



Study report

Study Title

Antibacterial Activity and Efficacy of Dupray's $NEAT^{TM}$ Steam Cleaner

Test Method

Custom Device Study Based on: Modified ASTM E1153

Study Identification Number

NG18486

Author

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Testing Facility

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Study Sponsor

Dupray USA LLC. (800) 881-8482 info@dupray.com

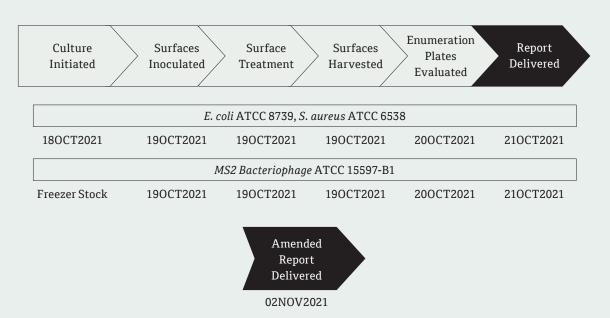
Purpose of the Study

The purpose of this study was to determine the antimicrobial efficacy of Dupray's canister-type steam cleaner model NEATTM DUP020WNA and its standard accessories.

Brief History of the Performing Laboratory

Microchem Laboratory is located in the greater Austin, Texas area. It is owned and operated by microbiologist Dr. Benjamin Tanner. The core of the company was founded by Dr. Tanner as Antimicrobial Test Laboratories in 2006. Antimicrobial Test Laboratories was later combined with a niche cosmetic testing lab and Microchem Laboratory, founded in 1988 by Dr. Norman Miner. The combined labs have operated under one roof as Microchem Laboratory since 2016. Microchem Laboratory is ISO 17025 accredited and offers testing in compliance with current Good Laboratory Practice (GLP) regulations as stipulated by EPA and FDA. Clients are always welcome to tour the lab, observe studies, and audit the lab's quality systems.

Study Timeline



Test Substance Information

Name of Test Device: NEAT[™] Steam Cleaner - DUP020WNA

Manufacturer: Dupray Industries

Mode of Active: Steam

The test device was received on 17 SEP 2021. An operations manual was included with the device.

Test Microorganism Information

The test microorganism(s) selected for this test:



Escherichia coli

This bacteria is a Gram-negative, rod shaped, facultative anaerobe commonly found in the gastrointestinal tract of mammals. Although most serotypes of this microorganism are harmless there are pathogenic groups of *E. coli* such as enterohemorrhagic (EHEC), verocytotoxin producing (VTEC) and Shiga-like toxin producing (STEC) that can cause a multitude of illnesses. *E. coli* is relatively susceptible to disinfection when dried on a surface, yet it can be a challenging microorganism to mitigate in solution.



Staphylococcus aureus 6538

This bacterium is a Gram-positive, spherical-shaped, facultative anaerobe. *Staphylococcus* species are known to demonstrate resistance to antibiotics such as methicillin. *S. aureus* pathogenicity can range from commensal skin colonization to more severe diseases such as pneumonia and toxic shock syndrome (TSS). *S. aureus* is commonly used in several test methods as a model for gram positive bacteria. It can be difficult to disinfect but does demonstrate susceptibility to low level disinfectants.



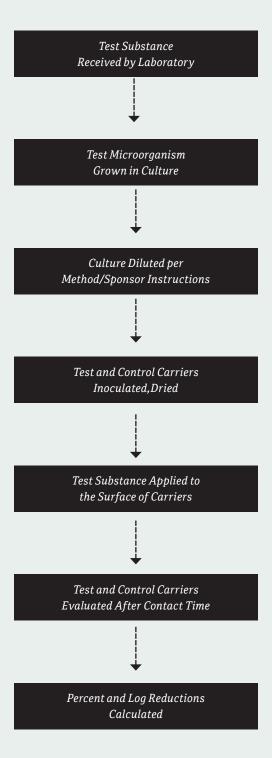
MS2 Bacteriophage (MS2), ATCC 15597-B1

This virus is a non-enveloped positive-stranded RNA virus of the bacteriophage family Leviviridae. Bacterial cells are the hosts

bacteriophages, and *E. coli* 15597 serves this purpose for MS2 bacteriophage. Its small size, icosohedral structure, and environmental resistance has made MS2 ideal for use as a surrogate virus (particularly in place of picornaviruses such as poliovirus and human norovirus) in water quality and disinfectant studies.

