MICROCHEM LABORATORY

STUDY REPORT

Study Title

Antimicrobial Efficacy of NanoTouch Non-porous Test Substance Modified for Viruses

Test Method

Japanese Industrial Standard Z 2801
Antibacterial Products – Test for Antibacterial Activity and Efficacy

Study Identification Number

NG6897

Study Sponsor

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Test Facility

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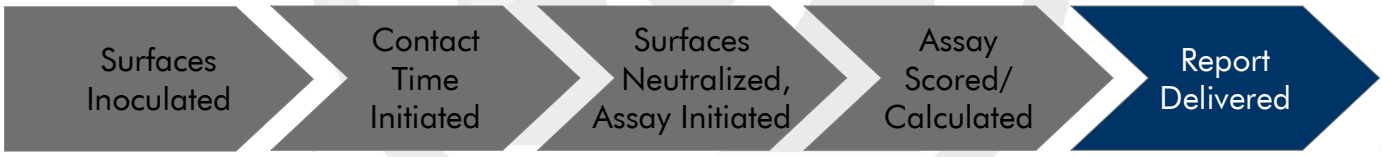
JIS Z 2801: General Information

The Japanese Industrial Standard Committee (JIS) is an international organization that develops and standardizes test methods for a variety of products and materials. The JIS method Z 2801 is a quantitative test designed to assess the performance of antimicrobial finishes on hard, non-porous surfaces and can be modified as needed to evaluate efficacy against viruses.. The method can be conducted using contact times ranging from ten minutes up to 24 hours. For a JIS Z 2801 test, non-antimicrobial control surfaces are used as the baseline for calculations of microbial reduction. The method is versatile and can be used to determine the antimicrobial activity of a diverse array of surfaces including plastics, metals, and ceramics.

Laboratory Qualifications Specific to JIS Z 2801

Microchem Laboratory has considerable experience in the proper execution of JIS Z 2801 tests modified for virucidal efficacy. The laboratory has performed many JIS Z 2801 tests in order to assess the virucidal efficacy of a broad spectrum of test surfaces. Each modified JIS Z 2801 test at Microchem Laboratory is performed in a manner appropriate to the test substances submitted by the Study Sponsor, while maintaining the integrity of the study.

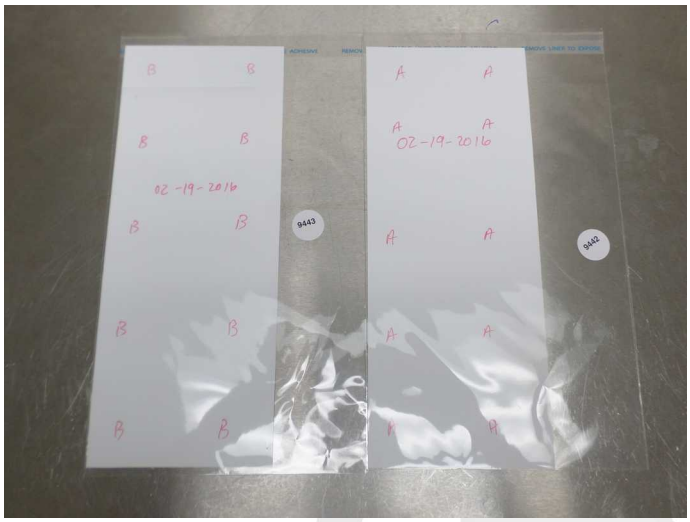
Study Timeline



15 MAR 2016	15 MAR 2016	15 MAR 2016	16 MAR 2016	18 MAR 2016

Test Substance Information

The test substance was received on 24 FEB 2016 and the following pictures were taken.

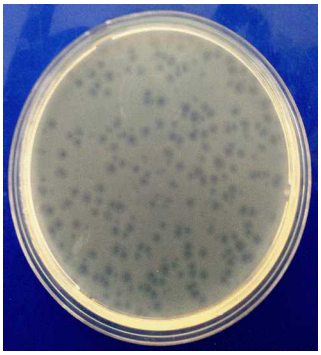


Test Substances Received: 02-19-2016 A, 02-19-2016 B

Test Substances arrived in dimensions that were not optimal for the conduct of the Study. Test substances were cut down to ideal sizes for the Study. Only one test substance (02-19-2016 B) was used for testing.

