Virucidal Activity of Puriton vs hCoV-OC43 Virus

Sponsor	UCI Medical Center
Sponsor Contact:	Jai Kim
Report Date:	March 17, 2020
Viruses Tested:	hCoV-OC43
Cell Line:	RD
Incubation:	1 hour room temperature 6 hours room temperature
Compounds Tested:	Puriton
Experiment #:	HCoV-027

Study Director:

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

Procedure

Human coronavirus (hCoV-OC43) stocks were previously prepared in MEM with 2% FBS and 50 μg/mL gentamicin.

Test compound was received from the sponsor in liquid form. The compound was tested at concentrations of 90% and 70% by adding virus stock directly to sample in triplicate tubes of each for each concentration. Media only was added to one tube of each prepared concentration to serve as toxicity and neutralization controls. Ethanol (70%) was tested in parallel as a positive control and water only to serve as the virus control.

Solution and virus were incubated at room temperature for 1 hour and 6 hours. The solution was then neutralized by a 1/10 dilution in culture media (MEM+5% FBS+50 μg/mL gentamicin) to each sample. Neutralized samples were serially diluted using eight log dilutions in test medium. Each dilution was added to 4 wells of a 96-well plate with 80-100% confluent RD cells. The toxicity controls were added to an additional 3 wells and infected with virus (50 CCID50) to serve as neutralization controls, ensuring that residual sample in the titer assay plated did not inhibit growth and detection of surviving virus. All plates were incubated at 37±2°C, 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.

Results

Virus titers and LRV for Puriton against hCoV-OC43 are shown in Table 1. Virus in control samples was between 2.4 - 2.8 $\log_{10}$ $CCID_{50}$, limiting our detection in reduction of virus to 1.7 - 2.1 $\log_{10}$ $CCID_{50}$.

Some toxicity was observed in the 1/10 dilution in both 70% and 90% Puriton samples, but it did not interfere with detection of virus.

Puriton was an effective virucidal after a 1-hour and 6-hour incubation against hCoV-OC43, reducing virus by 1.7-2.1 $\log_{10}$ $CCID_{50}$. Positive control and neutralization controls performed as expected.

Table 1. Virucidal efficacy of Puriton against hCoV-OC43 after 1-hour and 6-hour incubation with virus at 22 ± 2°C.

	[a]$CCID_{50}$/mL	[b]LRV
90% Virus Control, 1-hour	2.4	N/A
90% Puriton, 1-hour	<0.7***	1.7
70% Virus Control, 1-hour	2.8	N/A
70% Puriton, 1-hour	<0.7***	2.1
EtOH, 1-hour	<0.7***	2.1
Virus Control, 6-hour	2.7	N/A
90% Puriton, 6-hour	<0.7***	2.0
70% Virus Control, 6-hour	2.5	N/A
70% Puriton, 6-hour	<0.7***	1.8
EtOH, 6-hour	<0.7***	1.8

[a] Log_{10} $CCID_{50}$ of virus per mL, average of 3 replicates ± standard deviation

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

***P < 0.001 by one-way ANOVA and Dunnett post-test compared with untreated virus control (water). For statistical analysis "<" signs were ignored.

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