Permissive Host Cell System for MS2: Escherichia coli, 15597

Diagram of the Procedure



Summary of the Procedure

- The test microorganism is prepared, usually by growth in liquid culture medium or on an appropriate agar plate.
- The test culture may be supplemented with an artificial soil load, such as horse or fetal bovine serum, for one-step cleaner/sanitizer claims.
- Sterilized 1" x 3" glass slide carriers are inoculated with a volume of the test culture.
 Inoculated slides are dried. Only completely dried carriers are used in the test.
- Test carriers are treated with the test device and incubated for the predetermined contact time.
- Control carriers are harvested at appropriate intervals to accurately represent any reduction during the contact time.
- At the conclusion of the contact time, test and control carriers are chemically neutralized.
- Dilutions of the neutralized test substance are evaluated using appropriate growth media to determine the surviving microorganisms at the respective contact time.
- The effect of the test substance is compared to the effect of the control substance in order to determine microbial reductions.

Criteria for Scientific Defensibility of a Custom Device Study

For Microchem Laboratory to consider a Device Study study to be scientifically defensible, the following criteria must be met:

- 1. The average number of viable bacteria recovered from the time zero samples must be approximately 1×105 cells/carrier or greater.
- 2. Positive/Growth controls must demonstrate growth of the appropriate test microorganism.
- 3. Negative/Purity controls must demonstrate no growth of test microorganism.

Passing Criteria

Due to the modified nature of the study, passing criteria may be determined by the Study Sponsor.

Testing Parameters

Test Substance Mode of Use:

Steam

Replicates:

Single

Carriers (Size):

Glass Slides (1" x 3")

Culture Growth Time:

18-24 hours

Culture Growth

Media:

Tryptic Soy Broth

Culture

Supplement:

N/A

Inoculum Con-

centration:

≥1.0 x 106 CFU/Carrier

Inoculum Volume:

0.010 mL

Contact Time:

30 seconds

Contact Temperature:

Ambient

Neutralizer (Vol.):

PBS w/ 0.1% Tween-80 (20.0 mL)

Enumeration Media:

Tryptic Soy Agar (EC 8739), Nutrient Agar (SA 6538), 50% Tryptic Soy Agar (MS2)

Enumeration

Plate

Incubation
Temperature:

36°C ± 1°C

Enumeration Plate

12-18 hours (MS2)

Incubation Time:

24-48 hours (EC 8739, SA

6538)

Study Notes

Per study sponsor's request, the triangular tool and bonnet accessories were used for this test. Per study sponsor's request, the machine was filled with sterile tap water and plugged into a common electrical outlet. The unit was then turned on and preheated for approximately 10 minutes. 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 as the accessory of choice for the experiment. Steam was then released for an additional 5 seconds 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 experiment was conducted.

Study report was amended 02NOV2021 for further clarification of testing procedure.

Control Results

Neutralization Method: Valid Media Sterility: No Growth

Growth Confirmation: Pure and viable

Calculations

Percent Reduction=
$$(\frac{B-A}{B})$$
 X 100

Where:

B = Number of viable test microorganisms on the control carriers immediately after inoculation

A = Number of viable test microorganisms on the test carriers after the contact time

$$Log_{10}$$
 Reduction= $Log(\frac{B}{A})$

Where:

B = Number of viable test microorganisms on the control carriers immediately after inoculation

A = Number of viable test microorganisms on the test carriers after the contact time

Results of the Study

Table 1: Results of Escherichia coli ATCC 8739 test

Test Microorganism	Contact Time	Replicate CFU/Carrier	Percent Reduction Compared to Time Zero	Log ₁₀ Reduction Compared to Time Zero
Escherichia coli ATCC 8739	Time Zero Control	5.70E+05	N/A	
	30 seconds	<1.00E+01	>99.998%	>4.76

Table 2: Results of Staphylococcus aureus ATCC 6538 test

Test Microorganism	Contact Time	Replicate CFU/Carrier	Percent Reduction Compared to Time Zero	Log ₁₀ Reduction Compared to Time Zero
Staphylococcus aureus ATCC 6538	Time Zero Control	5.70E+06	N/A	
	30 seconds	<1.00E+01	>99.9998%	>5.76

Table 3: Results of MS2 Bacteriophage ATCC 15597-B1 test

Test Microorganism	Contact Time	Replicate PFU/Carrier	Percent Reduction Compared to Time Zero	Log ₁₀ Reduction Compared to Time Zero
MS2 Bacteriophage ATCC 15597-B1	Time Zero Control	1.50E+05	N/A	
	30 seconds	1.10E+02	99.93%	3.13

 $The \ results \ of \ this \ study \ apply \ to \ the \ tested \ substances (s) \ only. \ Extrapolation \ of \ findings \ to \ related \ materials \ is \ the \ responsibility \ of \ the \ Sponsor.$

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