ANTIMICROBIAL TEST LABORATORIES

Study Report

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Study Title

Antiviral Activity and Efficacy of Spectra254 Device Against Human Coronavirus 229E

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

NG5314-A2

Study Sponsor

George Lichtblau

Test Facility

Antimicrobial Test Laboratories
1304 W. Industrial Blvd Round
Rock, TX 78681
(512) 310-8378

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: Luisa Ikner, Ph.D

Luisa holds a Ph. D. in Soil, Water, and Environmental Science from the University of Arizona.

Luisa is a Senior Virologist at Antimicrobial Test Laboratories. She has contributed extensively to the field of virology by developing and publishing new techniques in peer-reviewed scientific journals. At the laboratory, she works on behalf of virucide manufacturers by maintaining reliable cell cultures and executing virucidal efficacy assays with a skillfulness that is evident in every study she completes. Luisa is friendly and eager to speak with customers in order to answer technical questions about their projects.

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

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

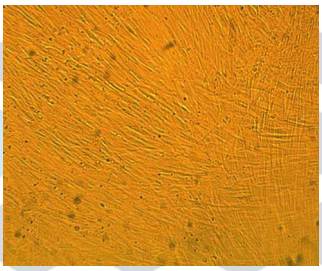
Test Substance Information

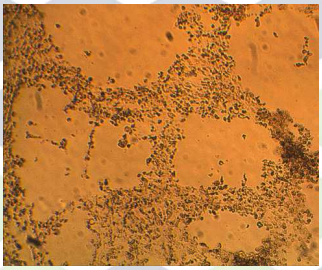
The test device was received on 01 MAY 2014 and the following pictures were taken:
(note: photo depicts the test device that was analyzed in this study)

Test Device arrived in shipping crate. New UV bulbs were sent to replace damaged bulbs. UVC dosage labels, and device remote were also included with device.

Test Microorganism Information

The test microorganism(s) selected for this test:





Human coronavirus 229E (HCoV), ATCC VR-740
HCoV is an enveloped, positive-sense RNA virus in the *Coronaviridae* family. Coronaviruses cause mostly mild to moderate upper respiratory infections year-round in humans. Zoonotic strains of coronavirus have also recently emerged (e.g. SARS-CoV and MERS-CoV) and become of great concern due to their virulence and high mortality rates. Similar to other respiratory viruses, coronaviruses are transmitted by inhalation of infective aerosols and person-to-person contact. Symptoms include cough, runny nose, sore throat, and mild fever. Immunocompromised persons and those with poor cardiovascular health may also develop pneumonia. Relative to other enveloped, respiratory viruses, coronaviruses are less vulnerable to inactivation during dessication, yet are similarly inactivated by a number of disinfectants.
Permissive Host Cell Line for HCoV: MRC-5 (Human Lung Fibroblast Cells), ATCC CCL-171

Summary of the Procedure

- Stock virus is thawed and may be supplemented with an organic soil load, if requested.
- Sterile 1" x 3" glass carriers 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 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 EME) 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 MRC-5 host cell monolayers in multi-well trays.
- Host cell-virus assay plates were incubated for seven days at 34 ± 1 °C in a 5% CO_2, humidified atmosphere.

Study Timeline

Amended report delivered on 11 NOV 2014
Amended report delivered on 07 JAN 2015

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 x 10^4 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 (Ready To Use)	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		

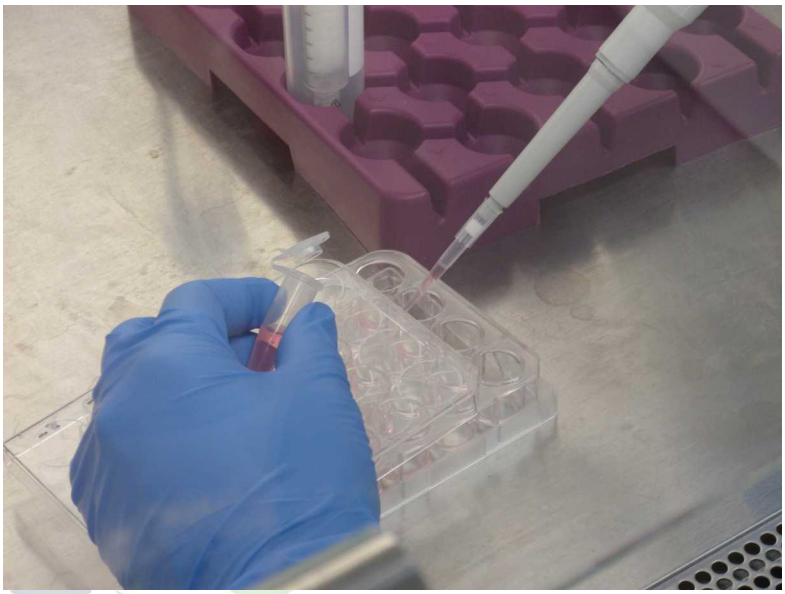
Viral Inoculum Volume:	0.02 ml	Carrier Inoculation Area:	1 in ^{2}
Carrier Dry Time:	15 Minutes	Carrier Dry Conditions:	Ambient
Contact Time(s):	5 Minutes	Contact Conditions:	Ambient
Host Cell Line:	MRC-5 (ATCC CCL-171)	Cell Passage Number:	29
Assay Medium:	2% FBS EMEM	Soil Load:	5% FBS
Incubation Period:	7 Days	Incubation Conditions:	34.0 °C; 5% CO_2

Study Notes

Two treatment distances were tested to evaluate virucidal efficacy at 5 feet and 10 feet from the device.

The selected test virus was chosen by the Study Sponsor because of its similarities to the Middle East respiratory syndrome coronavirus (MERS-CoV). Both MERS-CoV and human coronavirus 229E are positive-sense, single-stranded enveloped RNA viruses belonging to the virus family Coronaviridae.

Study Photographs



Suspensions from harvested carriers were serially diluted in the appropriate test medium and plated in quadruplicate onto MRC-5 host cell monolayers in multi-well trays.

Control Results

Virus Control Titer:	Mean 5.40 $\log_{10}$/Carrier	Cytotoxicity Titer:	N/A
Virus Stock Titer:	7.75 $\log_{10}$/0.100 ml	Sterility Controls:	Valid
Neutralization Effectiveness:	N/A		

Calculations

Viral and cytotoxicity titers ($TCID_{50}$/$TCLD_{50}$ and $TCCD_{50}$, respectively) were determined according to the method developed my Spearman-Karber:

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

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

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

Where:
B = Log_{10} of Virus Control Carrier
C = Log_{10} of Virus Test Carrier

Results of the Study

Table 1. Custom Device Test Evaluating Device-Generated Test Substance Against Human Coronavirus, Strain 229E, ATCC VR-740

Microorganism	Contact Time	Treatment Distance	Test Replicate	Log_{10} per Carrier*	Mean Log_{10} per Carrier*	Percent Reduction vs Control	Log_{10} Reduction vs Control
Human Coronavirus, Strain 229E, ATCC VR-740	Initial Numbers Control		1	5.23	5.40	N/A	
			2	5.73			
			3	5.23			
	5 Minutes	5 Feet	1	≤1.98	≤1.98	≥99.96%	≥3.42
			2	≤1.98			
			3	≤1.98			
		10 Feet	1	≤1.98	≤1.98	≥99.96%	≥3.42
			2	≤1.98			
			3	≤1.98			

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

Data included in the table above indicates the Spectra 1000 UV Device reduced the titer of viable viral particles to the limit of detection for this assay at both tested distances.

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