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Pathogen Testing Results Executive Summary

SMP recently invested substantial resources for independent testing to confirm kill rates of various pathogens in a real world application using our patent pending needlepoint bi-polar ionization technology. The test was designed to prove kill rates of MRSA, E.Coli, c. Diff and TB, in the space, which can be found in many applications including hospitals, schools, correctional facilities, airports and many others as well.

Background

Until recently, documented pathogen control had been limited to UV lights. It is well known that UV lights can kill airborne pathogens, but UV lights can only kill pathogens the UV light can shine on. Pathogen kill rates in systems utilizing UV lights are determined by the intensity of the UV light and the time or speed of the pathogen through the light. UV lights have several issues: first, from the time UV lights are energized, the output starts to diminish and for this reason UV lights are generally replaced annually; second, UV lights are not safe for service technicians because they can burn your skin and eyes; third, they contain mercury; fourth, UV lights will deteriorate filters, duct insulation, wiring insulation and any other type of organic material or rubber. While UV lights can work for pathogen control in recirculating systems (see Figure 2 below), they cannot actively kill pathogens in the space. In addition to not being adaptable to single pass systems without recirculation (see Figure 1 below), systems with UV lights have to rely on the ventilation effectiveness in getting the airborne pathogen back to the air handler for a chance to kill it. For the sake of argument, let's say a UV light system kills 100% of what passes by it; if only 20% of the pathogens return to the air handler from the space, then only 20% of the actual pathogens are controlled, even with a 100% effective kill rate. This is not beneficial if you are one of the occupants subjected to the remaining 80% pathogen load in the space.

Amazing Results of O2PRIME Needlepoint Ion Technology

Unlike UV light technology, O2PRIME needlepoint ion technology reaches down into the space and kills pathogens at the source before they can infect healthy occupants, see Figure 1 below. Based on the recent data provided by the world renowned testing agency, EMSL Laboratories, O2PRIME technology provided the following extraordinary results:

Pathogen	Time Exposed	Kill Rate Using Needlepoint Bi-Polar Ionization
E.coli	15 minutes	99.68%
MRSA	30 minutes	96.24%
c. Diff	30 minutes	86.87%
TB	60 minutes	69.01%

The benefits to O2PRIME ion technology are as follows:

1. Kills mold, bacteria, and virus on the surface and in the air
2. No replacement parts required
3. Keeps cooling coil clean when mounted on the air entering side of the coil (just like UV light would do on the air leaving side of the coil)
4. Kills pathogens in the space
5. Does not produce ozone per the UL867-2007 ozone chamber test
6. Requires essentially no maintenance in non-smoking applications
7. Shock free design
8. Fits any size system and any layout configuration
9. Little to no air pressure drop
10. Controls gases and odors in the space
11. Using the IAQ Procedure from ASHRAE 62, outside air may be reduced in most applications to 5 CFM per person or less

FINAL REPORT

Efficacy of a Bipolar Ionization System

ORDER Number
371106420

PREPARED FOR:

Sustainability Management Partners
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Evansville, IN 47714

EMSL Analytical, Inc.
200 Rt. 130 N, Cinnaminson, NJ 08077
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Certificate of Analysis

Client: O2PRIME

Contact: Darline Moore
Project: Bipolar Ionization System

Product: O2PRIME-IBAR-36
EMSL NO: 371106420

Sample received: 5/25/2011
Start date: 6/28/2011
Report date: 7/15/2011
Challenge Bacteria: *Mycobacterium terrae* ATCC 15755

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-IBAR-36 for its ability to disinfect (kill) bacteria in the air. The testing was conducted in our Cinnaminson Microbiology Laboratory.

Procedure:

Bacteria

Mycobacterium terrae (*M. terrae*) is commonly used as a surrogate test for *Mycobacterium turberculosis* as it demonstrates similar physical characteristics and is slightly more resistant but is far less dangerous. *M. terrae* first was innoculated on Tryptic Soy agar + 5% sheep blood (TSAB) and incubated at 35°C for 5 days under carbon dioxide conditions. A sterile inoculation loop was then used to collect colonies and place them into 5 mL of normal saline solution. This solution was then washed three times with Phosphate buffer at 3,000 x g for 20 min. Ten milliliter of this dilution was then placed into the base of the nebulizer and mixed with 90 mL of Phosphate buffer to create an additional 1:10 dilution.

