Coronavirus neutralisation performance of GERMii germicidal UVC lights

Final report

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1. Rationale

The control of a new pandemic virus is achieved through the cumulative contributions of multiple controls to reduce risk or prevent transmission. In addition to methods of limiting host contact (social distancing or lockdown measures), sanitising and sterilising high-traffic areas and sterilising contaminated surfaces is key in the prevention of spread.

Efficient and effective sterilisation of potentially contaminated equipment is problematic within intricate environments with complex surfaces or many moving parts such as dials, switches, levers, buttons and cables, or storage compartments containing multiple, frequently handled items.

To that effect, GERMii has developed a germicidal sterilisation system, utilising ultraviolet (UV) spectrum-sterilisation for viral and microbial neutralisation. The units tested herein include a handheld, portable, shielded UV light source (a germicidal wand) designed for the decontamination of intricate equipment in small spaces (such as aircraft) and a second, prototype chassis designed to encase a germicidal light source within a vacuum robot to neutralise microbes during automated cleaning cycles of public spaces.

2. Objective

The report will establish the effectiveness of the UVC application for sterilising a surface contaminated with coronavirus, H-CoV-OC43 – a β-coronavirus strain, closely related to SARS-CoV-2 (the causative agent of COVID-19).

Primary readout:

- Infectious virus remaining after UVC exposure
  - Using lamps with different power output and design (handheld unit or vacuum chassis)
  - At different distances from the light source (10, 20, 50, 100mm)
  - For different durations of exposure (30s, 60s, 90s, 120s)
3. Results

3.1. UVC exposure using GERMii UVC lamps neutralised coronavirus to undetectable levels

In separate experiments, the handheld and vacuum chassis units housing UVC lamps were tested for neutralisation of coronavirus strain H-CoV-OC43. In each experiment, a standardised viral droplet containing $2.5 \times 10^5$ TCID$_{50}$ (250 thousand infectious units) of virus in a 25µl droplet were exposed to UVC light on plastic, as per the stipulated matrix in section 6.1.

Experimental Overview.

Remaining virus was recovered into a final volume of 1mL of media, frozen at -80°C and TCID$_{50}$ assay was later performed on the recovered virus, using the permissive cell line (MRC-5) to determine the remaining titration of infectious units of coronavirus (Figure 1A-B).

![Prototype unit - neutralisation](image)

![Handheld unit - neutralisation](image)

Figure 1: GERMii UVC sterilisation products reduced viral load to undetectable levels.

2.5x10^5 infectious units of OC43 virus load were exposed to GERMii lamps in single droplets, in 3 experimental replicates, at the indicated distances after 30-120s of exposure to the vacuum chassis-mounted lamp (A) or the handheld lamp (B) and remaining virus was quantified by TCID$_{50}$. Data would show mean ± standard error, n = 3. ND = not detectable.
4. Key findings

GERMii germicidal lamps neutralised 100 of the virus on plastic after just 3s of exposure even at 100mm exposure distance with either the small, or large lamp.

5. Discussion and conclusions

Germicidal UVC lamps have been successfully used as a means for non-physical surface sterilisation in hospitals and laboratory spaces due to their ability to reduce live bacterial and fungal contaminants (1, 2) and their use as effective air disinfectant strategies has been known for decades, existing in multiple forms (3).

There is now a growing body of evidence showing that germicidal UVC lamps can effectively neutralise coronavirus, including SARS-CoV-2 on high-traffic, high-touch surfaces or even personal protective equipment including n95 respirators (4, 5). The dose of UVC required to achieve neutralisation of viral surface contaminants should be tested and confirmed. This report therefore set out to determine the virucidal performance of GERMii UVC lamps at various durations of exposure (UVC dose) at different distances.

The scientific aim of this report was to identify a dose-response curve upon the levels of infectious units of coronavirus remaining after exposure GERMii UVC lamps. The virucidal extent is dependent on the dose of UV and the dose is determined by the power of the lights, the duration of exposure and distance from the light source. However, the dose of UVC exposure in this experimental design was too strong to determine a dose-response curve even with the shortest duration time and the furthest distance from the even the smaller light source.

The germicidal capacity of UVC can be determined by the single pass inactivation efficacy equation and other equations (6) which can be estimated with high accuracy based on the virus-specific constants of closely-related viral strains. Regardless, the experiments demonstrate a powerful germicidal capacity of GERMii lamps, effective against coronavirus.
6. Materials and methods

6.1. Experimental overview

The design resulted in the following matrix:

<table>
<thead>
<tr>
<th>Number of lights</th>
<th>Duration of Light activation</th>
<th>Distance from the light</th>
<th>Virus Load</th>
<th>Materials</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lamp 1</td>
<td>4 times tests, 30 seconds to 2 minutes in 30 second intervals</td>
<td>4 distance tests, 10mm to 100mm away from the light (10mm, 20mm, 50mm, 100mm)</td>
<td>1 different virus loads on each piece of material</td>
<td>hard Plastic</td>
</tr>
<tr>
<td>Lamp 2</td>
<td></td>
<td></td>
<td>3 replicates required</td>
<td></td>
</tr>
<tr>
<td>Lamp 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lamps 2+3 are identical. Lamps 1+2 evaluated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Number of possible combinations (test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>1</td>
</tr>
</tbody>
</table>

