

ANTIMICROBIAL TEST LABORATORIES



Study Report



Study Title

Evaluation of the Antimicrobial Effect of Spectra254 Ultraviolet Radiating Room Disinfection Device
Against Airborne Microorganisms

Test Method

Custom Air Quality Study

Study Identification Number

NG6090-I

Study Sponsor

George Jay Lichtblau
Spectra 254
3 Corporate Dr
Danbury, CT 06810
(203) 796-5315
gjlichtblau@comnet.net

Test Facility

Antimicrobial Test Laboratories
1304 W. Industrial Blvd
Round Rock, TX 78681
(512) 310-8378

History of the Laboratory

Antimicrobial Test Laboratories was launched in 2006 to provide testing services to the antimicrobial industry. The company has grown considerably since then but its focus remains the same: Test antimicrobial agents, test them well, and test them fast! Antimicrobial Test Laboratories operates a 15,000+ square foot facility near Austin, Texas, where hundreds of studies are conducted annually by a staff of friendly, knowledgeable, and experienced microbiologists and virologists.

Laboratory Qualification Statement

Antimicrobial Test Laboratories was founded by microbiologist Dr. Benjamin Tanner. The laboratory ensures consistent, reproducible results by utilizing a well-trained and educated scientific staff who work from a comprehensive system of Standard Operating Procedures, official standard methods from ASTM, AOAC, and other organizations, and custom study protocols. The laboratory provides testing services to dozens of Fortune 500 companies and has been inspected for GLP compliance by the US government.

Scientist Qualifications

This study was designed, conducted, and reported by: Blake Rolland, B.S.

Blake graduated from the University of Oklahoma with a Bachelors of Science in Microbiology.

Blake is well-versed with regard to a variety of microbiological test methods and procedures. As a Microbiologist at Antimicrobial Test Laboratories, he has taken part in hundreds of studies and mastered several test methods. Blake enjoys seeing large projects through to completion. His scientific character, coupled with his strong work ethic bring a high degree of efficiency and care to every study he leads.



If you have any questions about your study, please don't hesitate to contact Blake at:

Blake@AntimicrobialTestLabs.com
or
(512) 310-8378

Test Device Information

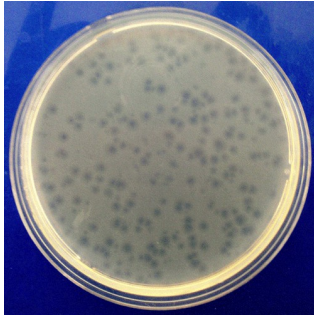
The following test device was received on 22APR2015:



Spectra254 1000A

Test Microorganism Information

The test microorganism(s) selected for this test:



MS2 Bacteriophage (MS2), ATCC 15597-B1

This virus is a non-enveloped positive-stranded RNA virus of the bacteriophage family Leviviridae. Bacterial cells are the hosts for bacteriophages, and *E. coli* 15597 serves this purpose for MS2 bacteriophage. Its small size, icosohedral structure, and environmental resistance has made MS2 ideal for use as a surrogate virus (particularly in place of picornaviruses such as poliovirus and human norovirus) in water quality and disinfectant studies.

Permissive Host Cell System for MS2: *Escherichia coli* ATCC 15597



***Staphylococcus aureus* 6538**

This bacterium is a Gram-positive, spherical-shaped, facultative anaerobe. *Staphylococcus* species are known to demonstrate resistance to antibiotics such as methicillin. *S. aureus* pathogenicity can range from commensal skin colonization to more severe diseases such as pneumonia and toxic shock syndrome (TSS). *S. aureus* is commonly used in several test methods as a model for gram positive bacteria. It can be difficult to disinfect but does demonstrate susceptibility to low level disinfectants.



