ANTIMICROBIAL
TEST LABORATORIES

Study Report

Study Title

Custom Microbiological Air Quality Study to determine the efficacy of an AtmosAir Solutions Ionization System against bacterial bioaerosols.

Test Method

Custom Aerosol Study

Study Identification Number

NG6275-A1

Study Sponsor

Steve Levine with AtmosAir Solutions
Clean Air Group, Inc.
418 Meadow Street, Suite 204
Fairfield, CT 06824
(203) 335-3700
Slevine@atmosair.com

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

Qualifications

Test Device Information

The following test device was received on 12AUG2015:

ATMOSAIR Matterhorn Series

Application (Write-up from *www.atmosair.com/commercial/commercial-products.html*):

The Matterhorn series ionization system, which encompasses models 1000, 1002, 880 and 882, are intended to be mounted in the supply duct or air handler of a heating, cooling, or ventilating system. The unit is intended to operate only when air flow is present, thus, power to the ionization unit should be interlocked with fan operation, or controlled via an air pressure switch. The size and number of ionization systems is dependent upon the airflow, size of the space, and severity of the pollution and odors. Ionization can be adjusted with a 5-step knob.

Test Microorganism Information

The test microorganism(s) selected for this test:
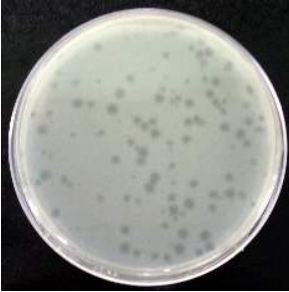
MS2 Bacteriophage (MS2), ATCC 15597-B1

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 saprophyticus ATCC 35552

This bacterium is a Gram-positive, spherical-shaped, facultative anaerobe. *Staphylococcus* species are known to demonstrate resistance to antibiotics such as methicillin. *S. saprophyticus* pathogenicity can range from commensal gastrointestinal and environmental flora to more severe diseases such as urinary tract infections. *S. saprophyticus* is used as a representative Gram-positive bacteria in testing as it is closely related to other *Staphylococcus* species and is largely non-pathogenic.
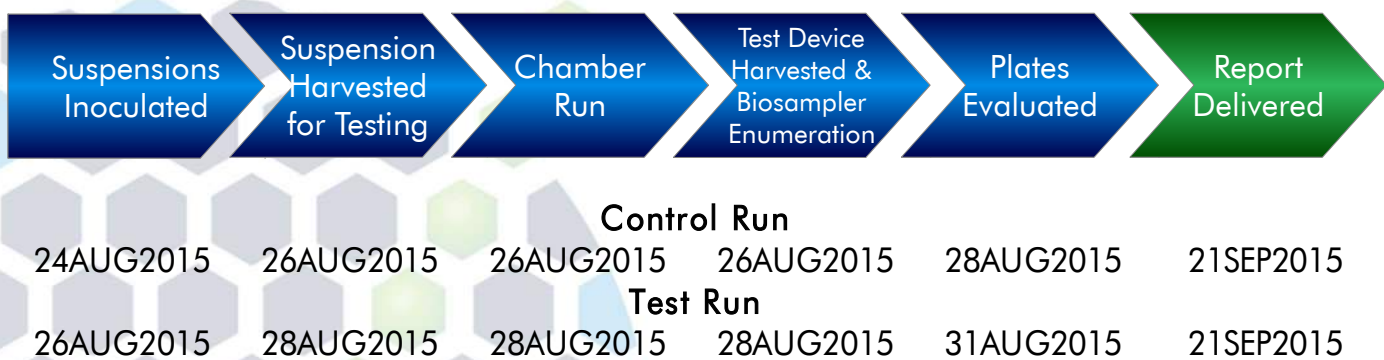
Escherichia coli K12

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

- 24 hour cultures of *E. coli* were washed from the surface of Tryptic Soy Agar with sterile R/O water, centrifuged to concentrate, re-suspended, struck for isolation, and stored at 4°C until needed for testing.
- A 48 hour culture of *S. saprophyticus* was centrifuged, decanted, and re-suspended with the prepared *E. coli* suspension to create a pooled concentrated bacterial suspension. An aliquot of suspension was removed to create test inoculum targeting $\geq 1.0 \times 10^8$ CFU/ml.
- The pooled bacterial suspension was then inoculated with an aliquot of MS2 (stock solution) to create final test inoculum targeting $\geq 1.0 \times 10^9$ PFU/ml.
- Test Device was positioned within the chamber (see Study Photographs).
- The test suspension was loaded into a Collison 6-Jet nebulizer for aerosolization and nebulized for 7 minutes and 30 seconds.
- An SKC biosampler was used to take an Initial Numbers Control sample to determine starting chamber concentration for baseline comparison.
- Device was activated immediately after the Time Zero sample was collected.
- An SKC biosampler was used to sample after 15 and 45 minute contact times.
- 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 Normalized Numbers Control concentrations at each contact time sampled. Normalized Numbers Control samples at each corresponding contact time are calculated using settling rates for each microorganism tested from data obtained from Control (Baseline) Run.

