MICROCHEM LABORATORY

STUDY REPORT

Study Title

Determination of the Antiviral Effectiveness of Advanced UV Systems Test Device Against Feline Calicivirus, ATCC VR-782

Test Method

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

Study Identification Number

NG12055

Study Sponsor

Wayne Clark
Advanced UV Systems
PO Box 57
South Hill, VA 23970

Test Facility

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

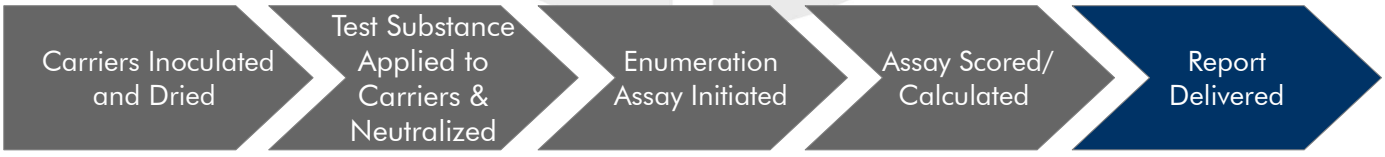
ASTM E1053: General Information

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

Microchem Laboratory 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 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



Test Substance Information

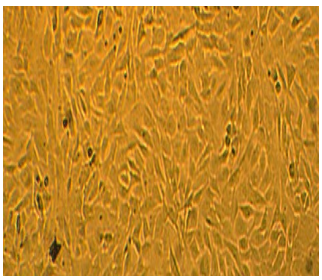
The test device was received on 11JAN2019 and the following picture was taken.
(Note: Photo depicts the test device that was analyzed in this study)

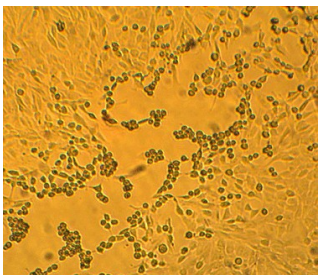
Test Device Received: KR615 UV Germicidal Enclosure Device

Test Device arrived ready to use.

Test Microorganism Information

The test microorganism(s) selected for this test:





Feline calicivirus (FCV), ATCC VR-782
This virus is a non-enveloped, positive-stranded RNA member of the genus *Vesivirus*, and a common cause of respiratory infections in cats. Symptoms of infection in felines include nasal discharge and mouth ulcers. As a member of the *Caliciviridae* viral family, FCV is closely related to human noroviruses, which cause acute gastroenteritis marked by nausea, vomiting, and diarrhea. Unlike human norovirus, however, a simple cell culture assay system is available for FCV. Therefore, feline calicivirus is the US EPA-approved surrogate microorganism for human norovirus label claims. Both FCV and human norovirus are able to remain viable on environmental surfaces for extended periods of time and are resistant to a number of disinfectant actives.
Permissive Host Cell Line Selected for FCV: CRFK (Crandell-Rees Feline Kidney Cells), ATCC CCL-94

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 4.80 $\log_{10}$ 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 (cont.)

- 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 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 viral films exposed to the test product relative to the titer obtained for the study control carrier(s), and reported to the Study Sponsor.

1304 W. INDUSTRIAL BLVD. ROUND ROCK, TX 78681 | © 2016 | CONTACT@MICROCHEMLAB.COM | 512.310.8378

Criteria for Scientific Defensibility of an ASTM E1053 Study

For Microchem Laboratory to consider a virucidal effectiveness test to be scientifically defensible, the following criteria must be met:

1. A minimum of 4.80 $\log_{10}$ 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. In the presence or absence of cytotoxicity, a $\geq$3.00 $\log_{10}$ reduction in viral titer is observed past the level of cytotoxicity relative to the virus control.

