Challenge Bacteria: *Clostridium difficile*

ATCC 70057

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 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 Polar Ionization Technology, 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 Polar Ionization Technology 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 itinto 9 mL of PBS. For each dilution 100µL was plated onto a TSAB plate. The inoculated plates were then incubated in anaerobic conditions at37°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 Polar Ionization Technology, 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 proprietary Polar Ionization Technology demonstrated the ability to disinfect *C. difficile* on a solid surface with an observed percent reduction of 86.87% in 30 minutes.