STUDY TITLE
Non-GLP Custom Device Test

Test Devices
Neat Plus
Home
Hill
Tosca

Test Microorganisms
Staphylococcus aureus ATCC 6538
Escherichia coli ATCC 8739
MS2 Bacteriophage ATCC 15597-B1

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TABLE OF CONTENTS

STUDY TITLE 1

PERSONNEL INVOLVED IN THE STUDY 2

TABLE OF CONTENTS 3

TEST DEVICE INFORMATION 4

TEST PARAMETERS 4

TEST SYSTEMS 4

TEST METHOD 5

STUDY PHOTOGRAPHS 6

STUDY SUCCESS CRITERIA 7

CALCULATIONS AND STATISTICAL ANALYSIS 7

STUDY NOTES 8

RESULTS 9

TEST DEVICE INFORMATION

Test Devices:	Neat plus Home Hill Tosca
Test Substance Manufacturer:	Dupray
Special Handling Requirements:	None

TEST PARAMETERS

Date of Start of Testing:	20DEC2024
Date of End of Testing:	22DEC2024
Contact Time(s):	30 seconds
Carrier Type:	1" x 3" glass slide
Testing Replicates:	Single Replicate
Harvest Fluid (Volume):	Dey-Engley Broth (10.0 mL)
Inoculum Target Concentration:	≥1 x 10^6 CFU/Surface for bacteria ≥1 x 10^6 PFU/Surface for bacteriophage
Inoculum Volume:	0.020 mL
Serial Dilution Media (Volume):	PBS (0.900 mL)

TEST SYSTEMS (Microorganisms)

Table A: Test Microorganisms Growth Medium and Incubation Conditions

Microorganisms	Growth Medium	Incubation Conditions
Staphylococcus aureus ATCC 6538	Tryptic Soy Broth (TSB)/ Tryptic Soy Agar (TSA)	36 ± 1°C Aerobic
Escherichia coli ATCC 8739	Tryptic Soy Broth (TSB)/ Tryptic Soy Agar (TSA)	36 ± 1°C Aerobic
Escherichia coli ATCC 15597 Host Culture	Tryptic Soy Broth (TSB)/ Tryptic Soy Agar (TSA)	36 ± 1°C Aerobic
MS2 Bacteriophage ATCC 15597-B1	50% Tryptic Soy Agar (TSA)	36 ± 1°C Aerobic

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TEST METHOD

Preparation of Test Cultures and Test Inocula

Prior to test initiation, test cultures for *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, and *Escherichia coli* ATCC 15597 Host Culture were initiated in Tryptic Soy Broth (TSB) and allowed to incubate under conditions necessary for sufficient growth of the target test microorganism. *MS2 Bacteriophage* ATCC 15597-B1 used in testing was from Microchem freezer stock.

Preparation of Harvest Media

Prior to test initiation, 10.0 mL of sterile Dey-Engley Broth were aliquoted into individual sterile conical tubes to be used for the harvest of microbial population control test carriers and device test carriers. The number of replicates and test microorganisms was considered to determine the number of sterile conical tubes to prepare.

Inoculation of Test Carriers

Individual test carriers (1" x 3" glass slides) were inoculated with 0.020 mL of each target test microorganism to achieve a target concentration of at least 1 x 10^6 CFU/Surface or at least 1 x 10^6 PFU/Surface. The number of replicates and test microorganisms was considered to determine the number of test carriers to prepare. The inoculated test carriers were then placed in an incubator until all microorganisms appeared visually dry.

Harvest of Control Test Carriers

Time Zero control carriers were placed into 10.0 mL Dey-Engley Broth and then vortexed for at least 60 seconds. The harvest media was then plated to achieve a sufficient range of countable colonies for each target microorganism.

