ANTIMICROBIAL TEST LABORATORIES

Study Report

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Study Title

Evaluation of the Effectiveness of Fresh-Aire Ultraviolet Lights Against Airborne Microorganisms

Test Method

Aerosol Efficacy Study

Study Identification Number

NG5999

Study Sponsor

Michael Walrath
Fresh-Aire UV
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Test Facility

Antimicrobial Test Laboratories
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Round Rock, TX 78681
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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

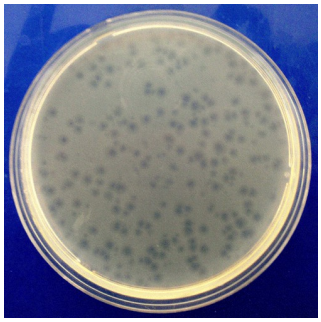
Test Device Information

UV Bulbs were received on 15JAN2015 and mounted on the ceiling of the Negative Pressure Aerosol Chamber (NPAC).

Sample Power Supply Identification: TUVCPS-46/60D Dual Lamp, powering (2) TUVC-L-160D 60" bulbs (Pictured) mounted on chamber ceiling.

Test Microorganism Information

The test microorganism(s) selected for this test:



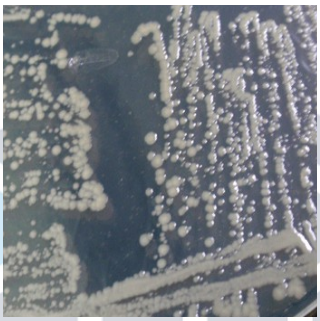
MS2 Bacteriophage (MS2), ATCC 15597-B1

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 epidermidis* ATCC 12228**

This bacteria is a Gram-positive, cocci-shaped, facultative anaerobe. *S. epidermidis* is part of the human bacterial flora, mostly located on skin. It is not usually pathogenic, however, antibiotic resistant strains have evolved. Most *Staphylococcus* species are a hardy microorganisms capable of surviving on surfaces and under dry conditions. This bacteria, specifically, is regularly used in quality control, media testing, and pharmacuetical/personal care products testing.



***Escherichia coli* ATCC 6538**

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 prepared in liquid growth medium.
- An aliquot of bacterial suspension was centrifuged and resuspended in 14 ml Phosphate Buffered Saline (PBS) per microorganism and supplemented with 1 ml MS2 stock to create the test inoculum.
- Initial Inoculum concentrations were determined prior to aerosolization.
- 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.
- UV bulbs were turned on immediately after Time Zero.
- An SKC biosampler was used to take samples 60 minutes and 240 minutes after UV bulbs were turned on.
- After the 240 minute contact time, the chamber was swabbed in various places to determine if viable microorganisms and/or bacteriophage are present after UV disinfection.
- All samples were diluted and plated using standard techniques. Plates were incubated for 24-48 hours.
- After incubation, microbial concentrations were determined, and reductions of microorganisms were calculated relative to concentration at Time Zero.

Study Timeline

02MAR2015	04MAR2015	04MAR2015	04MAR2015	06MAR2015	07APR2015

Criteria for Scientific Defensibility of the 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 x 10^6 PFU/m^3 or greater.
2. The average number of bacteria recovered from the samples taken at time zero must be approximately 1 x 10^5 CFU/m^3 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

Biosampler Medium, Vol.:	Phosphate Buffered Saline (PBS), 20 ml
Volume of Inoculum:	14 ml/Nebulizer (28 ml total)
Inoculum Supplement:	3.5% Simethicone (antifoaming agent)
Nebulization Duration:	60 Minutes
Contact Times:	Time Zero, 60 Minutes, and 240 Minutes
Sampling Rate and Time:	12.5 L/minute for 10 Minutes
Total Volume Sampled:	125 L

