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Certificate of Analysis

Client: Global Environmental Restoration, LLC

Contact: Steven Thibodeaux

Product: Sniper Broad Spectrum Disinfectant

Project: Sniper Disinfectant Testing

LA Testing Reference Number: 330600526

Testing Protocol:
The AOAC Official Method 961.02 Germicidal Spray Products as Disinfectants was performed with Sniper Broad Spectrum Disinfectant on *Legionella pneumophila* ATCC 33152 and *Leuconostoc mesenteroides* ATCC 8293.

Bacterial test cultures of *Leuconostoc mesenteroides* were prepared using nutrient broth at 30°C and *Legionella pneumophila* was prepared using Buffered Charcoal Yeast Extract agar, Mueller Hinton Broth with lysed horse blood and TAT broth at 37°C. The concentrations of the cultures was determined by standard plate count methods.

For each challenge organism, eleven sterile, glass slides were inoculated with 0.1milliliter (ml) of inoculum broth each. The slides were dried for 30 minutes at 37°C. One inoculated, untreated slide was used as a positive control and two uninoculated, sterile slides were included as negative controls. Sniper was sprayed onto 10 inoculated slides and one uninoculated slide for 10 seconds at a distance of one foot. After 10 minutes, the bacterial test and control slides were aseptically transferred to TAT broth and incubated for 48 hours at 30°C. A sterile blank tube was also included with each setup for media sterility verification. After the defined incubation periods, the tubes were observed for growth. Cloudiness in the broth indicates a positive sample.

Negative tubes were subcultured onto Trypticase Soy agar or Buffered Charcoal Yeast Extract agar to verify the negative results. They were also tested to rule out inhibitory effects as a cause for lack of growth. This was done by adding 0.1 ml of the challenge

organism to the negative tubes. These were incubated for an additional 48 hours. Growth of the challenge organism eliminates bacteriostasis as a cause for negative results.

Killing of the test organism in 10 out of 10 trials is presumptive evidence of disinfecting action. Viability (positive) controls should show growth and negative controls should have no growth.

Testing Results:
The results indicate that Sniper Broad Spectrum Disinfectant has a negative impact on both *Leuconostoc mesenteroides* and *Legionella pneumophila*. After 10 minutes of exposure to the product, all challenge organisms were killed in 10 out of 10 trials. The positive controls exhibited growth while the negative controls all showed no growth. After addition of each challenge organism to the negative tubes, growth occurred, ruling out stasis. Photos have been included of all the tested tubes.

Table 1:

Challenge Organism	Concentration of inoculum (CFU/ml)	Inoculum size of each slide (CFU)	Test samples and negative control (all tubes)	Positive control and stasis challenge (all tubes)
L. pneumophila	3.0×10^6	3.0×10^5	NG	G
L. mesenteroides	2.4×10^6	2.4×10^5	NG	G

NG = No Growth G = Growth CFU = Colony forming units

Analyst: Regina Norman, Microbiology Laboratory Manager

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