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Laboratory Testing Report

Antifungal Product Testing

Job # :	**050407-287/2**
Product Tested:	**MOLD BARRIER SYSTEM**
Date Received:	**05/01/09**
Date Analyzed/Tested:	**05/02/09 to 11/21/09**
Date Printed Report:	**11/22/09**
Sample Type:	**Antifungal Solutions (Liquids)**
Analysis: :	Standard Practice for Determining Resistance of Materials to Fungi
Client:	BGH International Inc.
Contact:	Brian G. Hubka

Protocol

The protocol consists of selection of specimens , inoculation of the specimens with suitable organisms, exposure of inoculated specimens under conditions favorable to growth, examination and rating for visual growth,

Nutrient-Salts Agar Medium prepared by dissolving in 1 L of distilled water the designated amounts of the following reagents:
Potassium dihydrogen orthophosphate (KH_2PO_4) 0.7 g
Magnesium sulfate ($MgSO_4 \cdot 7H_2O$) 0.7 g
Ammonium nitrate (NH_4NO_3) 1.0 g
Sodium chloride (NaCl) 0.005 g
Ferrous sulfate ($FeSO_4 \cdot 7H_2O$) 0.002 g
Zinc sulfate ($ZnSO_4 \cdot 7H_2O$) 0.002 g
Manganous sulfate ($MnSO_4 \cdot H_2O$) 0.001 g
Agar 15.0 g
Potassium monohydrogen orthophosphate (K_2HPO_4) 0.7 g
Test medium has been sterilized by autoclaving at 121°C (250°F) for 20 min. pH of the medium adjusted by the addition of 0.01 *N* NaOH solution so that after sterilization the pH is between 6.0 and 6.5.

Five fungi cultures were prepared by growing them on a 1.5% potato glucose agar plate (Fluka) at 25^{o}C for 7 days. The cultures were visually inspected before next step.

1. The fungal species tested included:
    a. *Aspergillus niger* ATCC 9642
    b. *Penicillium funiculosum* ATCC 11797
    c. *Chaetomium globosum* ATCC 6205
    d. *Gliocladium virens* ATCC 9645
    e. *Aureobasidium pullulans* ATCC 15233.
2. A spore suspension of each of the five fungi prepared by pouring into one subculture of each fungus a sterile 10-mL portion of basal salt solution. A sterile wooden toothpick was used each time to gently scrape the surface growth from the culture of the test organism.
3. Fungal suspension was further diluted and the final solution consisted of equal volumes of 5 mold spore suspensions in basal salt solution at a concentration of ~1,000,000±200,000 spores per ml. The final solution with mixed fungi placed into the aerosol producing bottle was used for spore dissemination.
4. Each sample was tested using Petri dishes (150 mm) that contained sterile nutrient salts agar (pH 6.5) and drywall (3'x4'). Negative control was a nutrient salt agar (no drywall present). Positive control was a potato glucose agar plate (no drywall present).
5. The drywall material consisted of the following:
    a. Gypsum ($CaSO_4$ > 85%)
    b. Recycled paper (<15%)
    c. Starch (<3%)
    d. Crystalline silica (<5%)
6. The 20x20 mm drywall pieces embedded in the solidified basal salt agar were sprayed with a spore solution to achieve consistent surface coverage of the plate. Same treatment was used for a negative and positive control without the drywall pieces.
7. The inoculated test specimens were incubated at 28 to 30°C (82 to 86°F) and not less than 85 % relative humidity. The length of the test was 200 days of incubation. The test was terminated in less than 7 days for control sample exhibiting an extensive growth rating of four.
8. One drywall squares not treated control sample was incubated for 7 days at the same conditions as in #9.
9. Two drywall squares treated with the sample solutions were incubated for 200 days in a high humidity chamber at 29 □□1^{o}C, and were examined at 40, 80, 120, 160, and 200 days for visible effects of mold growth. (See attached Table 1) The following rating system was used to score the test samples.

Table 1: Observed growth ratings

Observed Growth on Specimens Rating	**Rating**
None	0
Traces of growth (less than 10%)	1
Light growth (10 to 30%)	2*
Medium growth (30 to 60%)	3
Heavy growth (60% to complete coverage)	4

* Continuous cobwebby growth extending over the entire specimen, even though not obscuring the specimen, should be rated as a two".

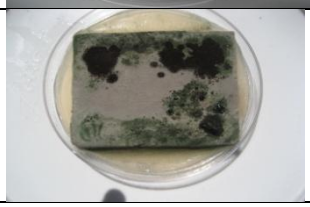
Table 2: Visible effect ratings of samples evaluated in the period of up to 200 days after inoculation.

Sample ID	**Rating**									
	40 Days		**80 Days**		**120 Days**		**160 Days**		**200 Days**	
Mold Barrier System 1 and 2	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Control	**n/a**		**n/a**		**n/a**		**n/a**		**n/a**	

Observations (see photographs below)

1. The Mold Barrier System treated drywall has no signs of mold growth on the Sample 1 200 days after the treatment.
2. The Mold Barrier System treated drywall has no signs of mold growth on the Sample 2 200 days after the treatment.
3. Control drywall sample not treated with Mold Barrier System shown heavy mold growth within first 5 days.

Table 3: Pictures of samples evaluated 200 days after inoculation and control sample evaluated 5 days after inoculation.

Mold Barrier System 1 sample	
Mold Barrier System 2 sample	
Control sample	

Test performed by _____*Signed*_____
Sergei Bibikov PhD

Scientist, Q.C. Officer ____ *Signed*_____
Jim Polansky

Date: November 22, 2009