Penta900 - The Best Solution!

<u>It doesn't just remove mold, — test labs report Penta900 is 100% effective.*</u>

Penta900 is human, animal and environmentally friendly and it complies with IICRC S520 standards. In many cases, with only one application, making it the fastest working, and as consistently reported in laboratory tests: repeatedly the most cost effective mold solution on the market.

BioCide Challenge Test

The biocide challenge is designed to test the ability of Penta-900 to inactivate mold spores in the presence of a soil load on Teflon disk carriers that represent environmental surfaces. This test treated various colonies with Penta-900P before incubating for 7 days. It demonstrated colonies are virtually eliminated after 5 minutes contact time in the case of the following:

Stachybotrys chartarum, Penicillium brevicompactum, and Cladosporium cladosporioides, and 10 minutes in the case of Aspergillus niger.

* See full laboratory test results for complete details – Available at: www.Penta900.com

Penta-900P BioCide Challenge

ASTM 2197 Methodology

The method herein is a modification of ASTM 2197 and is designed to evaluate the ability of liquid chemical germicides to inactivate fungal (mold) spores in the presence of a soil load on Teflon disk carriers that represent environmental surfaces. The test protocol does not include any wiping or rubbing action.

Edward Sobek, Ph.D.
9/16/2009

Summary of Test Method

A total of four fungi were challenged with Penta-900P and included: *Stachybotrys chartarum (ABC 226)*, *Aspergillus niger (ATCC 16888)*, *Penicillium brevicompactum* (FRR 862) and *Cladosporium cladosporoides (ATCC 6721)*. Pure spore concentrations were extracted from petri plate colonies and further diluted to provide a log-concentration series of spore suspensions: 10^6, 10^5, 10^4, 10^3 spores per ml. Each carrier (1.2 cm in diameter) was placed on the inside bottom surface of a sterile, 24-well microtiter plate. The carriers received 10 µL of the appropriate test suspension of spores with a soil load and allowed to air dry. Exactly 50 µL of Penta-900P was applied to the Teflon carriers for 30, 15, and 5 minute contact times. Control carriers received 50 µL of harmless phosphate buffer. The Penta-900P was then neutralized with a saturated activated carbon/phosphate buffer for 5 minutes in a rotary plate vortex. Following the vortex, the entire contents of each microtiter plate well was extracted and inoculated onto malt extract agar culture plates. Plates were incubated for 7 days at 27 ^{o}C and checked daily for growth. Colonies were counted and log10 reductions calculated.

Significance of Challenge

The design of this test minimizes any loss of viable organisms through wash off, thus making it possible to produce statistically valid data. The stringency in the test is provided by the use of a soil load, the microtopography of the carrier surface and the small ratio of disinfectant to surface area typical for many disinfectant applications. Thus the formulation under test is presented with a reasonable challenge while allowing for efficient recovery of the test fungal spores from the inoculated carriers with or without their exposure to the test formulation. The design of the Teflon carriers makes it possible to place onto each precisely measured volume of the test organism (10 µL) as well as the test formulation (50 µL). The inoculum is placed at the center of each disk whereas the volume of the test formulation covers nearly the entire disk surface thus eliminating the risk of any organisms remaining unexposed to the test formulation. 5.5 The relatively small ratio of 1:5 between the volume of the inoculum and that of the test formulation closely reflects many field applications of liquid chemical biocides.

