

Comprehensive Summary Report - Scientific Testing Performed on BioNeat NTS™ Toxicity Testing

As requested by BIO-NEAT INC, I would like to interpret some scientific facts regarding BioNeat NTS $^{\text{\tiny{M}}}$, the greenest, most effective cleaning product, based on their commercial laboratory test reports and my twelve years of scientific research experience as a colloid scientist with a doctorate (Ph.D.) in Chemistry.

BioNeat NTS™ is manufactured and distributed by BIO-NEAT INC. of Fort Lauderdale, Florida. It is formulated from all-natural, USEPA-satisfied, and USFDA-safe ingredients according to a proprietary process. The components of the BioNeat NTS™ product were completely analyzed by XENCO Laboratories (a NELAC-accredited testing laboratory) of Carrollton, Texas. The following analyses were performed:

- 1. Mercury by EPA 245.1
- 2. Metals by EPA 200.8
- 3. Pesticides and PCBs by EPA 608
- 4. VOA (Volatile Organic Analysis) GC/MS by EPA 624
- 5. Total Cyanide (Colorimetric, Automated UV) by SW-846 9012

For ALL substances tested (over 60 metals or volatile organic compounds), the amounts found were below reportable limits. No toxic heavy metals, organic solvents, PCBs or pesticides beyond the safety limit for everyday use were found. The contents of common heavy metals and chlorinated hydrocarbons were tested by Xenco Labs and their report indicates that BioNeat NTS™ is a completely safe product for humans and animals.

Table 1. Concentration of Heavy Metals and Chlorinated Hydrocarbons in BioNeat NTS™ (By Xenco Labs)

COMPOUND	CONCENTRATION (mg/kg)
Arsenic	0.0265
Cadmium	<0.005
Chromium	0.0985
Copper	<0.0150
Lead	<0.0100
Nickel	<0.0250
Zinc	0.2455
Cyanide	<0.300
Chlorinated Hydrocarbons	<5.0
Mercury	<0.500 μg/L



Marine Toxicity Testing

Analytical results from Bio-Aquatic Testing Inc. (a NELAC-accredited testing laboratory) of Carrollton, Texas, demonstrate as follows: In comparison to the majority of conventional, market-branded cleaning products, which usually contain the effective surfactant Sodium Laurel Sulfate, BioNeat NTS is *six to ten times less toxic* when the marine invertebrate species, *Mysidopsis bahia* (*Americamysis bahia*) and the marine vertebrate species, *Menidia beryllina* were used as subjects in the tests. During their 48 hours and 96 hours survival experiment, 50% of the *Menidia beryllina* was killed in 96 hours in the presence of a 12.19 ppm solution of sodium laurel sulfate (LC50 or median lethal concentration), while the same survival rate was achieved at concentrations even as high as 136.12 ppm of BioNeat NTS™ in the solution. Bio-Aquatic Testing Inc.'s results show that BioNeat NTS™ can be *up to 10 times less toxic* than other common cleaning products containing Sodium Laurel Sulfate or similar surfactants as part of their formulation.

Table 2. Surface Washing Agent Toxicity (by Bio-Aquatic Testing Inc.)

MATERIAL TESTED	SPECIES	LC50 (PPM)
BioNeat NTS™	Menidia beryllina	136.1
	Mysidopsis bahia	70.7
No. 2 Fuel Oil	Menidia beryllina	3.35
	Mysidopsis bahia	2.24
Product & No. 2 Fuel Oil	Menidia beryllina	4.73
	Mysidopsis bahia	2.24
Reference Toxicant: (Sodium Laurel	Menidia beryllina	12.19
Sulfate)	Mysidopsis bahia	10.53

BioNeat NTS™ is not only safe to be used for everyday cleaning, it is also a powerful, industrial-strength cleaning product that can even clean an oil-drilling rig or an oil spill. Its safety properties are highly related to its unique formulation. BioNeat NTS™ only contains FDA-approved (GRAS list) components and uses natural products in its formulation. Its superior cleaning capability is powered by modern bio-nanotechnology and science. BioNeat NTS™ forms particles four-nanometers in size when it is manufactured. This effective component of the BioNeat NTS™ formulation has extraordinary surface activity. These nanoparticles can surround oil drops, grease, and many other contaminants rapidly with their fatty tails and leaving their soluble heads outside. When water is applied, these oily contaminants can be easily rinsed away as a biodegradable biomass. Due to the extremely small size of these particles, BioNeat NTS™ is significantly more effective as a surfactant and emulsifier than other conventional soaps or detergents with larger-sized micelles.

