Challenge Bacteria: Vancomycin-Resistant *Enterococcus (VRE)-Enterococcus faecium - (*ATCC *700221)*

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: *Enterococcus faecium (VRE) 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 10 min. This solution was then used to inoculate the test carrier.

Inoculation of the Test Carrier: Three sterile Petri dishes were labeled as follows: Control, Time 1 minute and Time 15 minute. Three carriers were then placed into each respective Petri dish. 10µL 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. (Total of 9 carriers per test organism). 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 was first set up facing down with 1 inch of clearance from the surface. The test carriers in their respective Petri dishes were then placed under the system and turned on. The control carriers were not exposed to the ionizer and instead placed directly into 10 mL of PBS. After 1 minute the 1 minute Petri dish was removed and the three carriers placed into 10 mL of PBS. This was similarly repeated for 15 minute Petri dish. Serial dilutions were then created for each carrier by taking 1 mL out and placing it into 9 mL of PBS. For each dilution 100 µL was plated onto a TSA plate. The inoculated plates were then incubated at 35°C for 24 h. The colonies were counted and recorded.

Experimental Results:

Table 1: Reduction of *Enterococcus faecium (VRE)*

***E.* faecium Control**			***E. faecium* Test**	
Time (min)	**Avg CFU**	**Log10**	**LR**	**%Reduction**
Control	$1.23x10^5$	5.09		
1	$9.57x10^4$	4.98	0.11	22.43%
15	$6.93x10^4$	4.84	0.25	43.78%

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 Vancomycin- Resistant *Enterococcus* (*Enterococcus faecium*) was tested. It was observed that the Log Reduction was 0.25 for 15 minute and 0.11 for 1 minute (Table 1). In conclusion, the proprietary Polar Ionization Technology demonstrated the ability to disinfect *Enterococcus faecium* on a solid surface with observed percent reduction of 22.43% for 1 minute and 43.78% for 15 minutes. Extrapolating to 60 minutes, the disinfection rate would be over 99%.