Virucidal Activity of Xlear compounds vs SARS-CoV-2 Virus and Rhinovirus-16

Sponsor	XLEAR/Spry
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Viruses Tested:	SARS-CoV-2, Rhinovirus-16
Cell Line:	Vero 76, HeLa
Incubation:	15 minutes, room temperature
Experiment #:	SARS2-113, RV-0085

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Procedure

SARS-CoV-2, USA-WA1/2020 strain, virus stock was prepared prior to testing by growing 2 passages in Vero 76 cells. Culture media for prepared stock (test media) was MEM with 2% fetal bovine serum (FBS) and 50 µg/mL gentamicin. Human rhinovirus 16, strain 11757 purchased from ATCC, was grown in 3 passages of HeLa cells in MEM with 2% fetal bovine serum (FBS), 25 mM $MgCl_2$ and 50 µg/mL gentamicin. Test media is the growth media with 5% FBS.

Virucidal Assay.

Test compounds were received from the sponsor in liquid form and stored at room temperature. The test compound 11% xylitol in saline was diluted 1:2 with water prior to testing. Each solution was mixed directly with virus stock so that the final concentration was 90% of each individual test compound and 10% virus stock. A single concentration was tested in triplicate. Test media without virus was added to duplicate tubes of the compounds to serve as toxicity and neutralization controls. Ethanol (90%) was tested in parallel as a positive control and water only as a virus control.

The test solutions were incubated at room temperature (22 ± 2°C) for 15 minutes with SARS-CoV-2 or Rhinovirus-16. The solutions were then neutralized by a 1/10 dilution in the test media of each specific virus.

Virus Quantification.

Surviving virus from each sample was quantified by standard end-point dilution assay. Briefly, the neutralized samples were pooled and serially diluted using eight log dilutions in test medium. Then 100 µL of each dilution was plated into quadruplicate wells of 96-well plates containing 80-90% confluent Vero 76 (SARS-CoV-2) or HeLa cells (Rhino-16). The toxicity controls were added to an additional 4 wells of Vero 76 or HeLa cells and 2 of those wells at each dilution were infected with virus to serve as neutralization controls, ensuring that residual sample in the titer assay plate did not inhibit growth and detection of surviving virus. Plates were incubated at 37 ± 2°C with 5% CO_2 for 5 days for the SARS-CoV-2 assay and at 33 ± 2°C with 5% CO_2 for 4 days for the Rhinovirus-16 assay. Each well was then scored for presence or absence of infectious virus. The titers were measured using a standard endpoint dilution 50% cell culture infectious dose ($CCID_{50}$) assay calculated using the Reed-Muench (1948) equation and the log reduction value (LRV) of each compound compared to the negative (water) control was calculated.

Results

Virus titers and LRV of Rhinovirus-16 and SARS-CoV-2 when incubated with a single concentration of the Xlear solutions are shown in **Table 1**. After a 15-minute contact time, the Xlear Nasal Decongestant was effective at reducing infectious Rhino-16 virus. When tested against SARS-CoV-2, the test compound GSE 0.2% was the only compound effective at reducing >3 log_{10} $CCID_{50}$ infectious virus from, 3.67 log_{10} $CCID_{50}$/0.1 mL to an undetectable amount of infectious virus. The Xlear Nasal Decongestant and the GSE 0.2% had some toxicity in the top rows (1/10 dilution of the test sample) which may have contributed to the virucidal effect of the GSE. The 11% xylitol and 11% erythritol had no cytotoxicity. The positive control and neutralization controls performed as expected.

Table 1. Virucidal efficacy of Xlear compounds against Rhinovirus-16 and SARS-CoV-2 after a 15 minute incubation with virus at 22 ± 2°C.

	Tested Concentration	Virus Tested	Incubation Time	Virus Titer[a]	LRV[b]
Xlear Nasal Decongestant	90%	Rhino-16	15-minute	5.0	0
Ethanol	90%	Rhino-16	15-minute	1.5	3.17
Virus Control	na	Rhino-16	15-minute	4.67	na
Xlear Nasal Decongestant	90%	SARS-CoV-2	15-minute	3.0	0.67
GSE 0.2% in DI water	90%	SARS-CoV-2	15-minute	<0.67	3.0
Saline w/ 11% Xylitol	90%	SARS-CoV-2	15-minute	3.5	0.17
Saline w/ 11% Erythritol	90%	SARS-CoV-2	15-minute	4.3	0
Ethanol	90%	SARS-CoV-2	15-minute	<0.67	3.0
Virus Control	na	SARS-CoV-2	15-minute	3.67	na

[a] Log_{10} $CCID_{50}$ of virus per 0.1 mL. The assay lower limit of detection is 0.67 Log_{10} $CCID_{50}$/0.1 mL.
[b] LRV (log reduction value) is the reduction of virus compared to the virus control

Table 2. Virucidal efficacy of Xlear compounds against Rhinovirus-16 after a 20 minute incubation with virus at 22 ± 2°C.

	Tested Concentration	Incubation Time	Virus Titer[a]	LRV[b]
Xlear Nasal Spray	90%	20-minute	5.0	0
Xlear Rescue Spray	90%	20-minute	4.67	0.33
XRS +50 ppm silver	90%	20-minute	5.0	0
XyChlor Spray, pH 6.1	90%	20-minute	5.0	0
Chlorhexidine 0.03%	90%	20-minute	4.67	0.33
Chlorhexidine 0.015%	90%	20-minute	4.5	0.5
Ethanol	63%	20-minute	2.0	3.0
Virus Control	na	20-minute	5.0	na

[a] Log_{10} $CCID_{50}$ of virus per mL. The assay lower limit of detection is 0.67 Log_{10} $CCID_{50}$/mL.
[b] LRV (log reduction value) is the reduction of virus compared to the virus control