In December of 2013, BIO-NEAT INC. was granted the Champion Award as a manufacturer of only safer surfactants by the U.S. EPA's Design for the Environment/Safer Detergents Stewardship Initiative Program. In April of 2014, BioNeat NTS™ was granted organic certification by the Organic Materials Review Institute (OMRI).



Measurement of Micelle Size of BioNeat NTS™

Testing and Report prepared by Bio-Neat Inc. Consulting Chemist Shaoyong Yu, Ph.D.

The micelle size of BioNeat NTS^m was obtained by Dynamic Light Scattering (DLS) Technique. DLS measurements were performed on a Proton Correlation Spectrometer with a BI9000 AT Digital Correlator (Brookhaven Instruments) equipped with a Compass 315M-150 Laser (Coherent Technologies), which provides a green light source (λ =532nm).

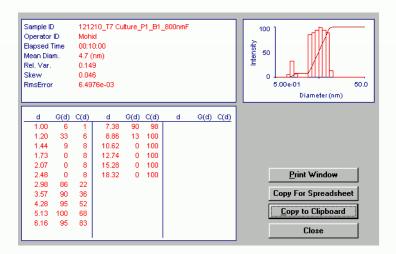
Data obtained from DLS can be interpreted in different ways using different mathematic models. The figure below, which uses a Cumulant model, is the easiest and most common interpretation of the measurement.

	Gamma (s ⁻¹)	Diff, Coef, (cm 2 s-1)	Eff. Diam. (nm)	Poly	Skew	Kurtosis
Linear:	4.808e+04	9.685e-07	5.1			
Quadratic:	6.063e+04	1.221e-06	4.0	0.187		
Cubic:	6.573e+04	1.324e-06	3.7	0.323	0.30	
Quartic:	6.957e+04	1.401e-06	3.5	0.453	0.62	3.20

Gamma is the average decay rate, and Diff. Coef. is the Diffusion Coefficient. Both of these values characterize the moving speed of the micelles in solution, i.e., Brownian movement or thermal diffusion. The larger the micelle, the lower the diffusion coefficient. The micelle size can be solved from the Cumulant model with four different orders of approximation. Each order provides a slightly different micelle size (Eff. Diam) in nanometer (nm) units, which is the DIAMETER of the micelles. Since the quadratic (4.0) or cubic (3.7) results are typically used, that result indicates that the BioNeat micelles are approximately 4 nm with polydispersity (Poly) index of about 0.2 to 0.3.

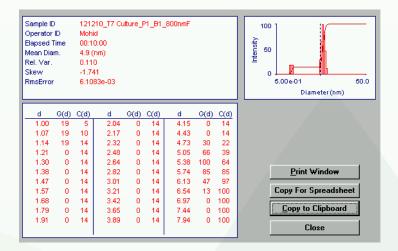
The following two figures are different interpretation of the measurement with more complicated models. CONTIN mode (below) is better when there is a polydispersed system.





This chart indicates that BioNeat NTS $^{\text{m}}$ micelles have two populations: one is about 1.2 nm and the other one is around 5nm, so the overall average (Mean Diam.) size from this model is 4.7 nm, which agrees well with the Cumulant result when polydispersity is considered.

NNLS (below) is another model to interpret the measurement, which gives a quite close value 4.9nm.



It is important to note that all of these results represent an average of statistical results, so the approximate level of 4-5 nm in all these results are sufficiently valid for all practical purposes.



OECD 301B Ready/Ultimate Biodegradability Assessment

Performed by Respirtek Laboratories, Biloxi, MS

Project ID: BIO-2413 Date: November 19, 2014

Study Summary

The test substance, BioNeat NTS™, was evaluated for ready and ultimate biodegradability in an aqueous medium, when exposed to an inoculum source according to procedures detailed in the OECD 301B methodology.

Based on the test method employed, the maximum biodegradability of the test materials is as follows:

Test Substance	Test Substance Percent Biodegradation	
BioNeat NTS™	61.7%	Ultimate

This value is the highest observed during the 28-day test for each substance.

