

## Analytical Laboratory Report

Fungal Culture

9313-R01

### FINAL REPORT

Project/PO: **Antifungal Testing, Rust Inhibitive Coating**

Control ID # **9313**

Received: **09-30-2005**

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**Analytical Laboratory Report**  
Antifungal Efficacy Testing  
Inhibitive Coating Product

Account Name:	Rust Bullet, LLC	Submitter:	David Ciglar
Project/P.O.:	Antifungal Testing, Rust Inhibitive Coating	Control ID#:	9313
Date Received:	09-30-2005	Date Reported:	10-28-2005

**Fungal Susceptibility/Product Testing Protocol:**

Goal: To test the efficacy of a rust inhibitive coating product to provide an effective encapsulant for wood with residual fungal spores left on wood surfaces and demonstrate absence of growth of fungi on the surfaces treated with the product.

1. Prepare fungal spore suspension using *Aspergillus fumigatus* to test for fungal growth or inhibition in samples.
  - 1.1. Suspend spores of *Aspergillus fumigatus* collected on sterile swab from pure culture, in sterile test tube containing 25 mL of sterile water with 0.1% tween 80.
2. Apply wet wipe of spore suspension to wood surfaces.
  - 2.1. Use one sample of 2x4 lumber and one sample of oriented strand board (OSB).
  - 2.2. Wet wipe spore suspension onto top surface.
  - 2.3. Prepare samples in biosafety cabinet and allow to air dry.
3. Test for residual surface contamination levels from application of fungal suspension.
  - 3.1. Aseptically test the surface using a Malt Extract Agar (MEA) contact plate.
  - 3.2. Press the contact plates onto the surface area along each wood sample.
  - 3.3. Repeat with 5 replicate plates.
4. Incubate/monitor plates.
  - 4.1. Incubate the plates at 25 C.
  - 4.2. Watch for development of fungal growth and record presence of fungal colonies (*Aspergillus fumigatus*).
    - 4.2.1. Enumerate fungal growth from the 5 replicate plates at 2-5 days.
5. Apply Rust Bullet Rust Inhibitive Coating to wood samples per manufacturers recommendations.
  - 5.1. Completely coat top surface with Rust Bullet encapsulant product.
6. Test for surface contamination levels after application of encapsulant product.
  - 6.1. Aseptically test the surface using a Malt Extract Agar (MEA) contact plate.
  - 6.2. Press the contact plates onto the surface area along each wood sample.
  - 6.3. Repeat with 5 replicate plates.
7. Incubate/monitor plates.
  - 7.1. Incubate the plates at 25 C.
  - 7.2. Watch for development of fungal growth and record presence of fungal colonies (*Aspergillus fumigatus*).
    - 7.2.1. Enumerate fungal growth from the 5 replicate plates at 2-5 days.

Report#: 9313-R01      Analysis Date: 10-26-2005  
Laboratory Results authorized by Sean P. Abbott, Ph.D., Analytical Director

Natural Link MOLD LAB, Inc. reports sample results as a record of the microbes identified by our analytical staff. Any guidance given with regards to sampling methods, interpretation of results, remediation, health effects, or other information given to the client, beyond microbial identification, is given as general information from published sources and is not an extension of liability to Natural Link MOLD LAB, Inc. Natural Link MOLD LAB, Inc. establishes responsibility over analysis completed in the laboratory but cannot establish responsibility for activities completed in the field by the client, other personnel associated with the samples submitted, or other activities beyond the laboratory. All reports are confidential and are not to be reproduced, except in whole, without the permission of Natural Link MOLD LAB, Inc.

**Sample Identification:** Before treatment, wood sample with spore suspension

<u>Sample Replicate Number</u>	<u>Fungal Growth Detected (CFU/25cm<sup>2</sup>)</u>	
	<u>Lumber</u>	<u>OSB</u>
1	375	375
2	325	275
3	300	250
4	375	300
5	275	300

**Summary of Findings:**

- ∞ Sensitivity: 1 CFU/sample = 1 CFU/25cm<sup>2</sup>.
- ∞ All sample plates taken from wood surfaces inoculated with fungal spore suspension exhibited extensive fungal growth at 2 and 5 days, indicating a surface spore level of approximately 250-375 CFU/25cm<sup>2</sup>.
- ∞ Fungal colonies isolated from sample were identified as *Aspergillus fumigatus*, corresponding to inoculated strain.

**Sample Identification:** After treatment, wood sample treated with Rust Bullet rust inhibitive coating

<u>Sample Replicate Number</u>	<u>Fungal Growth Detected (CFU/25cm<sup>2</sup>)</u>	
	<u>Lumber</u>	<u>OSB</u>
1	ND	ND
2	ND	ND
3	ND	ND
4	ND	ND
5	ND	ND


**Other Data**

ND = None Detected

**Summary of Findings:**

- ∞ Sensitivity: 1 CFU/sample = 1 CFU/25cm<sup>2</sup>.
- ∞ All sample plates taken from wood surfaces treated with Rust Bullet rust inhibitive coating exhibited no fungal growth at 2 and 5 days, indicating that the inoculated surface spore level was effectively sealed using the encapsulant coating.

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Laboratory Results authorized by Sean P. Abbott, Ph.D., Analytical Director



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## **Antifungal Testing, Rust Bullet Rust Inhibitive Coating**

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The Rust Bullet Rust Inhibitive Coating product was tested to determine if it could be applied as an effective encapsulant following mold remediation work. Recommended methods of mold remediation are found in the IICRC S520 Standard and Reference Guide For Professional Mold Remediation (2003). The premise of the testing was that on wood surfaces that have undergone remediation cleaning, some residual spores may be left behind and the product may be an effective sealant for exposed wood surfaces. In order to test this, we used samples of 2x4 lumber and oriented strand board (OSB). To the surface of each sample we applied a laboratory prepared spores suspension using the mold *Aspergillus fumigatus*. Levels of surface contamination were then tested, revealing a relatively heavy spore concentration of approximately 250-375 colonies per 25 square centimeters. The spore-treated wood surfaces were then coated with the Rust Bullet product in accordance with manufacturers recommendations. After curing of the product, the surfaces were again tested for residual fungal spores. Results indicated no growth of *Aspergillus fumigatus*, confirming the effectiveness of the product to act as a spore encapsulant under the testing conditions.