



BioVir Laboratories, Inc.

685 Stone Road, Unit 6 • Benicia, CA 94510 • (707) 747-5906 • 1-800-GIARDIA • FAX (707) 747-1751 • WEB: www.biovir.com

DATE: October 28, 2002

TO: Mr. Mike Harvey
Enviro Tech Chemical Services
P.O. Box 577470
Modesto, CA 95357
(209) 581-9576
(209) 581-9653 (fax)

FROM: Richard E. Danielson, Ph.D. 
Laboratory Director

SUBJECT: Evaluation of the efficacy of Peroxyacetic Acid on Microbiological Water Quality, BioVir Project # 020860.

Purpose. To perform a microbial challenge test (protocol provided by Enviro Tech) to evaluate the efficacy of 15% Peroxyacetic Acid (PAA) disinfectant at specified concentrations (1, 2, and 5 ppm). In this challenge, a bacteria (*Pseudomonas aeruginosa*) and a yeast (*Candida albicans*) were used as the challenge organisms. The effect of the disinfectant was measured by the reduction of culturable organisms assayed over a specified time period (8 hours).

Method

1. Materials and Conditions:

Test material: 15% Peroxyacetic Acid (PAA) Lot 33-14933
Test Concentrations: 1, 2, and 5 ppm
Diluent: AOAC "Hard Water" ca. 500 ppm (AOAC 6.10)
pH: 7.5 - 7.6
Test Temperature: 35°C (95°F)
Sodium Thiosulfate
Catalase

2. Test Organisms:

Pseudomonas aeruginosa (ATCC) cultured on tryptic soy broth agar (TSBA) and assayed with *Pseudomonas* Isolation Agar (PIA) (Difco).

Candida albicans (ATCC 90027) cultured and assayed onto Potato Dextrose Agar (PDA) (Difco).

Cultures of both organisms were started and serially transferred over a four day period in/on media specified above. A suspension of culture was prepared on the day of testing and added to the 1 liter of diluent to achieve a 10^8 cfu/mL of *P. aeruginosa* and a 10^4 cfu/mL of *C. albicans*.

3. Preparation of Disinfectant Suspension:

- a. One liter of diluent was added to each of three sterile Nalgene sample bottles. The bottles were labeled 5 mg/L; 2 mg/L and 1 mg/L. Before the addition of disinfectant the target

organisms were added to achieve the concentrations stated above. A 100 mL sample was taken from each bottle to represent "Time = 0" concentration.

- b. A 15% solution of PAA is equivalent to 150,000 mg PAA/L (Solution A). An initial 1:30 dilution (4 mL 15% PA per 120 mL diluent) was prepared (Solution B). This would yield a 5,000 mg/L solution or 5 mg/mL solution. 0.9 mL of Solution B was added to the remaining 900 mL of diluent and microbial suspension to yield 5 mg/L. For a 2 mg/L solution, 0.36 mL of Solution B was added to 900 mL diluent; and, for 1mg/L, 0.18 mL of Solution B was added to their respective bottles.

4. Sampling.

- a. There were two sample periods of interest, 3 and 8 hours of exposure.
- b. At Time = 3 hours a 100 mL sample was taken from each bottle, neutralized and assayed for the target organisms.
- c. At Time = 8 hours, a 100 mL sample was taken from each bottle, neutralized and assayed for the target organisms.
- d. Neutralization: Samples were neutralized following manufacturers' directions. 5 mL of a 0.8 M sodium thiosulfate solution (50 mg sodium thiosulfate/L) was added first, followed by 2 mL of a 0.1 g/100 mL solution of catalase (20 mg catalase)/L.

5. Assay.

- a. Dilutions from the samples were prepared and organisms applied to their specific growth media.
- b. For *P. aeruginosa*, samples were applied directly to Pseudomonas Isolation Agar (PIA). Plates were incubated for two days at 35°C and counted. *P. aeruginosa* appears with green colonies.
- c. For *C. albicans*, samples were applied directly to Potato Dextrose Agar (PDA). These plates were incubated for three days at 25°C. *C. albicans* appear as white, round, shiny to suede-looking colonies.

6. Results. The results of the testing is presented below in Table 1. From table 1 it can be seen that at least a two log reduction was achieved in all instances except for the 3-hour observation of *C. albicans* in 1 mg/L PAA.

Please contact me if you have any questions regarding this information.

TABLE 1

RESULTS OF PEROXYACETIC ACID (PAA) TESTING ON
PSEUDOMONAS AERUGINOSA AND CANDIDA ALBICANS.

| PAA 1 mg/L | <i>P. aeruginosa</i> (cfu/mL) | Log ₁₀ Reduction | <i>C. albicans</i> (cfu/mL) | Log ₁₀ Reduction |
|------------|----------------------------------|-----------------------------|--------------------------------|-----------------------------|
| T = 0 | 1,300,000 | | 40,000 | |
| T = 3 | 3900 | 2.52 | 1500 | 1.43 |
| T = 8 | 5300 | 2.38 | 150 | 2.42 |

| PAA 2 mg/L | <i>P. aeruginosa</i> (cfu/mL) | Log ₁₀ Reduction | <i>C. albicans</i> (cfu/mL) | Log ₁₀ Reduction |
|------------|----------------------------------|-----------------------------|--------------------------------|-----------------------------|
| T = 0 | 1,000,000 | | 40,000 | |
| T = 3 | 1100 | 2.95 | 180 | 2.34 |
| T = 8 | 1900 | 2.72 | 20 | 3.30 |

| PAA 5 mg/L | <i>P. aeruginosa</i> (cfu/mL) | Log ₁₀ Reduction | <i>C. albicans</i> (cfu/mL) | Log ₁₀ Reduction |
|------------|----------------------------------|-----------------------------|--------------------------------|-----------------------------|
| T = 0 | 1,200,000 | | 30,000 | |
| T = 3 | 1,100 | 3.03 | 90 | 2.52 |
| T = 8 | 900 | 3.12 | 10 | 3.48 |