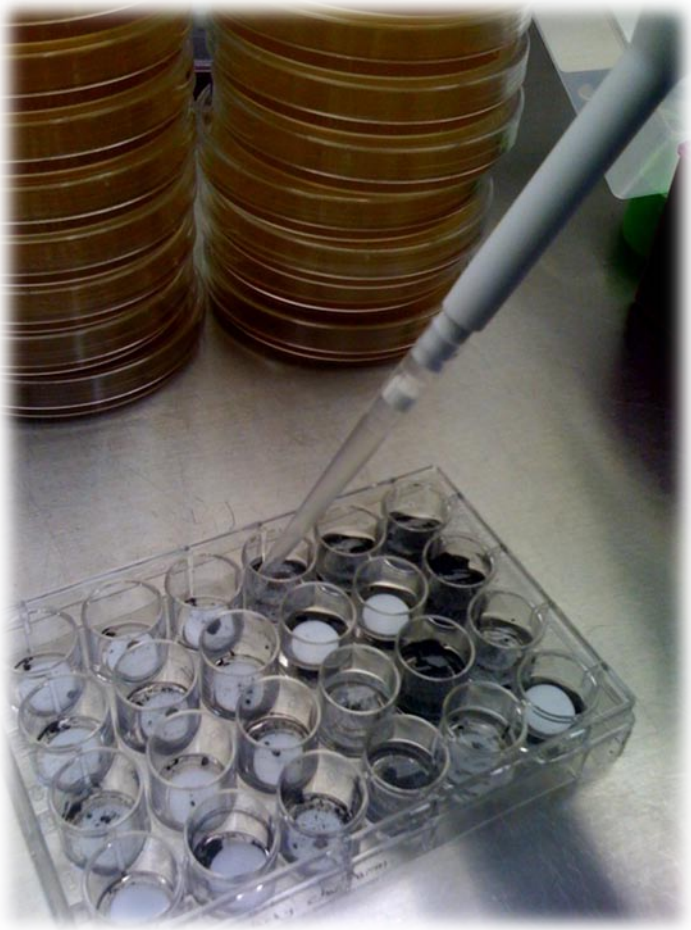
Soil Load

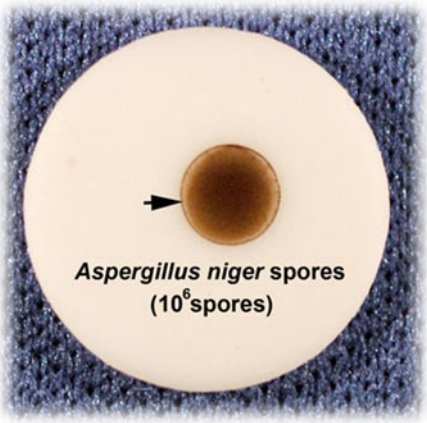
The soil load used in this test is a mixture of plant matter, recalcitrant organic matter, and inorganic matter, and was designed to represent the constituent dirt and debris from soil. Such soil fractions represent the common challenges that any biocide may encounter under field conditions. It is suitable for working with all types of test fungi included here. The components of the soil load are readily available and subject to much less variability than common soil mixtures.

The soil load incorporated in the suspension of the test fungal spores consisted of a mixture of the following stock solutions in phosphate buffer (pH 7.2):

a. 0.5 g of Cellibiose to 10 mL of phosphate buffer.
b. 0.5 g of Inulin to 10 mL of phosphate buffer.
c. 0.04 g of Celite to 10 mL of phosphate buffer.

The solutions were prepared separately and sterilize by passage through a 0.22 µm pore diameter membrane filter, fractioned into aliquots and stored at 4°C. The Celite was autoclaved but not filtered due to its course nature. The inoculum final volume was 500 µL and was obtained by adding 340 µL of the fungal spore suspension, 25 µL of Cellibiose, 100 µL of Inulin, and 35 µL of Celite stock.





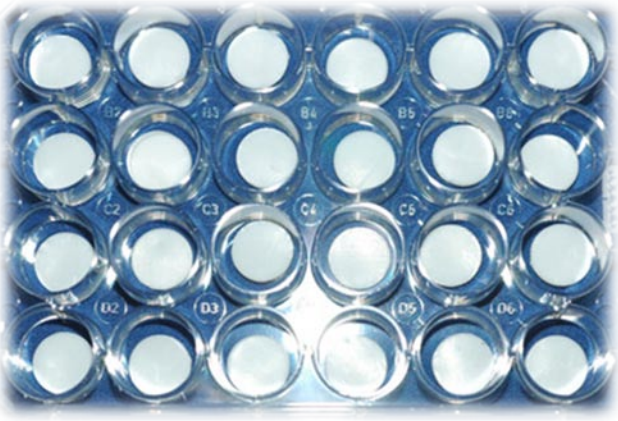


Table 1. Stock concentrations and carrier disk inoculum.

	Aspergillus niger			
Dilution	fungal Stock Conc.(ml)	Per ul	adjusted to 500ul	total on carrier (1x)
10^6	50,000,000	50000	34000	340000
10^5	5000000	5000	3400	34000
10^4	500000	500	340	3400
10^3	50000	50	34	340
	Stachybotrys chartarum			
Dilution	fungal Stock Conc.(ml)	Per ul	adjusted to 500ul	total on carrier (2x)
10^6	11,000,000	11000	7480	149600
10^5	1100000	1100	748	14960
10^4	110000	110	74.8	1496
10^3	11000	11	7.48	149.6
	Penicillium brevicompactum			
Dilution	fungal Stock Conc.(ml)	Per ul	adjusted to 500ul	total on coupon (2x)
10^6	13,000,000	13000	8840	176800
10^5	1300000	1300	884	17680
10^4	130000	130	88.4	1768
10^3	13000	13	8.84	176.8
	Cladosporium cladosporoides			
Dilution	fungal Stock Conc.(ml)	Per ul	adjusted to 500ul	total on carrier (2x)
10^6	12,000,000	12000	8160	163200
10^5	1200000	1200	816	16320
10^4	120000	120	81.6	1632
10^3	12000	12	8.16	163.2

Table 2. Log Reduction Time x Concentration exposure to Penta-900P

Aspergillus niger

Concentration	Time: 30 min Rep 1	30 min Rep 2	30 min Rep 3	15 min Rep 1	15 min Rep 2	15 min Rep 3	5 min Rep 1	5 min Rep2	5 min Rep 3
10^6	1	nd	nd	1	nd	nd	5	35	2
10^5	nd	nd	nd	nd	nd	nd	21	2	nd
10^4	nd	nd	nd	nd	nd	nd	14	5	200
10^3	nd	nd	nd	10	nd	nd	13	3	4

