

ANTIMICROBIAL TEST LABORATORIES



Study Report



Study Title

Virucidal Activity Assessment of Spectra254 1000 Device Using CDC Ebola Disinfection Guidelines for Selection of Appropriate Test Virus (Non-enveloped Virus, Feline Calicivirus)

Test Method

Custom Device Study Based on: ASTM International Standard Test Method E1053
Assessment of the Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces

Study Identification Number

NG5648

Study Sponsor

George Lichtblau

Test Facility

Antimicrobial Test Laboratories
1304 W. Industrial Blvd Round
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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

This study was designed, conducted, and reported by: Erika Guin, B.S.

Erika graduated from St. Edward's University with a Bachelors of Science in Biology.

Erika has a strong educational background in microbiology, and is a dedicated and enthusiastic professional. As a Microbiology Associate at Antimicrobial Test Labs she has assisted other microbiologists in the conduct of a wide range of custom studies. Her strong work ethic and interest in comprehensive and accurate testing make her an asset to the laboratory as well as a competent conductor of antimicrobial studies.



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

Erika@AntimicrobialTestLabs.com
or
(512) 310-8378

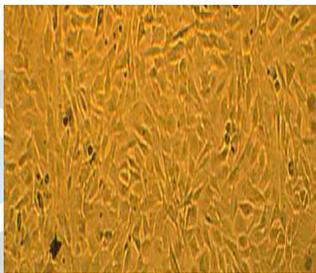
Test Substance Information

The test device was received on 01 MAY 2014. The following picture was taken prior to testing:



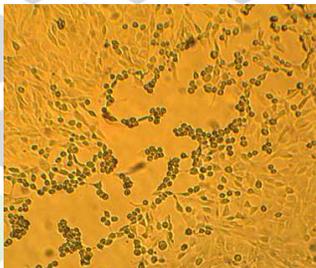
Test Microorganism Information

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

Summary of the Procedure

- Stock virus is thawed and prepared to the appropriate log density per carrier.
- Sterile 1" x 3" glass carriers are inoculated with a volume of virus suspension containing an adequate titer to recover a minimum of 6-log₁₀ 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 device was turned on by a trained operator and allowed to run for the designated exposure time (5 minutes).
- At the close of the designated exposure time, test carriers were immediately harvested by pipetting 3 ml of test medium (2% FBS EMEM) over the surface of each carrier. Sterile cell scrapers were used to mechanically detach the virus films.
- Suspensions from harvested carriers (control and test) were serially diluted ten-fold in the appropriate test medium and plated in quadruplicate onto CRFK host cell monolayers in multi-well trays.
- Host cell-virus assay plates were incubated for seven days at 37 ± 1 °C in a 5% CO₂, humidified atmosphere.

Study Timeline



Quantitative Evaluation of the Antimicrobial Effect of an Ultraviolet Light Emitting Device Protocol Number SP005

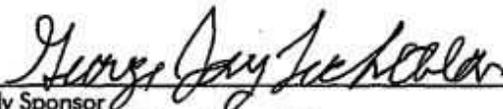
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Summary of parameters requested by the Study Sponsor, and incorporated into this Protocol by reference:

UV generated from the device below will be tested against Feline calicivirus dried on standard 1" x 3" glass microscope slides at a distance and duration diagrammed on page 8. Feline calicivirus was selected by the Study Sponsor as a representative of Ebola virus UV reactivity based on the CDCs Interim Guidance for Environmental Infection Control in Hospitals for Ebola Virus document, citing the greater endurance of non-enveloped viruses (ex. Feline calicivirus) compared to enveloped viruses (ex. Ebola virus).