Environmental Chamber

The environmental chamber was set-up as per the instructions included. One computer fan was placed in the center of the chamber to provide air movement and the two ionizers were placed on either side about 1 inch off the ground. Before testing began the entire chamber was disinfected with a disinfectant solution (5% Hydrogen peroxide with accompanying silver ionic solution), as well as cleaning the fans and ionizers with alcohol wipes. Additionally, between all

testing the disinfectant solution was sprayed throughout the chamber and allowed to air out with the fans running for at least 2 hr.

Inoculation of the Test Chamber

The nebulizer was connected to an air compressor with ¼ inch plastic tubing and to the environmental test chamber through one of the testing openings created. The fan was turned on to create an air flow in the chamber but the ionizers were not turned on until after the initial sampling. Once testing was ready to begin 60 psi of compressed air was pumped through the nebulizer, creating the release of 10.8 mL/h of aerosolized solution. This was run for 28 min allowing for a total of 5 mL of solution being aerosolized into the test chamber.

Organism Collection

Immediately, following inoculation of the test chamber an initial collection of the bacteria was taken without the use of the bipolar ionizer. The bacteria were collected with an Anderson impactor at the sample time points 1 min (100 L), 5 min (100 L), 15 min (100 L), 30 min (150 L) and 60 min (150 L) in order to determine the natural rate of decay for *M. terrae*. This data was then compared to the data collected when the ionizer was run to create a corrected Log Reduction. The test run was then completed identically the same with the exception that the bipolar ionizer was turned on. Bacteria were collected using TSAB plates and incubated at 35°C for 5 days under carbon dioxide conditions. Afterwards, colonies were counted and statistics were performed on the data. All samples were completed in triplicate.

Experimental Results:

Table 1: Reduction of *M. terrae*

***M. terrae* Control**			***M. terrae* Test**			
Time (min)	**CFU/m^3**	**Log10**	**CFU/m^3**	**Log10**	**Corrected LR**	**%Reduction**
1	$1.28x10^4$	4.11	$3.67x10^4$	4.56	0.00	0
5	$1.01x10^4$	4.01	$2.44x10^4$	4.39	0.18	33.58%
15	$8.50x10^3$	3.93	$1.47x10^4$	4.17	0.22	39.48%
30	$6.51x10^3$	3.81	$7.83x10^3$	3.89	0.38	57.99%
60	$4.61x10^3$	3.66	$4.08x10^3$	3.61	0.51	69.09%

Corrected LR = Log Reduction that has been compared to natural rate of decay for *M. terrae*
Log Reduction and %Reduction compares initial CFU and specified CFU
A negative LR or %Reduction is the result of an increase in cells

Conclusions/Observations:

The efficacy of the O2PRIME-IBAR-36, a bipolar ionization system, to disinfect the air against *M. terrae* was analyzed. After correcting for the natural rate of decay it was observed that there was a 0.38 log reduction after 30 min exposure and a
0.51 log reduction after 60 min exposure (Table 1).
In conclusion, the O2PRIME-IBAR-36 was observed to reduce *M. terrae* by 69.09%. Furthermore, these results demonstrate that the bipolar ionization system tested does not require direct line of sight to produce kill rates like ultraviolet light. The bipolar ionization system's kill rates are indicative of those in the entire space.