Study Timeline

Suspensions Inoculated → Suspension Harvested for Testing → Chamber Run → Test Device Harvested & Biosampler Enumeration → Plates Evaluated → Report Delivered

Control Run

| 24AUG2015 | 26AUG2015 | 26AUG2015 | 26AUG2015 | 28AUG2015 | 21SEP2015 |

Test Run

| 26AUG2015 | 28AUG2015 | 28AUG2015 | 28AUG2015 | 31AUG2015 | 21SEP2015 |

Criteria for Scientific Defensibility of a Custom Device Study

For Antimicrobial Test Laboratories to consider a Device Study study to be scientifically defensible, the following criteria must be met:

1. The average number of viable bacteria/bacteriophage recovered from the initial numbers control samples must be approximately 1×10^5 CFU/m^3 (bacteriophage: PFU/m^3) or greater.
2. Positive/Growth controls must demonstrate growth of the appropriate test microorganism.
3. Negative/Purity controls must demonstrate no growth of test microorganism.

Testing Parameters used in this Study

Test Parameters			
Test System (Microorganism)	MS2 Bacteriophage ATCC 15597-B1	*E. coli* K12	*S. saprophyticus* ATCC 35552
Culture Growth Media:	None (Stock Suspension)	~250ml Solidified TSA (Bacterial Lawns Harvested)	100 ml Tryptic Soy Broth
Culture Growth Time:	None	~24 Hours	~48 Hours
Culture Dilution Media:	Sterile RO Water		
Target Inoculum Concentration:	≥1.0 x 10⁹ PFU/ml	≥1.0 x 10⁸ CFU/ml	
SKC Biosampler Medium and Volume:	Phosphate Buffered Saline (PBS), 20 ml		
Volume of Inoculum Added to Nebulizer:	10 ml		
Nebulization Duration:	7 Minutes and 30 Seconds		
SKC Biosampler Sampling Time:	10 Minutes (Initial Numbers, 15 Minute Contact Time) 30 Minutes (45 & 90 Minute Contact Times)		
SKC Biosampler Liters (L) Sampled:	125 L (10 Minute Sample), 375 L (30 Minute Sample)		
Enumeration Plating Media:	50% Tryptic Soy Agar(Supplemented with log phase growth E. coli ATCC 15597)	Tryptic Soy Agar MacConkey Agar	Tryptic Soy Agar Mannitol Salt Agar
Enumeration Plate Incubation Time:	18 – 48 Hours		



Study Notes

Control (Baseline) Run Temperature & Relative Humidity: 23.1°C @ 53%
Test Run Temperature & Relative Humidity: 23.0°C @ 53%

A small fan was placed inside of the test chamber for both Control (Baseline) Run and Test Runs to aid with homogenization of the bioaerosols within the internal environment and allow for sufficient recycling of contaminated air through the device. Continuous airflow in front of the sampling port was ensured using a smoke emitter in place of nebulizer (bioaerosol dispersion point source) with device and fan turned on and positioned in testing configuration. Configuration pictured below.