Test Device Preparation and Use

Each steam cleaner device was filled with sterile tap water and plugged into a common electrical outlet. Each unit was turned on and preheated until each device indicated it was ready for use. The steam release trigger was then pressed for 15 seconds to heat up the hose and clear initial condensation. The triangular tool and bonnet were then attached to the hose of each device as the accessory of choice for the experiment. Steam was then released until the bonnet was warmed up. The triangular tool and bonnet were then placed directly over the inoculated carriers. The steam release trigger was then pressed, and the bonnet was allowed to contact the inoculated carrier for the duration of the contact time.

Harvest of Test Carriers

After the contact time, test carriers were placed into 10.0 mL Dey-Engley Broth and then vortexed for at least 60 seconds. The harvest media was then serially diluted and plated to achieve a sufficient range of countable colonies for each target microorganism.

Media Sterility and Microorganism Purity Controls

Sterility controls of all media used in the study were plated and incubated to confirm sterility of media used on each day of testing. A purity streak of each test microorganism used in the study was incubated along with test materials to confirm purity and viability of the test culture. An infectivity control was performed for *MS2 Bacteriophage* ATCC 15597-B1.

TEST METHOD (Continued)

Incubation of Test and Control Materials

All test and control plates were incubated for 18-24 hours for *Staphylococcus aureus* ATCC 6538 and *Escherichia coli* ATCC 8739 at incubation conditions optimal for the microorganism. All test and control plates were incubated for 12-18 hours for *MS2 Bacteriophage* ATCC 15597-B1 at incubation conditions optimal for the microorganism.

STUDY PHOTOGRAPHS

Figure 1: Triangular Tool Accessory

Figure 2: Microfiber Bonnet Attached to Triangular Tool

STUDY SUCCESS CRITERIA

The experimental success (controls) criteria follow:

- The initial microbial population, Time Zero control, must demonstrate a starting concentration of at least 1 x 10^6 CFU/surface or at least 1 x 10^6 PFU/surface.
- The media sterility controls demonstrate no growth.
- The test microorganism purity control plates demonstrate the presence of the target microorganism and absence of contaminant microorganisms.

CALCULATIONS AND STATISTICAL ANALYSIS

Average Plate Count
Average Plate Count = Y/X
Where:
Y = total of all plate counts
X = total number of plates

CFU/Surface
CFU/Surface = (Average Plate Count × Dilution Factor) × Neutralization Factor

PFU/Surface
PFU/Surface = (Average Plate Count × Dilution Factor) × Neutralization Factor

Log_{10} Reduction
$LR = Log_{10}(A) – Log_{10}(B)$
Where:
LR= the mean Log_{10} of surviving microbial population
A= the mean CFU/Surface recovered from the microbial population controls
B= the mean CFU/Surface recovered from the test substance at the contact time

Percent Reduction
$PR = 100 * (1\text{-}10^{-LR})$
Where:
PR = Percent Reduction versus microbial population control
LR = the mean Log_{10} of surviving microbial population

Percent Recovery Neutralization
% Recovery = (X/Y) * 100
Where:
X = the values associated with the neutralizer test A or B
Y = the values associated with the neutralizer test C

Statistical Analysis
No statistical analysis was performed.

STUDY NOTES

This study report has been amended to update the Results tables as the incorrect values for concentration per surface were inadvertently captured, resulting in a lower reduction. The tables in the Results section of this report have been updated and are representative of the efficacy of the device(s).

Additionally, the data tables have been amended to include further specificity as it pertains to percent reduction, as well as to clarify the units for limit of detection of microorganisms to be CFU/carrier or PFU/carrier as opposed to CFU/mL or PFU/mL as seen in the Results section below.

RESULTS

Table 1: *S. aureus* ATCC 6538 Percent Reduction and Log_{10} Reduction at 30 Seconds Compared to Time Zero for Neat Plus

Test Date	Test Microorganism	Test Device	Contact Time	CFU/Surface	Percent Reduction Compared to Time Zero	Log_{10} Reduction Compared to Time Zero
20DEC2024	*S. aureus* ATCC 6538	Microchem Control	Time Zero	1.27E+07	N/A	N/A
		Neat Plus	30 Seconds	<5.00E+00*	>99.99996%	>6.40
**Limit of detection. Plate counts fell below the limit of detection of <5.00E+00 CFU/surface.*						

