

Virucidal Assay

Sponsor: CofixRX
Sponsor Contact: Greg Shockley
Report Date: June 21, 2021
Viruses Tested: SARS-CoV-2
Influenza B
HRV-14
RSV
Compounds Tested: CofixRX Nasal Spray
Contact Time: 45 seconds
Experiment #: SARS2-526
FLU-1423
RV-107
RSV-415

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Procedure

Virus, Media, and Cells

SARS-CoV-2 virus stock was prepared by passaging virus in Vero 76 cells. Test media used was MEM supplemented with 2% FBS and 50 µg/mL gentamicin. Influenza B/Brisbane/60/2008 (Victoria) was passaged in MDCK cells and test media was MEM with 10 U/mL trypsin, 1 µg/mL EDTA, and gentamicin. Human rhinovirus-14 (HRV-14) was passaged in HeLa Ohio cells with test media of MEM with 5% FBS, 25 mM MgCl₂, and gentamicin. Respiratory syncytial virus (RSV) was passaged in MA-104 cell and test media was MEM with 5% FBS and gentamicin.

Virucidal Assay

CofixRX Nasal Spray was received from the sponsor as a solution and was tested in triplicate at full strength against each of the four viruses. Virus stock was added to triplicate tubes so that there was 10% virus solution by volume and 90% CofixRX 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 a single contact time of 45 seconds. 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 80-100% 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% CO₂. On day 5-7 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, CCID₅₀) 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 four viruses are shown in Tables 1-4. The virus control titer for each virus was used for comparison of test sample titers to determine LRV. Samples with <1 log reduction are not considered active for virucidal activity.

CofixRX Nasal Spray exhibited virucidal activity against SARS-CoV-2 (enveloped) and HRV-14 (non-enveloped), though it did not reduce virus below the limit of detection (LRV=2.9±0.4 and LRV=1.2±0.2, respectively; Tables 1 and 3).

CofixRX Nasal Spray reduced influenza B and RSV titers below the limit of detection (LRV>5.0 and LRV>2.0, respectively; Tables 2 and 4).

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. Ethanol as a positive control performed as expected, noting that it is not consistently active against the non-enveloped HRV-14.

Table 1. Virucidal activity against SARS-CoV-2 after incubation with virus at $22 \pm 2^\circ\text{C}$.

Compound	Concentration	Contact Time	Toxicity ^a	Neut. Control ^b	Virus Titer ^c	VC Titer ^c	LRV ^d
CofixRX Nasal Spray	100%	45 seconds	1/10	None	2.0	5.0	3.0
CofixRX Nasal Spray	100%	45 seconds	1/10	None	1.7	5.0	3.3
CofixRX Nasal Spray	100%	45 seconds	1/10	None	2.5	5.0	2.5
Ethanol	70%	45 seconds	None	None	<0.7	5.0	>4.3

^a Cytotoxicity indicates the highest dilution of the endpoint titer where full (>80%) 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} CCID₅₀ of virus per 0.1 mL

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

Table 2. Virucidal activity against influenza B virus after incubation with virus at $22 \pm 2^\circ\text{C}$.

Compound	Concentration	Contact Time	Toxicity ^a	Neut. Control ^b	Virus Titer ^c	VC Titer ^c	LRV ^d
CofixRX Nasal Spray	100%	45 seconds	None	None	<0.7	5.7	>5.0
CofixRX Nasal Spray	100%	45 seconds	None	None	<0.7	5.7	>5.0
CofixRX Nasal Spray	100%	45 seconds	None	None	<0.7	5.7	>5.0
Ethanol	70%	45 seconds	None	None	<0.7	5.7	>5.0

^a Cytotoxicity indicates the highest dilution of the endpoint titer where full (>80%) 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} CCID₅₀ of virus per 0.1 mL

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

Table 3. Virucidal activity against HRV-14 after incubation with virus at $22 \pm 2^\circ\text{C}$.

Compound	Concentration	Contact Time	Toxicity ^a	Neut. Control ^b	Virus Titer ^c	VC Titer ^c	LRV ^d
CofixRX Nasal Spray	100%	45 seconds	None	None	3.7	5.0	1.3
CofixRX Nasal Spray	100%	45 seconds	None	None	4.0	5.0	1.0
CofixRX Nasal Spray	100%	45 seconds	None	None	3.7	5.0	1.3
Ethanol	70%	45 seconds	None	None	5.3	5.0	0

^a Cytotoxicity indicates the highest dilution of the endpoint titer where full (>80%) 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} CCID₅₀ of virus per 0.1 mL

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

Table 4. Virucidal activity against RSV after incubation with virus at $22 \pm 2^\circ\text{C}$.

Compound	Concentration	Contact Time	Toxicity ^a	Neut. Control ^b	Virus Titer ^c	VC Titer ^c	LRV ^d
CofixRX Nasal Spray	100%	45 seconds	None	None	<0.7	2.7	>2.0
CofixRX Nasal Spray	100%	45 seconds	None	None	<0.7	2.7	>2.0
CofixRX Nasal Spray	100%	45 seconds	None	None	<0.7	2.7	>2.0
Ethanol	70%	45 seconds	None	None	<0.7	2.7	>2.0

^a Cytotoxicity indicates the highest dilution of the endpoint titer where full (>80%) 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} CCID₅₀ of virus per 0.1 mL

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