Based on the testing conducted in accordance with methods specified by OECD 301B Procedure, test product BioNeat NTS™ achieved 61.7% biodegradation. The product met method requirements for *Ultimate Biodegradability* classification.





Antibacterial Efficacy Testing #1

Performed by BCS Laboratories Inc. 4609 NW 6th St. Bldg. A, Gainesville, FL 32609

We have completed the antibacterial efficacy study on the provided sample of BioNeat NTS™. The testing was done according to the protocol we regularly use to assess antimicrobial efficacy of disinfectants.

The disinfectant efficacy was determined against *Escherichia coli* and *Salmonella typhimurium* as models of gram-negative human bacterial pathogens that are transmissible through feces. According to observed results, the tested formula exhibited excellent antibacterial efficacy. It should effectively reduce and control the transmission of the tested pathogens when used as directed. Following you will find a summary of the results of our analysis.

Table 1: The inactivation of fecal bacterial pathogens by the BioNeat Natural Soap Formula at a 10% concentration and following 5 and 15 minute exposure to the diluted formula.

Microorganism	Average colony forming units (cfu)/ml present initially. (untreated Control)#	Average cfu/ml following 5 minute exposure	Average cfu/ml following 15 minute exposure	Percent reduction
Escherichia coli	3.4 x 10 ⁵	<1.0	<1.0	>99.999%
Salmonella Typhimurium	1.8 x 10 ⁵	<1.0	<1.0	>99.999%

Conclusion: BioNeat NTS™ demonstrates average over 99% effectiveness in killing gram-negative pathogenic bacteria.



Antibacterial Efficacy Testing #2

Performed by BCS Laboratories Inc. 4609 NW 6th St. Bldg. A, Gainesville, FL 32609

We have conducted the exploratory study on the provided BioNeat NTS™ concentrate to determine the BioNeat NTS™ solution's potential efficacy on the reductions of *Listeria monocytogenes* from inoculated surfaces; BCS 1409054. The testing was conducted as per AOAC Method 961.02 (AOAC Official Methods of Analysis; 2005) and ASTM E2197-11 "Standard Quantitative Disk Carrier Test Methods for Determining Bactericidal, Virucidal, Fungicidal, Mycobactericidal and Sporicidal Activities of Chemicals." Following, you will find a summary of the results of our analysis.

Table 1. The efficacy of a spray application of diluted BioNeat NTS™ concentrate on the reduction of *Listeria monocytogenes*; Method 961.02 Germicidal Spray Products as Disinfectants (2005) using a 10 minute contact time.

Treatment Slide	Average microorganism recovered from positive control [#]	Average cfu/mL recovered from each slides sprayed*	Percent Reduction	Log ₁₀ Reduction	Average Percent Reduction	Average Log ₁₀ Reduction
1	5.2 x 10 ³ cfu/mL	<0.45	<99.991%	>4.1	<99.991%	>4.1
2	5.2 x 10 ³ cfu/mL	<0.45	<99.991%	>4.1	<99.991%	>4.1
3	5.2 x 10 ³ cfu/mL	<0.45	<99.991%	>4.1	<99.991%	>4.1

Conclusion: BioNeat NTS™ demonstrates average over 99% effectiveness in killing gram-positive pathogenic bacteria.



Antiviral Efficacy Testing #1

Performed by BCS Laboratories Inc. 4609 NW 6th St. Bldg. A, Gainesville, FL 32609

The virucidal efficacy of a spray application of diluted BioNeat NTS™ concentrate against Human poliovirus 1 (ATCC VR 1562) inoculated onto non-porous surfaces. Test was conducted as per adaptation of ASTM E1053 "Standard Test Method for Efficacy of Virucidal Agents Intended for Inanimate Environmental Surfaces" and ASTM E2197-11 "Standard Quantitative Disk Carrier Test Method for Determining Bactericidal, Virucidal, Fungicidal, Mycobactericidal, and Sporicidal Activities of Chemicals."

