MICROCHEM LABORATORY

STUDY REPORT

Study Title

Antiviral Efficacy of Bio-Care Technology Non-Porous Coated Test Surfaces

Test Method

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

Study Identification Number

NG7184

Study Sponsor

Bio-Care

Test Facility

Microchem Laboratory
1304 W. Industrial Blvd
Round Rock, TX 78681
(512) 310-8378

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

Viral Stocks Thawed	Surfaces Inoculated	Surfaces Incubated	Surfaces Harvested	Surfaces Evaluated	Report Delivered
20JUL2016	20JUL2016	20JUL2016	20JUL2016	27JUL2016	29JUL2016

Test Substance Information

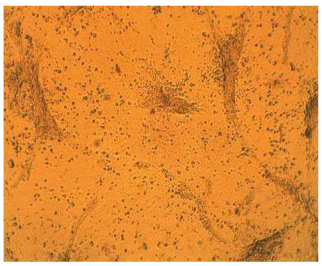
The test substance was received on 14JUL2016 and the following pictures were taken.

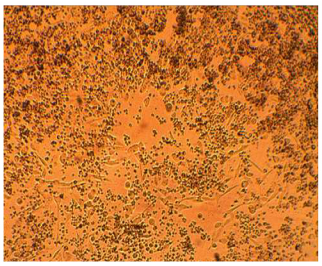
Test Substance Received: Coated Aluminum Sheet

Test Substances arrived in dimensions that were not optimal for the conduct of the Study. Test substances were cut to size for the Study.

Test Microorganism Information

The test microorganism(s) selected for this test:



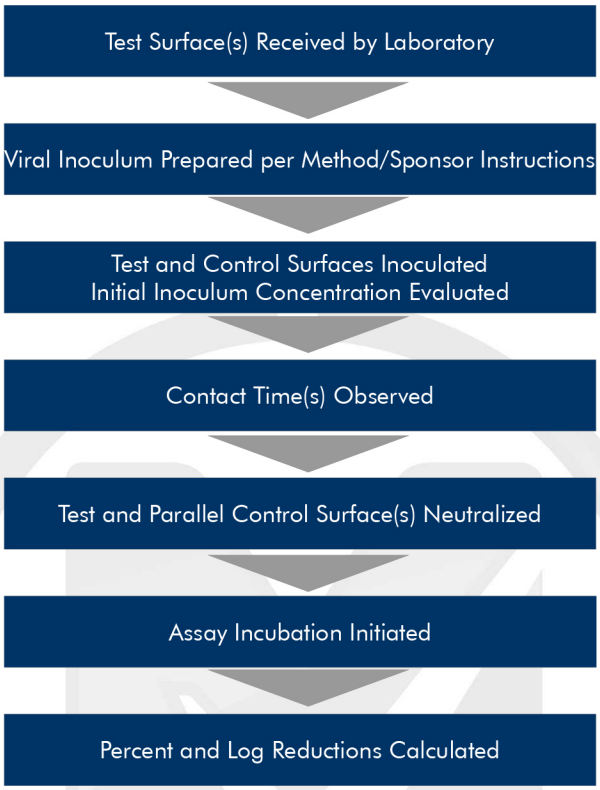


Influenza A (H1N1)

Influenza A virus is an enveloped, minus-stranded member of the family *Orthomyxoviridae*, and causative agent of the illness influenza (which is more widely recognized by the term 'flu'). Influenza is more serious than other seasonal mild, respiratory tract infections (e.g. the common cold) with symptoms that can last for upwards of several weeks. Young children and the elderly are particularly susceptible to severe illness and death due to infection. Influenza is readily transmitted via infective aerosols direct contact with infective respiratory secretions. Potential transmission by contaminated environmental surfaces (fomites) has increasingly become of interest, and Influenza virus is highly vulnerable to inactivation by drying and exposure to variety of disinfectant actives.

