ANTIMICROBIAL TEST LABORATORIES

Study Report

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Study Title

Determination of the Antiviral Effectiveness of KHG FiteBac Technology Test Substance Delivered via Pipette Against MS2 Bacteriophage

Test Method

ASTM International Standard Test Method E1053
Assessment of the Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces

Study Identification Number

NG5759-A1

Study Sponsor

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Test Facility

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History of the Laboratory

Antimicrobial Test Laboratories was launched in 2006 to provide testing services to the antimicrobial industry. The company has grown considerably since then but its focus remains the same: Test antimicrobial agents, test them well, and test them fast! Antimicrobial Test Laboratories operates a 15,000+ square foot facility near Austin, Texas, where hundreds of studies are conducted annually by a staff of friendly, knowledgeable, and experienced microbiologists and virologists.

Laboratory Qualification Statement

Antimicrobial Test Laboratories was founded by microbiologist Dr. Benjamin Tanner. The laboratory ensures consistent, reproducible results by utilizing a well-trained and educated scientific staff who work from a comprehensive system of Standard Operating Procedures, official standard methods from ASTM, AOAC, and other organizations, and custom study protocols. The laboratory provides testing services to dozens of Fortune 500 companies and has been inspected for GLP compliance by the US government.

Scientist Qualifications

Your Study was designed, conducted and reported by: Nicole Goulding, B.S.

Nicole graduated from the University of California at Santa Barbara with a Bachelors of Science in Biological Sciences.

Nicole is an experienced microbiologist with a broad and refined skill set. Her knowledge of microbiology enables her to conduct studies consistently and efficiently. Nicole frequently leads antimicrobial device studies and has a track record of seeing large, complex projects through to completion. She is perceptive of client goals and known within the laboratory for her professionalism and positive outlook

If you have any questions about your study, please don't hesitate to contact Nicole at:

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or
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ASTM E1053: General Information

ASTM International, formerly the American Society for Testing and Materials (ASTM), is an internationally recognized organization that develops and publishes product and testing standards. The ASTM E1053 test method is used to determine the virucidal effectiveness of liquid disinfectant products designed for use on hard, nonporous environmental surfaces. In an ASTM E1053 test, a viral inoculum is dried onto carriers, followed by exposure to a test formulation via spray device or pipette (modified use-dilution) for the specified contact time(s). Control carriers are concurrently processed using an equivalent volume of cell culture medium or other suitable buffer. Following neutralization, the carriers are enumerated using standard cell culture (e.g. $TCID_{50}$) or plaque assay techniques. Log_{10} and percent reduction values are calculated to determine the effectiveness of the test product relative to the control carriers. The ASTM E1053 test method for use with spray devices or pipette delivery is recognized by regulatory agencies as an approved method for claim substantiation.

Laboratory Qualifications Specific to the ASTM E1053 Test

Antimicrobial Test Laboratories has considerable experience in the proper execution of the ASTM E1053 test method. The laboratory has performed many ASTM E1053 tests in order to assess the virucidal efficacy of a broad spectrum of disinfectant products. In addition, the laboratory has experience modifying the method as needed to accommodate customer needs. Each ASTM E1053 test at Antimicrobial Test Laboratories is performed in a manner appropriate to the test substances submitted by the Study Sponsor, while maintaining the integrity of the study.

Study Timeline

15 JAN 2015 | 15 JAN 2015 | 15 JAN 2015 | 16 JAN 2015 | 26 JAN 2015

Amended report sent on 27 JAN 2015

Test Substance Information

The test substances were received on 14 JAN 2015 and the following picture was taken.

Test Substances Received: 2% K21; Control I, Control II (not used in Study NG5759)

Test Substances arrived and required dilution. Test substances were diluted in sterile reverse osmosis water prior to use in the study. The received 2% K21 test substance and Control I substance were further diluted by preparing three test dilutions (1:10, 1:100, 1:1000).

Test Microorganism Information

The test microorganism(s) selected for this test:

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 for 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

- Stock virus is thawed and may be supplemented with an organic soil load, if requested.
- Sterile glass Petri dish carriers (100 x 15 mm) are inoculated with a volume of virus suspension containing an adequate titer to recover a minimum of 4-$\log_{10}$ infectious viruses per carrier. A sufficient number of test and control carriers are prepared.
- Inoculated carriers are dried at room temperature under laminar flow conditions.
- The test substance is prepared according to the Study Sponsor's instructions as requested, and applied to the test carriers using a spray device or pipette. For spray tests, the distance, angle, and number of sprays applied are recorded. For use-dilution (pipette delivery) tests, the volume applied per carrier is recorded.
- The treated carriers are held for the predetermined contact time(s), and then neutralized in a manner appropriate for the test substance (e.g. dilution and/or gel filtration).
- The control carrier is harvested using an equivalent volume cell culture medium or other suitable buffer.