Test Replicates —	3
Test Device(s) —	Spectra1000
Test Microorganisms —	Feline calicivirus (US EPA-approved human norovirus surrogate) Strain F-9, ATCC VR-782
Organic Soil Load —	0% Soil Load
Target Concentration —	Approximately 2×10^6 infectious viruses /carrier
Treatment Time & Distance —	See Diagram (Page 8)
Test Temperature —	Ambient (Room) Temperature
Test Harvesting Media/Neutralizer—	2% FBS EMEM plus Antibiotics
Proposed Initiation Date —	09DEC2014
Proposed Completion Date —	16DEC2014

"I, the Study Sponsor, have read and understand the following protocol. By signing this protocol I am certifying that the information and parameters accurately describe the test(s) to be completed. I have also read, understand and agree to the terms and conditions listed in the protocol."


 Study Sponsor
 George Jay Litchblau, Spectra254

12/7/2014
 Date


 Principle Scientist
 Erika Guin, Antimicrobial Test Laboratories

09 DEC 2014
 Date

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I. Introduction

This document details the materials and procedure for evaluating the antimicrobial efficacy of a UV generating device. This study will be conducted using this protocol and modified parameters based on the AOAC Germicidal Spray Products and Disinfectants Test Method. This document also explains the terms and conditions of testing.

II. Purpose

The purpose of this study is to document the efficacy of the test device and/or test substances against the test systems (microorganisms) under the specified test parameters below.

III. Terms and Conditions

Prior to study initiation, Antimicrobial Test Laboratories must receive the approved and signed protocol, as well as all test substances and devices. Studies are scheduled at Antimicrobial Test Laboratories' discretion. Changes to the signed, approved protocol will require amendment and will incur additional fees. Cancellation of the study any time after the protocol has been signed will result in a cancellation fee of 50% of the total study cost.

Antimicrobial Test Laboratories will repeat studies, free of charge, in the event of unintended protocol non-conformance. If the neutralization system specified for a study is not adequate, the study will be deemed "inconclusive" and the Study Sponsor will be responsible for the cost of the study. In addition, the Study Sponsor is responsible for the cost of all studies performed to confirm the outcome of a previous study and for ensuring that the study will meet their regulatory objectives.

The Study Sponsor must obtain written consent from Antimicrobial Test Laboratories to use or publish its protocols, study reports (or parts thereof), logo or employee names for marketing purposes.

IV. Test Device Handling

Test device(s) are handled as follows:

- The test devices are stored at ambient (room) temperature under fluorescent lighting or in a closet.
- The test device is operated based on the manufacturer's instructions.
- The test substances and devices are handled safely in accordance with the chemical and other risks posed, stated on the MSDS or by the Study Sponsor during the course of pre-study or on-site communication.

V. Justification for the Selection of Test System (Microorganism)

The United States Environmental Protection Agency (US EPA) requires that specific claims made for antimicrobial products sold in the United States be supported by relevant test systems (microorganisms) and validated test methods, such as the AOAC Germicidal Spray Products as Disinfectants Test Method 961.02 (with modifications for viruses) as described in the US EPA Product Performance Test Pesticide Assessment Guidelines (OCSP 810.2200). The objective of the current study is to assess the antimicrobial efficacy of a UV-generating device against a non-enveloped virus, feline calicivirus ATCC VR-782. The test microorganism was selected by the Study Sponsor as an Ebola Virus surrogate to simulate a worse case scenario, as non-enveloped viruses are more difficult to disinfect than enveloped viruses (i.e. Ebola Virus).