Test Microorganism:	MS2 Bacteriophage 15597-B1	*S. epidermidis* 12228 and *E. coli* 8739
Culture Growth Media:	N/A (Stock Suspension)	Tryptic Soy Broth(TSB)
Culture Growth Time:	N/A	48 ± 6 Hours
Culture Dilution Media:	Phosphate Buffered Saline	Phosphate Buffered Saline
Target Concentration:	≥1.0 x 10^9 PFU/ml	≥1.0 x 10^8 CFU/ml (Pooled)
Plating Media:	50% Tryptic Soy Agar	Tryptic Soy Agar
Plate Incubation Time:	18 ± 6 Hours	48 ± 6 Hours

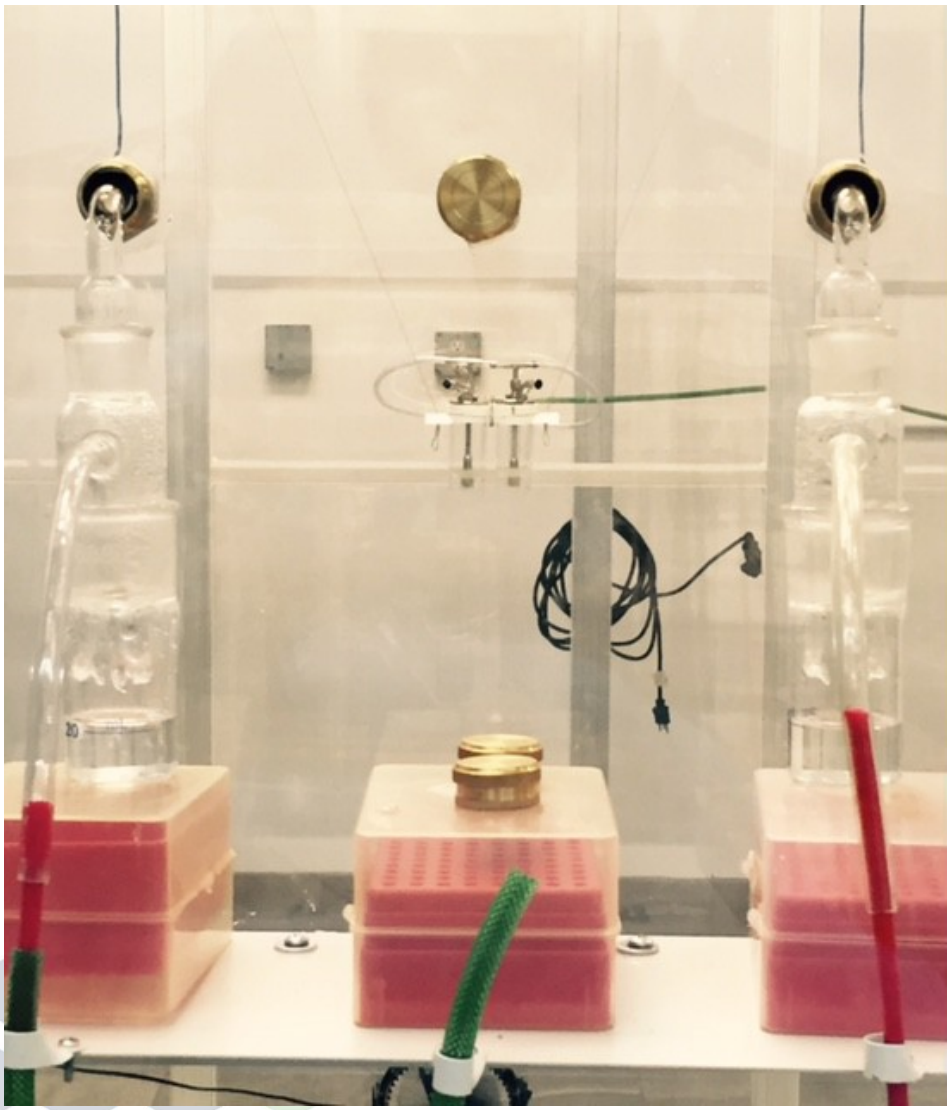
Study Notes

The Fresh-Aire bulbs functioned as anticipated, no problems were encountered.