Summary of the Procedure Continued

- Following neutralization of test and control carriers, the viral suspensions are quantified to determine the levels of infectious virus using standard cell culture (e.g. $TCID_{50}$) or plaque assay techniques.
- Assay trays/plates are incubated for the period most suitable for the virus-host cell system (e.g. 7 days).
- After the incubation period, the assay is scored by quantifying plaque-forming unites or observing the presence/absence of test virus. The appropriate calculations are performed to determine viral titers and levels of test substance cytotoxicity, where applicable.
- Log_{10} and percent reductions are computed for viral films exposed to the test product relative to the titer obtained for the study control carrier(s), and reported to the Study Sponsor.

Criteria for Scientific Defensibility of an ASTM E1053 Study

For Antimicrobial Test Laboratories to consider a virucidal effectiveness test to be scientifically defensible, the following criteria must be met:

1. A minimum of 4-$\log_{10}$ infectious viruses are recovered from the virus control carrier.
2. Viral cytopathic effects are distinguishable from cytotoxic effects caused by test substance exposure.
3. Neutralization effectiveness is demonstrated by recovery of comparable levels of infectious viruses from control (e.g. PBS), neutralizer (where applicable), and neutralized test substance.
4. Assay wells designated as sterility controls are absent of infectivity, contamination, and cytotoxicity.

Passing Criteria

ASTM International defines passing criteria to be:

1. Complete inactivation of the test virus at all dilutions.
2. If cytotoxicity is observed, a ≥3-log10 reduction in viral titer is observed past the level of cytotoxicity relative to the virus control.

Testing Parameters used in this Study

Test Substance Diluent:	Sterile R/O Water	Carrier Type:	Petri Dishes
Carriers Per Test:	1	Number of Sprays:	3
Spray Distance:	6-8 inches	Spray Angle:	45°
Use-dilution Volume:	Not Applicable		

Viral Inoculum Volume:	0.200 ml	Carrier Inoculation Area:	15 x 100 mm
Carrier Dry Time:	39 minutes	Carrier Dry Conditions:	Ambient
Contact Time(s):	10 minutes	Contact Conditions:	Ambient
Host Cell Line:	*E. coli* ATCC 15597	Cell Passage Number:	Not Applicable
Assay Medium:	Tryptic Soy Agar	Soil Load:	None Requested
Incubation Period:	18-24 Hours	Incubation Conditions:	36 ±1° C

Study Modifications

No further modifications were made to the method for this study.

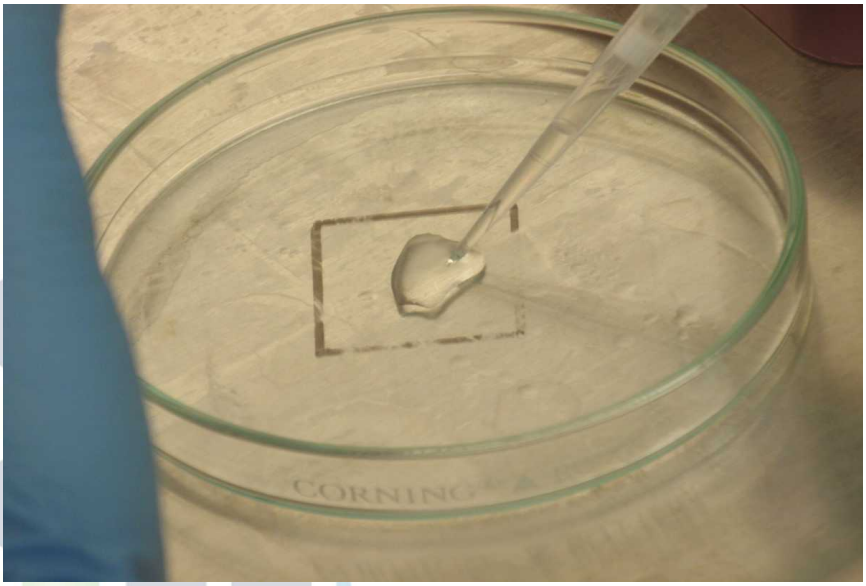
Study Notes

At the closure of the 10 minute contact time test carriers were neutralized by passing the test suspension through a pre-equilibrated Sephacryl S-1000 gel column. A secondary neutralization step was then performed where an aliquot of the test filtrate was neutralized in 9.9 ml of D/E neutralization broth.