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VI. Materials

- Test device supplied by the Study Sponsor
- Test microorganism feline calicivirus (US EPA-approved human norovirus surrogate), Strain F-9, ATCC VR-782.
- Sterile 1" x 3" glass microscope slides for use as test carriers.
- Sterile 100 x 15 mm plastic Petri dishes.
- Sterile phosphate-buffered saline (PBS) for dilution of the test culture prior to carrier inoculation, as needed.
- Surfactant for addition to test culture, to facilitate proper spreading of the test inoculum over the test carrier surface(s), as needed.
- 50 mL centrifuge test tubes containing 3 mL of 2% FBS EMEM plus antibiotics [100 µg/ml Kanamycin Sulfate solution and Antibiotic-Antimycotic solution (100 units/ml Penicillin G, 100 µg/ml Streptomycin, and 0.25 µg/ml Amphotericin B)].
- Sufficient sterile tubes containing sterile 0% FBS EMEM, for dilution of neutralized microbial suspensions prior to plating.
- Sufficient quantity of calibrated micropipettes and sterile micropipette tips (containing aerosol barriers) of the appropriate volumetric capacity.
- Sufficient quantity of disposable cell scrapers.
- Appropriate number of multi-well cell culture trays containing permissive host cell monolayers [Crandell-Rees Feline Kidney (CRFK) cells, ATCC CCL-94] prepared to suitable confluency.
- Incubator capable of maintaining the temperature range (37 ± 1 °C) and atmospheric conditions ($5 \pm 1\%$ CO₂) appropriate for feline calicivirus - CRFK host cell assay incubation.
- Appropriate volume of 95% ethanol or other suitable disinfectant.
- Forceps.
- Bunsen burner, microbiological incinerator, or micro-torch as appropriate to ensure rapid and complete flame-sterilization of forceps, loops, and bent glass rods.
- Automatic pipettor (PipetAid or similar) and sterile serological pipettes of the appropriate volumetric capacity.
- Certified satellite clock.
- Certified digital timer.

VII. Procedure

Preparation of the Stock Virus

The feline calicivirus strain (F-9) to be used in the study is obtained from the American Type Culture Collection (ATCC) located in Manassas, Virginia. Viral stocks are readied by combining the supernatants from multiple cell culture flasks displaying cytopathic effect on 90% of the host cell monolayers (CRFK). After subjection to several freeze-thaw cycles, the supernatants are centrifuged in order to remove cell debris. The supernatant is removed and stored in aliquots of 1 ml at approximately -70°C until the day of use. Prior to use in testing, one cryovial per stock lot is enumerated on the appropriate host cell line (CRFK) using standard cell culture techniques (e.g. TCID₅₀). On the date(s) of testing, the appropriate number of stock aliquots are removed, thawed, and used promptly in the assay.

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Preparation of Test Carriers

- Glass carriers are soaked in 95% ethanol and rinsed twice with distilled water (or equivalent).
- All test carriers are autoclaved and aseptically transferred to sterile Petri dishes prior to testing.

Preparation of Elution/Neutralization Broth

- Neutralization tubes are prepared by aseptically adding 3ml of sterile 2% FBS EMEM plus antibiotics to 50 mL conical centrifuge tubes.

Carrier Inoculation with Test Inoculum

- Each carrier is inoculated with 0.020 ml and the inoculum is spread to cover a surface area of approximately 15 cm² (and to within ~3 mm distance from the carrier perimeter).
- Inoculated test carriers are incubated in Petri dishes at room temperature for 10 minutes, or until visually dry.
- Only visually dry carriers are used for the test.

Assembly and Operation of Device

- The device is assembled according to manufacturer instructions received with the device, or direction from the Study Sponsor and/or representative at the time of test.

Treatment of Inoculated and Dried Test Carriers with Device (Triple Replicate) – 5 Minute Contact Time

- After inoculation and drying, test carriers are placed according to the diagram on page 8.
- The device is operated in accordance with the Study Sponsor's instructions:
 - The 5 minute button on the supplied remote is pressed to begin the UVC treatment cycle.
 - The device is allowed to shut off automatically.
- A certified digital timer is started when the 5 minute button is pressed and stopped when the device automatically shuts off. The total cycle time is recorded.

Initial Numbers Control

- At the start of the test and within 2 hours of drying, 3 dried test carriers are selected and transferred individually to 3 ml of sterile 2% FBS EMEM. Harvested carriers are washed via pipette aspiration, and a sterile cell scraper is used to mechanically detach remaining infectious viruses from the carriers.
- Serial dilutions (1:10) are performed on the eluates, and the dilutions plated in quadruplicate onto CRFK host cell monolayers prepared to suitable confluency in multi-well trays.

