CLEANING LABORATORY EVALUATION SUMMARY

SCL #:	23
DateRun:	11/08/2023
Experimenters:	Namrata Chauhan, Serena Burkinshaw
ClientType:	Cleaner Manufacturer
ProjectNumber:	Project #1
Substrates:	Stainless Steel
PartType:	Coupon
Contaminants:	MS2 Bacteriophage
Cleaning Methods:	Pour Plate
Analytical Methods:	Organism count
Purpose:	To test the efficacy of GMA NaDCC tablets at 200ppm to inactivate MS2 Bacteriophage on a clean, non-porous surface.

Experimental Procedure:

Single Pour Plate Method - MS2 Bacteriophage

Six hours before one run (26 plates), E.coli 15597 was 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, 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 petri dishes were labeled as 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 pipetted with 1000µl of the cleaning solution onto each coupon for 30 seconds before immediately placing them in the conical tube with 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.

Results:

Product Name	Concentration (ppm)	Log Reduction	% Reduction
GMA NaDCC	200	5.36	99.9915

Summary:

Conclusion: GMA NaDCC at 200ppm inactivated MS2 Bacteriophage at a 5.36 Log Reduction (99.9915%) on a cleaned, stainless steel surface after 30 seconds of contact.