Microorganism	Number of Replicates Tested	Contact Time (Minutes)	Average Infectious Units Control Slides	Average Recovered From Each Sprayed Slide	Percent Reduction	Average Percent Reduction	${ m Log_{10}}$ Reduction
			1.6 x 10 ⁴				
Human Poliovirus 1	3	10	iu/ml	790 iu/ml	95.1%	95.9%	1.4
"	44	٠,	44	940 iu/ml	94.1%	دد	"
"	66	66	44	230 iu/ml	98.6%	66	"

Conclusion: BioNeat NTS™ demonstrates average 95.9% effectiveness in killing Human Poliovirus.



Antiviral Efficacy Testing #2

Performed by BCS Laboratories Inc. 4609 NW 6th St. Bldg. A, Gainesville, FL 32609

The virucidal efficacy of a spray application of diluted BioNeat NTS™ concentrate against Murine Norovirus 1 (MNV-1, PTA-5935) inoculated onto non-porous surfaces. Test was conducted as per adaptation of ASTM E1053 "Standard Test Method for Efficacy of Virucidal Agents Intended for Inanimate Environmental Surfaces" and ASTM E2197-11 "Standard Quantitative Disk Carrier Test Method for Determining Bactericidal, Virucidal, Fungicidal, Mycobactericidal, and Sporicidal Activities of Chemicals."

Microorganism	Number of Replicates Tested	Contact Time (Minutes)	Average Infectious Units Control Slides	Average Recovered From Each Sprayed Slide	Percent Reduction	Average Percent Reduction	Log ₁₀ Reduction
			85,000				
Murine Norovirus 1	5	10	iu/ml	1,640 iu/ml	80.7%	82.5%	0.76
"	"	"	دد	1,520 iu/ml	82.1%	"	"
"	"	"		1,260 iu/ml	85.2%	"	"
"	"	"	44	1,480 iu/ml	82.6%	"	"
"	"	"	"	1,533 iu/ml	82.0%	"	66

Conclusion: BioNeat NTS™ demonstrates average 82.5% effectiveness in killing Murine Norovirus.



Antifungal/Mold Efficacy Testing

Performed by BCS Laboratories Inc. 4609 NW 6th St. Bldg. A, Gainesville, FL 32609

We have conducted the fungicidal efficacy testing on the provided Bio-Neat NTS concentrate. The testing was conducted as per AOAC Method 961.02 (AOAC Official Methods of Analysis; 2005) and ASTM E2197-11 "Standard Quantitative Disk Carrier Test Method for Determining Bactericidal, Virucidal, Fungicidal, Mycobactericidal, and Sporicidal Activities of Chemicals."

Table 1. The fungicidal efficacy of a spray application of diluted Bio-Neat NTS™ concentrate on *Aspergillus niger;* Method 961.02 Germicidal Spray Products as Disinfectants (2005) using a 10-minute contact time.

Treatment Slide	Average microorganisms cfu/ml recovered from control	Average cfu/ml recovered from each of slides sprayed	Percent Reduction	Log ₁₀ Reduction	Cumulative Percent Reduction	Cumulative Log ₁₀ Reduction
1	5.0 x 10 ³	575	88.5%	0.9	98.4%	1.9
2	и	24.1	99.5%	2.3	и	и
3	u	7.3	99.9%	2.8	и	u
4	и	20.9	99.6%	2.4	и	и
5	u	19.1	99.6%	2.4	u	u
6	u	20	99.6%	2.4	u	u
7	u	37.3	99.3%	2.1	и	u
8	u	19.1	99.6%	2.4	и	и
9	и	45.6	99.1%	2.1	и	и
10	u	40.9	99.2%	2.1	и	u

Conclusion: BioNeat NTS™ demonstrates average 98.4% effectiveness in killing fungus/mold spores.



Cytotoxicity Testing

Performed by BCS Laboratories Inc. 4609 NW 6th St. Bldg. A, Gainesville, FL 32609

We have conducted the ASTM F895-11 (Standard Test Method for Agar Diffusion Cell Culture Screening for Cytotoxicity) preliminary screening study as per your request. The analysis is equivalent to ISO 10993-5. The study was performed on the provided "BioNeat NTSTM Concentrate" sample (BCS # 1409054) received on September 09, 2014. In the following pages, you will find a brief description of the methodology used and the results of our analyses. Based on the observed results, the diluted solution exhibited **Negligible Toxicity** on the CCL-1 cell line tested as per the agar diffusion method.

