MICROCHEM LABORATORY

Study Update

Sponsor: Gary Russoti, SAM Air Systems **Study ID:** NG16192

Date: 13 NOV 2020

The basic structure of C. difficile spores is similar to the spores of Bacillus subtilis.

Many orthologs to key proteins that constitute the B. subtilis cortex and spore coat are not encoded in C. difficile and the receptors and regulatory pathways that govern spore germination. highlighting the differences between C. difficile spores and other bacterial spores

Test Microorganism	Run Type	Treatment Time Point	Replicate	CFU/m³	Percent Reduction Compared to Time Zero	Log_{10} Reduction Compared to Time Zero	Adjusted Log_{10} Reduction [1] Compared to Baseline
B. subtilis ATCC 19659	Baseline	Time Zero	Replicate 1	5.81E+07	N/A		
		15 Minute	Replicate 1	4.97E+07	14.39%	0.07	N/A
		30 Minute	Replicate 1	4.22E+07	27.27%	0.14	N/A
		60 Minute	Replicate 1	4.14E+07	28.79%	0.15	N/A
		90 Minute	Replicate 1	2.07E+07	64.30%	0.45	N/A
	Test	Time Zero	Replicate 1	9.41E+07	N/A		
		15 Minute	Replicate 1	2.42E+06	97.429%	1.59	1.52
		30 Minute	Replicate 1	6.89E+04	99.9268%	3.14	3.00
		60 Minute	Replicate 1	8.40E+01	99.99991%	6.05	5.90
		90 Minute	Replicate 1	< 8.64E+01	> 99.99991%	> 6.04	> 5.59

The limit of detection for this assay is 8.00+01 CFU/m³ and values below the limit of detection are noted as "<8.00E+01" in the data table.
[1] The Log reductions for the Test Runs are adjusted to account for natural die-off and gravitational settling observed in the Control Run.

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STUDY REPORT

Study Title

Evaluation of Bioaerosols and Antimicrobial Efficacy of SAM Air System's Test Device

Test Method

Custom Aerosol Study

Study Identification Number

NG16192

Study Sponsor

Cynthia Bell
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Scientific Air Management
1301 West Copans Road suite C6
Pompano Beach, Florida

Test Facility

Microchem Laboratory
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Round Rock, TX 78681
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Report Author: Samuel Hanley, B.S.

Purpose of the Study

The purpose of this study is to document the antimicrobial efficacy of SAM Air System's Test Device

Study Timeline

Devices Received	Cultures Initiated	Chamber Run	Nebulization Initiated and Treatment	Enumeration Plates Evaluated	Report Delivered
Baseline: *Bacillus subtilis* ATCC 19659					
01 SEP 2020	05 NOV 2020	05 NOV 2020	05 NOV 2020	06 NOV 2020	20 NOV 2020
Test: *Bacillus subtilis* ATCC 19659					
01 SEP 2020	10 NOV 2020	10 NOV 2020	10 NOV 2020	12 NOV 2020	20 NOV 2020
Baseline: *Mycobacterium smegmatis* ATCC 607					
01 SEP 2020	03 NOV 2020	13 NOV 2020	13 NOV 2020	16 NOV 2020	20 NOV 2020
Test: *Mycobacterium smegmatis* ATCC 607					
01 SEP 2020	12 NOV 2020	16 NOV 2020	16 NOV 2020	18 NOV 2020	20 NOV 2020

Test Device Information

Name of Test Device: SAM S400
Manufacturer: SAM Air Systems

Note: The image above depict the test device, SAM S400, which was provided by the Study Sponsor for use in testing.

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Test Microorganism Information

The following test microorganisms were selected for this test:

Bacillus subtilis

This bacteria is Gram-positive, rod shaped, capable of forming endospores. Endospores of *Bacillus subtilis* can tolerate harsh environmental conditions such as UV exposure and high temperatures. Typically found in soil, this species is not known to cause disease in healthy individuals, but can be considered an opportunistic pathogen among the immuno-compromised. *Bacillus subtilis* endopores serve as one of the models for evaluating the effectiveness of sporicides and sterilants.

Mycobacterium smegmatis ATCC 607: This bacteria is an acid-fast, bacillus-shaped, aerobic microorganism that is commonly used a surrogate model for *M. tuberculosis* and is found in soil, plants, and water. *M. Smegmatis* is non-pathogenic to humans except in rare cases, and is considered saprophytic. Unlike other pathogenic *Mycobacterium*, *M. Smegmatis* isn't dependent on living in animals. *M. smegmatis* shares a number of morphological traits with *M. Tuberculosis* including the distinctive waxy cell wall that provides a robust resistance to chemical disinfectants and sanitizers. The quick growth rate of this microorganism is ideal for in-vitro testing, as other bacteria in this Genus may take several weeks to demonstrate growth. Due to the non-pathogenic nature of this organism, it is used as a *M. Tuberculosis* model for aerosol disinfection testing.

Criteria for Scientific Defensibility of a Custom Device Study

For Microchem Laboratory to consider a Device Study study to be scientifically defensible, the following criteria must be met:

1. The average number of viable bacteria, fungi, or bacteriophage recovered from the time zero samples should be approximately 1 x 10^5 cells/m^3.
2. Positive/Growth controls must demonstrate growth of the appropriate test microorganism.
3. Negative/Purity controls must demonstrate no growth of test microorganism.
4. The neutralization test suspension must be ≥ 70% of that recorded for the neutralization control suspension count.