Test Microorganism Information

The test microorganism(s) selected for this test:

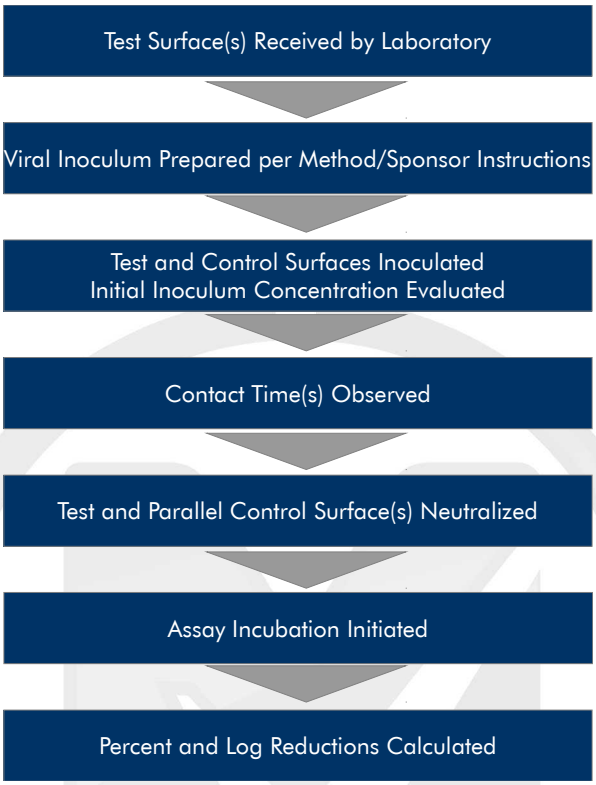


MS2 Bacteriophage (MS2), ATCC 15597-B1

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

- Test virus is thawed from frozen stock. Virus may be supplemented with an organic soil load.
- Test and Control surfaces are inoculated with the test virus. A thin, sterile film is used to cover and spread the inoculum, preventing evaporation and ensuring close contact with the test surface.
- The viral concentration is determined at "Time Zero" to verify the starting virus concentration.
- The Test and Control surfaces are held for the contact time(s) specified by the Study Sponsor, and then harvested using an appropriate neutralizing solution and/or gel filtration.
- Following neutralization, the carrier 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.
- After the incubation period, the assay is scored for the presence/absence of test virus and cytotoxic effects. The appropriate calculations are performed (e.g. Spearman-Karber) to determine viral titers and levels of test substance cytotoxicity, where applicable.
- Log_{10} and percent reductions are computed for Test surfaces relative to the timed Control surfaces, and reported to the Study Sponsor.

Criteria for Scientific Defensibility of a Modified JIS Z 2801 Study

For Microchem Laboratory to consider a JIS Z 2801 study modified for viruses to be scientifically defensible, the following criteria must be met:

1. A minimum of 4-Log_{10} infectious viruses are recovered from the both the "Time Zero" and timed virus control surfaces.
2. Viral cytopathic effects are distinguishable from cytotoxic effects caused by test surface exposure.
3. Effectiveness of the neutralization method (dilution and/or gel filtration) is demonstrated.
4. Assay wells designated as sterility controls are absent of infectivity, contamination, and cytotoxicity.

Passing Criteria

JIS specifies a performance criteria for antimicrobial efficacy of greater than or equal to a 2 Log_{10} or 99% reduction in in the test microorganisms when comparing the treated surface to the control surface after the contact time.

Alternatively, passing criteria may be determined by the Study Sponsor in accordance with pertinent governmental regulations. Federal regulatory agencies such as the US EPA specify the following passing criteria for virucidal efficacy: Complete inactivation of the test virus at all dilutions. If cytotoxicity is observed, a ≥3-Log_{10} reduction in viral titer is observed past the level of cytotoxicity relative to the virus control.