6.2. Materials

UVC lights of two sizes with appropriate power supply, were provided by GERMii. Infectious coronavirus (OC43) was obtained from ATCC (ATCC number VR-1558) and provided by the lab of A/Prof Nathan Bartlett. The Bartlett lab also provided the MRC-5 cell line, and project consumables such as tissue culture plates, cell media, tips, waste disposal cleaning consumables and research personnel.
6.3. **Experimental setup:**

6.3.1. Virus samples

Viral stock concentration of $2.3 \times 10^8$/mL TCID$_{50}$ was used, and diluted to $1 \times 10^7$ (23x dilution)

$V_2 = 50 \times 25 = 1.250$ mL (per experiment... will make up 1500µL), $C_2 = 10^7$, $V_1 = x$, $C_1 = 2.3 \times 10^8$

$V_1 = C_2 \times V_2 / C_1 = 1 \times 1500 / 23 = 65.2174$

Add $65.22$uL of OC43 stock to $(1500 - 65.22 = 1434.78)$

That is $10^7$/ml TCID$_{50}$. 25uL per inoculum

We then add $975$uL of 1% EMEM to remove the virus. So, assuming no inactivation the final concentration would be $1 \times 10^7 \times 0.025 = 250,000.0$ ($2.5 \times 10^5$ TCID$_{50}$)

6.3.2. Virus sample exposure

For each assay, a 25uL drop of virus diluted in viral assay media applied to the sterile surface of an inverted tissue culture plate lid (flat, clear plastic surface) in a sterile class II BSC. Pedestals of each stipulated height were stacked using standardised 10mm increments to achieve the exposure distances of 10, 20, 50 and 100mm between the sample and the UVC light source. After UV exposure, samples were collected in 975 µL of TCID media and immediately transferred to a sterile 1.7mL tube. All samples and initial virus inoculums were stored at -80°C upon harvest, until TCID$_{50}$ infectivity assay.
6.4. Cell culture methods

6.4.1. Cell-lines and media
MRC-5 cells were used to propagate and assay viral titres for OC43 Growth and viral assay media are below. MRC-5s were propagated in T175 flasks with EMEM containing supplements and 10% FCS until sufficient cell numbers were achieved to assay an entire experiment with TCID₅₀. Culture passages were maintained between 20-25 passages to negate variability across many passages.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Media</th>
<th>Supplements</th>
<th>Use</th>
</tr>
</thead>
</table>
| MRC-5     | Eagles’ Minimal essential medium – alpha modification (EMEM)² | • Penicillin/Streptomycin (100ug/µL)¹  
• Sodium Pyruvate (1mM)¹  
• L-Glutamine (2mM)¹  
• Non-Essential amino acids (0.1mM)¹  
• Sodium Bicarbonate (0.18%)¹ | 10% FCS MRC-5 maintenance |

1% FCS OC43 viral assay media

Suppliers: ¹Life technologies, ²Sigma.

6.5. Measure of viral infectivity using TCID₅₀ assay
OC43 infectivity following exposure to material was assessed in MRC-5 cells. Cells were first seeded into 96 well plates at a final concentration of 1 x 10⁶ cells/10 mL in growth media (1 x 10⁴ cells/well) and incubated overnight at 37°C/5%CO₂, until 30-40% confluence.

The next day, the UV-exposed viral samples were serially diluted in 10-fold dilution series in viral assay media in replicates of 4 per sample. Serial dilutions were then transferred onto the cells, starting with undiluted sample in the first row of the plate, and subsequent 10-fold dilutions in the next 6 rows. Viral assay media alone was added in the last row of the 96 well plate and was used as control for the assay.

Plates were incubated for 5 days at 35°C/5%CO₂. Finally, cytopathogenic effect (CPE) was observed using light microscopy and the tissue culture infectious dose (TCID₅₀) was calculated using the Karber method.
7. Appendix 1: TCID$_{50}$ concept

Step 1: 180uL 1% EMEM rows B-G

(OC43 viral assay media)

Step 2: 140uL x 4 wells for each sample in row A

Step 3: dilution series down the plate, A-G (mix 20uL into 180uL)
Step 4: tip media off plate of cells

(MRC-5 maintenance media)

Step 5: Clean fresh 1% EMEM media into row H of cells (100µL)

Then transfer 100µL of each dilution (low to high concentration) onto the cells going from row G to A

Step 6: incubate plates as above and look for virus-induced cell death (cytopathic effect / CPE) after 5 days. Enter fraction of CPE wells at each dilution for each sample into the Karber equation to determine final TCID$_{50}$. 