FINAL REPORT

Efficacy of a Bipolar Ionization System

ORDER Number
151508127

PREPARED FOR:

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Certificate of Analysis

Client: O2PRIME
Contact: Darline Moore
Project: O2PRIME-2400 Efficacy Testing

Product: O2PRIME-2400
EMSL NO: 151508127-rev1
Sample received: 10/1/15
Start date: 10/7/15
Report date: 10/14/15
Challenge Bacteria: *Legionella pneumophila*

Experimental Summary:

The testing procedure was designed after discussions between EMSL Analytical, the testing company, and the client, O2PRIME. The testing was conducted on the O2PRIME-2400 system for its ability to disinfect (kill) bacteria on a solid surface. The testing was conducted in our Houston Microbiology Laboratory.

Procedure:

Bacterial Inoculum Preparation

Legionella pneumophila (L. pneumophila) was inoculated onto buffered charcoal yeast extract agar (BCYE) and incubated at 35°C for 48 hours. Colonies were harvested, suspended in phosphate buffer water, and vortexed for 1 minute to ensure homogenization. This suspension was then used to inoculate the test carriers.

Inoculation of the Test Carriers

Sterile Petri dishes were labeled as follows: Control, Time 5 minutes, Time 15 minutes, and Time 30 minutes. Carriers were placed in each labeled Petri dish, and then 10µL of the bacterial solution was placed in the middle of the carrier and spread evenly. This was repeated in triplicate for each time point and the control (Total of 12 carriers). The inoculated carriers were then allowed to air dry (~15 minutes) inside a biological safety cabinet.

Efficacy Testing

The O2PRIME-2400 system was set up facing down with 1 inch of clearance from the surface. The test carrier in its respective Petri dish was then placed under the system and turned on. After 5 minutes, the Petri dish was removed and the carrier placed into 10 mL of PBS for washing. This was repeated for each

replicate and each additional exposure time of 15 minutes and 30 minutes. After exposure the carriers were placed into 10 mL of phosphate buffer water (PBS) for washing and bacteria recovery. The control carriers were not exposed to the ionizer and instead placed directly into 10 mL of PBS.
Serial dilutions were prepared from each of the carrier washings by taking 1 mL out and placing it into 9 mL of PBS. For each dilution 100 µL was plated onto a BCYE agar plate. The inoculated plates were incubated at 35°C for 5 days and then any recovered colonies were counted.

Experimental Results:

Table 1: Reduction of *L. pneumophila*

***L. pneumophila* Control**			***L. pneumophila* Test**	
Time (min)	**Avg CFU**	**Log10**	**LR**	**%Reduction**
Control	$3.73x10^5$	5.57		
5	$3.24x10^5$	5.51	0.06	13.13
15	$6.43x10^3$	3.81	1.76	98.28
30	$1.07x10^3$	3.03	2.54	99.71

Log Reduction and %Reduction compares initial CFU and treated CFU recovered.
Any negative LR or %Reduction is the result of an increase in cells. ND=none detect <100.
Blank controls had no growth, Limit of detection = 100 CFU.

Conclusions/Observations:

The efficacy of the O2PRIME-2400 system to disinfect a solid surface against *Legionella pneumophila* was tested. The O2PRIME-2400 system demonstrated the strongest efficacy after 30 minutes of exposure by killing 99.71% of the *L. pneumophila* bacteria.

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Rev1: report was revised 10/15/15 to correct the product name