Challenge Bacteria: *Escherichia coli* ATCC 8739

Experimental Summary:

The testing procedure was designed after discussions between EMSL Analytical, the testing company, and the client. The testing was conducted on the proprietary Polar Ionization Technology 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.

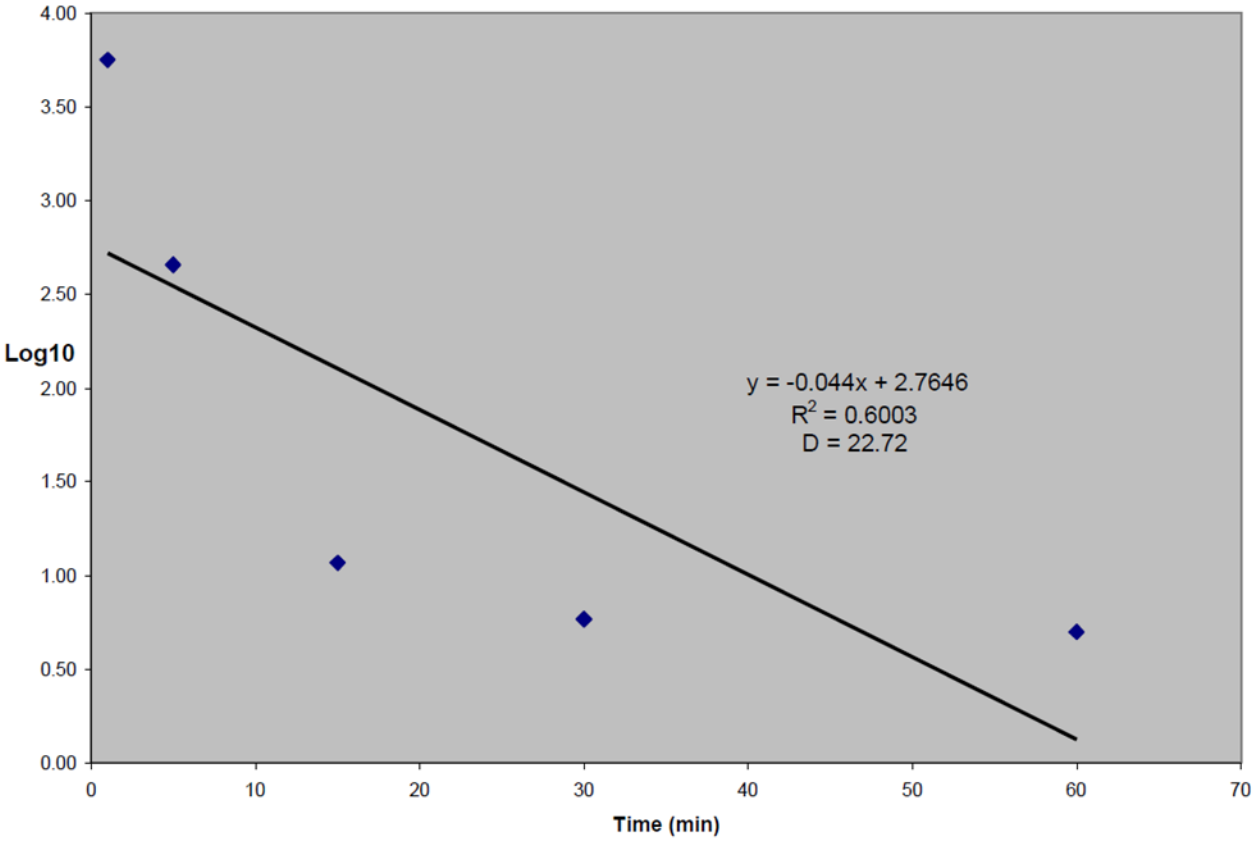
Experimental Results:

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	6.50×10^3	3.81	5.65×10^3	3.75	0.06	13.03
5	6.27×10^3	3.80	4.55×10^2	2.66	1.08	91.65%
15	4.25×10^3	3.63	1.17×10^1	1.07	2.50	99.68%
30	1.47×10^3	3.17	5.83×10	0.77	2.34	99.54%
60	7.46×10^2	2.87	5.0×10	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 proprietary Polar Ionization Technology 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 Polar Ionization Technology 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.