CUSTOM TEST

Sanitizer Test for Inanimate Surfaces based on the methods from ASTM E1153-14

FINAL REPORT: R2020-383

Prepared for:

OC Clean LLC - KleenzDri

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Testing Provided by:

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Objective:

To evaluate two samples for antimicrobial efficacy when applied to a pre-cleaned, inanimate, hard, non-porous surface inoculated with test organism. The methods are based on ASTM E1153-2014 with customer modifications.

Test Sample Identification:

1. KleenzDri S1
2. KleenzDri S2

Test Procedure Summary:

A volume of the working inoculum was evenly distributed across the substrate surface. The organisms were dried by incubating at 35°C with 40% Relative Humidity (~20-40 minutes). After drying, the number of organisms present were enumerated to establish a baseline.

Test Variables

Test Organisms:	*Klebsiella aerogenes*[1] ATCC#13048
Organism Preparation:	Transfer 1: Tryptic Soy Broth (TSB), 35°C for 24±2 hours Transfer 2: TSB, 35°C for 24±2 hours Transfer 3: TSB, 35°C for 48-54 hours Top 2/3 of culture used for working inoculum
Soiling:	5% Heat Inactivated Fetal Bovine Serum Albumin in Working Inoculum
Working Inoculum Concentration:	*K. aerogenes* ATCC#13048: 8.7 x 10^8 CFU/mL
Volume Inoculum:	0.03mL
Non-porous Substrate Used:	Glass slide (25mm x 25mm area)
Substrate Pre-Clean Method:	Dipped in 70% Isopropyl Alcohol, dipped in Sterile Deionized Water, and then air dried
Substrate Sterilization Method:	UV
Baseline Recovery of Organism Dried to Substrate Surface:	*K. aerogenes* ATCC#13048: 8.3 x 10^6 CFU/mL

[1] *Klebsiella aerogenes* formerly classified as *Enterobacter aerogenes*

Condition 1: The sample test solution was evenly sprayed onto the dried inoculated substrate. Immediately after application, the surface was wiped clean. The same procedure was also conducted with the control solution. Dilution medium was added and mixed thoroughly to facilitate removal of organisms. Serial dilutions were made, pour plated and then plates were incubated. After incubation, colonies of recovered bacteria were counted and used to determine log and percent reductions.

Condition 1

Test Solution:	Sample as supplied by customer in spray bottle
Control Solution:	Sterile Deionized H_2O (Same type of spray bottle was pre-cleaned with 70% Isopropyl Alcohol and dried prior to delivery of control solution)
Application:	One spray from a distance ~6 inches from substrate
Wiping Procedure:	A fresh gauze pad for each sample was used to wipe the surface; gentle even pressure was applied for two horizontal strokes across the surface; the pad was folded in half to expose a clean section and used to wipe two vertical strokes across the surface; let dry until there was no visible residue (~10 seconds)
Dilution Medium Used:	10 mL Phosphate Buffered Saline
Plating Medium:	Standard Methods Agar
Organism Incubation Temperature:	28-30°C
Organism Incubation Time:	24-48 hours

Condition 2: The sample solution was evenly sprayed onto the dried inoculated substrate. Immediately after application, the surface was wiped clean. Then a volume of the solution was re-applied to the surface for a specified amount of time. The same procedure was also conducted with the control solution. Dilution medium was added and mixed thoroughly to facilitate removal of organisms. Serial dilutions were made, pour plated and then plates were incubated. After incubation, colonies of recovered bacteria were counted and used to determine log and percent reductions.

Condition 2

Test Solution:	Sample as supplied by customer in spray bottle
Control Solution:	Sterile Deionized H_2O (Same type of spray bottle was pre-cleaned with 70% Isopropyl Alcohol and dried prior to delivery of control solution)
First Application:	One spray from a distance ~6 inches from substrate
Wiping Procedure:	A fresh gauze pad for each sample was used to wipe the surface; gentle even pressure was applied for two horizontal strokes across the surface; the pad was folded in half to expose a clean section and used to wipe two vertical strokes across the surface; let dry until there was no visible residue (~10 seconds)
Second Application:	1mL Solution was applied to the substrate surface for 5 minutes
Dilution Medium Used:	10 mL Phosphate Buffered Saline
Plating Medium:	Standard Methods Agar
Organism Incubation Temperature:	28-30°C
Organism Incubation Time:	24-48 hours

Results:

The results below pertain only to the samples tested. The Colony Forming Units per milliliter (CFU/mL) results are an average of five replicates for the treated treatment and an average of three replicates for the control. Percent and log reductions were determined by comparing the organism recovery from the treatment after the condition to the organism recovery from the control after the condition. The detection limit on this test is 10 CFU/mL due to the 10x dilution that occurs with the addition of the Phosphate Buffered Dilution Medium. Therefore, < 10 means there were no test organisms found on the lowest dilution plate.

Condition 1: *Percent reduction against Klebsiella aerogenes ATCC#13048*

Sample	Average Recovered Bacteria from the Control	Average Recovered Bacteria from the Sample	Log Reduction	Percent Reduction
KleenzDri S1	6.9×10^4	< 10	> 3.8	> 99.98%
KleenzDri S2	6.9×10^4	< 10	> 3.8	> 99.98%

Condition 2: *Percent reduction against Klebsiella aerogenes ATCC#13048*

Sample	Average Recovered Bacteria from the Control	Average Recovered Bacteria from the Sample	Log Reduction	Percent Reduction
KleenzDri S1	1.4×10^5	< 10	> 4.2	> 99.993
KleenzDri S2	1.4×10^5	< 10	> 4.2	> 99.993

Percent reduction is translated into log reduction by the following:

90% reduction = 1 log reduction; i.e. 1,000,000 reduced to 100,000 is a 1 log reduction
99% reduction = 2 log reduction; i.e. 1,000,000 reduced to 10,000 is a 2 log reduction
99.9% reduction = 3 log reduction; i.e. 1,000,000 reduced to 1,000 is a 3 log reduction
99.99% reduction = 4 log reduction; i.e. 1,000,000 reduced to 100 is a 4 log reduction