MICROCHEM LABORATORY

STUDY REPORT

## Study Title

Evaluation of Bioaerosols and Antimicrobial Efficacy of Fresh Light's Test Device

## Test Method

Custom Aerosol Study

## Study Identification Number

NG18094

## Study Sponsor

Steve Collins
Fresh Light LLC.
steve.collins@freshlightllc.com

## Test Facility

Microchem Laboratory
1304 W. Industrial Blvd
Round Rock, TX 78681
(512) 310-8378
Report Author: Samuel Hanley, B.S.

## Purpose of the Study

The purpose of this study is to document the antimicrobial efficacy of Fresh Light's Test Device

## Study Timeline

| Devices Received | Cultures Initiated | Chamber Run | Nebulization Initiated and Treatment | Enumeration Plates Evaluated | Report Delivered |
|---|---|---|---|---|---|
| Control Run | | | | | |
| 21APR2021 | 21JUN2021 | 21JUN2021 | 21JUN2021 | 22JUN2021 | 09JUL2021 |
| Test Run | | | | | |
| 21APR2021 | 01JUL2021 | 01JUL2021 | 01JUL2021 | 02JUL2021 | 09JUL2021 |





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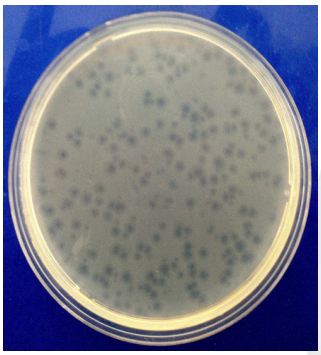
## Test Device Information

**Name of Test Device:** Ionizer
**Manufacturer:** Fresh Light

## Test Microorganism Information

The following test microorganisms were 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*, 15597

# 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<br>15 minutes<br>30 minutes<br>60 minutes<br>90 minutes |
| **Sampling Type** | Impingers,<br>SKC biosamplers | **Enumeration Media** | 50% Tryptic Soy Agar |
| **Incubation Temperature** | 36±1°C | **Incubation Time** | 18-24 hours |

PARAMETERS



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## Summary of the Procedure

- Test microorganisms were grown on appropriate media.
- Culture 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 and added to the appropriate number of nebulizers. Liquid culture should not exceed 20 ml per nebulizer.
- The device was setup per protocol requirements and operated per manufacturer's instructions.
- 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 PFU/$m^3$. Once the concentration was 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:

Test device was set up per study sponsor instructions, Ionizer was hung from ceiling of chamber approximately 6 feet above the ground.





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## Control Results

Neutralization Method: Confirmed
Growth Confirmation: Confirmed
Media Sterility: 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)

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

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

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

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

# Results of the Study

| Baseline | | | | | | |
|---|---|---|---|---|---|---|
| Test Microorganism | Test Device | Treatment Time Point | Replicate | PFU/m³ | Percent Reduction Compared to Time Zero | $Log_{10}$ Reduction Compared to Time Zero |
| MS2 Bacteriophage ATCC 15597-B1 | N/A | Inoculum | N/A | 4.70E+09 | N/A | N/A |
| | | Time Zero | Replicate 1 | 9.16E+07 | | |
| | | 15 minutes | Replicate 1 | 4.35E+07 | 52.48% | 0.32 |
| | | 30 minutes | Replicate 1 | 1.26E+07 | 86.26% | 0.86 |
| | | 60 minutes | Replicate 1 | 8.30E+06 | 90.93% | 1.04 |
| | | 90 minutes | Replicate 1 | 2.93E+06 | 96.80% | 1.49 |

*Inoculum is enumerated as PFU/ml. All other enumerations are PFU/m³*
*[1]The Log reductions for the Test Runs are adjusted to account for natural die-off and gravitational settling observed in the Control Run.*

| Test 1 | | | | | | | |
|---|---|---|---|---|---|---|---|
| Test Microorganism | Test Device | Treatment Time Point | Replicate | PFU/m³ | Percent Reduction Compared to Time Zero | $Log_{10}$ Reduction Compared to Time Zero[1] | Adjusted $Log_{10}$ Reduction [1] Compared to Baseline |
| MS2 Bacteriophage ATCC 15597-B1 | Fresh Light Ionizer | Inoculum | N/A | 2.50E+10 | N/A | N/A | N/A |
| | | Time Zero | Replicate 1 | 8.44E+07 | | | |
| | | 15 minutes | Replicate 1 | 6.99E+06 | 91.72% | 1.08 | 0.76 |
| | | 30 minutes | Replicate 1 | 2.60E+06 | 96.92% | 1.51 | 0.65 |
| | | 60 minutes | Replicate 1 | 4.58E+05 | 99.46% | 2.27 | 1.22 |
| | | 90 minutes | Replicate 1 | 1.11E+05 | 99.87% | 2.88 | 1.38 |

*Inoculum is enumerated as PFU/ml. All other enumerations are PFU/m³*
*[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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# Additional Observations

Table 1: Chamber Temperature and Humidity

| Chamber Run | Chamber Temperature (Start/End) | Humidity (Start/End) |
|---|---|---|
| Baseline | 24.9°C/25.9°C | 47%/51% |
| Test 1 | 23.1°C/23.8°C | 52%/53% |



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