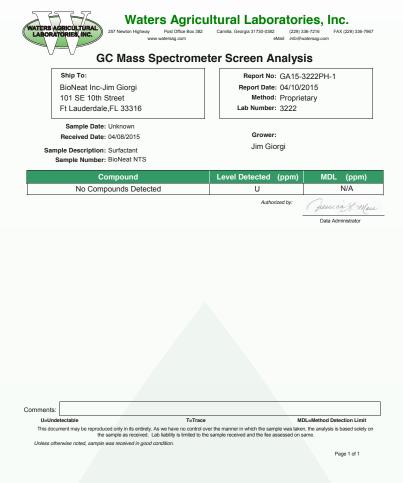
Conclusion: BioNeat NTS™ demonstrated to be of "Negligible Toxicity" to human and animal tissue.



Gas Chromatograph/Mass Spectrometer (GC/MS) Testing

Performed by Waters Agricultural Laboratories, 257 Newton Hwy. P.O. Box 382, Camilla, GA 31730

BioNeat NTS $^{\text{\tiny{M}}}$ was tested using Gas Chromatography/Mass Spectrometer instrumentation to determine the presence of any contaminating compounds such as heavy metals, pesticides, VOCs, etc. Results indicate that NO contaminants of any kind were present in the sample tested.



This Comprehensive Scientific Summary Report was prepared by Yaoshong Yu, Ph.D., Consulting Chemist for BIONEAT INC. Copies of full laboratory reports are available upon request.





BIOLOGICAL CONSULTING SERVICES OF NORTH FLORIDA, INC.

November 21, 2014

Jim Giorgi Bio-Neat Inc 101 SE 10th Street Ft. Lauderdale, FL 33316 954-729-1220 jim@bioneat.com

RE: Study report of the fungicidal efficacy testing of Bio-Neat™ NTS concentrate; BCS 1409054

Dear Mr. Giorgi,

We have conducted the fungicidal efficacy testing on the provided Bio-Neat NTS concentrate. The testing was conducted as per AOAC Method 961.02 (AOAC Official Methods of Analysis; 2005) and ASTM E2197-11 "Standard Quantitative Disk Carrier Test Method for Determining Bactericidal, Virucidal, Fungicidal, Mycobactericidal, and Sporicidal Activities of Chemicals."

In the following pages, you will find a summary of the methodology used and the results of our analysis. Should you have any questions or concerns, please do not hesitate to contact me.

Best Regards,

George Lukasik, Ph.D. Laboratory Director

Greap lebon

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FL DOH LABORATORY #E82924, EPA# FL01147

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Fungal Culture Preparation

Aspergillus niger (ATCC 6275) stock culture was obtained from Microbiologics (MN, USA) and maintained as per the supplier's recommendations. The culture was propagated on Sabouraud Dextrose Agar (SDA, Neogen, USA). For the preparation of the spore suspension, the culture was spread onto SDA plates and allowed to incubate for 14 days at 25.0°C. Following, the fungal growth on the plates was saturated PBW w/Tween 80 and the mycelial mats were removed using a sterile spatula and placed into a centrifuge tube. The culture was macerated and filtered through Whatman® #4 paper. The spore suspension was then centrifuged and suspended in PBW as per ASTM 2197-11. The suspension was enumerated by spread plating serial 1000-fold dilutions onto Sabouraud Dextrose Agar and incubating at 25.0°C for 7-8 days. The suspension was used in the challenge study within 30 days of harvest.

Test Article/Product: Bio-Neat NTS Concentrate

On September 09, 2014, a bottle of Bio-Neat™ NTS concentrate solution was delivered to BCS Laboratories. The solution was issued BCS identifier 1409054. The solution's directions were followed based on the client's recommendations. The concentrate was diluted with distilled or demineralized water to recommended working solution. Briefly, a

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1/1 dilution was made with 50ml of laboratory grade reagent water and 50 ml Concentrate. The solution was again diluted 1/1 with an additional 100ml of laboratory grade reagent water. The final diluted solution was placed in a sterile spray bottle and used within 30 minutes of preparation for the microbial spray studies. The temperature of the diluted solution prior to application and during the efficacy testing was maintained at 20-22°C. All tests were conducted in a Class II biological cabinet.

Study Date for *Aspergillus niger*: Study initiated November 14, 2014 and completed November 25, 2014.