Table 2: *E. coli* ATCC 8739 Percent Reduction and Log_{10} Reduction at 30 Seconds Compared to Time Zero for Neat Plus

Test Date	Test Microorganism	Test Device	Contact Time	CFU/Surface	Percent Reduction Compared to Time Zero	Log_{10} Reduction Compared to Time Zero
20DEC2024	*E. coli* ATCC 8739	Microchem Control	Time Zero	3.45E+06	N/A	N/A
		Neat Plus	30 Seconds	<5.00E+00*	>99.99986%	>5.84
**Limit of detection. Plate counts fell below the limit of detection of <5.00E+00 CFU/surface.*						

Table 3: *MS2 Bacteriophage* ATCC 15597-B1 Percent Reduction and Log_{10} Reduction at 30 Seconds Compared to Time Zero for Neat Plus

Test Date	Test Microorganism	Test Device	Contact Time	PFU/Surface	Percent Reduction Compared to Time Zero	Log_{10} Reduction Compared to Time Zero
20DEC2024	*MS2 Bacteriophage* ATCC 15597-B1	Microchem Control	Time Zero	1.27E+07	N/A	N/A
		Neat Plus	30 Seconds	<5.00E+00*	>99.99998%	>6.71
**Limit of detection. Plate counts fell below the limit of detection of <5.00E+00 PFU/surface.*						

RESULTS (Continued)

Table 4: *S. aureus* ATCC 6538 Percent Reduction and Log_{10} Reduction at 30 Seconds Compared to Time Zero for Home

Test Date	Test Microorganism	Test Device	Contact Time	CFU/Surface	Percent Reduction Compared to Time Zero	Log_{10} Reduction Compared to Time Zero
20DEC2024	*S. aureus* ATCC 6538	Microchem Control	Time Zero	1.27E+07	N/A	N/A
		Home	30 Seconds	<5.00E+00*	>99.99996%	>6.40
**Limit of detection. Plate counts fell below the limit of detection of <5.00E+00 CFU/surface.*						

Table 5: *E. coli* ATCC 8739 Percent Reduction and Log_{10} Reduction at 30 Seconds Compared to Time Zero for Home

Test Date	Test Microorganism	Test Device	Contact Time	CFU/Surface	Percent Reduction Compared to Time Zero	Log_{10} Reduction Compared to Time Zero
20DEC2024	*E. coli* ATCC 8739	Microchem Control	Time Zero	3.45E+06	N/A	N/A
		Home	30 Seconds	<5.00E+00*	>99.99986%	>5.84
**Limit of detection. Plate counts fell below the limit of detection of <5.00E+00 CFU/surface.*						

Table 6: *MS2 Bacteriophage* ATCC 15597-B1 Percent Reduction and Log_{10} Reduction at 30 Seconds Compared to Time Zero for Home

Test Date	Test Microorganism	Test Device	Contact Time	PFU/Surface	Percent Reduction Compared to Time Zero	Log_{10} Reduction Compared to Time Zero
20DEC2024	*MS2 Bacteriophage* ATCC 15597-B1	Microchem Control	Time Zero	1.27E+07	N/A	N/A
		Home	30 Seconds	<5.00E+00*	>99.99998%	>6.71
**Limit of detection. Plate counts fell below the limit of detection of <5.00E+00 PFU/surface.*						

RESULTS (Continued)

Table 7: *S. aureus* ATCC 6538 Percent Reduction and Log_{10} Reduction at 30 Seconds Compared to Time Zero for Hill

Test Date	Test Microorganism	Test Device	Contact Time	CFU/Surface	Percent Reduction Compared to Time Zero	Log_{10} Reduction Compared to Time Zero
20DEC2024	*S. aureus* ATCC 6538	Microchem Control	Time Zero	1.27E+07	N/A	N/A
		Hill	30 Seconds	<5.00E+00*	>99.99996%	>6.40
**Limit of detection. Plate counts fell below the limit of detection of <5.00E+00 CFU/surface.*						

