CLEANING LABORATORY EVALUATION SUMMARY

SCL #:	2021
DateRun:	01/14/2021
Experimenters:	Alicia McCarthy, Zoe Lawson
ClientType:	Cleaner Manufacturer
ProjectNumber:	Project #1
Substrates:	Stainless Steel
PartType:	Coupon
Contaminants:	Staphylococcus aureus ATCC 6539, MS2 Bacteriophage
Cleaning Methods:	Low Pressure Spray
Analytical Methods:	Organism count
Purpose:	To test the disinfection efficacy of NCIR Sanitizing Water Professional Strength (3 pH) and Extra Strength (2.5 pH) products on MS2 bacteriophage and Staphylococcus aureus.
Experimental Procedure:	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 the 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 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 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 treated with one spray amount of the product solution onto each coupon for five minutes 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 labeled 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. Spread Plating Method - Staphylococcus aureus Twenty-four hours prior to one run (26 plates), bacteria were 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, 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 treated with one spray amount of the product solution onto each coupon for five minutes before immediately placing them in the conical tube with an autoclaved forceps. The conical tubes were then placed on the shaker for 10 minutes.

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During this time, using the 1000µl pipette, 900µl of 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. 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 labeled 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:

Product**:** Professional Strength pH 3

Organism	Application	Contact Time	Substrate	Positive Plates Average Log	Average Log Reduction	Average % Reduction
MS2 Bacteriophage	1 spray	5 Minutes	Stainless Steel	5.44	1.1087	75.9409
Staphylococcus aureus				6.50	0.0879	15.9145

Product: Extra Strength pH 2.5

Organism	Application	Contact Time	Substrate	Positive Plates Average Log	Average Log Reduction	Average % Reduction
MS2 Bacteriophage	1 spray	5 Minutes	Stainless Steel	5.43	0.2005	31.0249
Staphylococcus aureus				7.21	0.1898	34.3318

S. aureus was measured based on SOP protocols to have a 0.132 mg/ml absorbency before applying to substrate and air-dried for 15 minutes before disinfecting on the stainless-steel coupon.

Summary:

Substrates:	Stainless Steel				
Contaminants:	Staphylococcus aureus ATCC 6539, MS2 Bacteriophage				
Company Name:	**Product Name:**	**Conc.:**	**Efficiency:**	**Effective:**	**Observations:**
NCIR Sanitizing Water	Professional Strength 3 pH	100%		☐	
NCIR Sanitizing Water	Extra Strength 2.5 ph.	100%		☐	

Conclusion:

Both products were ineffective at disinfecting MS2 Bacteriophage and Staphylococcus aureus with a five-minute contact time on a stainless-steel surface.