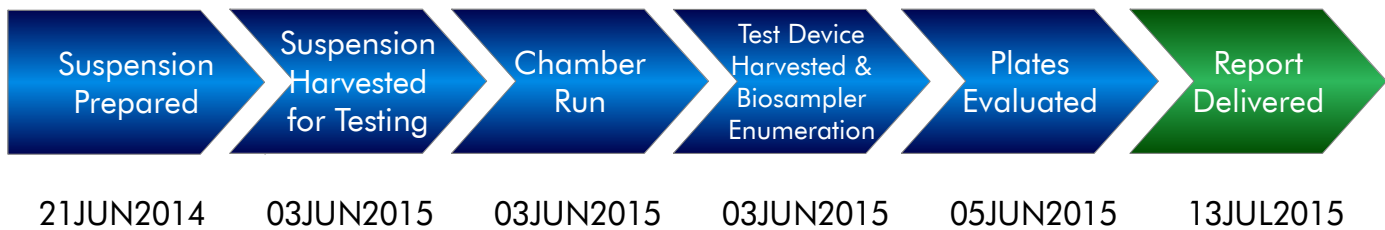
Escherichia coli

This bacteria is a Gram-negative, rod shaped, facultative anaerobe commonly found in the gastrointestinal tract of mammals. Although most serotypes of this microorganism are harmless there are pathogenic groups of *E. coli* such as enterohemorrhagic (EHEC), verocytotoxin producing (VTEC) and Shiga-like toxin producing (STEC) that can cause a multitude of illnesses. *E. coli* is relatively susceptible to disinfection when dried on a surface, yet it can be a challenging microorganism to mitigate in solution.

Summary of the Procedure

- Bacterial test microorganisms were grown for 48 hours on appropriate solid/liquid media.
- Bacterial cultures used for test inoculum were washed and concentrated in sterile RO water upon harvesting.
- Bacterial cultures and a virus stock were pooled to target concentrations.
- 14 ml of test inoculum was added to each nebulizer (total of 28 ml) and nebulized for 60 minutes.
- An SKC biosampler was used to take a Time Zero sample to determine starting chamber concentration for baseline comparison.
- Device was activated for 15 minutes then an air sample was taken.
- Device was activated for 15 minutes (a total of 30 minutes UV) then an air sample was taken.
- Samples were enumerated using standard dilution and plating techniques.
- Microbial concentrations were determined after 48 hours of incubation
- Reductions of microorganisms were calculated relative to concentration at Time Zero.

Study Timeline



Criteria for Scientific Defensibility of a Custom Device Study

The following criteria must be met in order for ATL to consider this study scientifically defensible:

1. The average number of bacteriophage recovered from the samples taken at time zero must be approximately 1×10^6 PFU/m³ or greater.
2. The average number of bacteria recovered from the samples taken at time zero must be approximately 1×10^5 CFU/m³ or greater.
3. Positive/Growth controls must demonstrate growth of the appropriate test microorganism.
4. Negative/Purity controls must demonstrate no growth of test microorganism.

