MICROCHEM LABORATORY

STUDY REPORT

Study Title

Evaluation of Bioaerosols Efficacy for LUV System's UVC Device

Test Method

Custom Aerosol Study

Study Identification Number

NG21481

Study Completion Date

20JUL2023

Study Sponsor

LUV System, Inc.
Sandy Seth
sandy@luvsystems.com

Test Facility

Microchem Laboratory
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(512) 310-8378 #6
Reported by: Alex Noyola, B.S.

Purpose of the Study

The purpose of this study is to document the antimicrobial efficacy of LUV System's "the halō" UVC device. Model Number: 5R/M

Study Timeline

Test Device Received	Cultures Initiated	Chamber Run	Nebulization Initiated and Treatment	Enumeration Plates Evaluated	Report Delivered
		Control			
29JUN2023	05JUL2023	05JUL2023	05JUL2023	06JUL2023	20JUL2023
		Test			
29JUN2023 (15 Mins)	29JUN2023	29JUN2023	29JUN2023	30JUN2023	20JUL2023
29JUN2023 (24 Mins)	03JUL2023	03JUL2023	03JUL2023	05JUL2023	20JUL2023
29JUN2023 (30 Mins)	30JUN2023	30JUN2023	30JUN2023	01JUL2023	20JUL2023

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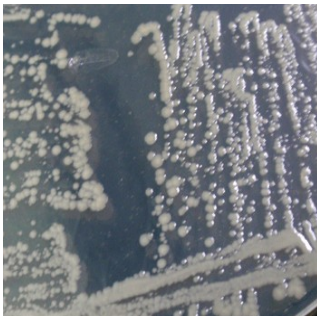
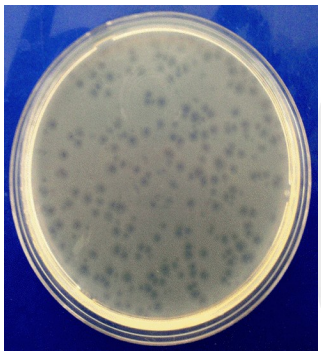
Test Microorganism Information

The following test microorganisms were selected for this test:



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.



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*, 15597

[1] Based on EPA data collected over MS2 aerosol particle size, "The count median diameter of aerosolized particle was 46 nm (0.046 µm) at beginning of each test (time = 0 minutes) and increased over the duration of test to 100 nm (0.1 µm) at end time of 120 minutes".

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Criteria for Scientific Defensibility of a Custom Device Study

For Microchem Laboratory to consider a Test Substance 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 CFU/m^3 or PFU/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 Each Nebulizer	14 mL	**Nebulization Time**	10 minutes
Sampler Media (Volume)	Phosphate buffered saline (20 mL)	**Neck Rinse Media (Volume)**	Phosphate buffered saline with (5 mL)
Sampling Time	10 minutes	**Contact Times**	Time zero 15 Minutes 24 Minutes 30 Minutes
Sampling Type	Impingers, SKC biosamplers	**Enumeration Media**	50% Trypic Soy Agar (Virus)
Incubation Temperature	36±1°C	**Incubation Time**	12-24 Hours

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Summary of the Procedure

- Test microorganisms were grown on appropriate media.
- Cultures used for test inoculum were evaluated for sterility, washed, and concentrated in sterile phosphate buffered saline upon harvesting.
- The test inoculum was split into two equal parts of 14.0 mL and added to the appropriate (2)two Six-jet Collison nebulizers.
- The Test Device was setup per Study Sponsor requirements and operated per Sponsor instructions.
- The chamber size measures 19'9" L x 25'4" W x 10' H or ~ 141.2 m^3
- The chamber was setup and the safety checklist was completed prior to test initiation.
- Test was initiated by aerosolizing the microorganisms per the nebulizers and allowing the concentration to reach the required CFU/m^3 or PFU/m^3. Once the concentration was reached, a time zero sample was taken then the device was run for the specified contact time and an additional sample was taken for each contact time.
- At the conclusion of testing all UV lights equipped in the chamber, not including test device, were activated to facilitate decontamination. The decontamination process was run, 2-4 hours of UV exposure, prior to any scientists entering the testing chamber.
- Samples were enumerated using standard dilution and plating techniques.
- Microbial concentrations were determined after appropriate incubation times.
  Reductions of microorganisms were calculated relative to concentration of the time zero or corresponding control run sample as applicable.

