Virucidal Assay against Common Respiratory Viruses

Sponsor:	NanoTech Solutions Norway AS	
Sponsor Contact:	Christian Sauvageau	
Report Date:	October 2,	
Viruses Tested:	2020 HCoV-229E	Influenza A(H1N1)pdm09
	HCoV-OC43	Influenza A(H3N2)
	PIV-3	Influenza A(H5N1)
	HRV-14	Adenovirus-5
	HRV-16	
Cell Line:	Vero 76	
Compounds Tested:	NanoSanis (3D Microbiota Surface Barrier Spray)	
Experiment #:	HCOV-068	FLU-1373
	PIV-167	Flu-Hi-104
	RV-091	ADV-215
	FLU-1372	

Study Director:

Michelle Mendenhall
Utah State University, Institute for Antiviral Research
5600 Old Main Hill
Logan, UT 84322
michelle.mendenhall@usu.edu

Procedure

Viruses, media and cells.

Virus stocks were prepared prior to testing by growing hCoV-229E virus stock in Huh7 cells, hCoV-OC43 in RD cell, parainfluenza virus-3 (PIV-3) in MA-104 cells, human rhinoviruses (HRV-14 and -16) in HeLa Ohio cells, influenza viruses (A(H1N1)pdm09, and A(H5N1)) in MDCK cells, adenovirus-5 (Ad5) in A549 cells. Culture test media was MEM with 5% fetal bovine serum and 50 μg/mL gentamicin for hCoV viruses, PIV-3, and HRV viruses. For influenza viruses, test media was MEM with 10 u/mL trypsin, 1 μg/mL EDTA and gentamicin. And for Ad5, tes media was DMEM with 2% FBS and gentamicin.

Virucidal Assay.

NanoSanis (3D Microbiota Surface Barrier) was received from sponsor as a solution and tested at full strength. Compounds was mixed directly with virus solution in three tubes at a volume ratio of 90% prepared compound and 10% virus solution. Test media only 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 (3 wells pooled) 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 3-6 post-infection once virus reached maximum CPE, 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 log reduction value (LRV) for NanoSanis against several respiratory viruses when in contact for 1 minute are shown in Table 1. The average of virus control samples were used for comparison of test samples to determine the LRV. Samples with <1 log reduction of virus compared to the virus control were considered not active for virucidal activity. Virus control titers varied by virus as indicated in Tables 1-3. LRV can be converted to a percentage of virus reduction as follows: LRV=1, 90%; LRV=2, 99%; LRV=3, 99.9%; LRV=4, 99.99%; etc.

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. For instance, when cytotoxicity was seen in the 1/10 dilution the limit of detection was 1.7 logs, in 1/100 it was 2.7 logs, and so forth.

NanoSanis exhibited cytotoxicity in some cell types and the limit of detection was accordingly either 0.7 or 1.7 log CCID50 per 0.1 mL. When in contact with virus for 1 minute, NanoSanis demonstrated virucidal activity against enveloped viruses, reducing virus below the limit of detection and LRV>1 for all enveloped viruses tested (exact LRV values in Table 1). NanoSanis did not inactivate non-enveloped viruses (HRV and adenovirus).

Positive controls performed as expected (Table 2). Ethanol is not as effective against non-enveloped viruses (HRV viruses and Ad5).

Table 1. Virucidal efficacy of NanoSanis against a panel of respiratory viruses after a 1-minute contact time with virus at 22 ± 2°C.

Virus	Cell Type	Test Concentration	Cytotoxicity[a]	Neutralization Control[b]	Virus Titer[c]	VC Titer[c]	LRV[d]
hCoV-229E	Huh7	100%	1/10	None	<1.7	3.0	>1.3
hCoV-OC43	RD	100%	1/10	None	<1.7	3.3	>1.6
PIV-3	MA-104	100%	None	None	<0.7	2.5	>1.8
HRV-14	HeLa	100%	1/10	None	4.7	5.0	0.3
HRV-16	HeLa	100%	1/10	None	5.3	5.7	0.4
Flu A(H1N1)pdm09	MDCK	100%	None	None	<0.7	4.7	>4.0
Flu A(H5N1)	MDCK	100%	1/10	None	<1.7	2.7	>1.0
Adenovirus-5	A549	100%	None	None	6.5	6.5	0

[a] Cytotoxocity indicates the highest dilution in endpoint virus titers where full (80-100%) cytotoxicity was observed

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

[c] 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

5600 Old Main Hill Logan, UT 84322 PH: (435) 797-4134 e-mail: michelle.mendenhall@usu.edu www.usu.edu/iar

Table 2. Virucidal efficacy of ethanol as a positive control against a panel of respiratory viruses after a 1-minute contact time with virus at 22 ± 2°C.

Virus	Cell Type	Test Concentration	Cytotoxicity[a]	Neutralization Control[b]	Virus Titer[c]	VC Titer[c]	LRV[d]
hCoV-229E	Huh7	70%	None	None	<0.7	3.0	>2.3
hCoV-OC43	RD	70%	None	None	<0.7	3.3	>2.6
PIV-3	MA-104	70%	None	None	<0.7	2.5	>1.8
HRV-14	HeLa	70%	None	None	3.5	5.0	1.5
HRV-16	HeLa	70%	None	None	2.5	5.7	3.2
Flu A(H1N1)pdm09	MDCK	70%	None	None	<0.7	4.7	>4.0
Flu A(H5N1)	MDCK	70%	1/10	None	<1.7	2.7	>1.0
Adenovirus-5	A549	70%	None	None	2.5	6.5	4.0

[a] Cytotoxocity indicates the highest dilution in endpoint virus titers where full (80-100%) cytotoxicity was observed
[b] Neutralization control indicates the highest dilution in endpoint virus titers where compound inhibited virus CPE in wells following neutralization (ignored for calculation of virus titer and LRV)
[c] 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