EVALUATION OF THE ACTIVITY OF TWO ACCELERATED HYDROGEN PEROXIDE-BASED FORMULATIONS AGAINST THE SPORES OF BACILLUS ANTHRACIS AND BACILLUS SUBTILIS USING A QUANTITATIVE CARRIER TEST

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**OBJECTIVE OF THE STUDY**

The objective of this study was to test the activity of two accelerated hydrogen peroxide-based formulations against the spores of a virulent strain *Bacillus anthracis* and the surrogate organism *Bacillus subtilis* using the second tier of a quantitative carrier test (QCT-2) developed in our lab; the method is now a standard (#E-2197) of ASTM International (ASTM 2002).

**MATERIALS AND METHODS**

**Test Facility**

The experimental work for this study was performed in a facility certified by Health Canada for handling Biohazard Level 3 infectious agents by a technician fully trained in working with the spores as well as the test protocol.

**The Test Formulations**

The test formulations were shipped to us directly by the Sponsor and upon receipt they were stored at room temperature in a secure area with controlled access. The product effectiveness criterion was arbitrarily set at a minimum $\geq 6$ log$_{10}$ reduction in viable spores.

**The Spores**

The spores of the test bacterium were grown in a 1:10 dilution of Columbia Broth for 72 hours at 37°C. The bacterial suspensions were heated at 80°C for 10 minutes to ensure the absence of any vegetative cells.

**Soil load**

The test spores 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 spore suspension. The soil load mixture contains a level of protein roughly equal to that in 5% serum.

**Test Procedure:**

Stainless steel disks (1 cm in diameter) were used as the carriers in QCT-2 developed at CREM (Springthorpe and Sattar, 2003). The test spores were first suspended in the soil load and 10 $\mu$L of it placed on each metal disk. Teflon vials were used to hold the disks. The inoculum was allowed to become visibly dry under ambient conditions. The dried inoculum on each disk was then overlaid with 50 $\mu$L of the test formulation and control carriers received an equivalent volume of normal saline. The desired minimum number of viable spores in the dried inoculum on each carrier was $\geq 6$ log$_{10}$. Each test incorporated 3 control and five test carriers.

The contact time between the dried spore inoculum and the test formulation was 10 minutes at 20°C. At the end of the contact time, each vial received 10 mL a diluent/eluent containing Letheen broth+ 1% sodium thiosulfate as the neutralizer. The contents of the vial were vortexed for 30 seconds and the eluate passed through a 47 mm diameter filter membrane (0.22 $\mu$m pore diameter). The vial with the disk was rinsed with saline several times and the washes were also...
filtered. The total volume of saline for each carrier was about 100 mL. The filters were placed on
trypticase soy agar medium as the recovery medium. The plates were examined periodically over
a period of 5 days to get the final count of colony forming units (CFU). Log_{10} reductions were
then calculated.

**Results and Discussion**

As can be seen from the data summarized in Table 1, the formulation labeled AHP 5 (lot
#12400) showed good activity against the spores of *B. anthracis* and *B. subtilis* used as the
challenge organisms here. The contact time was 10 minutes at 20°C. The titre of viable spores on
the control carriers was roughly similar for both organisms and was also higher than the target
value of 10^6. No survivors were detected in any of the five test carriers for *B. anthracis* and thus
the log_{10} reduction obtained was almost 7 log_{10}. For *B. subtilis* two of the test carriers showed
some survivors. However, the mean log_{10} reduction was still higher than the target value of 10^6.

In the evaluation of the test formulation labeled Accel (lot #13114) the titre of the spores of
*B. anthracis* on the control carriers was 3.2 X 10^6 but that on the *B. subtilis* controls was nearly
10-fold lower. Survivors were recovered from two of the test carriers for *B. anthracis* and this
gave the mean log_{10} reduction for this organism as 5.55. No survivors were found on the test
carriers for *B. subtilis* thus giving a log_{10} reduction of 5.37.

The test facility was made available to us for a very limited time during which it was
necessary for us to develop the basic procedures for working with *B. anthracis* and then conduct
the microbicide tests. In view of this, only one test with each formulation could be run at only
one contact time. However, these preliminary findings show that the two test formulations have
nearly the same level of activity against both the spore-formers tested. This means that the results
obtained with *B. subtilis* could be regarded as predictive of the activity of the formulation against
*B. anthracis* as well.

As far as we are aware, this is the first study where the spores of both organisms were grown
and processed in an identical manner and the microbicide testing was also carried out using the
same test protocol. This makes it possible to directly compare the results against the two spore-formers. The test method used in this study meets the requirements of the Canadian General
Standard Board’s national standard (CGSB 1997).

**LITERATURE CITED**

Determining the Bactericidal, Virucidal, Fungicidal, Mycobactericidal and Sporicidal Activities
of Liquid Chemical Germicides, Document #E 2197-02. ASTM International, West
Conshohocken, PA.

for Use on Environmental Surfaces and Medical Devices*. Document number CAN/CGSB-2.161-
M97, CGSB, Ottawa, Ontario, Canada.

Centre for Research on Environmental Microbiology (CREM), University of Ottawa, Ottawa,
ON, Canada.

CREM’s report to Virox on sporicidal activity against *B. anthracis* and *B. subtilis*, April 2004.
Table 1. Sporicidal Activity of AHP-Based Formulations

<table>
<thead>
<tr>
<th>Sample</th>
<th>Titre of viable spores in log_{10}/carrier</th>
<th>Log_{10} Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B. anthracis</td>
<td>B. subtilis</td>
</tr>
<tr>
<td>AHP 5 - Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carrier 1</td>
<td>1.21 X 10^7</td>
<td>1.03 X 10^7</td>
</tr>
<tr>
<td>Carrier 2</td>
<td>1.19 X 10^7</td>
<td>1.11 X 10^7</td>
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<tr>
<td>Carrier 3</td>
<td>4.50 X 10^6</td>
<td>1.31 X 10^7</td>
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<tr>
<td>Mean titre on controls</td>
<td>9.50 X 10^6</td>
<td>1.15 X 10^7</td>
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<tr>
<td>AHP 5 (Lot #12400) - Test</td>
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<td></td>
</tr>
<tr>
<td>Carrier 1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Carrier 2</td>
<td>0</td>
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<tr>
<td>Carrier 3</td>
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<td>0</td>
</tr>
<tr>
<td>Carrier 4</td>
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<td>Carrier 5</td>
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<td>1.43 X 10^2</td>
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<tr>
<td>Mean log_{10} reduction</td>
<td>6.98</td>
<td>6.43</td>
</tr>
<tr>
<td>Accel - Control</td>
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<td></td>
</tr>
<tr>
<td>Carrier 1</td>
<td>2.5 X 10^6</td>
<td>1.6 X 10^5</td>
</tr>
<tr>
<td>Carrier 2</td>
<td>3.9 X 10^6</td>
<td>1.9 X 10^5</td>
</tr>
<tr>
<td>Carrier 3</td>
<td>-</td>
<td>3.5 X 10^5</td>
</tr>
<tr>
<td>Mean titre on controls</td>
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<td>2.33 X 10^5</td>
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<tr>
<td>Accel (Lot #13114) - Test</td>
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</tr>
<tr>
<td>Carrier 1</td>
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<td>0</td>
</tr>
<tr>
<td>Carrier 2</td>
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</tr>
<tr>
<td>Carrier 3</td>
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<td>Carrier 4</td>
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<tr>
<td>Carrier 5</td>
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<tr>
<td>Mean log_{10} reduction</td>
<td>5.55</td>
<td>5.37</td>
</tr>
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