Testing Parameters used in this Study

Test Substance Diluent:	N/A	Carrier Type:	Glass Petri Dish
Carriers Per Test:	2 (Test Carriers)	Number of Sprays:	N/A
Spray Distance:	N/A	Spray Angle:	N/A
Use-dilution Volume:	N/A		
Neutralization Method:	2.0 ml of neutralizer was applied to each test carrier after exposure		

Viral Inoculum Volume:	0.200 ml	Carrier Inoculation Area:	10-in^2
Carrier Dry Time:	15±3 Minutes	Carrier Dry Conditions:	See Notes
Contact Time(s):	~ 1 Minute (See Notes)	Contact Conditions:	Ambient
Host Cell Line:	CRFK ATCC CCL-94	Cell Passage Number:	221
Assay Medium:	2% FBS EMEM	Soil Load:	N/A
Incubation Conditions:	36 ± 2°C, 6±1% CO_2	Incubation Period:	9 Days

1304 W. INDUSTRIAL BLVD. ROUND ROCK, TX 78681 | © 2016 | CONTACT@MICROCHEMLAB.COM | 512.310.8378

Study Modifications

This study was modified to accommodate the use of a UV device for treatment of inoculated carriers.

Prior to inoculation of the test carriers, the stock virus was diluted to 6.00 $\log_{10}TCID_{50}$/0.100 ml by performing two 1:10 dilutions with 1 part stock virus to 9 parts test media per the Study Sponsor's request.

Plate recovery controls were left inside the Biological Safety Cabinet, covered, and harvested at the conclusion of the contact time (1 minute).

Study Notes

A handheld timer was used to measure the actual contact time of the device. The timer read 59 seconds when the device completed the contact time.

Testing was performed in double replicate, per the Study Sponsor's request.

The following is a description of the temperature and relative humidity level observed during the drying time of the inoculated carriers:

Drying conditions

Temperature: 24.5-25.0°C
Relative humidity: 42.0-45.0%

2.0 ml of 2% FBS EMEM was used as Neutralizer for this assay.

Study Photographs

No study photographs were taken during the conduct of the study.

1304 W. INDUSTRIAL BLVD. ROUND ROCK, TX 78681 | © 2016 | CONTACT@MICROCHEMLAB.COM | 512.310.8378

Control Results

Virus Control Titer:	See Results	Cytotoxicity Titer:	None observed
Virus Stock Titer:	8.00 $\log_{10}$ $TCID_{50}$/0.100 ml	Sterility Controls:	Confirmed
Neutralization Effectiveness:	Confirmed		

Calculations

- The Spearman-Kärber Method is used to calculate the Plate Recovery Control titer ($TCID_{50}$), the viral titer following test substance exposure ($TCLD_{50}$), and the titer of host cell cultures exhibiting cytotoxicity following test substance exposure (TCD_{50}).
- The $TCID_{50}$ (Tissue Culture Infectivity Dose) represents the endpoint dilution where 50% of the cell cultures exhibit cytopathic effects due to infection by the test virus. The dose required to kill 50% of the test viruses after the given exposure time is referred to as the Tissue Culture Lethal Dose ($TCLD_{50}$), and the endpoint dilution at which 50% of the host cell monolayers exhibit cytotoxicity is termed the Tissue Culture Dose (TCD_{50}). The $TCID_{50}$, $TCLD_{50}$, and TCD_{50} are determined according to the method of Spearman-Kärber as follows:

  Negative logarithm of $TCID_{50}$ endpoint titer = [– Log of first dilution inoculated] – [((sum of % mortality at each dilution/100) – 0.5) x Logarithm of dilution]

  - The result of this calculation is expressed as $TCID_{50}$/0.1 ml (or volume of dilution inoculated)
  - Determination of viral titer per carrier is established by accounting for the volume of viral inoculum per carrier. This is calculated by dividing 1.0 ml by the total viral inoculum per carrier.