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Final Numbers Control

- During the test and after completion of device treatment for the final exposure time-kill 3-replicate set, 3 dried test carriers are transferred individually to 3 ml of sterile 2% FBS EMEM. Harvested carriers are washed via pipette aspiration, and a sterile cell scraper is used to mechanically detach remaining infectious viruses from the carriers.
- Serial dilutions (1:10) are performed on the eluates, and the dilutions plated in quadruplicate per dilution onto CRFK host cell monolayers prepared to suitable confluency in multi-well trays.

Cell Culture Infectivity Assay

The permissive host cell line for the test system (feline calicivirus) is of feline kidney origin [Crandell-Rees Feline Kidney (CRFK), ATCC CCL-94]. For both the test and control groups previously described, sample aliquots (0.1 ml) of each dilution are inoculated in replicates of four onto healthy monolayers prepared to suitable confluency in cell culture treated multi-well plates. A minimum of two cell viability (sterility) control sets (also performed in quadruplicate) are dispersed throughout the assay trays, and receive an inoculum of test/cell culture medium only (2% FBS EMEM plus antibiotics). The trays are incubated at $37 \pm 1^\circ\text{C}$ ($5 \pm 1\% \text{CO}_2$) for a minimum of 30 minutes to facilitate virus-host cell adsorption. The trays may also be placed upon an orbital rotator during this incubation period, if feasible. Following incubation, each well receives ~1.0 ml of test/cell culture medium via pipette delivery. The cell culture assay trays are incubated at $37 \pm 1^\circ\text{C}$ for 7 days in a humidified CO_2 incubator. Assay trays are examined regularly, with changes to healthy monolayers including viral cytopathic effects (CPE), cytotoxicity, and contamination clearly documented as such changes are observed. Enumeration and Plating of Test Carriers

VII. Determination of Viral Titers

The Spearman-Kärber Method is used to calculate the Numbers Control titers (TCID_{50}) and the viral titers following exposure to the test device (TCLD_{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 a given exposure time is referred to as the Tissue Culture Lethal Dose (TCLD_{50}). The TCID_{50} and $\text{TCLD}_{50} \log_{10}$ values per replicate are determined according to the method of Spearman-Kärber as follows:

$$-\text{Log of 1st dilution inoculated} - \left[\left(\frac{\text{Sum of \% mortality at each dilution}}{100} \right) - 0.5 \times (\text{logarithm of dilution}) \right]$$

Initial Numbers Control

$$\text{Initial Numbers Control Mean} = \frac{(\text{Control Carrier 1 TCID}_{50}) + (\text{Control Carrier 2 TCID}_{50}) + (\text{Control Carrier 3 TCID}_{50})}{3}$$

Final Numbers Control

$$\text{Final Numbers Control Mean} = \frac{(\text{Control Carrier 1 TCID}_{50}) + (\text{Control Carrier 2 TCID}_{50}) + (\text{Control Carrier 3 TCID}_{50})}{3}$$

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Calculation of the Mean Numbers Control

- The number of microorganisms present on the untreated carriers after drying is determined using the following formula:

$$\text{Mean Numbers Control} = \frac{(\text{Initial Numbers Control Mean TCID}_{50}) + (\text{Final Numbers Control Mean TCID}_{50})}{2}$$

Enumeration of Test Carriers

Per 3-replicate set (per contact time and distance):

$$\text{Mean Log}_{10} \text{ Viral Titer per Replicate Set} = (\text{Log}_{10}C_1 + \text{Log}_{10}C_2 + \text{Log}_{10}C_3) / 3$$

Where: C = Carrier Replicate Number

Determination of Log₁₀ Reduction

- The following formula is used to determine the Log₁₀ Reduction of the test carriers following treatment relative to the Mean Numbers Control:

$$\text{Log}_{10} \text{ Reduction} = \text{Mean Log}_{10} \text{ of Numbers Control} - \text{Mean Log}_{10} \text{ Test Carriers Per Distance and Exposure Time}$$

Determination of Percent Reduction

- The following formula is used to determine the Percent Reduction compared to the Mean Numbers Control:

$$P_r = 100 [(B - A) / B]$$

P_r = Percent Reduction

A = Mean log₁₀ of infectious viral units recovered from the treated test carriers