Penicillium brevicompactum

Concentration	Time: 30 min Rep 1	30 min Rep 2	30 min Rep 3	15 min Rep 1	15 min Rep 2	15 min Rep 3	5 min Rep 1	5 min Rep2	5 min Rep 3
10^6	nd	nd	nd	nd	nd	nd	nd	nd	nd
10^5	nd	nd	nd	nd	nd	nd	nd	nd	nd
10^4	nd	nd	nd	nd	nd	nd	nd	nd	nd
10^3	nd	nd	nd	10	nd	nd	nd	nd	nd

Stachybotrys chartarum

Concentration	Time: 30 min Rep 1	30 min Rep 2	30 min Rep 3	15 min Rep 1	15 min Rep 2	15 min Rep 3	5 min Rep 1	5 min Rep2	5 min Rep 3
10^6	nd	nd	nd	nd	nd	nd	nd	nd	nd
10^5	nd	nd	nd	nd	nd	nd	nd	nd	nd
10^4	nd	nd	nd	nd	nd	nd	nd	nd	nd
10^3	nd	nd	nd	1	nd	nd	nd	nd	nd

Cladosporium cladosporoides

Concentration	Time: 30 min Rep 1	30 min Rep 2	30 min Rep 3	15 min Rep 1	15 min Rep 2	15 min Rep 3	5 min Rep 1	5 min Rep2	5 min Rep 3
10^6	16	1	nd	nd	nd	nd	nd	nd	nd
10^5	nd	nd	nd	nd	nd	nd	nd	nd	nd
10^4	nd	nd	nd	nd	nd	nd	nd	nd	nd
10^3	nd	nd	nd	nd	nd	nd	nd	nd	nd

Table 3. Control Coupons Concentrations

		Aspergillus niger								
		Time								
		30 min			**15 min**			**5 min**		
Concentration		Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
	10^6	150	170	250	120	130	210	110	110	210
	10^5	110	180	270	170	120	140	130	140	120
	10^4	150	190	230	220	230	120	120	170	220
	10^3	110	100	180	110	100	140	130	120	120
		Penicillium brevicompactum								
		Time								
		30 min			**15 min**			**5 min**		
Concentration		Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
	10^6	150	170	140	150	160	170	110	210	140
	10^5	170	210	220	180	150	160	180	140	150
	10^4	140	110	140	130	120	200	240	170	140
	10^3	150	150	130	100	100	110	170	150	140
		Stachybotrys chartarum								
		Time								
		30 min			**15 min**			**5 min**		
Concentration		Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
	10^6	140	150	210	220	210	210	120	170	140
	10^5	170	110	100	150	150	130	140	140	150
	10^4	140	150	140	120	140	210	140	140	150
	10^3	140	150	170	120	120	140	220	150	150
		Cladosporium cladosporoides								
		Time								
		30 min			**15 min**			**5 min**		
Concentration		Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
	10^6	160	120	120	150	120	160	210	220	170
	10^5	110	100	130	150	140	150	180	150	140
	10^4	150	140	170	150	170	110	210	200	130
	10^3	170	150	150	170	140	150	170	140	150

REFERENCES

Sattar, S.A. and Springthorpe, V.S., New methods for efficacy testing of disinfectants and antiseptics, In Disinfection, Sterilization and Antisepsis: Principles and Practices in Healthcare Facilities, W.A.Rutala ed., Association for Practitioners in Infection Control and Epidemiology, Inc., Washington, DC 2001, p. 173–186.

Lloyd-Evans, N., Springthorpe, V.S., and Sattar, S.A., Chemical disinfection of human rotavirus-contaminated inanimate sur-faces, J. Hyg. 97:163-173, 1986.

CDC-NIH, Biosafety in Microbiological and Biomedical Laboratories, 4th ed., U.S. Department of Health and Human Services, Washington, DC, 1999.

Sharpe, A.N. and Perterkin, P.I., (1988), Membrane Filter Food Microbiology, John Wiley & Sons, New York, N.Y.

American Chemical Society, Reagent Chemicals, American Chemical Society Specifications, Washington, DC, 1993.

United States Pharmacopoeia and National Formulary, Analar Standards for Laboratory Chemicals, U.S. Pharmacopoeial Convention, Inc. (USPC), Rockville, MD, 1979.

Freshney, R.I., Culture of Animal Cells: A Manual of Basic Techniques, Alan R. Liss, Inc., New York, NY, 1987.

Lennette, E.H., Lennette, D.A., and Lennette, E.T., Diagnostic Procedures for Viral, Rickettsial, and Chlamydial Infections, 7th ed., Am. Pub. Hlth. Assoc., Washington, DC, 1995.

Sattar, S.A. and Springthorpe, V.S., (2001), Methods for testing the virucidal activity of chemicals, In Disinfection, Sterilization and Preservation, 5th ed., Block, S.S. (ed.), Lippincott Williams & Wilkins, Philadelphia, PA. 1391-1412.

Am. Public Health Assoc. Standard Methods for the Examination of Water and Wastewater, Washington, DC, 1998.

DeVries, T. A. and Hamilton, M.A., Estimating the antimicrobial log reductions: Part 1: Quantitative assays. Quant Microbiol 1: 29-45, 1999.