

# CLEANING LABORATORY EVALUATION SUMMARY

SCL #: 2021

DateRun: 05/18/2021

Experimenters: Aditi Patel

ClientType:

ProjectNumber: Project #2

Substrates: Stainless Steel

PartType: Coupon

Contaminants: MS2 Bacteriophage, Virus - Non-Enveloped

Cleaning Methods: Low Pressure Spray

Analytical Methods: Organism count

Purpose: To evaluate the disinfection efficacy of a Viking Pure Solutions HOCL Solution product to inactivate a surrogate virus for COVID-19, MS2 Bacteriophage, on a clean stainless steel surface.

**Experimental Procedure:** Pour Plate Method - MS2 Bacteriophage

Six hours prior to 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 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 pipetted with 1000µl of the product solution onto each coupon for a contact time of 30 seconds before immediately placing them in the conical tube using 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<sup>-4</sup> 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	ORP (mV)	pH	Total Chlorine (ppm)	Average Log Reduction	Average Percent Reduction
	Viking Pure Solutions HOCL Solution	943	6.39	107	2.2779	99.4334

Summary:	Substrates:	Stainless Steel				
	Contaminants:	MS2 Bacteriophage, Virus - Non-Enveloped				
	Company Name:	Product Name:	Conc.:	Efficiency:	Effective:	Observations:
	Viking Pure Solutions	Viking Pure Solutions HOCL Solution	100%	99.43	<input type="checkbox"/>	

**Conclusion:** Viking Pure Solutions HOCL Solution product was ineffective in inactivating MS2 Bacteriophage with a 5-6 Log reduction within 30 seconds.