Virucidal Assay against SARS-CoV-2

Sponsor:	NanoTech Solutions Norway AS
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Viruses Tested:	SARS-CoV-2,
Cell Line:	USA-WA1/2020 Vero 76
Compounds Tested:	VitaCoat
Experiment #:	SARS2-246

Study Director:

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Procedure

Virus, media and cells.

SARS-CoV-2, USA-WA1/2020 strain, virus stock was prepared prior to testing by growing in Vero 76 cells. Culture media for prepared stock (test media) was MEM with 2% fetal bovine serum and 50 μg/mL gentamicin.

Virucidal Assay.

VitaCoat was received from sponsor as solution and tested at full strength. Sample was mixed directly with virus solution at a volume ratio of 90% prepared compound and 10% virus solution. Each concentration was tested in triplicate. Test media was added to one tube of each prepared concentration to serve as toxicity controls. Ethanol (70%) was tested in parallel as a positive control and water only as a virus control.

Solution and virus were incubated at room temperature for 1 minute. The solutions were then neutralized by a 1/10 dilution in test media.

Virus Quantification.

Surviving virus was quantified by standard end-point dilution assay. Neutralized samples were pooled for quantification 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 80-100% confluent Vero 76 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 6 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 titers and LRV for VitaCoat against SARS-CoV-2 are shown in Table 1. The average of virus control samples was used for comparison of test samples and contained 4.0 log CCID50 per 0.1 mL. Samples with <1 log reduction of virus compared to the virus control were considered not active for virucidal activity.

Full toxicity was observed in the top dilution of VitaCoat (1/10). Because of this toxicity, presence of virus could not be ruled out and therefore the limit of detection was 1.7 $\log_{10}$ $CCID_{50}$ of virus per 0.1 mL. When in contact with SARS-CoV-2 for 1 minute, VitaCoat demonstrated virucidal activity, reducing virus titer below the limit of detection (LRV>2.3, >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. Virus controls and positive controls performed as expected.

Table 1. Virucidal efficacy against SARS-CoV-2 after a 1-minute contact time with virus at 22 ± 2°C.

Compound	Concentration	Virus Titer[a]	LRV[b]	Cytotoxicity[c]
VitaCoat	100%	<1.7	>2.3	1/10
Ethanol	70%	<1.7	>2.3	
Virus Control	n/a	4.0	-	none

[a] Log_{10} $CCID_{50}$ of virus per 0.1 mL

[b] LRV (log reduction value) is the reduction of virus compared to the virus control

[c] Highest dilution of test sample where full cytotoxicity was observed in endpoint dilutions

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