Farbod Nekouei, M.S., Laboratory Manager
or Other Approved Signatory

FINAL REPORT

Efficacy of a Cold Plasma System

ORDER Number
371106420

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Certificate of Analysis

Client: O2PRIME
Contact: Darline Moore
Project: Cold Plasma - Needlepoint Bipolar Ionization System

Product : O2PRIME-IBAR-36
EMSL NO: 371106420

Sample received: 5/25/2011
Start date: 6/2/2011
Report date: 6/13/2011
Challenge Bacteria: Methicillin Resistant *Staphylococcus aureus* (MRSA) ATCC 33591

Experimental Summary: The testing procedure was designed after discussions between EMSL Analytical, the testing company, and the client, 02PRIME. The testing was conducted on the O2PRIME-IBAR-36 for its ability to disinfect (kill) bacteria in the air. The testing was conducted in our Cinnaminson Microbiology Laboratory.

Procedure:

Bacteria

Methicillin Resistant *S. aureus* (MRSA) was inoculated on Tryptic Soy agar (TSA) and incubated at 35°C for 24 h. A single isolated colony was then taken and inoculated into Tryptic Soy broth (TSB) and incubated at 35°C for 24 h. This solution was then washed three times with Phosphate buffer at 3,000 ug for 20 min. A one to ten dilution was then made by removing 1 mL of inoculated TSB and placing it into 9 mL of Phosphate buffer. One milliliter of this dilution was then placed into the base of the nebulizer and mixed with 99 mL of Phosphate buffer to create an additional 1:100 dilution.

Environmental Chamber

The environmental chamber was set-up as per the instructions included. One computer fan was placed in the center of the chamber to provide air movement and the two ionizers were placed on either side about 1 inch off the ground. Before testing began the entire chamber was disinfected with a disinfectant solution (5% Hydrogen peroxide with accompanying silver ionic solution), as well as cleaning the fans and ionizers with alcohol wipes. Additionally, between all testing the disinfectant solution was sprayed throughout the chamber and allowed to air out with the fans running for at least 2 hr.

Inoculation of the Test Chamber

The nebulizer was connected to an air compressor with ¼ inch plastic tubing and to the environmental test chamber through one of the testing openings created. The fan was turned on to create an air flow in the chamber but the ionizers were not turned on until after the initial sampling. Once testing was ready to begin 60 psi of compressed air was pumped through the nebulizer, creating the release of 10.8 mL/h of aerosolized solution. This was run for 28 min allowing for a total of 5 mL of solution being aerosolized into the test chamber.

Organism Collection

Immediately, following inoculation of the test chamber an initial collection of the bacteria was taken without the use of the bipolar ionizer. The bacteria were collected with an Anderson impactor at the sample time points 1 min (50 L), 5 min (75 L), 15 min (100 L) and 30 min (150 L) in order to determine the natural rate of decay for MRSA. This data was then compared to the data collected when the ionizer was run to create a corrected Log Reduction. The test run was then completed identically the same with the exception that the cold plasma generator was turned on. Bacteria were collected using TSA plates and incubated at 35°C for 24 h. Afterwards, colonies were counted and statistics were performed on the data. All samples were completed in triplicate.

