CLEANING LABORATORY EVALUATION SUMMARY

SCL #:	2024
DateRun:	01/01/1970
Experimenters:	Alicia McCarthy, Serena Burkinshaw
ClientType:	Lab
ProjectNumber:	Project #12
Substrates:	Stainless Steel
PartType:	Coupon
Contaminants:	MS2 Bacteriophage
Cleaning Methods:	Pour Plate
Analytical Methods:	Organism count
Purpose:	To evaluate the efficacy of Clorox brand cleaners with inactivating MS2 on a hard surface without agitation.

Experimental Procedure:

Pour Plate Method - MS2 Bacteriophage

Six hours prior to the run, E.coli 15597 was subcultured into three milliliters of tryptic soy broth (TSB) screw-cap tubes and incubated at 37°C (98.6°F). 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 cleaning solution onto each coupon for 30 seconds 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.

Results:

Product	Log Reduction	% Removal
Clorox Healthcare Hydrogen Peroxide Cleaner Disinfectant	5.1347	99.9720
Clorox EcoClean Disinfecting Cleaner	6.4684	99.9947
Clorox Bleach (.1%)	7.2667	100.0000

Summary:

Conclusion: Clorox Bleach (.1%) had the highest log reduction at 7.2667 and percent reduction at 100.0000%. Clorox EcoClean, which contains Lactic acid had a percent removal of 99.9947%. Clorox Healthcare Hydrogen Peroxide Cleaner Disinfectant had a percent removal of 99.9720%.