CLEANING LABORATORY EVALUATION SUMMARY

SCL #:	2020
DateRun:	08/31/2020
Experimenters:	Alicia McCarthy, Zoe Lawson, Adorrah-Le Khan, Aditi Patel
ClientType:	Cleaning Equipment Mfr
ProjectNumber:	Project #1
Substrates:	Stainless Steel
PartType:	Part
Contaminants:	
Cleaning Methods:	Manual spreading
Analytical Methods:	Organism count
Purpose:	To test the Electrolysis Generator Sanitizing System to evaluate the disinfection efficacy of MS2 Bacteriophage, Staphylococcus aureus ATCC 6539, Candida Albicans, E.coli ATCC 29214, and Salmonella on non-porous surfaces.
Experimental Procedure:	The Electrolysis Generator Sanitizing System was prepared using one scoop of salt, 400ml of tap water, and one full cycle. Total chlorine of the tap water, pre-cycle, and post cycle using a Lovibond MD100 instrument set to the CLHR mode. The pH and total dissolved solids (TDS) of the tap water, pre-cycle, and post cycle were measured using an APERA Instrument. The client requested to test at five seconds, 10 seconds, 20 seconds, and 30 seconds up to one to three minutes, if necessary. The range of total chlorine for testing needed to be between 80-125ppm; higher ppm could be used and documented. Each run consisted of one contact time on one surface and had 26 plates that included two of each of the following dilutions: Negative 1:1; Positive 1:1, 1:10, 1:100, 1:1000; Test 1 1:1, 1:10, 1:100, 1:1000; and Test 2 1:1, 1:10, 1:100, 1:1000. Pour Plate Method - MS2 Bacteriophage Six hours prior to one run (26 plates), E.coli 15597 was sub cultured into three milliliters of tryptic soy broth (TSB) screw-cap tubes and incubated at 37°C (98.6°F). Four glass petri dishes, each containing one stainless steel coupon, along with 27 screw-cap tubes filled with 10ml of 0.5X tryptic soy agar (TSA) were autoclaved. The biosafety cabinet (BSC) was sprayed with 70% v/v isopropyl alcohol using a paper towel before spraying any items going into the BSC. Once autoclaving was complete, the TSA tubes were placed into a 45°C (113°F) D.I. water bath inside biosafety cabinet (BSC). The four glass petri dishes were marked using a black sharpie to designate the positive (P+), negative (N-), Test 1 (T1), and Test 2 (T2). Ten microliters of the organism was pipetted onto the P+, T1, and T2 stainless steel coupons and air dried for 15 minutes. A motorized pipette with 10ml tips was used to pipet 15 ml of Dey-Engley (D/E) neutralizing broth into four separate 50ml conical tubes labeled P+, N-, T1, and T2. Once the MS2 bacteriophage dried on the coupons, the P+ coupon was placed into the conical tube. The N-, T1, and T2 were pipetted with 1000µl of the Electrolysis Generator Sanitizing Sprayer solution onto each coupon for the desired contact time before immediately placing them in the conical tube with an autoclaved forceps. The conical tubes were then placed on the shaker for 10 minutes. During this time, using the 1000ml pipette, 900ml of 1x phosphate-buffered saline (PBS) was pipetted into nine autoclaved dilution tubes, and serial dilutions were made for P+, T1, and T2 up to 10^{-4} using 100µl of the shaken D/E broth. Once the six hour sub time was complete, the E. coli 15597 subculture was removed from the incubator for use. For each variable (N-, P+, T1, and T2), 100µl of the stock and serial dilutions of MS2 bacteriophage, and 100µl of the E.coli 15597 subculture were combined into an empty dilution tube. A screwcap tube of 0.5X TSA was removed from the water bath, wiped with a paper towel to remove moisture, and poured into the dilution tube. The mixture was immediately poured into a sterile polystyrene petri dish; swirled to cover the entire plate surface; and then air dried before covering. Dried petri dishes were placed into a clean labelled zip lock bag that was partially closed and incubated at 37°C overnight. Plates were counted the following day based on the clear lysis zones in the bacterial lawn of growth (1 plate forming unit) to calculate log reduction and percent removal.