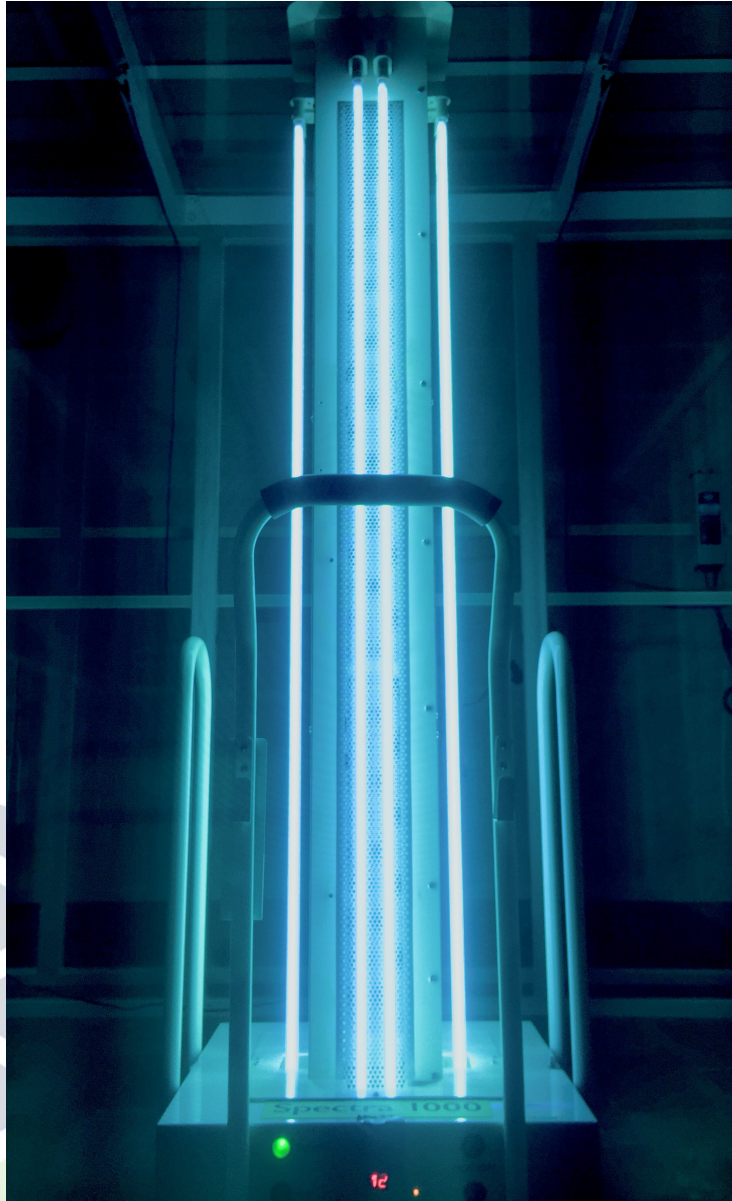
Testing Parameters used in this Study

Test Microorganism:	MS2 Bacteriophage 15597-B1	<i>S. aureus</i> 6538	<i>E. coli</i> 8739
Culture Growth Media:	N/A (Stock Suspension)	Tryptic Soy Broth (TSB)	Tryptic Soy Agar
Culture Growth Time:	N/A	48 ± 6 Hours	48 ± 6 Hours
Culture Dilution Media:	Reverse Osmosis (RO) Water	Reverse Osmosis (RO) Water	Reverse Osmosis (RO) Water
Target Concentration:	≥ 1.0 x 10 ⁸ PFU/ml	≥ 1.0 x 10 ⁸ CFU/ml (Pooled)	≥ 1.0 x 10 ⁸ CFU/ml (Pooled)
Enumeration Plating Media:	50% Tryptic Soy Agar	Mannitol Salt Agar	MacConkey Agar
Enumeration Plate incubation Time:	18 ± 6 Hours	24 ± 6 Hours	24 ± 6 Hours
Volume of Inoculum Added to Nebulizer:	14 ml/Nebulizer (28 ml total)		
SKC Biosampler Medium and Volume:	20 ml Phosphate Buffered Saline		
Nebulization Duration:	60 Minutes		
SKC Biosampler Sampling Time	10 Minutes		
Sampling Time Points:	Time Zero, after each 15 minute cycle		
SKC Biosampler Sampling Rate (L):	12.5 L/minute		
SKC Biosampler Liters (L) Sampled:	125 L		

Study Notes

No additional observations or notations were made for this study.

Study Photographs



Above: Spectra254 1000A. Picture taken mid-cycle.

Calculations

$$\text{Percent Reduction} = \left(\frac{B - A}{B} \right) \times 100$$

Where:

B = Number of viable test microorganisms on the control carriers immediately after inoculation

A = Number of viable test microorganisms on the test carriers after the contact time

$$\text{Log}_{10} \text{Reduction} = \text{Log} \left(\frac{B}{A} \right)$$

Where:

B = Number of viable test microorganisms on the control carriers immediately after inoculation

A = Number of viable test microorganisms on the test carriers after the contact time

$$\text{CFU/m}^3 = 1000 \times \left(\frac{\frac{\text{CFU}}{\text{ml}} \times (V_s)}{T_s (12.5)} \right)$$

Where:

V_s = Biosampler volume (ml)

T_s = Time sampled (min)