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Study Photos:

Study Notes:

- The test device was allowed to be primed ≥10 minutes prior to nebulization to act as a warm up cycle.

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Control Results

Harvest Media: Sterile
Growth Conformation: Pure

PBS Dilution: Sterile
Enumeration Media: Sterile

Calculations

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

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

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

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

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

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

Adjusted Log Reduction = Log reduction of test – the log reduction of parallel baseline

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Results of the Study

Table 1: Results from MS2 baseline chamber run performed on 05JUL2023.

Baseline							
Test Microorganism	Test Device	Treatment Time Point	Replicate	PFU/m^3	Average PFU/m^3	Percent Reduction Compared to Time Zero	Log_{10} Reduction Compared to Time Zero
MS2 Bacteriophage ATCC 15597-B1	The Halo 5R/M	Time Zero	Replicate 1	4.80E+05	4.35E+05	N/A	N/A
			Replicate 2	4.90E+05			
			Replicate 3	3.34E+05			
		30 Minutes	Replicate 1	1.32E+05	1.49E+05	65.68%	0.46
			Replicate 2	1.52E+05			
			Replicate 3	1.64E+05			

Table 2: Results from MS2 test chamber run performed on 29JUN2023.

Test							
Test Microorganism	Test Device	Treatment Time Point	Replicate	PFU/m^3	Average PFU/m^3	Percent Reduction Compared to Time Zero	Log_{10} Reduction Compared to Time Zero
MS2 Bacteriophage ATCC 15597-B1	The Halo 5R/M	Time Zero	Replicate 1	9.36E+05	7.81E+05	N/A	N/A
			Replicate 2	8.51E+05			
			Replicate 3	5.54E+05			
		15 Minutes	Replicate 1	3.89E+04	4.25E+04	94.55%	1.26
			Replicate 2	4.34E+04			
			Replicate 3	4.53E+04			

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Results of the Study (cont.)

Table 3: Results from MS2 chamber run performed on 03JUL2023.

Test							
Test Microorganism	Test Device	Treatment Time Point	Replicate	PFU/m^3	Average PFU/m^3	Percent Reduction Compared to Time Zero	Log_{10} Reduction Compared to Time Zero
MS2 Bacteriophage ATCC 15597-B1	The Halo 5R/M	Time Zero	Replicate 1	5.76E+05	5.52E+05	N/A	N/A
			Replicate 2	7.13E+05			
			Replicate 3	3.68E+05			
		24 Minutes	Replicate 1	3.94E+03	8.85E+03	98.40%	1.80
			Replicate 2	1.21E+04			
			Replicate 3	1.05E+04			

Table 4: Results from MS2 chamber run performed on 30JUN2023.

Test							
Test Microorganism	Test Device	Treatment Time Point	Replicate	PFU/m^3	Average PFU/m^3	Percent Reduction Compared to Time Zero	Log_{10} Reduction Compared to Time Zero
MS2 Bacteriophage ATCC 15597-B1	The Halo 5R/M	Time Zero	Replicate 1	4.77E+05	5.11E+05	N/A	N/A
			Replicate 2	5.99E+05			
			Replicate 3	4.57E+05			
		30 Minutes	Replicate 1	1.51E+03	1.72E+03	99.66%	2.47
			Replicate 2	1.88E+03			
			Replicate 3	1.78E+03			