Table 8: *E. coli* ATCC 8739 Percent Reduction and Log_{10} Reduction at 30 Seconds Compared to Time Zero for Hill

Test Date	Test Microorganism	Test Device	Contact Time	CFU/Surface	Percent Reduction Compared to Time Zero	Log_{10} Reduction Compared to Time Zero
20DEC2024	*E. coli* ATCC 8739	Microchem Control	Time Zero	3.45E+06	N/A	N/A
		Hill	30 Seconds	<5.00E+00*	>99.99986%	>5.84
**Limit of detection. Plate counts fell below the limit of detection of <5.00E+00 CFU/surface.*						

Table 9: *MS2 Bacteriophage* ATCC 15597-B1 Percent Reduction and Log_{10} Reduction at 30 Seconds Compared to Time Zero for Hill

Test Date	Test Microorganism	Test Device	Contact Time	PFU/Surface	Percent Reduction Compared to Time Zero	Log_{10} Reduction Compared to Time Zero
20DEC2024	*MS2 Bacteriophage* ATCC 15597-B1	Microchem Control	Time Zero	1.27E+07	N/A	N/A
		Hill	30 Seconds	5.00E+00	99.99998%	6.71

RESULTS (Continued)

Table 10: *S. aureus* ATCC 6538 Percent Reduction and Log_{10} Reduction at 30 Seconds Compared to Time Zero for Tosca

Test Date	Test Microorganism	Test Device	Contact Time	CFU/Surface	Percent Reduction Compared to Time Zero	Log_{10} Reduction Compared to Time Zero
20DEC2024	*S. aureus* ATCC 6538	Microchem Control	Time Zero	1.58E+07	N/A	N/A
		Tosca	30 Seconds	<5.00E+00*	>99.99997%	>6.50
**Limit of detection. Plate counts fell below the limit of detection of <5.00E+00 CFU/surface.*						

Table 11: *E. coli* ATCC 8739 Percent Reduction and Log_{10} Reduction at 30 Seconds Compared to Time Zero for Tosca

Test Date	Test Microorganism	Test Device	Contact Time	CFU/Surface	Percent Reduction Compared to Time Zero	Log_{10} Reduction Compared to Time Zero
20DEC2024	*E. coli* ATCC 8739	Microchem Control	Time Zero	8.75E+06	N/A	N/A
		Tosca	30 Seconds	<5.00E+00*	>99.99994%	>6.24
**Limit of detection. Plate counts fell below the limit of detection of <5.00E+00 CFU/surface.*						

Table 12: *MS2 Bacteriophage* ATCC 15597-B1 Percent Reduction and Log_{10} Reduction at 30 Seconds Compared to Time Zero for Tosca

Test Date	Test Microorganism	Test Device	Contact Time	PFU/Surface	Percent Reduction Compared to Time Zero	Log_{10} Reduction Compared to Time Zero
20DEC2024	*MS2 Bacteriophage* ATCC 15597-B1	Microchem Control	Time Zero	1.58E+07	N/A	N/A
		Tosca	30 Seconds	<5.00E+00*	>99.99997%	>6.50
**Limit of detection. Plate counts fell below the limit of detection of <5.00E+00 PFU/surface.*						

RESULTS (Continued)

Table 13: Media Sterility and Microorganism Purity Controls

Date	Control	Result
20DEC2024	*S. aureus* ATCC 6538 Purity	Pure Growth
	E. coli ATCC 8739 Purity	Pure Growth
	E. coli ATCC 15597 Host Culture Purity	Pure Growth
	MS2 Bacteriophage ATCC 15597-B1	No Plaque Formation
	MS2 Bacteriophage ATCC 15597-B1 and *E. coli ATCC 15597*	Plaque Formation
	Tryptic Soy Agar Sterility	No Growth
	Dey-Engley Broth Sterility	No Growth
	Serial Dilution Medium Sterility	No Growth

The results of this study apply to the tested substance(s), article(s), and/or device(s) only. Extrapolation of findings to related materials is the responsibility of the Sponsor.