CLEANING LABORATORY EVALUATION SUMMARY

Spread Plating Method - Staphylococcus aureus, E.coli 29214, Salmonella

Twenty-four hours prior to one run (26 plates), bacteria were subcultured into three milliliters of tryptic soy broth (TSB) screw-cap tubes and incubated at 37°C (98.6°F). Four glass petri dishes, each containing one stainless steel coupon, were autoclaved. The biosafety cabinet (BSC) was sprayed with 70% v/v isopropyl alcohol using a paper towel before spraying any items going into the BSC. After autoclaving, the four glass petri dishes were marked using a black sharpie to designate the positive (P+), negative (N-), Test 1 (T1), and Test 2 (T2). Ten microliters of the organism were pipetted onto the P+, T1, and T2 stainless steel coupons and air dried for 15 minutes. A motorized pipette with 10ml tips was used to pipet 15 ml of D/E neutralizing broth into four separate 50ml conical tubes labeled P+, N-, T1, and T2. Once the bacteria dried on the coupons, the P+ coupon was placed into the conical tube. The N-, T1, and T2 were pipetted with 1000µl of the Electrolysis Generator Sanitizing Sprayer solution onto each coupon for the desired contact time before immediately placing them in the conical tube with an autoclaved forceps. The conical tubes were then placed on the shaker for 10 minutes. During this time, using the 1000µl pipette, 900µl of were pipetted into nine autoclaved dilution tubes, and serial dilutions were made for P+, T1, and T2 up to 10^{-4} using 100µl of the shaken D/E broth. Using a pipette, 100µl of the stock and serial dilutions were each plated and spread evenly over the surface of the solid TSA petri dish plate using a metal spreader. Finished plates were placed into a clean labelled zip lock bag that was partially closed and incubated at 37°C overnight. Isolated colonies were counted the following day to calculate log reduction and percent removal.

Results:

Salmonella and E.coli 29214 at 10 seconds was skipped due to the very low log reduction. The TDS, pH, and Total Cl (ppm) for MS2 bacteriophage was the only testing that wasn't done at the time of testing due to performing this testing before the request.

MS2 Bacteriophage

Contact Time	TDS (ppm)	pH	Total Cl (ppm)	Avg Log Red	Avg % Red
30 sec	5.32*	8.52*	76*	6.03	100
20 sec				6.39	100
10 sec				6.30	100
5 sec				6.03	100

**Not taken at time of testing – post measurements using 1 cycle, 400ml, and 1g salt.*

Staphylococcus aureus

Contact Time	TDS (ppm)	pH	Total Cl (ppm)	Avg Log Red	Avg % Red
30 sec	2.53	8.48	132	6.67	100
30 sec	2.74	8.32	126	7.00	100
30 sec	2.24	9.07	89	4.88	99.98
20 sec	2.24	9.07	89	2.75	99.79
5 sec	3.25	8.41	123	1.34	94.14

E. coli 29214

Contact Time	TDS (ppm)	pH	Total Cl (ppm)	Avg Log Red	Avg % Red
30 sec	1.29	8.45	103	5.32	99.99
30 sec	2.49	8.90	91	2.04	97.17
20 sec	2.49	8.90	91	6.00	100
20 sec	2.53	8.48	132	6.42	100
5 sec	4.20	8.85	105	0.9439	87.16

Salmonella

Contact Time	TDS (ppm)	pH	Total Cl (ppm)	Avg Log Red	Avg % Red
30 sec	2.41	8.62	125	5.12	99.99
20 sec	2.41	8.62	126	4.52	99.91
5 sec	2.65	8.97	86	0.85	86.34

Candida Albicans

Contact Time	TDS (ppm)	pH	Total Cl (ppm)	Avg Log Red	Avg % Red
60 sec	2.13	8.40	132	1.68	99.99
60 sec	2.14	9.01	93	0.9218	99.91
30 sec	2.14	9.01	126	1.42	86.34

CLEANING LABORATORY EVALUATION SUMMARY

20 sec	2.18	9.04	78	N/A	N/A
10 sec	4.03	9.18	72	0.8047	87.22
5 sec	4.20	8.85	105	0.9734	80.92

*Barely any growth on positive plates to provide an average log and average percent reduction

Based on the higher pH of the post-cycle solution, the electrolyzing process of this unit is most likely producing more sodium hypochlorite than hypochlorous acid. Higher Total Cl concentrations had a higher log reduction and reduced contact time for effective disinfection of the stainless steel surfaces.

Summary:

Conclusion: The device tested in the lab should relate closely with the following models, but specific testing has not been conducted on all models listed.

1) AVS-SQ60U

2) AVS-SQ300U

3) AVS-SQ360P (prior was SQ400)

4) AVS-PRO400P (prior was PRO450)

5) AVS-191A

6) AVS-185B