Study Title
Antiviral Activity and Efficacy of a MRSA-UV Device Tandem Set-Up

Test Method
Custom Device Study Based on: ASTM E1053

Study Identification Number
NG5967

Study Sponsor
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Chris@MRSA-UV.com

Test Facility
Antimicrobial Test Laboratories
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(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: 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 Microbiologist at Antimicrobial Test Labs she has the lead in a wide range of virology and 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 devices were received on 09 JAN 2015 and 07 APR 2015, and the following pictures were taken.

Test Substances Received: MRSA-UV Tandem Use UV Devices
  Left and Center: Received 09 JAN 2015
  Right: Received 07 APR 2015

Test Substances arrived ready to use for the conduct of the study.

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

- Test microorganism stock suspension is retrieved from -70°C freezer storage and thawed.
- The stock suspension is diluted in phosphate buffered saline as appropriate to reach the desired inoculum titer per carrier.
- Test and control carriers are inoculated with an aliquot of prepared viral suspension and allowed to dry, producing viral films.
- At the conclusion of the dry time control carriers are harvested and enumerated to determine the “time zero” or control carrier viral titer.
- Test carriers are then exposed to the specified treatment cycle(s).
- At the conclusion of the treatment cycle(s) the test carriers are harvested in a small volume of cell culture maintenance media.
- Harvested carriers are diluted and plated to allow for enumeration of viral titers.

Study Timeline

<table>
<thead>
<tr>
<th>Event</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carriers Inoculated and Dried</td>
<td>13 MAY 2015</td>
</tr>
<tr>
<td>Testing and Neutralization</td>
<td>13 MAY 2015</td>
</tr>
<tr>
<td>Enumeration Assay Initiated</td>
<td>13 MAY 2015</td>
</tr>
<tr>
<td>Assay Scored/Calculated</td>
<td>20 MAY 2015</td>
</tr>
<tr>
<td>Report Delivered</td>
<td>22 MAY 2015</td>
</tr>
</tbody>
</table>
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. A minimum of \(4-\log_{10}\) infectious viruses are recovered from the virus control carrier.
2. Viral cytopathic effects are distinguishable from cytotoxic effects caused by test substance or harvest media exposure.
3. Assay wells designated as sterility controls are absent of infectivity, contamination, and cytotoxicity.

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 Mode of Use: Two UV Devices Used in Tandem on Vertical Carriers
Carriers (Size): Stainless Steel (1” x 3”) Replicates: Two

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inoculum Vol.</td>
<td>0.02 ml</td>
</tr>
<tr>
<td>Inoculum Supplements</td>
<td>None</td>
</tr>
<tr>
<td>Contact Time</td>
<td>45, 60 Minutes</td>
</tr>
<tr>
<td>Contact Humidity</td>
<td>See Study Notes</td>
</tr>
<tr>
<td>Harvest Volume</td>
<td>2.0 ml</td>
</tr>
<tr>
<td>Incubation Conditions</td>
<td>34 ± 2°C, 5% CO₂</td>
</tr>
<tr>
<td>Inoculum Area</td>
<td>1” x 1”</td>
</tr>
<tr>
<td>Inoculum Titer Goal</td>
<td>6.00 (\log_{10}) per Carrier</td>
</tr>
<tr>
<td>Contact Temperature</td>
<td>See Study Notes</td>
</tr>
<tr>
<td>Harvest Media</td>
<td>2% FBS EMEM</td>
</tr>
<tr>
<td>Assay Media</td>
<td>2% FBS EMEM</td>
</tr>
<tr>
<td>Incubation Period</td>
<td>7 Days</td>
</tr>
</tbody>
</table>
Study Notes

Carrier Dry Conditions:
Carrier drying was initiated at 1256 on 13 MAY 2015. Carriers were dried at reduced temperatures (approximately 9°C) to avoid viral loss from the drying process. Carriers were stored and transported in sterile plastic Petri dishes, and the dry period consisted of exposing each carrier to open air via the jarring or removal of the Petri dish cover. The carrier dry period concluded at 1320 on 13 MAY 2015. At the conclusion of the dry time carriers not being designated for immediate testing or harvest remained in reduced temperature conditions to avoid excessive viral reduction.

Study Set-Up:
The tandem UV devices were placed at the prescribed distance(s) from the vertically oriented stainless steel test carriers. The arms of the devices were raised to an approximate 45° angle relative to the ground for testing. The UV devices were placed in such a way that one set of arms from each device crossed one set of arms from the other device, resulting in the devices being approximately 12-18 inches from one another as measured from the edge of the base of each device (see study photographs).

Contact Conditions:
Each contact time initiated with the use of a Study Sponsor provided remote turning on both devices, and includes any device warm up time prior to UV light treatment initiation.

5 Foot Distance Treatment (45 Minutes):
23.3°C, 63% Relative Humidity

7.5 Foot Distance Treatment (60 Minutes):
24.1°C, 64% Relative Humidity
Study Photographs

**Photo 1.** Tandem UV device set-up. The arms of each device were raised to approximately 45° relative to the ground. Devices were placed in such a way as to allow one set of arms from each device to cross over one set of arms from the other device.

**Photo 2.** A stainless steel carrier inoculated over an area of approximately 1” x 1” with Human Coronavirus.
Control Results

Virus Control Titer: 5.05 log_{10} per Carrier
Virus Stock Titer: 6.50 log_{10} per 0.1 ml
Neutralization Effectiveness: Not Applicable

Cytotoxicity: None Observed
Sterility Controls: Validated

Calculations

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

\[-\log_{10} \text{ of 1st Dilution} - \left(\frac{\text{sum of } \% \text{ mortality at each dilution}}{100}\right) - 0.5\]

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

\[\text{Percent Reduction} = 1 - \left(\frac{C}{B}\right) \times 100\]

Where:
- \(B = \log_{10} \text{ of Virus Control Carrier}\)
- \(C = \log_{10} \text{ of Virus Test Carrier}\)
Results of the Study

Table 1. Custom UV Device Study Determining the Antiviral Efficacy of MRSA-UV Tandem Device Treatment Against Human Coronavirus

<table>
<thead>
<tr>
<th>Test Microorganism</th>
<th>Test Device Designation</th>
<th>Contact Time and Distance</th>
<th>Replicate</th>
<th>Log_{10} Infectious Units per Carrier</th>
<th>Mean Log_{10} Infectious Units per Carrier</th>
<th>Log_{10} Reduction vs. Control</th>
<th>Percent Reduction vs. Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human Coronavirus ATCC VR-740</td>
<td>Plate Recovery Control</td>
<td></td>
<td>1</td>
<td>4.80</td>
<td>5.05</td>
<td>Not Applicable</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>5.30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tandem UV Devices</td>
<td></td>
<td>45 Minutes at 5 Feet</td>
<td>1</td>
<td>≤1.80</td>
<td>≤1.80</td>
<td>≥3.25</td>
<td>≥99.94%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>≤1.80</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 Hour at 7.5 Feet</td>
<td>1</td>
<td>≤1.80</td>
<td>≤1.80</td>
<td>≥3.25</td>
<td>≥99.94%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>≤1.80</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* "≤" indicates a viral titer at or below the limit of detection for this assay

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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