Study Photographs
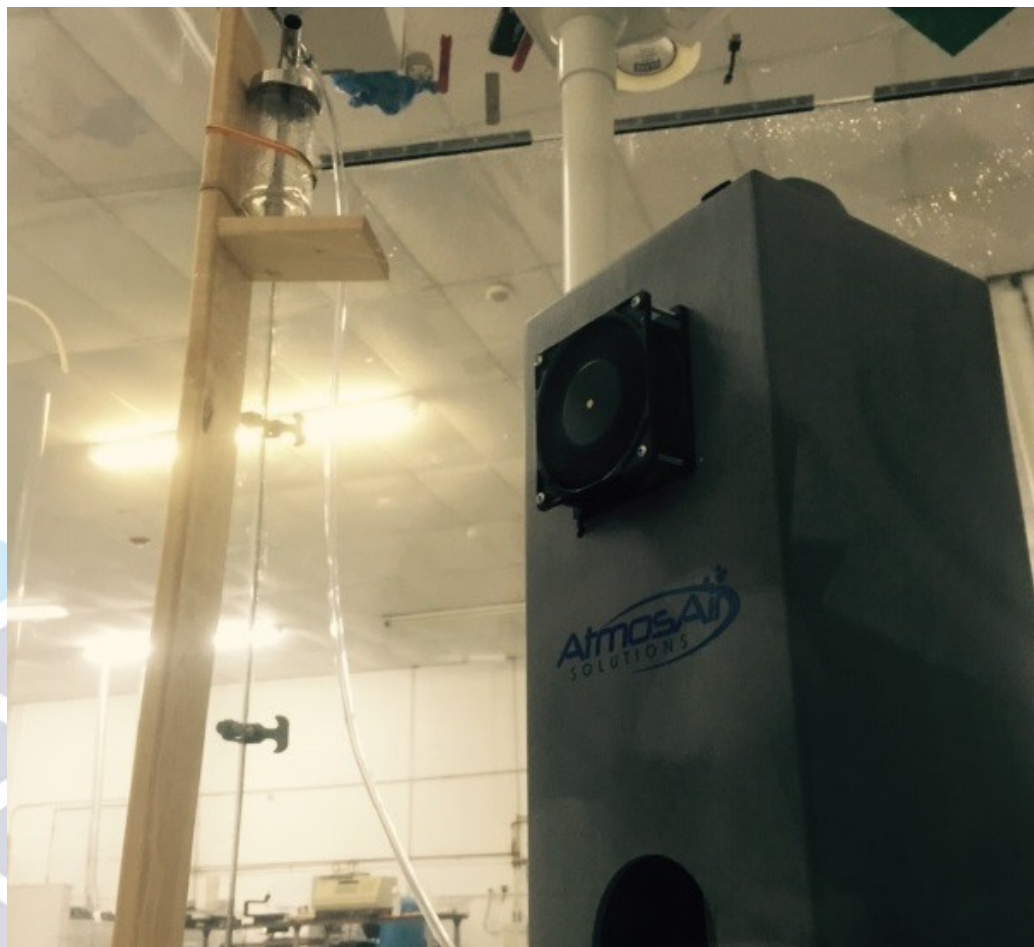
Pictured Above: AtmosAir Matterhorn activated during treatment of air after aerosolization of microorganisms via Collison 6-Jet Nebulizer (Top Left). Testing configuration is such that the built-in fan (inflow) of the device is positioned near the point source of aerosolization and exhaust vent serves to re-suspend bioaerosols after treatment.

Calculations

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

Where:
V_S = Biosampler volume (ml)
T_S = Time sampled (min)

$$Normalization\,Factor\,(N_f) = \left(\frac{CFU/m^3\,Recovered}{CFU/m^3\,Initial} \right)$$

Note: Normalization Factor is per Contact Time (i.e. 15 Minute N_f obtained from Control (Baseline) Run is used for 15 Minute calculations only)

Normalized Numbers Controls = (Initial Numbers Control Recovery (Test Run)) x Corresponding N_f

$$Percent\,Reduction = (\frac{B-A}{B}) \times 100$$

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

$$Log_{10}\,Reduction = Log(\frac{B}{A})$$

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

www.AntimicrobialTestLabs.com - Info@AntimicrobialTestLabs.com - (512) 310-8378

Study Controls and Calculations

Results of the Study: Controls

Microorganism	Test Device	Sampling Time Point	Recovery (CFU/m³)
S. saprophyticus ATCC 35552	N/A	Initial Numbers	4.32E+07
	None Control Run	15 Minutes	3.53E+06
		45 Minutes	4.66E+05

Microorganism	Test Device	Sampling Time Point	Recovery (CFU/m³)
E. coli K12	N/A	Initial Numbers	1.12E+06
	None Control Run	15 Minutes	3.84E+04
		45 Minutes	5.26E+03

Microorganism	Test Device	Treatment Time Point	Recovery (PFU/m³)
MS2 Bacteriophage ATCC 15597-B1	N/A	Initial Numbers	1.17E+08
	None Control Run	15 Minutes	4.13E+07
		45 Minutes	1.55E+07

Neutralization Method: N/A   Media Sterility: Sterile
Growth Confirmation: Positive

www.AntimicrobialTestLabs.com - Info@AntimicrobialTestLabs.com - (512) 310-8378

Results of the Study: Test Run

Microorganism	Test Device	Initial Numbers Control (CFU/m³)	Sampling Time Point	Recovery (CFU/m³)		Percent Reduction vs. Normalized Numbers Control	Log Reduction vs. Normalized Numbers Control
				Normalized Numbers Control	Test Data		
S. saprophyticus ATCC 35552	Matterhorn	4.14E+08	15 Minutes	3.39E+07	2.31E+05	99.32%	2.17
			45 Minutes	4.48E+06	<2.27E+01	99.9995%	5.29

Note: The Limit of Detection (LOD) for this germ is 22.7 CFU/m³. Values below the LOD are represented as <2.27E+01 in the chart above and 0 in the graph below.

