

# CLEANING LABORATORY EVALUATION SUMMARY

SCL #: 2019

DateRun: 08/19/2019

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ClientType:

ProjectNumber: Project #1

Substrates: Glass/Quartz

PartType: Coupon

Contaminants: Bacteria

Cleaning Methods: Immersion/Soak

Analytical Methods: Organism count

Purpose: To determine the minimum amount of time necessary to obtain 99.9% reduction using the EMop device supplied by the company

Experimental Procedure: We were initially requested to determine the minimum amount of time necessary to obtain 99.9% reduction using the EMop device supplied by the company. The request was to begin with a 60 second contact time, and if 99.9% achieved, then reduce the contact time to 30 sec, and if not achieved, extend to 90 sec. After providing preliminary data, we were requested to perform additional testing with a procedural modification (dissolving the salt in the water before cycling the device).

## Materials and Methods

### Bacterial strains

#### Strain used:

- *S. aureus* 6538 as a representative gram-positive organism

### Surface

Stainless steel was used as the test surface.

### Device Operation

Following the instructions provided by the company (via email, none provided with unit), 3 g salt was added to the unit, which was filled with 450 mL DI water and cycled. Electrolyzed water was used within 30 minutes of cycle completion. Free chlorine based on salt usage and manufacturer's supplied information, for each run was ~310 ppm. When not properly shaken, value was recorded in lab at ~200ppm.

### Disinfection Method

Overnight growth (10 mL) of bacteria (*S. aureus*) was spotted to the coupon and allowed to air dry for 30 minutes in the incubator. Freshly prepared electrolyzed water (500 mL) was pipetted onto each coupon, to fully cover the bacteria. After the appropriate contact time, the entire coupon was placed in a conical tube containing 15 mL DE Neutralizing broth to stop the disinfectant activity. Conical tubes were shaken on a wrist action shaker for 10 minutes followed by a 30 min incubation. Serial dilutions were spread plated in duplicate on tryptic soy agar and incubated overnight at 37°C. After incubation, colonies were counted and CFU/mL calculated.

### Controls

Positive (no treatment) and negative (no bacteria) controls were included with each run.

### Percent and Log Reduction Calculations

Percent and log reduction were calculated based on the reduction of the test coupons from the positive control. In each run, duplicate test coupons were included and averaged. For each time point, two separate runs were performed and averaged. If the two duplicates or runs did not agree, an additional run was performed.

Results: The results of the initial runs showed that for *S. aureus* was difficult to reduce, with ~60% reduction at both 60 and 90 seconds contact time.

Table 1. Data Summary *S. aureus* (Average of 2 Runs)

Bacteria	Contact Time	Avg % Reduction	Avg Log Red
<i>S. aureus</i>	30 sec	ND*	ND
<i>S. aureus</i>	60 sec	66.9438	0.63

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<i>S. aureus</i>	90 sec	57.8936	0.62
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Not performed as 60/90 sec <99.9% reduction.

Revised Protocol

After reviewing preliminary data from Tables 1 above, the company requested we perform selected additional testing with a procedural variation: the salt and water were mixing until the salt dissolved before initiating the device cycle. Using this protocol, the free chlorine increased to ~300 ppm for each run. In order to determine if this made a significant difference, we performed duplicate runs using *S. aureus* with a 5-minute contact time (to ensure maximum likelihood of achieving 99.9% reduction). For the second *S. aureus* run, we mixed the salt and water in a separate container before pouring into the unit and running the cycle.

Preliminary Data

Table 2. *S. aureus*, 5 min contact time, with mixing (average of 2 runs)

Bacteria	Contact Time	Avg % Reduction	Avg Log Red
<i>S. aureus</i>	5 min	99.9911	5.41

Summary:

<b>Substrates:</b>	Glass/Quartz				
<b>Contaminants:</b>	Bacteria				
<b>Company Name:</b>	<b>Product Name:</b>	<b>Conc.:</b>	<b>Efficiency:</b>	<b>Effective:</b>	<b>Observations:</b>
TCI America	Dicyclopentadiene	3 g salt		<input checked="" type="checkbox"/>	<i>S. aureus</i> 5 min 99.9911 5.41

Conclusion:

We achieved the minimum 99.9% reduction for *S. aureus* when utilizing the pre-mixing of the salt before cycling, and with a 5-minute contact time.