Permissive Host Cell Line Selected for Influenza A (H1N1): MDCK (Madin Darby Canine Kidney Cells), ATCC CCL-34

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 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 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):	Yes (40 mm x 40 mm)
Replicates:	Single		

Viral Inoculum Volume:	400µl	Target Inoculum:	5.00 log_{10}/carrier
Dilution Medium:	PBS	Soil Load:	None requested
Contact Time(s):	4 Hours	Contact Conditions:	24.0° C, 48% RH
Host Cell Line:	MDCK (ATCC CCL-34)	Cell Passage Number:	p. 167
Assay Medium:	Influenza Infection Medium	Neutralizer:	IIM
Enumeration Plate Incubation Conditions:	34°C ± 2°C, 5 ± 1% CO_2	Enumeration Plate Incubation Period:	7 Days

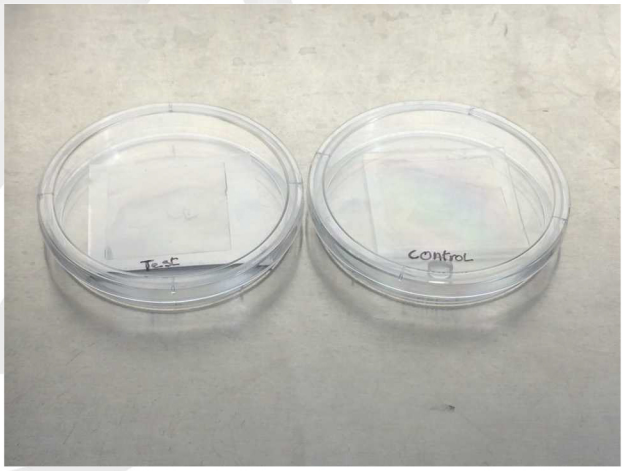
Study Modifications

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

Study Notes

Testing was performed using the coated side of the treated carrier. Coating was confirmed upon visual inspection based on information provided by the Study Sponsor (presence of an un-coated portion on the upper edge of each coated surface).

Study Photographs



Photograph 1. Test and control carriers with sterile cover films applied following inoculation with the prepared viral suspension.

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Control Results

Neutralization Method: Confirmed
Virus Control Titer: 3.80 $\log_{10}$ per carrier
Media Sterility: Confirmed
Cytotoxicity Titer: N/A

Calculations

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

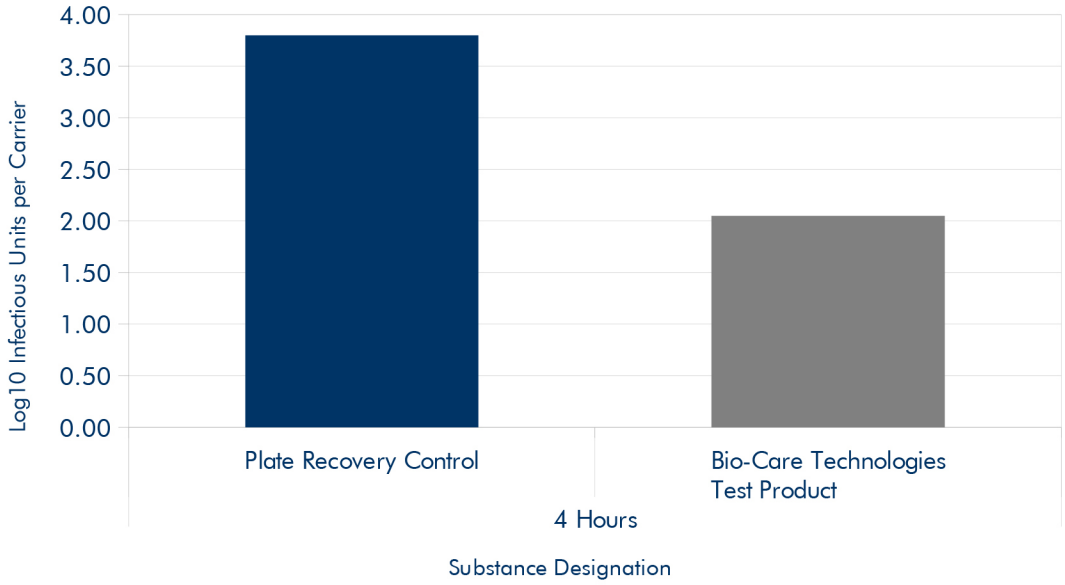
Where:
B = Number of viable test microorganisms on the control carriers after the contact time
A = Number of viable test microorganisms on the test carriers after the contact time

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

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	Surface Designation	Log_{10} Infectious Units per Carrier	Log_{10} Reduction vs. Plate Recovery Control	Percent Reduction vs. Plate Recovery Control
Influenza A (H1N1) ATCC VR-1736	4 Hours	Plate Recovery Control	3.80	N/A	
		Bio-Care Technologies Test Product	2.05	1.75	98.22%



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