



LA Testing Inc.
Microbiology Division
10772 Noel Street Los Alamitos, CA 90720
(800) 755-1794
www.latesting.com & www.emsl.com



Certificate of Analysis

Client: Global Environmental Restoration, LLC

Contact: Steven Thibodeaux

Product: Sniper Broad Spectrum Disinfectant

Project: Sniper Disinfectant Testing

LA Testing Reference Number: 330502348

Testing Protocol:

The AOAC Official Method 961.02 Germicidal Spray Products as Disinfectants was performed with Sniper Broad Spectrum Disinfectant on *Staphylococcus aureus* (Methicillin Resistant) American Type Culture Collection (ATCC) 33591, *Pseudomonas aeruginosa* ATCC 15442, *Vibrio vulnificus* ATCC 27562, *Aspergillus niger* ATCC 6275, *Stachybotrys chartarum* ATCC 9182, *Chaetomium globosum* ATCC 6205 and *Trichophyton mentagrophytes* ATCC 9533.

S. aureus was prepared using synthetic broth at 37°C, *P. aeruginosa* was prepared using nutrient broth at 37°C and *V. vulnificus* was prepared using alkaline peptone water at 37°C. The concentrations of the cultures were determined by standard plate count methods. (Table 1)

Fungal organisms were cultured on Malt Extract agar with the exception of *T. mentagrophytes*, which was cultured on Sabouraud Dextrose agar. These cultures were incubated 7 to 10 days at 25°C. Spores were harvested in sterile, normal saline and a spore count was performed to determine final concentration of each inoculum. (Table 1)

For each challenge organism, eleven sterile, glass slides were inoculated with 0.1 milliliter (ml) of inoculum broth each. The slides were dried for 30 minutes at 37°C. For each challenge organism, 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 37°C. Fungal test and control slides were aseptically transferred to Sabouraud Dextrose broth after 10 minutes, and incubated for 7 days at 25°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.



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Cloudiness in the broth indicates a positive sample for bacteria. Fungal positive samples exhibit white, hyphal growth floating in the tubes.

Negative tubes were subcultured onto Trypticase Soy agar (bacteria) or Malt Extract agar (fungi) 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 0.1ml of the challenge organism to the negative tubes. These were incubated for an additional 48 hours at 37°C for bacteria or for 7 days at 25°C for fungi. Growth of the challenge organism eliminates bacteriostasis or fungistasis 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 all tested organisms. 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 organisms to the negative tubes, growth occurred, ruling out stasis. Photos have been included of the tested tubes.

Table 1:

Challenge Organism	Concentration of inoculum (CFU/ml)	Inoculum size on each slide (CFU)	Test samples and negative control (all tubes)	Positive control and stasis challenge (all tubes)
<i>S. aureus (MRSA)</i>	2.1×10^9	2.1×10^8	NG	G
<i>P. aeruginosa</i>	3.0×10^9	3.0×10^8	NG	G
<i>V. vulnificus</i>	1.6×10^9	1.6×10^8	NG	G
<i>A. niger</i>	8.4×10^6	8.4×10^5	NG	G
<i>S. chartarum</i>	1.5×10^6	1.5×10^5	NG	G
<i>C. globosum</i>	1.4×10^6	1.4×10^5	NG	G
<i>T. mentagrophytes</i>	2.8×10^6	2.8×10^5	NG	G

NG= No growth G= growth CFU= colony forming units

Analyst: Regina Norman
Regina Norman, Microbiology Laboratory Manager

Date: December 20, 2005