The results harvested from the neutralization validation control indicate that neutralization was not validated at the 1:10 and 1:100 preparations of the 2% K21 test substance. Neutralization using the neutralization scheme specified above was validated at the 1:1000 dilution of the 2% K21 test substance.

The *E. coli* ATCC 15597 host culture prepared for this testing was inoculated in Trypic Soy Broth on 14 JAN 2015 and allowed to incubate at 36 ± 1 °C for 18-24 hours prior to use.

Study Photographs



0.200 ml of the test virus was applied to the surface of a sterile Petri Plate and evenly spread over the entire area (10-in^2 equivalent). The virus films were allowed to dry under ambient conditions until the surface appeared visibly dry.

Control Results

Virus Control Titer:	2.80 x 10^4 PFU/carrier	Cytotoxicity Titer:	None Detected
Virus Stock Titer:	2 x 10^{10} PFU/ml	Sterility Controls:	Sterility Confirmed
Neutralization Effectiveness:	Not Validated; See Study Notes on Page 9		

Calculations

$$\text{Percent Reduction} = \left(\frac{B-A}{B}\right) \times 100$$

Where:
B = Number of viable test microorganisms on the control carriers after the contact time
A = Number of viable test microorganisms on the test carriers after the contact time

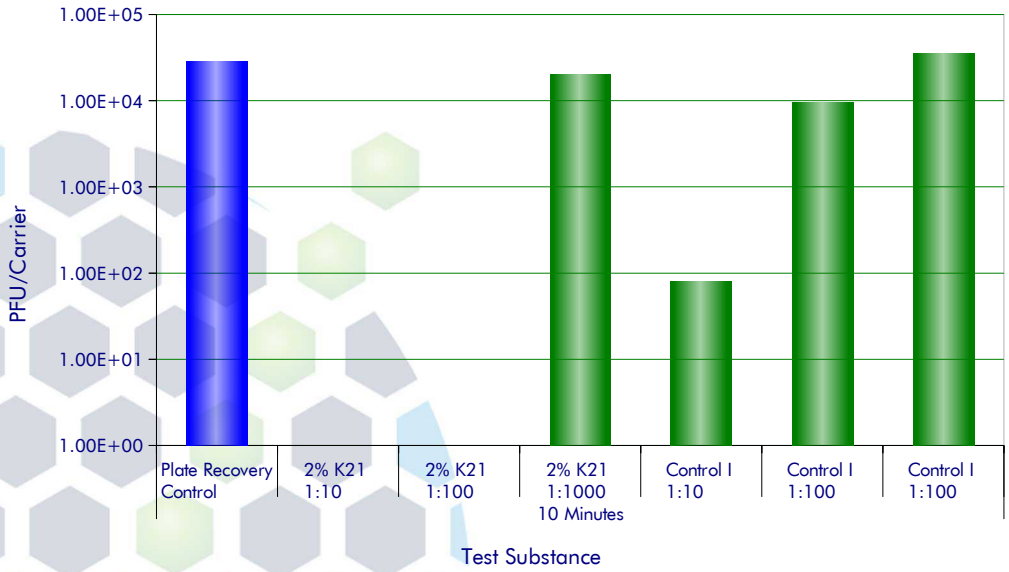
$$\text{Log}_{10}\text{Reduction} = \text{Log}\left(\frac{B}{A}\right)$$

Where:
B = Number of viable test microorganisms on the control carriers after the contact time
A = Number of viable test microorganisms on the test carriers after the contact time

Results of the Study

Table 1. ASTM E1053 Evaluation of the Efficacy of 2% K21 Prepared at Three Test Dilutions Against MS2 Bacteriophage

Test Microorganism	Contact Time	Test Substance and Dilution Factor	Replicate PFU/Carrier	Percent Reduction Compared to Plate Recovery Control	Log_{10} Reduction Compared to Plate Recovery Control
MS2 Bacteriophage 15597-B1	10 Minutes	Plate Recovery Control	2.80E+04	N/A	
		2% K21 1:10	<2.00E+01	>99.93%	>3.15
		2% K21 1:100	<2.00E+01	>99.93%	>3.15
		2% K21 1:1000	2.00E+04	28.57%	0.15
		Control I 1:10	8.00E+01	99.71%	2.54
		Control I 1:100	9.40E+03	66.43%	0.47
		Control I 1:100	3.50E+04	No Reduction	



Note: The limit of detection for this assay was 2.00E+01.
Values below the limit of detection are represented as zero in the chart above.

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