B = Mean log₁₀ of infectious viral units recovered from the Mean Numbers Control carriers

VIII. Success Criteria

- A minimum average of 6.00 log₁₀ infectious viruses (TCID₅₀) are recovered from the Mean Numbers Control carriers.
- Quantification of the control and test carriers is conducted at a minimum of four determinations per dilution for the assay system.
- The TCLD₅₀ values are calculated and provided for each test assay.
- The test results are calculated by a statistical method (e.g. Spearman-Kärber).
- Assay wells designated as cell viability (sterility) controls be absent of infectivity, contamination, and cytotoxicity.

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IX. Reporting

Results are reported accurately and fully. A standard report including all relevant parameters, results, and calculations will be issued within 3-4 days of test completion.

X. Data and Sample Retention

- The study report and corresponding data sheets will be held in the archives of Antimicrobial Test Laboratories for at least 2 years after the date of the final report and then may be destroyed.
- The test device and other relevant materials may be returned to the Study Sponsor at Sponsor's request and expense within 30 days of study completion.

XI. Quality Control

The study will be conducted in accordance with Antimicrobial Test Laboratories' Quality Management System. All protocol amendments will be fully recorded and reported, as well as any deviations from the protocol.

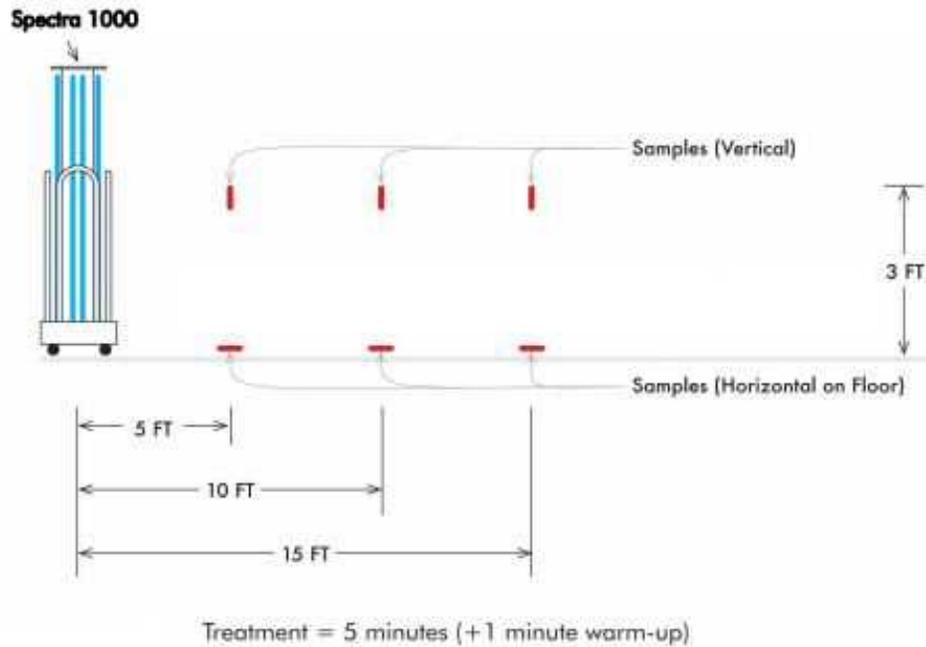
XII. References

1. *Interim Guidance for Environmental Infection Control in Hospitals for Ebola Virus* [Internet]. Atlanta (GA): Centers for Disease Control and Prevention; [2014 Nov 14, cited 2014 Dec 08]. Available from: <http://www.cdc.gov/vhf/ebola/hcp/environmental-infection-control-in-hospitals.html>
2. *Official Methods of Analysis of the AOAC International*, Chapter 6, Disinfectants, Official Method 961.02 Germicidal Spray Products as Disinfectants, Current edition. AOAC International, Suite 500, 481 North Frederick Avenue, Gaithersburg, MD 20877-2417.

