# Persistence Test for Core-19 X30 Surface Sanitizer/Disinfectant against a Surrogate Virus for SARS-CoV-2

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## 1.0 Objective

The overall objective of this study was to quantify the persistence kill properties of the Core-19 X-30 Surface Sanitizer / Disinfectant product provided by Ipro Medical LLC against SARS-CoV- 2 surrogate virus on stainless steel coupon at regular intervals up to 30 days after the initial treatment.

## 2.0 Protocol Overview

The murine hepatitis virus, MHV-S, a CDC recognized surrogate virus for SARS-CoV-2 testing, was grown in a mouse liver cell line, NCTC clone 1469. Stainless steel discs were sprayed on different dates with the surface sanitizer per use instructions and allowed to dry at room temperature in a sterile hood. 30 days after the first discs were treated the test of virucidal activity was performed. Virus was placed onto each disc for 10 minutes at room temperature. Following the inoculation with virus the discs were dropped into tubes containing the neutralizer solution and 150 mg of glass beads. The tubes were vortexed for 30s and then 500 µl of each sample was loaded onto a 3 ml Sephadex LH-20 column and centrifuged. Serial 10-fold dilutions of the column eluates were used to inoculate NCTC clone 1469 cells in a 96 well plate. After 6 days the wells were examined for cytopathological effects (CPE) in the cells and scored accordingly.

## 3.0 Materials and Methods

### 3.1 Growth of stock virus

#### 3.1.1 Cell culture

Mouse liver cell line NCTC Clone 1469 (ATCC® CCL-9.1™) was maintained in DMEM with 4500 g/l glucose plus L-gln and 1.5g/l sodium bicarbonate, pH 7.3, supplemented with 10% Donor Horse Serum (Biotechne, Minneapolis, MN) in a humidified incubator at 37ºC and 5% $CO_2$. Cells were passaged by scraping cells from the flask surface, centrifuging and re-suspending in new growth media. 5 x $10^4$ cells/well were plated in DMEM + 10% horse serum in a 96 well plate 24 hours before the assay and incubated as above.

#### 3.1.2 Virus preparation

Murine Hepatitis virus, MHV-S (ATCC VR-766™), was used to inoculate NCTC Clone 1469 cells at a moi of about 1.0 following published procedures (Leibowitz et al., 2011). Virus was harvested after 48 hours as per Leibowitz et al. 2011). Isolated virus was stored at -80ºC in 1.0 ml

aliquots. Virus titer was determined using the endpoint dilution procedure to obtain the $TCID_{50}$ on the NCTC Clone 1469 cells.

**3.2** Surface Test Protocol

3.2.1 Disc treatment

Stainless steel alloy 304 discs, 0.5 in diameter, 16 ga thickness, with #4 grained finish from Metal remnants, Inc., Salt lake City, UT were rinsed in 70% alcohol to remove surface oil and then autoclaved. For each time point a disc was placed on a glass plate in a sterile hood and surface sanitizer was applied to it using the sprayer provided by the manufacturer at a 45$^{o}$ angle from 8 inches away as per the use instructions. The sanitizer was allowed to dry on the disc in the hood for about 60 minutes. The disc was then placed into a well of a sterile 24 well plate until the assay date.

3.2.2 Virucidal Assay

On 7/20/20 NCTC Clone 1469 cells were plated at 5 x $10^4$cells per well in a 96 well plate in DMEM + 10% horse serum as above and incubated for 24 hours at 37$^{o}$C and 5% $CO_2$. On 7/21/20 50 µl of stock virus was pipetted onto one disc for each time point as well as one untreated disc. An extra disc treated for one day was not inoculated with virus to be used for the cytotoxicity control. The contact time for virus on the disc was 10 minutes at 24$^{o}$C. Each disc was then dropped into an ice cold tube to which had previously been added 1.0 ml of BBP++ neutralizer (Butterfield's buffered phosphate + surfactants) and 150 mg of glass disruption beads, 0.1mm diameter, Research Products International, which had been washed, dried and autoclaved as per manufacturer's instructions. Tubes were placed back on ice and then each was vortexed at the number 1 vortex speed for 30s. 500µl from the liquid in each tube was layered onto a 3.0 ml packed Sephadex LH-20 column and centrifuged at 4$^{o}$C at the top setting of an IEC clinical centrifuge. Serial 10-fold dilutions in DMEM + 2% Horse serum (DMEM-2) were made with the eluate of each column. Media was removed from the NCTC clone 1469 cells in the 96 well plates and 100 µl of each dilution was added to quadruplicate wells. Control wells received only fresh medium. The plates were then incubated at 37$^{o}$C and 5% $CO_2$ for 2 hrs. The media was removed and 100 µl of DMEM-2 was added and then removed from each well as a wash. Fresh 100µl of DMEM-2 was then added to each well and the plates were incubated for 6 days. Plates were scored for cytopathological effects (CPE) using a Zeiss inverted microscope.

**4.0 Results – Test done on 7/21; Plates scored on 7/27**

| Dilution ($Log_{10}$) | Virus control | Test Product – Days on disc | | | | | Cytotoxic ity (1 day on disc) | Neutralizer Control |
|---|---|---|---|---|---|---|---|---|
| | | 1 | 5 | 7 | 14 | 30 | | |
| -2 | ++++ | CT | CT | CT | CT | CT | ++++ | ++++ |
| -3 | ++++ | ++++ | ++++ | 0000 | 0000 | 0000 | 00++ | ++++ |
| -4 | ++++ | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 | ++++ |
| -5 | ++++ | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 | 00++ |
| -6 | ++++ | 0000 | 0000 | 0000 | 0000 | 0000 | | |
| -7 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 | | |
| $Log_{10}$ $TCID_{50}$ | 6.5 | ≤ 3.5 | ≤ 3.5 | ≤2.5 | ≤ 2.5 | ≤ 2.5 | 3.0 | 5.0 |
| $Log_{10}$ Reduction | | ≥3.0 | ≥3.0 | ≥ 4.0 | ≥ 4.0 | ≥4.0 | NA | NA |
| % kill | | ≥99.9 | ≥99.9 | ≥99.99 | ≥99.99 | ≥99.99 | NA | NA |

| | |
|---|---|
| + | CPE (cytopathic/cytotoxic effect) present per well |
| **0** | CPE (cytopathic/cytotoxic effect) not detected |
| **N/A** | Not applicable |
| **CT** | Cytotoxicity |

A neutralizer cytotoxicity control, run separately, showed no CPE at any dilutions of the BBP++. Formaldehyde killed all cells to the $10^{-4}$ dilution.

**5.0 Summary**

The untreated virus $TCID_{50}$ (log 10) was determined to be 6.5
The virus $TCID_{50}$ (log 10) after 1 and 5 days was at least 3.5.
The virus $TCID_{50}$ (log 10) after 7, 14 and 30 days was at least 4.5.

**In summary, the Core-19 X30 Surface Disinfectant remained active on stainless steel coupons with a confirmed 3 log reduction (99.9%) kill percentage against the SARS-CoV-2 virus up to 5 days after initial application, and a confirmed 4 log reduction (99.99%) against the SARS-CoV-2 virus from 7 to 30 days after the initial application.**

References:

Leibowitz, J., Kaufman, G and Liu, P. *Coronaviruses: Propagation, Quantification, Storage and Construction of Recombinant Mouse Hepatitis Virus.* Current Protocols in Microbiology; John Wiley and Sons, Wiley Online Library; May, 2011, Supplement 21, CH 15.