

FINAL REPORT

Textile Material Biocidal Efficacy Testing

PROTOCOL Modified ASTM E2315

> ORDER Number 371101724

PREPARED FOR:

21st Global Pty LTD 3365 Nelson Bay Rd Bobs Farm, NSW, Australia 2316

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EMSL Analytical, Inc.



Certificate of Analysis

Client: 21st Global Pty, LTD

Contact: Robert Goldworthy **Project:** Product Efficacy on Fungi

Product: GM 2000 EMSL NO: 371101724

Sample received: 2/16/2011 Start date: 2/21/2011 Report date: 3/11/2011 Challenge Fungi: Stachybotrys chartarum and Aspergillus niger

Experimental Summary: The testing procedure was designed after discussions between EMSL Analytical, the testing company, and the client, 21st Global Pty, LTD. The testing procedure is based on ASTM E2315, with the testing conducted on a cleaning solution for its ability to disinfect (kill) fungus. The testing was conducted in our Cinnaminson Microbiology Laboratory.

Procedure:

Stock fungal cultures of *Stachybotrys chartarum* and *Aspergillus niger* were used to inoculate Malt Extract Agar (MEA) and incubated for 7 days at 25°C. After incubation, 10 mL of 0.1% Tween was poured onto the agar plates and spores were removed with a sterile plastic loop. The spore solution was then pipetted into a sterile 50 mL centrifuge tube for storage. The concentration of each spore solution was determined by counting the spores with a hemacytometer. The spore solution was then diluted to make a ~10⁶ concentration.

The testing product was made per instructions given by the client; 10 mL of part A and 10 mL of part B were mixed together in 180 mL of water. Once the solution was created 10 mL aliquots were placed into 15 mL centrifuge tubes for testing. One milliliter of each respective spore solution was added separately to the test solution and incubated for 24 h at room temperature (19°C).

Simultaneously, a time zero (initial spore count) was determined by inoculating 9 mL of 0.1% tween with 1 mL of each respective spore solution separately. Dilutions were made and plated onto MEA. The agar plates were then incubated at 25°C for 7 days.

After the 24 h exposure to the test solution, the test sample was vortexed and 1 mL was removed and placed in 9 mL of 0.1% tween. Serial dilutions were made and MEA plates were inoculated and incubated at 25°C for 7 days. After the appropriate incubation period fungal colonies were counted. All tests were completed in triplicate and appropriate calculations were completed.



Experimental Results:

Organism	Time (h)	Avg CFU	Log10	LR	%Reduction
A. niger	0	9.7 x 10 ⁶	6.96		
	24	ND	0.00	6.96	>99.99998
S. chartarum	0	2.5 x 10 ⁶	6.39		
	24	ND	0.00	6.39	>99.99996

ND = None Detected <10 CFU/mL

LR = Log Reduction; %Reduction = Percent difference between Log10/CFU after 24 h – Log10/CFU after 0 h

Conclusions/Observations:

In conclusion, the test product, GM 2000, provided by 21st Global Pty, LTD was able to effectively disinfect (kill) both *A. niger* and *S. chartarum* after 24 h exposure at room temperature. Specifically, GM 2000 was able to reduce *A. niger* by >99.99998% and *S. chartarum* by >99.99996% after exposure for 24 h.

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