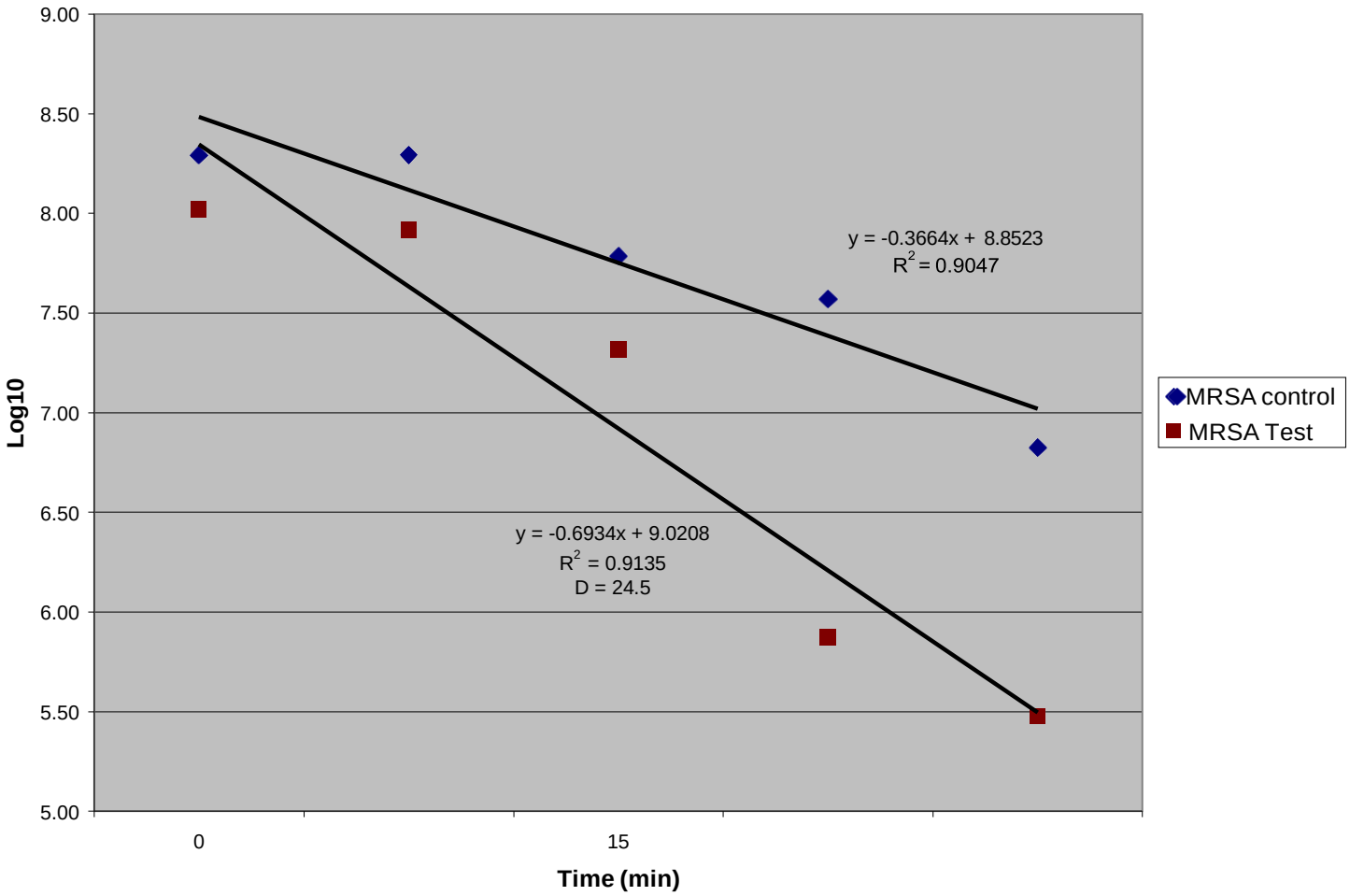
Experimental Results:

Table 1: Reduction of MRSA

MRSA Control			MRSA Test			
Time (min)	**CFU/m^3**	**Log10**	**CFU/m^3**	**Log10**	**Corrected LR**	**%Reduction**
1	1.96×10^8	8.29	1.05×10^8	8.02	0.00	0
5	1.97×10^8	8.29	8.28×10^7	7.92	0.10	21.37%
15	6.11×10^7	7.79	2.07×10^7	7.32	0.20	36.88%
30	3.72×10^7	7.57	7.50×10^5	5.88	1.43	96.24%

Corrected LR = Log Reduction that has been compared to natural rate of decay for MRSA
Log Reduction and %Reduction compares initial CFU and specified CFU

Figure 1: Reduction of MRSA over time when exposed to the O2PRIME-IBAR-36



Conclusions/Observations:

The efficacy of the O2PRIME-IBAR-36, a bipolar ionization (cold plasma) system, to disinfect the air of MRSA was analyzed. After correcting for the natural rate of decay it was observed that there was a 1.43 log reduction after 30 min exposure (Table 1). Furthermore, a D-value was calculated using the reciprocal of the slopes in Figure 1 and a linear regression was computed from log D-value versus time giving us a D-value of 24 min.

An expected 90% reduction (1 log) of MRSA will occur every 24 min.

