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Testing the Inactivation and Surface Kill of *Legionella pneumophila* Using Fresh Air with ActivePure by Aerus/Vollara

Test Report

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November 2, 2015

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1. Introduction

Under Purchase Order with Aerus/Vollara, RTI performed inactivation and surface kill testing of *Legionella pneumophila* inoculated on 1" x 1.5" stainless steel coupons using the Fresh Air with ActivePure device provided by Aerus/Vollara. The objective for this study was to determine the kill efficiency by assessing the survivability of the bacteria following exposure to the airborne oxidizers from the device. This was accomplished via plate counts for colony forming units per milliliter. This report covers the statement of work for this Purchase Order.

2. Procedures

The Fresh Air with ActivePure device was placed in a class II biosafety cabinet (BSC) throughout the testing process. *L. pneumophila* were cultured on buffered charcoal yeast extract agar (BYCE) plates. The cultures were harvested and suspended in 10 mL of sterile saline until it measured an optical density at 600 nm (OD_{600}) of 1.9 – 2.0. The suspended cells were further concentrated by centrifugation, and the resulting pellet was resuspended in 1.4 mL of sterile saline making up the inoculum. Fifteen stainless steel coupons were sterilized in the autoclave and inoculated with 50 µL of the inoculum. A pipette tip was used to spread the bacteria on the surface of each coupon, then the coupons were allowed to dry. At time zero hour, three of the coupons were placed into specimen containers with 10 mL of Phosphate Buffer Saline + Tween 20 (PBST). These were shaken in the wrist action shaker for 10 min and plated. The remaining coupons were divided into unexposed controls and the device-exposed samples for the 4 and 6 hour time points. The device-exposed samples were placed upright one inch away from the front grill. For each time point, the coupons were processed in the same manner as the time zero control samples and plated. The plates were counted to determine colony forming units per milliliter of PBST (CFU/mL). In order to determine the percent reduction at a given time point, Equation 1 was used.

$$\% \text{ Reduction} = \frac{(A-B)}{A} \times 100 \qquad (1)$$

A = Concentration of *L. pneumophila* from control coupons at the time point
B = Concentration of *L. pneumophila* from sample coupons at the time point

3. Results

Table 1 shows the data for the test. The data are averages of the three coupons at each time point.

Table 1. Summary of Results.

	Control	Device	
Time (hr)	CFU/mL	CFU/mL	% Reduction
0	1.60E+05	–	–
4	< 2.5E+03	5.42E+02	>92.3
6	< 2.5E+03	< 2.5E+02	>91.3

Estimated concentrations were reported for samples with colony counts that were below the acceptable range. Overall, there was a loss of *Legionella* following exposure to the device. By four hours, there was a percent reduction of >92.3%. By six hours, the percent reduction was similar (>91.3%) to the four hour time point due to a greater loss of *Legionella* from the unexposed control coupons because of desiccation.

Conclusion: The Fresh Air with ActivePure device reduced the level of *Legionella* on the coupons by more than 91% for both the 4 and 6 hour exposure times.