Testing Parameters used in this Study

Test Substance Size:	50 mm x 50 mm	Film Used? (Size):	No
Replicates:	Two		

Viral Inoculum Volume:	10µl	Target Inoculum:	2 x 10^5 PFU/Carrier
Dilution Medium:	PBS	Soil Load:	None Requested
Contact Time(s):	12 Hours	Contact Conditions:	Ambient
Host Cell Line:	*E. coli* 15597	Cell Passage Number:	N/A
Assay Medium:	50% TSA	Neutralizer:	D/E Broth (10 ml)
Enumeration Plate Incubation Conditions:	36°C ± 1°C	Enumeration Plate Incubation Period:	24 ± 6 hours

Study Modifications

The inoculation method for this study was modified to facilitate contact between the test microorganism and the test surface, as well as to conform with previously performed virucidal efficacy testing. Both the inoculum volume and method of contact were modified from the method standard (0.400 ml inoculum volume over a 40 mm x 40 mm area with a wet inoculum held in contact by a sterile plastic film):

Viral Inoculum Volume: 10 µl
Viral Inoculum Area: 1"x1"
Mode of Contact: Dried viral film

Study Notes

The contact time was observed under controlled lighting conditions (1000-1400 lux).

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Control Results

Neutralization Method: N/A
Virus Control Titer: 2.80×10^5 PFU/Carrier
Media Sterility: Confirmed
Cytotoxicity Titer: N/A

Calculations

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

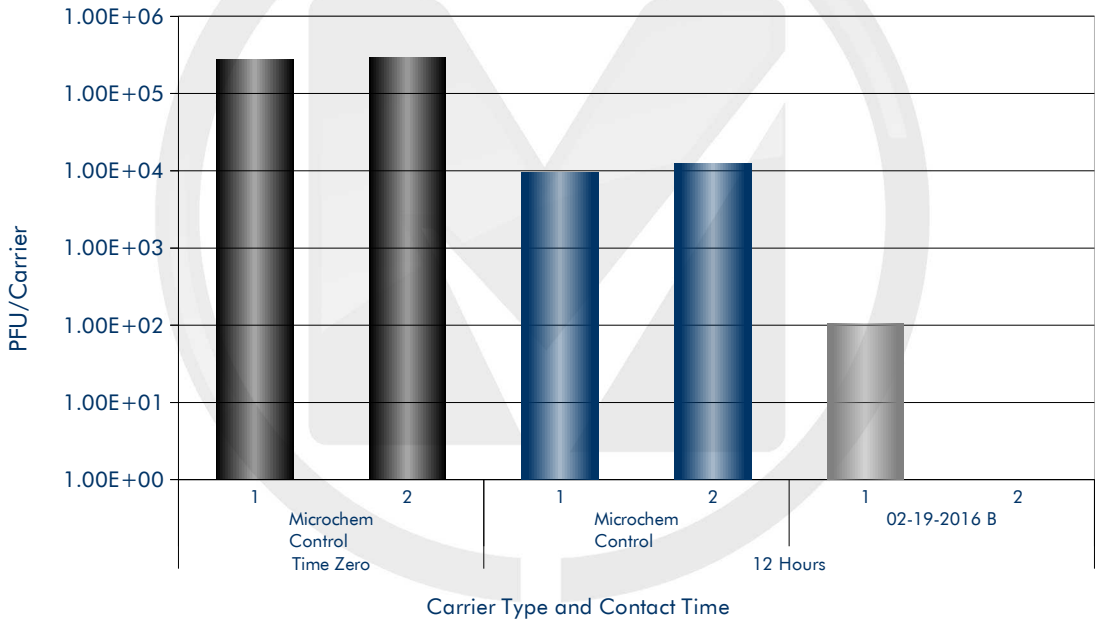
Where:
B = Number of viable test microorganisms on the control carriers after the contact time
A = Number of viable test microorganisms on the test carriers after the contact time

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

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

Test Microorganism	Contact Time	Carrier Type	Replicate Number	PFU/Carrier	Mean PFU/Carrier	Percent Reduction Compared to Control at Contact Time	Log_{10} Reduction Compared to Control at Contact Time
MS2 Bacteriophage ATCC 15597-B1	Time Zero	Microchem Control	1	2.75E+05	2.80E+05	N/A	
			2	2.85E+05			
	12 Hours	Microchem Control	1	9.50E+03	1.10E+04	N/A	
			2	1.25E+04			
		02-19-2016 B	1	1.05E+02	<5.50E+01	>99.50%	>2.30
			2	<5.00E+00			



**Note: Values below the limit of detection for this assay are designated with "≤" in the table above, and are depicted as a value of zero in the graph 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.