  Viral Titer per Carrier, where:

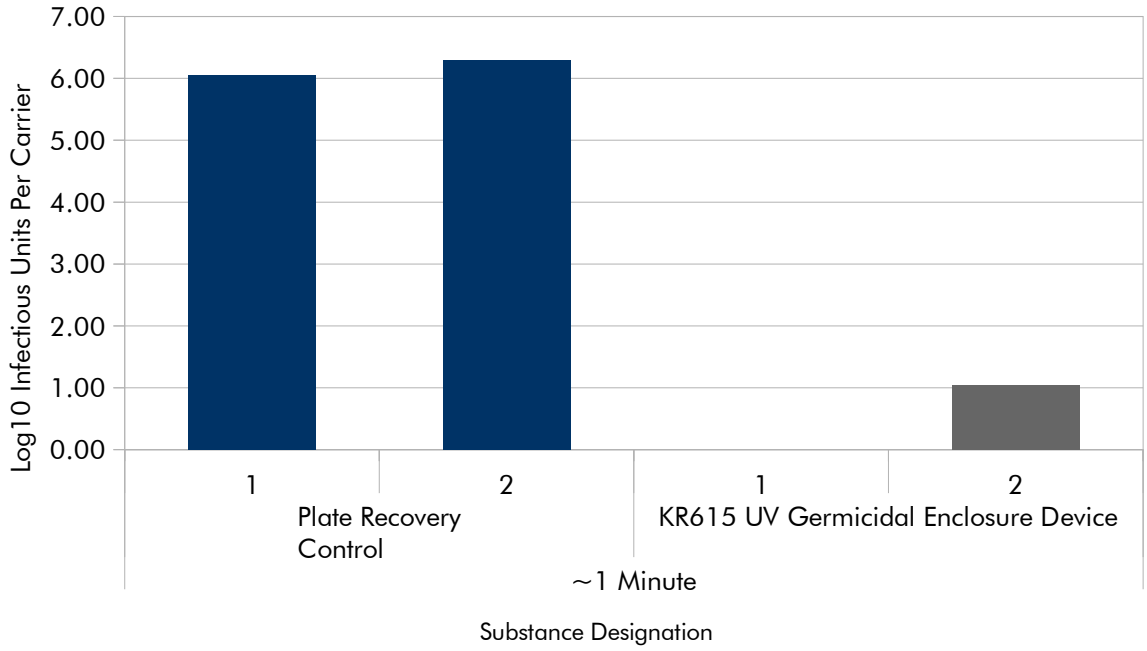
  1. Factor of Volume per carrier = Log_{10}[Volume per Carrier / 0.1 ml]
  2. Viral Titer per Carrier = $TCID_{50}$/0.1 ml + Factor of Volume per carrier

  Calculation of Virus Inactivation Due to Test Substance Exposure

  Plate Recovery Control Log_{10} $TCID_{50}$ – Virus-Test Substance Film Log_{10} $TCLD_{50}$ = Log_{10} Reduction of Virus Due to Inactivation by Test Substance

Results of the Study

Test Microorganism	Contact Time	Substance Designation	Replicate	Log_{10} Infectious Units per Carrier	Average Log_{10} Infectious Units per Carrier	Average Log_{10} Reduction Compared to Recovery Control	Average Percent Reduction Compared to Recovery Control
Feline Calicivirus ATCC VR-782 (EPA-Approved Human Norovirus Surrogate)	~1 Minute	Plate Recovery Control	1	6.05	6.18	N/A	
			2	6.30			
		KR615 UV Germicidal Enclosure Device	1	≤0.80	≤0.93	≥5.25	≥99.999%
			2	1.05			



Note: The limit of detection for this assay was 0.80 log_{10} $TCID_{50}$/carrier. Observations below this limit of detection are presented as ≤0.80 in the table and 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.

Copyright © Microchem Laboratory, 2019. Reproduction and ordinary use of this study report by the entity listed as "Sponsor" is permitted. Other copying and reproduction of all or part of this document by other entities is expressly prohibited, unless prior permission is granted in writing by Microchem Laboratory.