# *Custom Test*

## *Sanitizer Test for Inanimate Surfaces based on methods from ASTM E1153*

*FINAL REPORT: R2024-492-2*
**Amendment to R2024-492*

Prepared for:
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## Objective:

To evaluate a sanitizer test solution for efficacy in organism removal from a soiled, hard, non-porous surface. The methods are based on ASTM E1153 with customer modifications to include a wiping procedure and test organism recovery from the surface after wiping.

## Test Sample Identification:

1. 16oz bottle KleenzDRI
2. Sterile Deionized $H_2O$ (Control)

## Test Procedure Summary:

A volume of the test organism (working inoculum) was evenly distributed across the substrate surface. The inoculum was dried to the surface by incubating at 35°C with 40% Relative Humidity (~20-40 minutes). After drying, the substrate surface was subjected to the wiping procedure.

*Substrate and Organism Variables*

| | |
|---|---|
| **Test Organisms:** | *Staphylococcus aureus* MRSA ATCC#43300<br>*Escherichia coli* O157:H7 ATCC#35150 |
| **Organism Preparation:** | Tryptic Soy Broth (TSB), 35°C for 48-54 hours<br>Culture tube was vortexed and let settle for ≥ 15 minutes<br>Top 2/3 of culture used for working inoculum |
| **Soiling:** | 5% Heat Inactivated Fetal Bovine Serum Albumin added to working inoculum |
| **Working Inoculum Concentration:** | *S. aureus* MRSA ATCC#43300: 8.3 $Log_{10}$ CFU/mL<br>*E. coli* O157:H7 ATCC#35150: 8.8 $Log_{10}$ CFU/mL |
| **Comparison Item:** | Baseline - inoculated, dried and unwiped substrate |
| **Volume of Working Inoculum:** | 0.03mL |
| **Non-Porous Substrate:** | Glass slide (25mm x 25mm area) |
| **Substrate Pre-Clean/Sterilization Method:** | Dipped in 70% Isopropyl Alcohol, dipped in sterile Deionized Water, air dried, then UV prior to use. |

## Test Procedure Summary (cont.):

The test solution was evenly sprayed onto the dried inoculated substrate. Immediately after application, the surface was wiped. The procedure was conducted with the control solution in the same manner. One set of slides was incubated for 24 hours prior to organism recovery and the remaining set of slides was plated after the wipe procedure for organism recovery as follows. Dilution media was added and mixed thoroughly to facilitate removal of organisms from the surface. Serial dilutions were made, pour plated and then plates were incubated. In addition, at each plating time (T=0 and T=24 hours) an inoculated, dried and unwiped substrate (baseline) was evaluated. After incubation, colonies of recovered bacteria were counted and used to determine log recovery and percent recovery differences.

*Wipe Procedure & Organism Recovery Variables*

| | |
|---|---|
| **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 Media:** | Standard Methods Agar |
| **Organism Incubation Temperature:** | 35°C |
| **Organism Incubation Time:** | 24-48 hours |

## Results:

The results below pertain only to the samples tested. The number of recovered bacteria per replicate were averaged and converted to common logarithm ($Log_{10}$). Results are an average of five replicates for the treatment, an average of three replicates for the control, and one replicate for the baseline. The average log values were used to determine log and percent differences by comparing test organism recovery of the baseline (inoculated, dried and unwiped substrate) versus the test organism recovery from the wiped test or control substrates after each contact time*.

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 Media. Therefore, <10 means there were no test organisms found on the lowest dilution plate.

### ***Staphylococcus aureus MRSA ATCC#43300*:***

*Plated After Wipe Procedure:*

*Recovery Difference for the Test and Control Compared to Baseline Recovery*

| Clean Procedure Solution | $Log_{10}$ Recovered Bacteria from the Baseline | $Log_{10}$ Recovered Bacteria from the Solution | $Log_{10}$ Recovery Difference | Percent Difference |
|---|---|---|---|---|
| 16oz bottle KleenzDRI | 5.7 | 3.0 | 2.7 | 99.8 |
| Sterile Deionized $H_2O$ | | 4.1 | 1.7 | 98 |

*Plated 24-Hours After Wipe Procedure:*

*Recovery Difference for the Test and Control Compared to Baseline Recovery*

| Clean Procedure Solution | $Log_{10}$ Recovered Bacteria from the Baseline | $Log_{10}$ Recovered Bacteria from the Solution | $Log_{10}$ Recovery Difference | Percent Difference |
|---|---|---|---|---|
| 16oz bottle KleenzDRI | 6.7 | <1.0 | >5.7 | >99.9998 |
| Sterile Deionized $H_2O$ | | 5.1 | 1.6 | 98 |

***Escherichia coli O157:H7 ATCC#35150*:***

## *Plated After Wipe Procedure:*

*$Log_{10}$ Recovery Difference for the Test and Control Compared to Baseline Recovery*

| Clean Procedure Solution | $Log_{10}$ Recovered Bacteria from the Baseline | $Log_{10}$ Recovered Bacteria from the Solution | $Log_{10}$ Recovery Difference | Percent Difference |
|---|---|---|---|---|
| 16oz bottle KleenzDRI | 4.5 | <1.0 | >3.5 | >99.97 |
| Sterile Deionized $H_2O$ | | 3.6 | 0.9 | 88 |

## *Plated 24-Hours After Wipe Procedure:*

*$Log_{10}$ Recovery Difference for the Test and Control Compared to Baseline Recovery*

| Clean Procedure Solution | $Log_{10}$ Recovered Bacteria from the Baseline | $Log_{10}$ Recovered Bacteria from the Solution | $Log_{10}$ Recovery Difference | Percent Difference |
|---|---|---|---|---|
| 16oz bottle KleenzDRI | 3.4 | <1.0 | >2.4 | >99.6 |
| Sterile Deionized $H_2O$ | | 2.6 | 0.8 | 86 |