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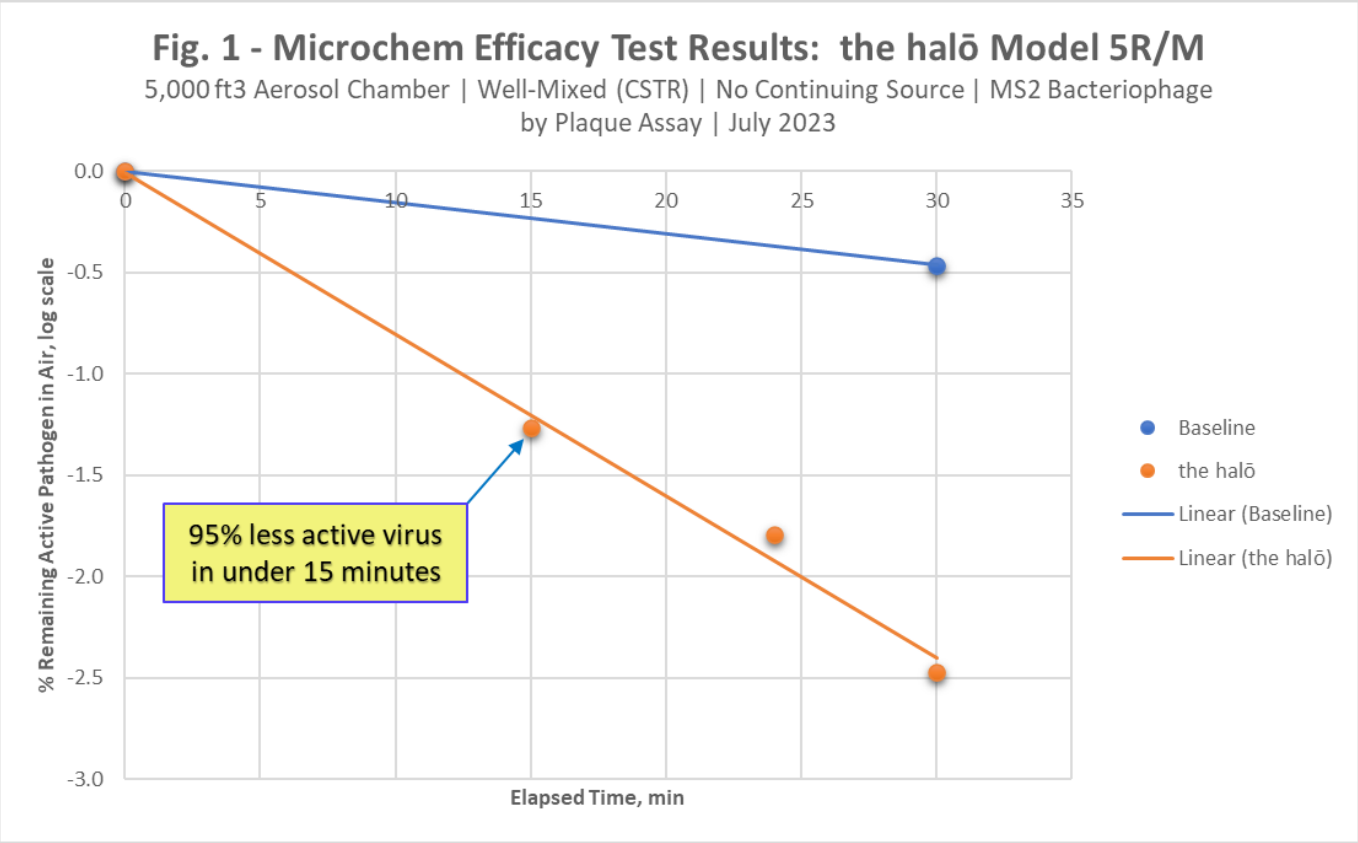
Results of the Study (cont.)