Study Photographs

Two TUVC-L-160D 60" bulbs mounted on the ceiling of the NPAC

Study Photographs



SKC Biosampler Setup in relation to Nebulization setup

Calculations

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

Where:
B = Number of viable test microorganisms collected in biosampler at Time Zero
A = Number of viable test microorganisms collected in biosampler at Contact Time

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

Where:
B = Number of viable test microorganisms collected in biosampler at Time Zero
A = Number of viable test microorganisms collected in biosampler at 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)

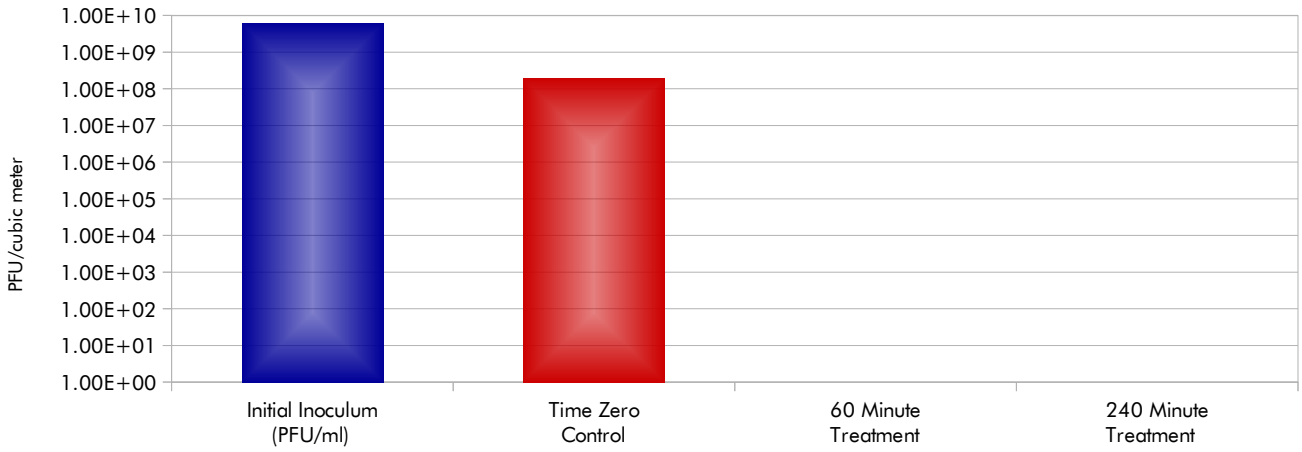
Control Results

Neutralization Method: N/A
Growth Confirmation: Positive

Media Sterility: Sterile

Results of the Study

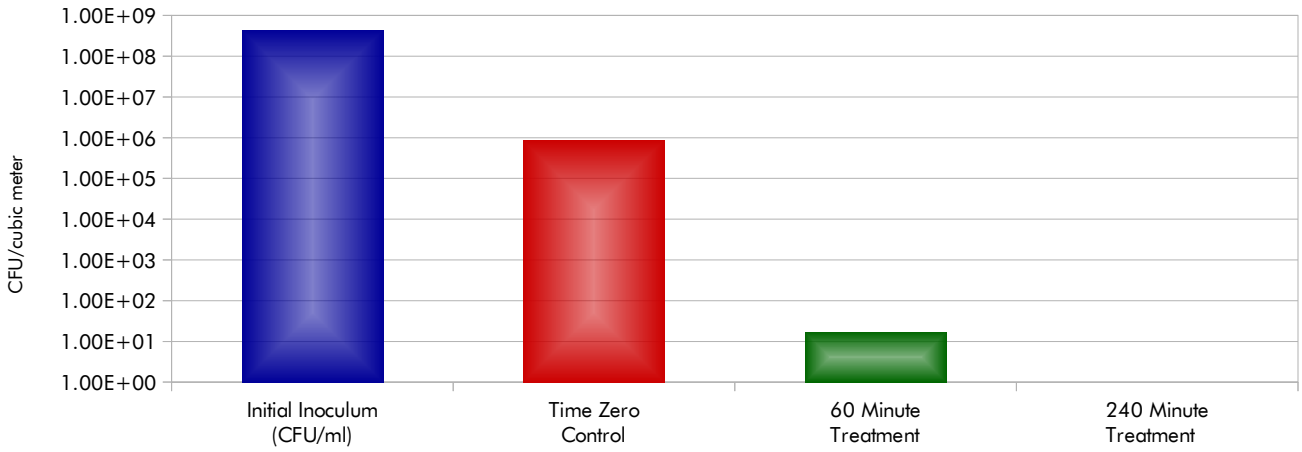
Test Microorganism	Initial Inoculum (PFU/ml)	Treatment	Test Group	Recovery (PFU/m³)	Percent Reduction vs. Time Zero	Log_{10} Reduction vs. Time Zero
MS2 Bacteriophage 15597-B1	5.85E+09	None	Time Zero	1.91E+08	N/A	
		TUVC-L-160D 60" Bulbs	60 Minutes	<8.00E+00	>99.999996%	>7.38
			240 Minutes	<8.00E+00	>99.999996%	>7.38