Control Results

Neutralization Method: N/A

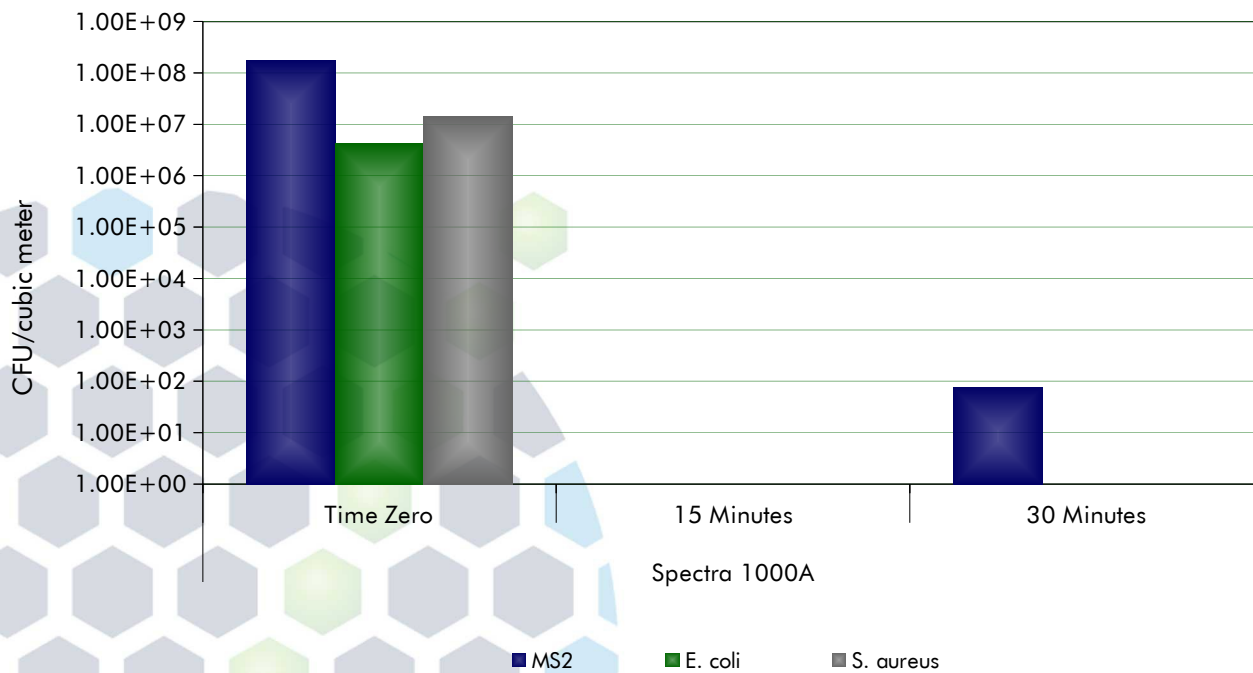
Growth Confirmation: Confirmed (Morphology)

Media Sterility: Sterile

Results of the Study

Test Device	Microorganism	Inoculum Concentration (CFU/ml)	Treatment Time Point	Recovery (CFU/m ³)	Percent Reduction vs. Time Zero	Log ₁₀ Reduction vs. Time Zero
Spectra 1000A	MS2 Bacteriophage ATCC 15597-B1	3.00E+09	Time Zero	1.73E+08	N/A	
			15 Minutes	<7.92E+01	>99.99995%	>6.34
			30 Minutes	7.68E+01	99.99996%	6.35
	<i>E. coli</i> ATCC 8769	5.95E+09	Time Zero	4.22E+06	N/A	
			15 Minutes	<7.92E+01	>99.9981%	>4.73
			30 Minutes	<7.68E+01	>99.9982%	>4.74
	<i>S. aureus</i> ATCC 6538	8.38E+07	Time Zero	1.44E+07	N/A	
			15 Minutes	<7.92E+01	>99.99945%	>5.26
			30 Minutes	<7.68E+01	>99.99947%	>5.27

Note: The limits of detection for this study vary by sample time points. The limit of detection for 15 minutes is 7.92E+01 CFU/m³, and the limit of detection for 30 minutes is 7.38E+01 CFU/m³. Values below the limit of detection are represented as <7.92E+01 or <7.68E+01 in the chart above and 0 in the graph below.



The results of this study apply to the tested substances(s) only. Extrapolation of findings to related materials is the responsibility of the Sponsor.

Copyright © Antimicrobial Test Laboratories, 2015. Reproduction and ordinary use of this study report by the entity listed as "Sponsor" is permitted. Other copying and reproduction of all or part of this document by other entities is expressly prohibited, unless prior permission is granted in writing by Antimicrobial Test Laboratories.