In conclusion, the O2PRIME-IBAR-36 demonstrated the ability to disinfect MRSA from the air with a 96.24% reduction after 30 min exposure. Furthermore, these results demonstrate that the bipolar ionization system tested does not require direct line of sight to produce kill rates like ultraviolet light. The bipolar ionization system's kill rates are indicative of those in the entire space.

Farbod Nekouei, M.S., Laboratory Manager
or Other Approved Signatory

FINAL REPORT

Efficacy of a Bipolar Ionization System

ORDER Number
371106420

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Certificate of Analysis

Client: O2PRIME
Contact: Darline Moore
Project: Bipolar Ionization System

Product : O2PRIME-IBAR-36
EMSL NO: 371106420

Sample received: 5/25/2011
Start date: 6/7/2011
Report date: 7/21/2011
Challenge Bacteria: *Escherichia coli* ATCC 8739

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-IBAR-36 for its ability to disinfect (kill) bacteria in the air. The testing was conducted in our Cinnaminson Microbiology Laboratory.

Procedure:

Bacteria

Escherichia coli (E. coli) was inoculated on Tryptic Soy agar (TSA) and incubated at 35°C for 24 h. A single isolated colony was then taken and inoculated into Tryptic Soy broth (TSB) and incubated at 35°C for 24 h. This solution was then washed three times with Phosphate buffer at 3,000 x g for 20 min. A one to ten dilution was then made by removing 1 mL of inoculated TSB and placing it into 9 mL of Phosphate buffer. One milliliter of this dilution was then placed into the base of the nebulizer and mixed with 99 mL of Phosphate buffer to create an additional 1:100 dilution.

Environmental Chamber

The environmental chamber was set-up as per the instructions included. One computer fan was placed in the center of the chamber to provide air movement and the two ionizers were placed on either side about 1 inch off the ground. Before testing began the entire chamber was disinfected with a disinfectant solution (5% Hydrogen peroxide with accompanying silver ionic solution), as well as cleaning the fans and ionizers with alcohol wipes. Additionally, between all testing the disinfectant solution was sprayed throughout the chamber and allowed to air out with the fans running for at least 2 hr.

Inoculation of the Test Chamber

The nebulizer was connected to an air compressor with ¼ inch plastic tubing and to the environmental test chamber through one of the testing openings created. The fan was turned on to create an air flow in the chamber but the ionizers were not turned on until after the initial sampling. Once testing was ready to begin 60 psi of compressed air was pumped through the nebulizer, creating the release of 10.8 mL/h of aerosolized solution. This was run for 28 min allowing for a total of 5 mL of solution being aerosolized into the test chamber.

Organism Collection

Immediately, following inoculation of the test chamber an initial collection of the bacteria was taken without the use of the bipolar ionizer. The bacteria were collected with an Anderson impactor at the sample time points 1 min (75 L), 5 min (100 L), 15 min (100 L), 30 min (150 L) and 60 min (200 L) in order to determine the natural rate of decay for *E. coli*. This data was then compared to the data collected when the ionizer was run to create a corrected Log Reduction. The test run was then completed identically the same with the exception that the bipolar ionizer was turned on. Bacteria were collected using TSAB plates and incubated at 35°C for 24 h. Afterwards, colonies were counted and statistics were performed on the data. All samples were completed in triplicate.