RESULTS

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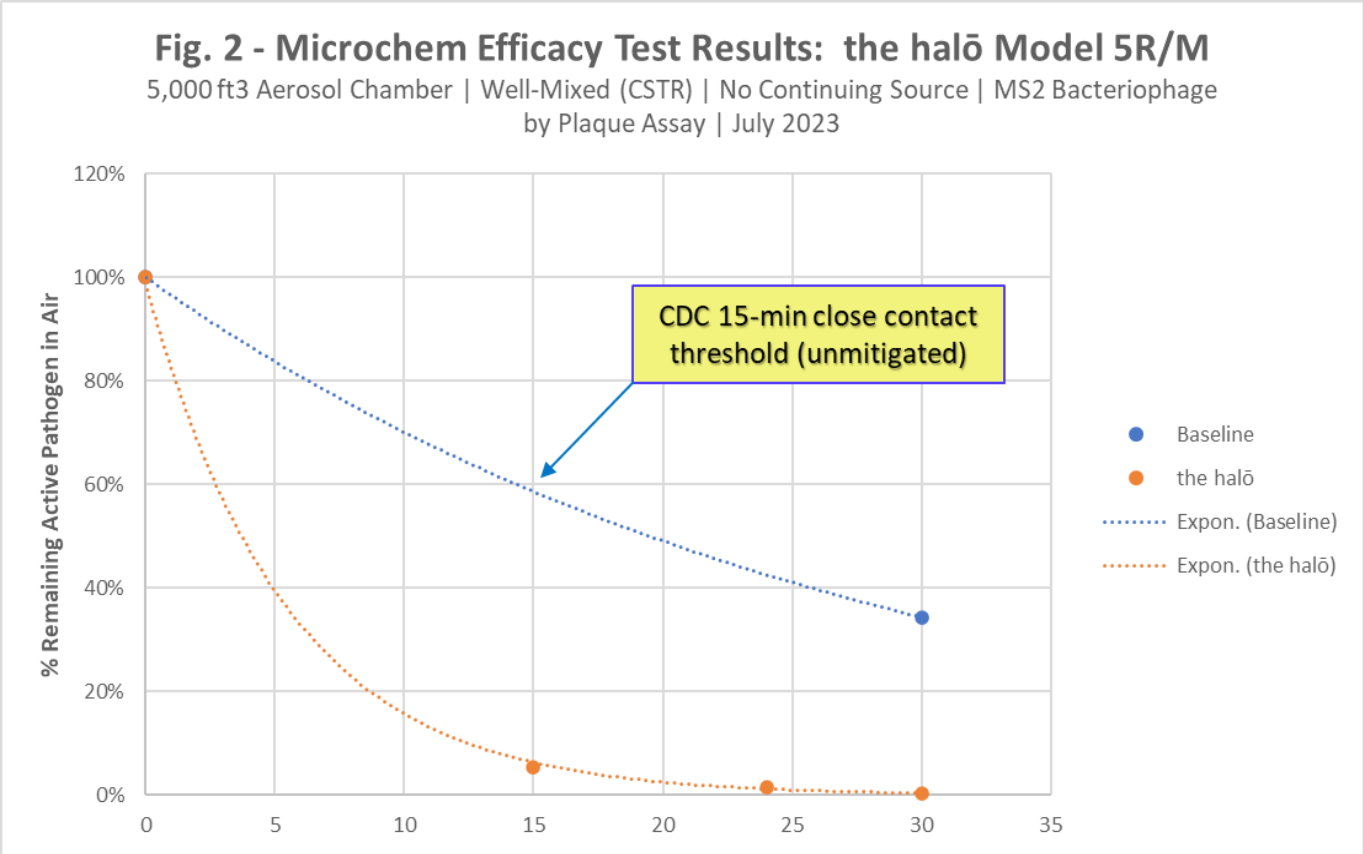
Results of the Study (cont.)



References

[1]*PuriFi AMP technology tested by the EPA's Homeland Security Research Division under highly rigorous HVAC mechanical conditions, 2022.*

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

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