Microorganism	Test Device	Initial Numbers Control (CFU/m³)	Sampling Time Point	Recovery (CFU/m³)		Percent Reduction vs. Normalized Numbers Control	Log Reduction vs. Normalized Numbers Control
				Normalized Numbers Control	Test Data		
E. coli K12	Matterhorn	3.42E+07	15 Minutes	1.18E+06	<7.68E+02	>99.94%	3.19
			45 Minutes	1.61E+05	<2.27E+01	>99.986%	>3.85

Note: The Limit of Detections (LOD) for this germ are 768 CFU/m³ and 22.7 CFU/m³ for 15 and 45 minutes, respectively. Values below the LOD are represented as <7.68E+02 and <2.27E+01 in the chart above and 0 in the graph below.

Microorganism	Test Device	Initial Numbers Control (CFU/m³)	Sampling Time Point	Recovery (CFU/m³)		Percent Reduction vs. Normalized Numbers Control	Log Reduction vs. Normalized Numbers Control
				Normalized Numbers Control	Test Data		
MS2 Bacteriophage ATCC 15597-B1	Matterhorn	2.50E+08	15 Minutes	8.84E+07	1.65E+06	98.13%	1.73
			45 Minutes	3.32E+07	2.27E+01	99.99993%	6.17

Note: The Limit of Detection (LOD) for this germ is 22.7 CFU/m³. Values below the LOD are represented as <2.27E+01 in the chart above and 0 in the graph below.

Relative Performance of AtmosAir Matterhorn when Tested Against Bioaerosolized Microorganisms
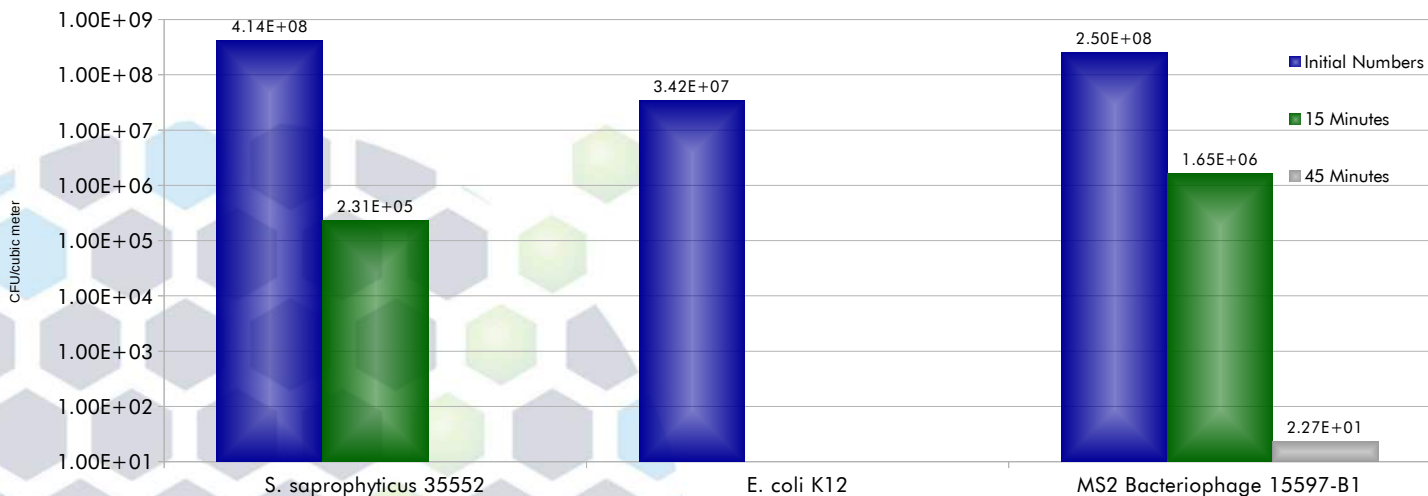
The results of this study apply to the tested substances(s) only. Extrapolation of findings to related materials is the responsibility of the Sponsor.

www.AntimicrobialTestLabs.com - Info@AntimicrobialTestLabs.com - (512) 310-8378