

Analytical Laboratory Report

Bulk Sample

Antifungal/Antibacterial Testing

35581-R01

FINAL REPORT

Project/PO: **Penta-900P Comparison**

Control ID # **35581**

Received: **06-23-2015**

July 10, 2015

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Texas Department of State Health Services, Mold Analysis Laboratory License Number: LAB0146



Report submitted to:

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Analytical Laboratory Report
Antifungal/Antibacterial Testing
Bulk sample

Account Name:	Portinscale, LLC	Control ID#:	35581
Project/P.O.:	Penta-900P Comparison	Date Received:	06-23-2015
Submitter:	Adam Smith	Date Reported:	07-10-2015

Purpose:

To test the efficacy of three antimicrobial products, Penta-900W Peppermint Oil, Benefect Botanical Disinfectant and Microban Disinfectant Spray Plus to inhibit the growth of fungal spores and bacterial cells. The fungal organism selected for this trial was *Aspergillus sydowii* taken from stock cultures and grown on Malt Extract Agar (MEA). The bacterial organism selected for this trial was *Bacillus cereus* taken from stock cultures and grown on Tryptic Soy Agar (TSA). Natural Link Mold Lab cultures used: SA 1533 (*Aspergillus sydowii*) and SA B1-41 (*Bacillus cereus*).

Fungal Susceptibility/Product Testing Protocol:

1. Prepare fungal and bacterial suspensions.
 - 1.1. Swab surface of fungal colonies (*Aspergillus sydowii*) from stock cultures with a sterile stick and vortex in 1.00 mL sterile 0.1% Tween 80 solution. This master suspension will be used to prepare the primary suspensions.
 - 1.2. Swab surface of bacterial colonies (*Bacillus cereus*) from stock cultures with a sterile stick and vortex in 1.00 mL sterile 0.1% Tween 80 solution. This master suspension will be used to prepare the primary suspensions.
 - 1.3. Prepare 5 mL sterile centrifuge tube with 0.90 mL 0.1% Tween 80 for each organism.
 - 1.4. Prepare 5 mL sterile centrifuge tube with 0.90 mL Penta-900W Peppermint Oil stock solution for each organism.
 - 1.5. Prepare 5 mL sterile centrifuge tube with 0.90 mL Benefect Botanical Disinfectant stock solution for each organism.
 - 1.6. Prepare 5 mL sterile centrifuge tube with 0.90 mL Microban Disinfectant Spray Plus stock solution for each organism.
 - 1.7. Add 0.10 mL from one of the master suspensions to the previously prepared 0.1% Tween 80 (control), Penta-900W Peppermint Oil, Benefect Botanical Disinfectant and Microban Disinfectant Spray Plus stock solution centrifuge tubes. This will bring the total volume to 1.00 mL. Do the same with the other master suspensions. These are the primary suspensions and will be used in the serial dilutions to follow.
2. Prepare dilution series and incubate.
 - 2.1. Prepare serial dilutions and plate out the fungal organism on MEA plates to appropriate levels at 1 minutes, 10 minutes, and 30 minutes after preparing the primary suspensions.
 - 2.2. Prepare serial dilutions and plate out the bacterial organism on TSA plates to appropriate levels at 1 minutes, 10 minutes, and 30 minutes after preparing the primary suspensions.
 - 2.3. Plate out 1.00 mL 0.1% Tween 80, 1.00 mL Penta-900W Peppermint Oil stock solution, 1.00 mL Benefect Botanical Disinfectant stock solution and 1.00 mL Microban Disinfectant Spray Plus stock solution onto separate MEA and TSA plates. This is a control to ensure the sterility of the 0.1% Tween 80 as well as the antimicrobial products stock solutions used in the trials.
 - 2.4. Incubate plates for 96 hours (4 days) at 25° C.

Report#: 35581-R01 Analysis Date: 07-08-2015
Laboratory Results authorized by Sean P. Abbott, Ph.D., Analytical Director

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3. Count colonies and report.
 - 3.1. Visually and microscopically confirm fungal colonies recovered are the challenge organisms.
 - 3.2. Count colonies on appropriate dilution plates and calculate CFUs/mL. Report counts and percent reduction in CFU/mL from the Penta-900W Peppermint Oil stock solution (challenge), Benefect Botanical Disinfectant stock solution (challenge) and Microban Disinfectant Spray Plus stock solution (challenge) versus the Tween 80 solution (control).

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Results:

Sample Identification: *Aspergillus sydowii*

<u><i>Aspergillus sydowii</i></u>	<u>CFU/mL</u>	<u>Percent Reduction (%)</u>
Control	1 000 000 000	
Penta-900W Peppermint Oil 1 minute	2 000 000	99
Penta-900W Peppermint Oil 10 minutes	490 000	99
Penta-900W Peppermint Oil 30 minutes	320 000	99

Sample Identification: *Aspergillus sydowii*

<u><i>Aspergillus sydowii</i></u>	<u>CFU/mL</u>	<u>Percent Reduction (%)</u>
Control	1 000 000 000	
Benefect Botanical Disinfectant 1 minute	740 000	99
Benefect Botanical Disinfectant 10 minutes	< 100	100*
Benefect Botanical Disinfectant 30 minutes	< 100	100*

*Percent reduction is representative of the dilutions plated and limited to a sensitivity of 100 CFU/mL.