Note: The limit of detection for this study is 8.00E+00 PFU/m³. Values below this limit are represented as <8.00E+00 in the chart above and zero in the graph above.

Results of the Study

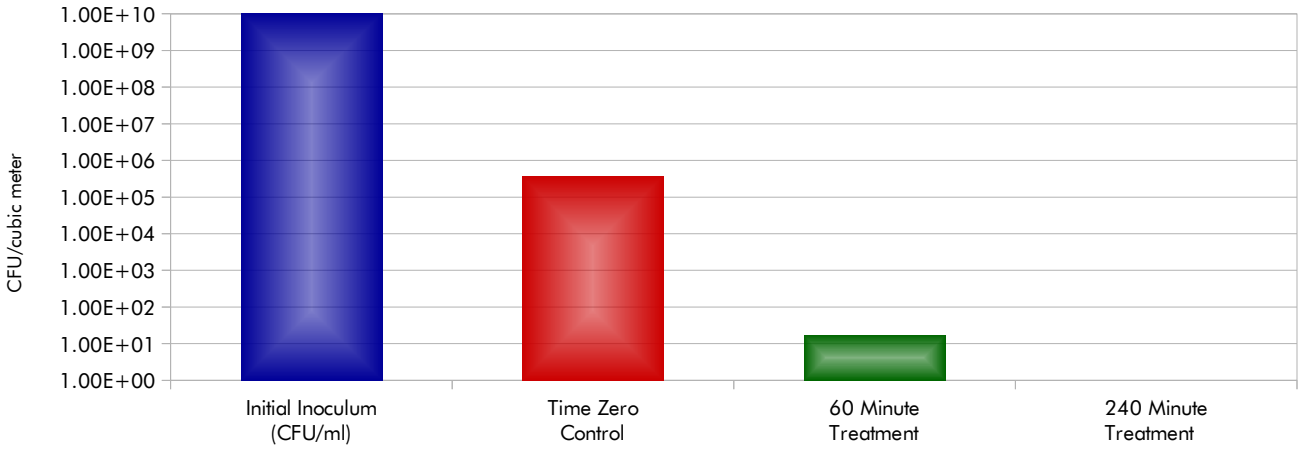
Test Microorganism	Initial Inoculum (CFU/ml)	Treatment	Test Group	Recovery (CFU/m³)	Percent Reduction vs. Time Zero	Log_{10} Reduction vs. Time Zero
S. epidermidis 12228	4.15E+08	None	Time Zero	8.57E+05	N/A	
		TUVC-L-160D 60" Bulbs	60 Minutes	1.60E+01	99.9981%	4.73
			240 Minutes	<8.00E+00	>99.9991%	>5.03



Note: The limit of detection for this study is 8.00E+00 CFU/m³. Values below this limit are represented as <8.00E+00 in the chart above and zero in the graph above.

