/control Carriers	CFU/test Carrier	Log <sub>10</sub> Reduction
3635-reg	12/11/04	1 minute	10	7.03 X 10 <sup>6</sup>	11	4.80
3646-reg	12/11/04	1 minute	10	7.03 X 10 <sup>6</sup>	7	5.02
3647-reg	12/11/04	1 minute	10	7.03 X 10 <sup>6</sup>	11	4.80

**Activity of ACCEL-TB against the conidia of *T. mentagrophytes*:** As can be seen from Table 2, the product was able to bring about a >5 log<sub>10</sub> reduction in the viability titre of *T. mentagrophytes* in a contact time of 3 minutes at room temperature, indicating fungicidal activity against this organism.

**Table 2: The Activity of ACCEL-TB against the conida of *T. mentagrophytes***

<b>Lot Number</b>	<b>Date of Experiment</b>	<b>Contact Time</b>	<b>Number of Carriers</b>	<b>CFU/control Carriers</b>	<b>CFU/test Carrier</b>	<b>Log<sub>10</sub> Reduction</b>
3635-reg	24/11/04	3 minute	10	9.23 X 10 <sup>5</sup>	1	5.67
3646-reg	24/11/04	3 minute	10	9.23 X 10 <sup>5</sup>	1	5.67
3647-reg	24/11/04	3 minute	10	9.23 X 10 <sup>5</sup>	1	5.67

## **E. CONCLUSION**

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

## **F. LITERATURE CITED**

- ASTM International (2000). Standard Quantitative Carrier Test Method to Evaluate The Bactericidal, Fungicidal, Mycobactericidal, and Sporocidal Potencies of Liquid Chemical Germicides. Document # E-2111-00, ASTM, West Conshohocken, PA.
- AOAC (1990). Official Methods of Analysis of the AOAC. AOAC, Washington, D.C.
- Canadian General Standards Board. (1991). Assessment of Efficacy of Antimicrobial Agents for Use on Environmental Surfaces and Medical Devices. Document; #CAN/CGSB-2.161-M91. CGSB, Ottawa, Canada.