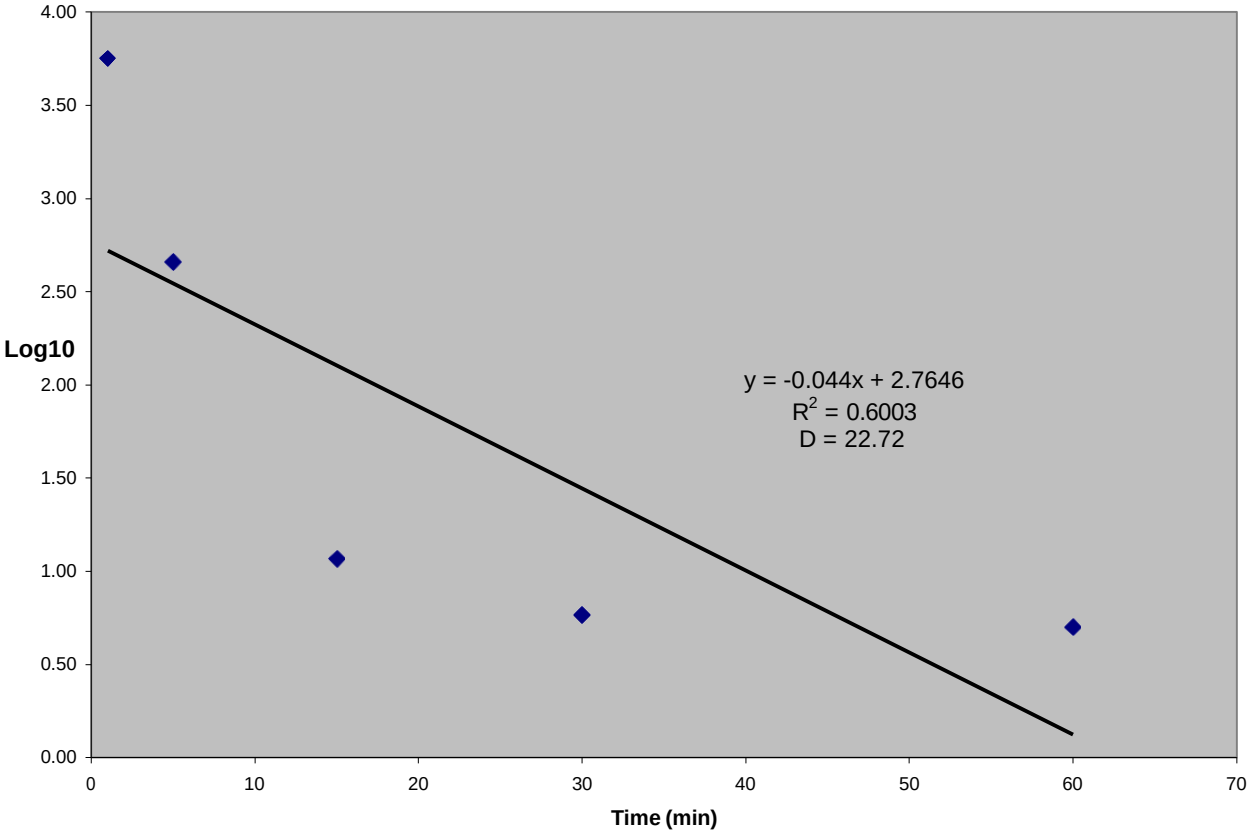
Experimental Results:

Table 1: Reduction of *E. coli*

***E. coli* Control**			***E. coli* Test**			
Time (min)	**CFU/m^3**	**Log10**	**CFU/m^3**	**Log10**	**Corrected LR**	**%Reduction**
1	$6.50x10^3$	3.81	$5.65x10^3$	3.75	0.06	13.03%
5	$6.27x10^3$	3.80	$4.55x10^2$	2.66	1.08	91.65%
15	$4.25x10^3$	3.63	$1.17x10^1$	1.07	2.50	99.68%
30	$1.47x10^3$	3.17	5.83x10	0.77	2.34	99.54%
60	$7.46x10^2$	2.87	5.0x10	0.77	2.11	99.23%

Corrected LR = Log Reduction that has been compared to natural rate of decay for *E. coli*
Log Reduction and %Reduction compares initial CFU and specified CFU
A negative LR or %Reduction is the result of an increase in cells

Figure 1.1: Reduction of *E. coli*



D value = amount of time it takes for *E. coli* to be reduced by 1 log

Conclusions/Observations:

The efficacy of the O2PRIME-IBAR-36, a bipolar ionization system, to disinfect the air of *E. coli* was analyzed. After correcting for the natural rate of decay it was observed that there was a 2.34 log reduction after 30 min exposure and a 2.11 log reduction after 60 min exposure (Table 1). Furthermore, a D-value was calculated using the reciprocal of the slopes in Figure 1 and a linear regression was computed from log D-value versus time giving us a D-value of 22.72 min. In laymen terms with the use of the bipolar ionization device an expected 90% reduction (1 log) of *E. coli* will occur every 24 min, until a maximum reduction is achieved.

