Virucidal Assay

Sponsor:	Immunetec Proof Ltd.
Sponsor Contact:	Zsofia Hornok
Report Date:	March 4, 2021
Viruses Tested:	SARS-CoV-2
Compounds Tested:	Immunetec Antimicrobial Hand and Skin Care Cream (Silver-ion hand sanitizer)
Contact Time:	10 minutes, room temperature
Experiment #:	SARS2-438

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Procedure

Virus, Media, and Cells

SARS-CoV-2 virus stock was prepared by growing virus in Vero 76 cells. Test media used was MEM supplemented with 2% FBS and 50 g/mL gentamicin.

Virucidal Assay

Silver-ion hand sanitizer was received from the sponsor as a solution and was tested at full strength. SARS-CoV-2 virus solution was added to triplicate wells containing sample so that there was 10% virus solution by volume and 90% prepared sample. Media only was added to one tube of each prepared concentration to serve as toxicity controls. Ethanol was tested in parallel as a positive control and water only to serve as the virus control.

Compound and virus were incubated at room temperature for 10 minutes. Following the contact period, the solutions were neutralized by a 1/10 dilution in test media.

Virus Quantification.

Surviving virus was quantified by standard end-point dilution assay. Neutralized samples were combined for quantification for the average of triplicate tests. Samples were serially diluted using eight 10-fold dilutions in test medium. Each dilution was added to 4 wells of a 96-well plate with 60-100% confluent cells. The toxicity controls were added to an additional 4 wells and 2 of these wells were infected with virus to serve as neutralization controls, ensuring that residual sample in the titer assay plated did not inhibit growth and detection of surviving virus.

Plates were incubated at 37 +/-2 C with 5% CO_2. On day 5 post-infection plates were scored for presence or absence of viral cytopathic effect (CPE). The Reed-Muench method was used to determine end-point titers (50% cell culture infectious dose, $CCID_{50}$) of the samples, and the log reduction value (LRV) of the compound compared to the negative (water) control was calculated.

Controls

Virus controls were tested in water and the reduction of virus in test wells compared to virus controls was calculated as the log reduction value (LRV). Toxicity controls were tested with media not containing virus to see if the samples were toxic to cells. Neutralization controls were tested to ensure that virus inactivation did not continue after the specified contact time, and that residual sample in the titer assay plates did not inhibit growth and detection of surviving virus. This was done by adding toxicity samples to titer test plates then spiking each

well with a low amount of virus that would produce an observable amount of CPE during the incubation period.

Results

Virus titer and log reduction value (LRV) for samples tested against SARS-CoV-2 are shown in Table 1. The average virus control titer was 5.2 or 4.7 log CCID50 per 0.1 mL, respectively, and this was used for comparison of all test sample titers to determine LRV. Samples with <1 log reduction are not considered active for virucidal activity.

The limit of detection of virus for samples that did not exhibit cytotoxicity when plated for endpoint dilution assay was 0.7 log CCID50 per 0.1 mL. When >80% cytotoxicity was observed in wells of diluted samples, presence of virus could not be ruled out and therefore the limit of detection was altered. With the Silver-ion hand sanitizer sample, cytotoxicity was seen in the 1/100 dilution and therefore the limit of detection was 2.7 logs.

Silver-ion hand sanitizer exhibited virucidal activity against SARS-CoV-2, an enveloped virus, reducing virus titer below the limit of detection (LRV>2.0, >99%).

Neutralization controls demonstrated that residual sample did not inhibit virus growth and detection in the endpoint titer assays in wells that did not have cytotoxicity. Positive controls performed as expected.

Table 1. Virucidal activity against SARS-CoV-2 after incubation with virus at 22 +/-2 C.

Compound	Concentration	Contact Time	Toxicity[a]	Neut. Control[b]	Virus Titer[c]	VC Titer[c]	LRV[d]
Silver-ion hand sanitizer	100%	10 min	1/100	None	<2.7	4.7	>2.0
Ethanol	70%	10 min	None	None	<0.7	4.7	>4.0

[a] Cytotoxicity indicates the highest dilution of the endpoint titer where full (80-100%) cytotoxicity was observed

[b] Neutralization control indicates the highest dilution of the endpoint titer where compound inhibited virus CPE in wells after neutralization (ignored for calculation of virus titer and LRV)

[c] Virus titer of test sample or virus control (VC) in $\log_{10}$ $CCID_{50}$ of virus per 0.1 mL

[d] LRV (log reduction value) is the reduction of virus in test sample compared to the virus control