Sample Identification: *Aspergillus sydowii*

<u><i>Aspergillus sydowii</i></u>	<u>CFU/mL</u>	<u>Percent Reduction (%)</u>
Control	1 000 000 000	
Microban Disinfectant Spray Plus 1 minute	1 200 000	99
Microban Disinfectant Spray Plus 10 minutes	110 000	99
Microban Disinfectant Spray Plus 30 minutes	2 000	99

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Sample Identification: *Bacillus cereus*

<u><i>Bacillus cereus</i></u>	<u>CFU/mL</u>	<u>Percent Reduction (%)</u>
Control	3 200 000 000	
Penta-900W Peppermint Oil 1 minute	1 200 000	99
Penta-900W Peppermint Oil 10 minutes	600 000	99
Penta-900W Peppermint Oil 30 minutes	370 000	99

Sample Identification: *Bacillus cereus*

<u><i>Bacillus cereus</i></u>	<u>CFU/mL</u>	<u>Percent Reduction (%)</u>
Control	3 200 000 000	
Benefect Botanical Disinfectant 1 minute	420 000	99
Benefect Botanical Disinfectant 10 minutes	440 000	99
Benefect Botanical Disinfectant 30 minutes	390 000	99

Sample Identification: *Bacillus cereus*

<u><i>Bacillus cereus</i></u>	<u>CFU/mL</u>	<u>Percent Reduction (%)</u>
Control	3 200 000 000	
Microban Disinfectant Spray Plus 1 minute	660 000	99
Microban Disinfectant Spray Plus 10 minutes	440 000	99
Microban Disinfectant Spray Plus 30 minutes	350 000	99

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Summary of Findings:

- Spores of *Aspergillus sydowii* treated with Penta-900W Peppermint Oil were able to grow on Malt Extract Agar (MEA) at reduced levels relative to the control at 1 minute and 10 minutes of exposure. Viability of spores of *Aspergillus sydowii* treated with Penta-900W Peppermint Oil for 30 minutes was reduced by 99%.
- Cells of *Bacillus cereus* treated with Penta-900W Peppermint Oil were able to grow on Tryptic Soy Agar (TSA) at reduced levels relative to the control at 1 minute and 10 minutes of exposure. Viability of cells of *Bacillus cereus* treated with Penta-900W Peppermint Oil for 30 minutes was reduced by 99%.
- Spores of *Aspergillus sydowii* treated with Benefect Botanical Disinfectant were able to grow on Malt Extract Agar (MEA) at reduced levels relative to the control at 1 minute and 10 minutes of exposure. Viability of spores of *Aspergillus sydowii* treated with Benefect Botanical Disinfectant for 30 minutes was reduced by 100% within the limitations of the dilutions plated.
- Cells of *Bacillus cereus* treated with Benefect Botanical Disinfectant were able to grow on Tryptic Soy Agar (TSA) at reduced levels relative to the control at 1 minute and 10 minutes of exposure. Viability of cells of *Bacillus cereus* treated with Benefect Botanical Disinfectant for 30 minutes was reduced by 99%.
- Spores of *Aspergillus sydowii* treated with Microban Disinfectant Spray Plus were able to grow on Malt Extract Agar (MEA) at reduced levels relative to the control at 1 minute and 10 minutes of exposure. Viability of spores of *Aspergillus sydowii* treated with Microban Disinfectant Spray Plus for 30 minutes was reduced by 99%.
- Cells of *Bacillus cereus* treated with Microban Disinfectant Spray Plus were able to grow on Tryptic Soy Agar (TSA) at reduced levels relative to the control at 1 minute and 10 minutes of exposure. Viability of cells of *Bacillus cereus* treated with Microban Disinfectant Spray Plus for 30 minutes was reduced by 99%.
- Fungi not treated (control) with the antimicrobial products exhibited extensive fungal growth on Malt Extract Agar (MEA).
- Bacteria not treated (control) with the antimicrobial products exhibited extensive bacterial growth on Tryptic Soy Agar (TSA).
- Sterility Tests: No growth was detected on the untreated 0.1% Tween 80 solution. No growth was detected on the untreated antimicrobial products.

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Chain-of-Custody Form

Account name PORTINSCALE, LLC
 Sampling date 06/18/15
 Project / P.O. Penta-900P Comparison

Submitter ADAM SMITH
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Sample identification, description, and/or location	Sample volume	Analysis *				Alternative / additional analysis requested:	RUSH				
		FME	NFME	FC	BC		EC	24hr	48hr		
PENTA-900 w Peppermint Oil				✓							
Benefect Botanical Disinfectant				✓							
Microban Disinfectant Spray Plus											

(*) FME, Fungal Microscopic Examination -- NFME, Non-Fungal Microscopic Exam -- FC, Fungal Culture -- BC, Bacterial Culture -- EC, E.coli (coliforms) ID

Submitter's Signature Adam Smith Date 06/18/15 Time am pm
 Receiver's Signature [Signature] Date 06/23/15 Time 1:35 am pm

Submitter's Signature _____ Date _____ Time _____ am pm
 Receiver's Signature _____ Date _____ Time _____ am pm

Lab use: _____ Control #: 35581