In conclusion, the O2PRIME-IBAR-36 demonstrated the ability to disinfect *E. coli* from the air with a 99.54% reduction after 30 min exposure and a 99.23% reduction after 60 min exposure. Furthermore, these results demonstrate that the bipolar ionization system tested does not require direct line of sight to produce kill rates like ultraviolet light. The bipolar ionization system's kill rates are indicative of those in the entire space.

Farbod Nekouei, M.S., Laboratory Manager
or Other Approved Signatory

EMSL

FINAL REPORT

Efficacy of a Bipolar Ionization System

ORDER Number
371208933

PREPARED FOR:

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Certificate of Analysis

Client: O2PRIME
Contact: Darline Moore
Project: Bipolar Ionization System

Product : O2PRIME-IBAR-36
EMSL NO: 371208933

Sample received: 6/11/2011
Start date: 6/18/2011
Report date: 6/26/2011
Challenge Bacteria: *Clostridium difficile* ATCC 70057

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-IBAR-36 for its ability to disinfect (kill) bacteria on a solid surface. The testing was conducted in our Cinnaminson Microbiology Laboratory.

Procedure:

Bacteria

Clostridium difficile (*C. difficile*) was inoculated on Tryptic Soy agar + 5% sheep blood (TSAB) and incubated at 35°C for 48 h under anaerobic conditions. A single isolated colony was then taken and inoculated into Reinforced Clostridium Medium (RCM) and incubated at 35°C for 24 h under anaerobic conditions. This solution was then washed three times with Phosphate buffer at 3,000 x g for 10 min. This solution was then used to inoculate the test carrier.

Inoculation of the Test Carrier

Two sterile Petri dishes were labeled as follows: Control and 30 minutes. Two carriers were then placed into each respective Petri dish. 100µL of the bacterial solution was then placed into the middle of the carrier and spread evenly. This was repeated in triplicate for each time point and the control (a total of 6 slides). The Petri dish containing the inoculated carriers was then allowed to dry for 4 hours in a biological hood.

Efficacy Testing

The O2PRIME-IBAR-36, a bipolar ionization system, was first set up facing down with 5 cm of clearance from the surface. The test carriers in their respective Petri dishes were then placed under the O2PRIME-IBAR-36 and system was turned on.

The control was not exposed to the ionizer and instead placed directly into 10 mL of PBS. After 30 minutes the 30 min Petri dish was removed and the three carriers placed into 10 mL of PBS.
Serial dilutions were then created for each carrier by taking 1mL out and placing it into 9 mL of PBS. For each dilution 100µL was plated onto a TSAB plate.
The inoculated plates were then incubated in anaerobic conditions at 37°C for 48 – 72 h. The colonies were counted and recorded.

Experimental Results:

Table 1: Reduction of *C. difficile*

***C. difficile* Control**			***C. difficile* Test**	
Time (min)	**Avg CFU**	**Log10**	**LR**	**%Reduction**
Control	$1.07x10^4$	4.03		
30	$1.40x10^3$	3.15	0.88	86.87%

Log Reduction and %Reduction compares initial CFU and specified CFU
A negative LR or %Reduction is the result of an increase in cells.

Conclusions/Observations:
The efficacy of the O2PRIME-IBAR-36, a bipolar ionization system, to disinfect a solid surface against *C. difficile* was tested. It was observed that the Log Reduction was 0.88 for 30 min, refer to Table 1.
In conclusion, the O2PRIME-IBAR-36 demonstrated the ability to disinfect *C. difficile*
on a solid surface with an observed percent reduction of 86.87% in 30 minutes.

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Jason Dobranic, Ph.D.
National Director of Microbiology