Results of the Study

Test Microorganism	Initial Inoculum (CFU/ml)	Treatment	Test Group	Recovery (CFU/m³)	Percent Reduction vs. Time Zero	Log_{10} Reduction vs. Time Zero
E. coli 8739	9.87E+09	None	Time Zero	3.67E+05	N/A	
		TUVC-L-160D 60" Bulbs	60 Minutes	1.60E+01	99.996%	4.36
			240 Minutes	<8.00E+00	>99.9978%	>4.66

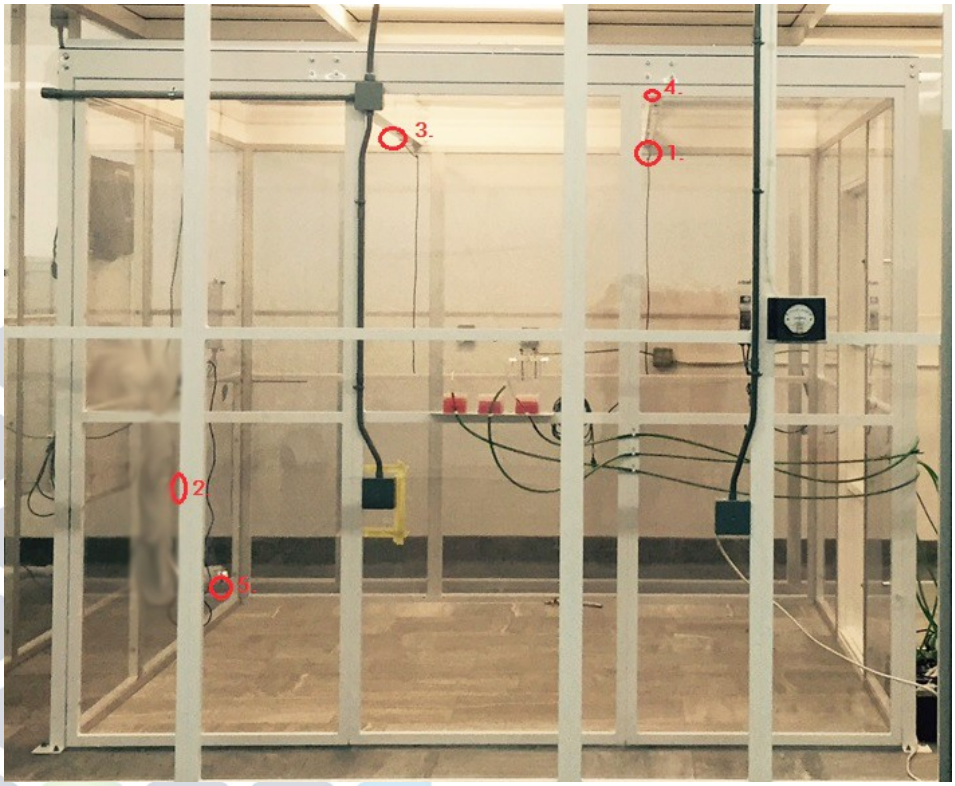


Note: The limit of detection for this study is 8.00E+00 CFU/m³. Values below this limit are represented as <8.00E+00 in the chart above and zero in the graph above.

Results of the Study: Post UV Treatment Swab

Areas outside the direct line of UV exposure were swabbed after bulbs ran for 4 hours (240 minutes) to determine if microorganisms in hard-to-reach areas evade UV disinfection. Location of swabs are detailed below:

Location	Description	Bacterial Counts	Bacteriophage Counts
1	North Beam (Underside of Beam)	11 CFU	None
2	Outlet (Fan Plug-in)	None	None
3	South Beam/West Wall Junction (South Face)	1 CFU	None
4	North Beam/East Wall Junction (North Face)	None	None
5	Outer Bottom Lip of Snorkel Inlet	None	None



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