Passing Criteria

Because of the nature of the study, passing criteria may be determined by the Study Sponsor.

Testing Parameters used in this Study

Volume of inoculum added to Nebulizer	20.0 ml	**Nebulization Time**	60 minutes
Sampler Media (Volume)	Phosphate buffered saline (20.0 ml)	**Neck Rinse Media (Volume)**	Phosphate buffered saline (5.0 ml)
Sampling Time	10 minutes	**Contact Times**	Time zero 15 minutes 30 minutes 60 minutes 90 minutes
Sampling Type	Impingers, SKC biosamplers	**Enumeration Media**	BHIA (MS607) TSA (BS19569)
Incubation Temperature	36±1°C	**Incubation Time**	24-72 hours

PARAMETERS

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Summary of the Procedure

-
- Test microorganisms are grown on appropriate media.
- Culture used for test inoculum are evaluated for sterility, washed and concentrated in sterile phosphate buffered saline upon harvesting.
- The test inoculum is split into two equal parts and added to the appropriate number of nebulizers. Liquid culture should not exceed 20 ml per nebulizer.
- The device is setup per protocol requirements and operated per manufacturer's instructions.
- The chamber is setup and the safety checklist is completed prior to test initiation.
- Test is initiated by aerosolizing the microorganisms per the nebulizers and allowing the concentration to reach the required CFU/m^3. Once the concentration is reached, a time zero sample is taken then the device is run for the specified contact time and an additional sample is taken for each contact time.
- The decontamination process is run, 4 hours of UV exposure, prior to any scientists entering the testing chamber.
- Samples are enumerated using standard dilution and plating techniques.
- Microbial concentrations are determined after appropriate incubation times.
  Reductions of microorganisms are calculated relative to concentration of the time zero or corresponding control run sample as applicable.

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Study Notes:

4.0 ml of *Bacillus subtilis* ATCC 19659 spore stock was added to 46.0 ml of Phosphate Buffered Saline and mixed. 20.0 ml of inoculum was added to each nebulizer on testing that occurred on 05 NOV 2020 and 10 NOV 2020.

30.0 ml of *Mycobacterium smegmatis* ATCC 607 culture was added to 15.0 ml of Phosphate Buffered Saline and mixed. 20.0 ml of inoculum was added to each nebulizer on testing that occurred on 13 NOV 2020.

32.0 ml of *Mycobacterium smegmatis* ATCC 607 culture was added to 12.0 ml of Phosphate Buffered Saline and mixed. 20.0 ml of inoculum was added to each nebulizer on testing that occurred on 16 NOV 2020.

Per Study Sponsor instruction a small fan was placed in the upper corner of the NPAC chamber and allowed to run during the chamber runs to simulate the air circulation of an AC system.

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Study Photos

Image 1: Test set up on 10 NOV 2020.

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Control Results

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

Calculations

CFU/ml = (Average plate count) x 1:10 serial dilution factor

$CFU/m^3 = [(CFU/ml \times V_s) \div (T_s \times 12.5\ L/min)] \times (1000\ L/m^3)$

Where:
V_S = Bio-sampler volume (ml)
T_S = Time sampled (min)

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

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

Where:
B = Number of viable test microorganisms at time zero after nebulization
A = Number of viable test microorganisms after the contact time

Results of the Study

Test Microorganism	Run Type	Treatment Time Point	Replicate	CFU/m³	Percent Reduction Compared to Time Zero	Log_{10} Reduction Compared to Time Zero	Adjusted Log_{10} Reduction [1] Compared to Baseline
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Test Microorganism	Run Type	Treatment Time Point	Replicate	CFU/m³	Percent Reduction Compared to Time Zero	Log_{10} Reduction Compared to Time Zero	Adjusted Log_{10} Reduction [1] Compared to Baseline
M. Smegmatis ATCC 607	Baseline	Time Zero	Replicate 1	5.29E+07	N/A		
		15 Minute	Replicate 1	4.41E+07	16.7%	0.08	N/A
		30 Minute	Replicate 1	4.51E+07	14.78%	0.07	N/A
		60 Minute	Replicate 1	3.42E+07	35.27%	0.19	N/A
		90 Minute	Replicate 1	2.55E+07	51.75%	0.32	N/A
	Test	Time Zero	Replicate 1	3.10E+08	N/A		
		15 Minute	Replicate 1	4.19E+05	99.86%	2.87	2.79
		30 Minute	Replicate 1	2.96E+05	99.905%	3.02	2.95
		60 Minute	Replicate 1	9.59E+03	99.997%	4.51	4.32
		90 Minute	Replicate 1	< 8.56E+01	> 99.99997%	> 6.56	> 6.24

The limit of detection for this assay is 8.00E+01 CFU/m³ and values below the limit of detection are noted as "<8.00E+01" in the data table.
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Additional Observations

Table 1. Chamber Temperature and Humidity

Chamber Run	Chamber Temperature (Start/End)	Humidity (Start/End)
Baseline *B. subtilis* ATCC 19659	23.6°C/23.6°C	31%/36%
Test *B. subtilis* ATCC 19659	23.5°C/23.4°C	33%/37%
Baseline *M. Smegmatis* ATCC 607	23.7°C/23.7°C	32%/36%
Test *M. Smegmatis* ATCC 607	21.1°C/21.8°C	26%/29%

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