AOAC Official Method 961.02 Germicidal Spray Products as Disinfectants (2005)

Test Methodology Narrative:

On the day of study, the cultures used were prepared as described previously and as per Method 961.02. The fungal population in each of the cultures was determined to be greater than 10⁶ cfu/mL.

An aliquots of the microbial suspension was spread onto each of 12 sterile 22 mm² glass slides (Propper Scientific, NY). The inoculum was allowed to dry in a covered chamber at 37°C for 50-60 minutes. Ten of the twelve inoculated slides were sprayed

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for 10 seconds from a distance of approximately 12" with an even fine mist the prepared solution. The glass slides were completely saturated with solution. The slides were t allowed to incubate at 20-22°C for 10 minutes. Immediately following, each of the slides were picked up with sterile forceps, the excess liquid was allowed to run off, and the slide was placed into a sterile glass tube containing 10 ml of D/E neutralization buffer with tween (Neogen, USA). Additionally, un-inoculated slides were sprayed and incubated to serve as negative and neutralization controls. The remaining inoculated slides that were not exposed to the spray were used as positive recovery controls. The positive, neutralization, and negative controls slides were eluted in the same manner. The samples were agitated on a tabletop rotary shaker at a medium speed for 10 minutes. Following, the solution was assayed for fungal species by spread plating onto SDA as per Lab Standard Operating Procedure: SOP-1. Each of the treatment samples, neutralization control and negative control samples were analyzed by plating duplicate 0.1 ml and 1.0 ml samples of the solution directly. Positive controls were plated as described at 1/100 and 1/1000 dilutions. The Sabouraud Dextrose Agar plates were incubated at 25°C ±1 for 10 days. Following incubation, the colonies on the respective plates were enumerated. Neutralization control recoveries confirmed the efficient neutralization of and antimicrobial residual.

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Study data are summarized in the provided table(s). Positive, negative, and process controls were performed as outlined in the method and as per Good Laboratory Practices. All analyses were performed in accordance with laboratory practices and procedures set-forth by our NELAP/TNI accreditation standards (ISO 17025) unless otherwise noted. BCS makes no express or implied warranty regarding the ownership, merchantability, safety or fitness for a particular purpose of any such property or product. The results presented pertain only to the study conducted on the test articles/samples provided by the client (or client representative). The study was authorized and commissioned by the client. The results presented pertain only to the samples analyzed and identifier number(s) indicated. The data provided is strictly representative of the study conducted using the material/samples/articles provided by the client (or client's representative) and it's (their) condition and homogeneity when received and at the time of test. Thus, the data may not be representative of the lot or batch number or other samples. Consequently, the data may not necessarily justify the acceptance or rejection of a lot or batch, a product recall, or support legal proceedings. It is the responsibility of the client to provide all information relevant to the analysis requested. The study and data obtained under the laboratory conditions may not be representative or indicative of a real-life process and/or application. This

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report does not imply that BCS Laboratories has been engaged to consult upon the consequences of the analysis and for any action that should be taken as a result of the analysis.

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Table 1. The fungicidal efficacy of a spray application of diluted Bio-Neat™ NTS concentrate on <u>Aspergillus niger</u>; Method 961.02 Germicidal Spray Products as Disinfectants (2005) using a 10 minute contact time.

Treatment Slide	Average microorganism cfu/mL recovered from control #	Average cfu/mL recovered from each of slides sprayed*	Percent Reduction	Log ₁₀	Cumulative Percent Reduction	Cumulative Log ₁₀ reduction
1		575	88.5%	0.9		
2		24.1	99.5%	2.3		
3		7.3	99.9%	2.8		
4	5.0 x 10 ³	20.9	99.6%	2.4		
5		19.1	99.6%	2.4	00.49/	4.0
6		20	99.6%	2.4	98.4%	1.9
7		37.3	99.3%	2.1		
8		19.1	99.6%	2.4		
9		45.6	99.1%	2.1		
10		40.9	99.2%	2.1		

[#] This number represents the average number of microorganisms (colony forming units/ milliliter) recovered from glass slides inoculated, dried, and not exposed to spray treatment (positive control).

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^{*} Glass slides were inoculated with the indicated microorganisms and allowed to dry. Slides were sprayed to saturation with the solution and allowed to incubate at 20-22.0°C for ten minutes. Slides were eluted and examined for growth as described in the methodology section.