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XIII. Diagram of Study Setup



Note: Test carriers in the vertical position will be placed so that the dried inoculum side of the carrier is facing the device. Test carriers in the horizontal position will be positioned so that the dried inoculum side of the carrier is facing the ceiling. The 5, 10, and 15 ft distance measurements will be taken from the front edge of the base of the device to the center of the test carrier.

Criteria for Scientific Defensibility of a Custom Device Study

For Antimicrobial Test Laboratories to consider a Device Study study to be scientifically defensible, the following criteria must be met:

1. The average number of viable infectious units are recovered from the time zero samples must be approximately 1×10^6 infective units/carrier or greater.
2. Negative/Purity controls must demonstrate no growth of test microorganism.

Passing Criteria

Because of the nature of the study, passing criteria may be determined by the Study Sponsor.

Testing Parameters used in this Study

Test Substance Diluent:	N/A	Carrier Type:	1" x 3" glass slides
Carriers Per Test:	3	Number of Sprays:	N/A
Spray Distance:	N/A	Spray Angle:	N/A
Harvest Volume:	3.0 ml, 2% FBS EMEM		

Viral Inoculum Volume:	0.02 ml	Carrier Inoculation Area:	15 cm ²
Carrier Dry Time:	14 Minutes	Carrier Dry Conditions:	Ambient
Contact Time(s):	5 Minute Cycle	Contact Conditions:	Ambient
Host Cell Line:	CRFK	Cell Passage Number:	p. 197
Assay Medium:	2% FBS EMEM	Soil Load:	None
Incubation Period:	7 Days	Incubation Conditions:	37 °C; 5% CO ₂

Study Notes

Two treatment heights and two treatment orientations were tested to evaluate virucidal efficacy at 5, 10, and 15 feet from the device: floor height and horizontal; 3 feet from the floor and vertical (see page 16 for a diagram of the study set-up). The distance between the device and test carriers was measured from the front edge of the device to the center of the carrier.

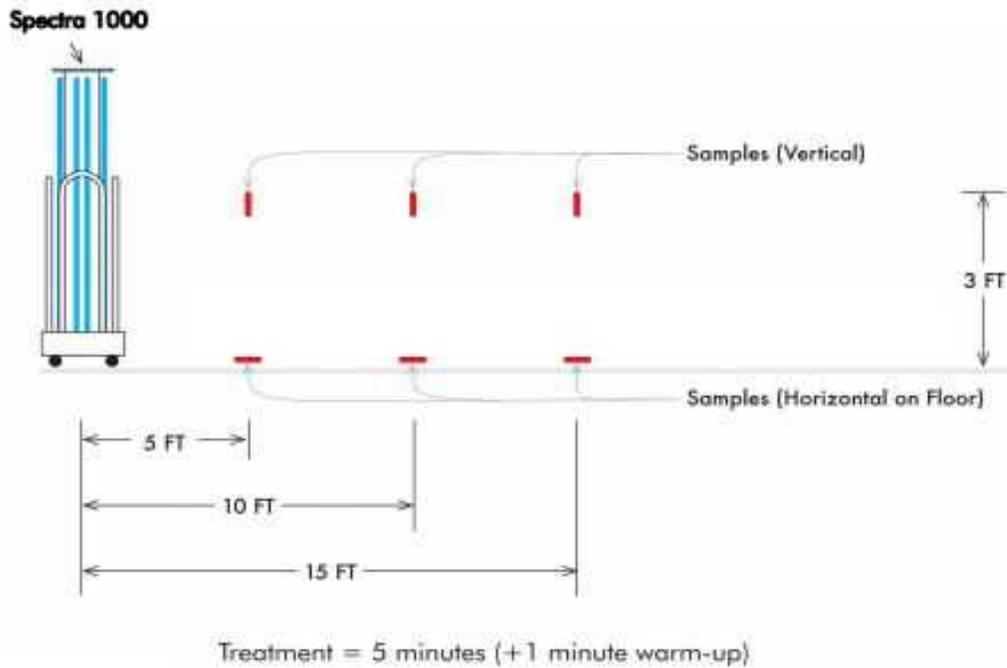
The 5 minute cycle was initiated by pressing the 5 minute cycle button on the provided remote and allowed to terminate automatically. Treatment time included a “warm up” period and a five minute “treatment” period. The full device cycle time from the time the cycle button was pressed to the time the cycle automatically terminated was 6 minutes and 39 seconds.

To facilitate spreading of the viral inoculum across the carrier surface, the inoculum diluent (PBS) was supplemented with 0.1% Triton X-100.

This study was performed in compliance with the Study Sponsor approved protocol SP005.

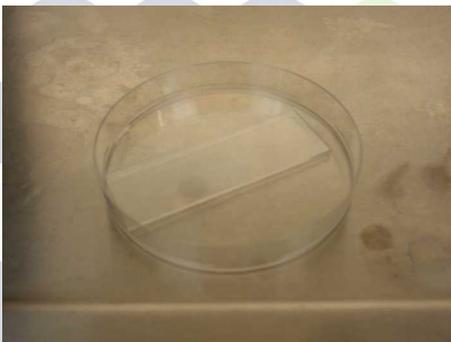
Study Setup

XIII. Diagram of Study Setup



Note: Test carriers in the vertical position will be placed so that the dried inoculum side of the carrier is facing the device. Test carriers in the horizontal position will be positioned so that the dried inoculum side of the carrier is facing the ceiling. The 5, 10, and 15 ft distance measurements will be taken from the front edge of the base of the device to the center of the test carrier.

Study Photographs



Left: An inoculated carrier drying under ambient conditions within a laminar flow hood.

Control Results

Virus Control Titer: 6.11 log₁₀/Carrier

Cytotoxicity Titer: N/A

Neutralization Effectiveness: N/A

Sterility Controls: Sterility Validated

Calculations

Viral and cytotoxicity titers (TCID₅₀/TCLD₅₀ and TCCD₅₀, respectively) were determined according to the method developed by Spearman-Kärber:

$$-\text{Log}_{10} \text{ of 1st Dilution} - \left(\frac{\text{sum of \% mortality at each dilution}}{100} \right) - 0.5$$

Percent Reduction of Virus is determined according to the following formula:

$$\text{Percent Reduction} = 1 - \left(\frac{C}{B} \right) * 100$$

Where:

B = Log₁₀ of Virus Control Carrier

C = Log₁₀ of Virus Test Carrier

Spectra 1000 UV Device Results

Table 1. Custom Device Test Evaluating UV-Generating Device Against Feline Calicivirus ATCC VR-782 Applied to Horizontally or Vertically Positioned Test Carriers

Microorganism	Contact Time	Carrier Position	Treatment Distance	Test Replicate	Log ₁₀ per Carrier	Mean Log ₁₀ per Carrier	Percent Reduction vs Control	Log ₁₀ Reduction vs Control			
Feline Calicivirus ATCC VR-740	Initial Numbers Control			1	6.48	6.11	N/A				
				2	6.48						
				3	6.98						
				Final Numbers Control					1	5.48	
									2	5.73	
									3	5.48	
	5 Minutes	Horizontal		5 Feet	1	≤ 1.98	≤ 1.98	≥ 99.993%	≥ 4.13		
					2	≤ 1.98					
					3	≤ 1.98					
				10 Feet	1	2.98	2.81	99.95%	3.30		
					2	2.73					
					3	2.73					
				15 Feet	1	2.98	3.31	99.84%	2.80		
					2	3.73					
					3	3.23					
		Vertical		5 Feet	1	≤ 1.98	≤ 2.06	≥ 99.991%	≥ 4.05		
					2	≤ 1.98					
					3	2.23					
				10 Feet	1	≤ 1.98	≤ 1.98	≥ 99.993%	≥ 4.13		
					2	≤ 1.98					
					3	≤ 1.98					
				15 Feet	1	2.23	2.65	99.97%	3.46		
					2	3.23					
					3	2.48					

**≤" No viral cytopathic effects (CPE) observed; therefore infectious viral titers at or below the limit of detection.

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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